

EFFECT OF COTRIMOXAZOLE PROPHYLAXIS TAKEN BY HUMAN IMMUNODEFICIENCY VIRUS (HIV)–INFECTED PERSONS ON THE SELECTION OF SULFADOXINE-PYRIMETHAMINE–RESISTANT MALARIA PARASITES AMONG HIV-UNINFECTED HOUSEHOLD MEMBERS

SAMUEL S. MALAMBA,* JONATHAN MERMIN, ARTHUR REINGOLD, JOHN R. LULE, ROBERT DOWNING, RAY RANSOM, AMINAH KIGOZI, BEN M. HUNT, ALAN HUBBARD, PHILIP J. ROSENTHAL, AND GRANT DORSEY
Centers for Disease Control and Prevention–Uganda, Global AIDS Program, National Center for HIV, STD and TB Prevention, Entebbe, Uganda; Division of Epidemiology, School of Public Health, University of California, Berkeley, California; Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California

Abstract. The purpose of this prospective cohort study was to assess the effect of cotrimoxazole prophylaxis taken by human immunodeficiency virus (HIV)–infected persons on the selection of sulfadoxine-pyrimethamine (SP)–resistant malaria parasites among HIV-uninfected household members. A total of 2,567 HIV-uninfected persons from 605 households were followed and blood specimens were collected each time an episode of *Plasmodium falciparum* malaria was diagnosed. Study participants were living in households where HIV-infected persons were either taking (exposed) or not taking (unexposed) cotrimoxazole prophylaxis. From all malaria episodes diagnosed, 50% of the specimens were randomly selected and tested for the presence of five key mutations known to mediate resistance to SP (dihydrofolate reductase [*dhfr*] Asn-108, Ile-51, and Arg-59, and dihydropteroate synthase [*dhps*] Gly-437 and Glu-540). *Plasmodium falciparum* isolates were recovered from 163 specimens in the exposed households and 113 specimens in the unexposed households, with similar proportions containing the *dhfr* triple mutant (37% versus 45%; $P = 0.18$), the *dhps* double mutant (64% versus 62%; $P = 0.81$), and the *dhfr/dhps* quintuple mutant (30% versus 32%; $P = 0.74$). The HIV-uninfected persons living with HIV-infected household members taking cotrimoxazole prophylaxis had a lower incidence of malaria (incidence rate ratio [IRR] = 0.64, 95% confidence interval [CI] = 0.50–0.83, $P = 0.001$) and fewer malaria episodes due to parasites containing the *dhfr/dhps* quintuple mutant (IRR = 0.61, 95% CI = 0.41–0.91, $P = 0.014$). Cotrimoxazole prophylaxis taken by HIV-infected persons did not select for SP-resistant malaria parasites among HIV-uninfected household members, and was associated with a lower overall incidence of SP-resistant malaria among household members.

INTRODUCTION

Cotrimoxazole (trimethoprim-sulfamethoxazole) prophylaxis reduces morbidity and mortality among persons infected with human immunodeficiency virus (HIV).^{1–4} In 2000, the World Health Organization (WHO) and the United Nations program on HIV/AIDS recommended the use of cotrimoxazole prophylaxis for HIV-infected patients in Africa with symptomatic disease or CD4 cell counts < 500 cells/ μ L.⁵ However, there has been concern that the widespread use of cotrimoxazole for prophylaxis might accelerate the spread of resistance to sulfadoxine-pyrimethamine (SP), another antifolate combination that is one of the most widely available and affordable antimalarials in Africa.^{6,7} Sulfadoxine-pyrimethamine used alone or in combination is the first-line treatment of uncomplicated malaria in several African countries and is currently the only recommended drug for the prevention of malaria in pregnancy.⁸

Resistance to SP among *Plasmodium falciparum* is mediated by point mutations that accumulate at several sites in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes, resulting in increasing levels of drug resistance *in vitro*.⁹ In Africa, clinical failure after treatment with SP either alone or in combination has been associated with the *dhfr* triple mutant (Asn-108 + Ile-51 + Arg-59) and the *dhps*

double mutant (Gly-437 + Glu-540).¹⁰ Laboratory studies have shown that *P. falciparum* isolates with the *dhfr* triple mutant have high-level resistance to both trimethoprim and pyrimethamine.¹¹ However, there are few clinical studies evaluating whether cotrimoxazole leads to the selection of parasites resistant to SP. It has been argued that the clinical benefits of cotrimoxazole prophylaxis among HIV-infected patients outweigh potential risks related to the selection of SP resistant parasites.¹² However, concerns that cotrimoxazole use by HIV-infected patients might increase the spread of SP-resistant parasites in the community have delayed implementation of prophylaxis guidelines.

To evaluate the potential association between taking cotrimoxazole prophylaxis and the spread of SP-resistant malaria, we analyzed data collected during a study of daily cotrimoxazole prophylaxis among a cohort of HIV-infected persons living in rural Uganda.³ During the study, all HIV-infected and HIV-uninfected household members were followed-up with weekly home visits to detect clinical malaria, and blood specimens were collected on filter paper from each episode of possible malaria for resistance testing. We evaluated the hypothesis that HIV-uninfected persons residing in households where HIV-infected household members were taking cotrimoxazole prophylaxis would have a higher risk of developing malaria caused by parasites containing molecular markers of SP resistance than those in households where HIV-infected household members were not taking cotrimoxazole.

METHODS

Study participants and clinical study. The study was reviewed and approved by the Science and Ethics Committee of

* Address correspondence to Samuel S. Malamba, Division of Epidemiology, School of Public Health, University of California, 1918 University Avenue, Fourth Floor, Berkeley, CA 94720 and Centers for Disease Control and Prevention–Uganda, Global AIDS Program, National Center for HIV, STD and TB Prevention, c/o Uganda Virus Research Institute, PO Box 49, Entebbe, Uganda. E-mails: malambas@berkeley.edu and zqc2@ug.cdc.gov

the Uganda Virus Research Institute, the Uganda National Council of Science and Technology, and the Institutional Review Board of the CDC.

The cohort and study methods have been described previously, and are abbreviated below.³ During two time periods, between April and May 2001 and between January and April 2002, we enrolled HIV-infected clients of The AIDS Support Organization (TASO) and their household members in Tororo District, eastern Uganda, in a prospective cohort study. After written informed consent was provided, study staff visited clients' homes to conduct a census, obtain informed consent from household members, and administer an individual, standardized questionnaire. A household was defined as persons who shared their meals and slept in the same house or cluster of houses for at least five days of the week for ≥ 3 months before the baseline survey. Households enrolled in 2001 were followed-up for a period of approximately five months before HIV-infected participants were given treatment with daily cotrimoxazole (adults, 160 mg of trimethoprim/800 mg of sulfamethoxazole; children between 5–12 years of age, 80 mg of trimethoprim/400 mg of sulfamethoxazole; and children less than five years of age, syrup adjusted to weight). To increase sample size, a second group of households were enrolled in 2002 using identical inclusion criteria and consent procedures. Neither consent for HIV testing nor receiving test results were requirements for enrollment in the study, but only persons for whom HIV test results were known were included in analyses. In this study, we used the time period when HIV-infected participants from the first enrollment period were taking cotrimoxazole and HIV-infected participants from the second enrollment period were not yet taking cotrimoxazole (Figure 1). This overlap period allowed us to compare two parallel populations controlling for any temporal trends.

Households were visited weekly for one year by study staff who administered a questionnaire to all household members about their seven-day medical history. In the case of reported fever, thick and thin blood smears were made and blood was collected on filter paper. An episode of malaria was defined as a history of fever in the previous seven days and Plasmodia detected by thick blood smear. The HIV-uninfected study participants were not using cotrimoxazole prophylaxis and

were asked not to take the cotrimoxazole given to their HIV-infected household members. First-line treatment of uncomplicated malaria in both HIV-infected and HIV-uninfected participants was SP and chloroquine per Uganda Ministry of Health policy guidelines. Severe illness requiring hospitalization was treated with quinine.

Given that 113 malaria cases in the exposed group and 163 malaria cases in the unexposed group were available for resistance testing, we estimated that with 80% power, an alpha error of 0.05 and the prevalence of either *dhps* double mutant (Gly-437 + Glu-540) or *dhfr* triple mutant (Asn-108 + Ile-51 + Arg-59) of 0.4, we would be able to detect a difference in prevalence $\geq 8.3\%$ for the *dhps* double mutant and for the *dhfr* triple mutant outcomes.

Laboratory methods. Films for malaria parasites were treated with Leishman's stain and parasite counts were measured per 200 white blood cells. Thin smears were used to identify *Plasmodium* species. We randomly selected 50% of filter paper specimens from all malaria episodes diagnosed to test for the presence molecular markers of SP resistance. We assessed for the presence of three mutations in the *dhfr* gene (*dhfr* Asn-108, Ile-51, and Arg-59) and two mutations in the *dhps* gene (*dhps* Gly-437 and Glu-540) commonly found in east Africa and one mutation in the *dhfr* gene (Leu-164) rarely found in Africa, but associated with high-level SP resistance.¹³ Parasite DNA was isolated from filter paper using the Chelex extraction method,¹⁴ and genotypes were determined using nested polymerase chain reaction amplification followed by restriction enzyme digestion and visualization using gel electrophoresis as previously described.^{15,16} Specimens were classified as wild-type, pure mutant, or mixed (both mutant and wild-type alleles detected in the same specimen).

Statistical methods. Data were entered using EpiInfo (Centers for Disease Control and Prevention [CDC], Atlanta, GA)¹⁷ and analyzed using STATA (Stata Corporation, College Station, TX). We compared the prevalence of mutations in those exposed and unexposed to cotrimoxazole using the chi-square test. A Poisson distribution using a log-link function was used to compare the incidence of malaria episodes controlling for age, sex, and potential temporal trends associated with calendar period. We used rainfall data collected in

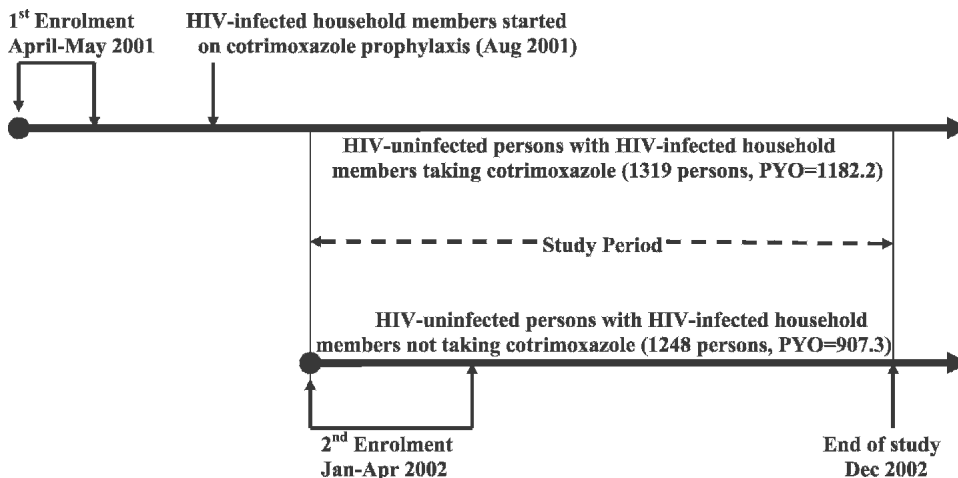


FIGURE 1. Schematic of study design. HIV = human immunodeficiency virus; PYO = person years of observation.

the geographic area during 2001 and 2002 to derive calendar periods that might have higher mosquito populations. Generalized estimating equation methods with an exchangeable correlation structure model were used for analysis of repeated outcome measures among the same individuals. To compare the incidence of malaria due to parasites containing the *dhfr/dhps* quintuple mutant, we first applied the proportions derived from the specimens tested for mutation to the whole study population. We then used the hotdeck imputation method¹⁸ to correct for missing mutation results. Variables with a significance level of 0.05 were included in the final model.

RESULTS

Characteristics of study participants. Between January and December 2002, 1,319 HIV-uninfected participants were followed-up for 1,182.2 person years in 320 households in which cotrimoxazole prophylaxis was administered to at least one HIV-infected household member and 1,248 HIV-uninfected study participants were followed-up for 907.3 person years in 285 households with HIV-infected household members who were not receiving cotrimoxazole (Figure 1). The HIV-uninfected household members exposed and unexposed to household cotrimoxazole use were similar in terms of median age (11 versus 10 years) and the proportion that were female (53% versus 49%). In both groups, the median number of HIV-infected and uninfected persons in a household was one and three, respectively.

Prevalence of molecular markers of SP resistance. Of the 224 episodes of malaria diagnosed among HIV-uninfected patients exposed to household cotrimoxazole use, 113 (50%) filter paper specimens were randomly selected for molecular analysis and 105 (93%) were successfully assayed for all mutations tested. Of the 323 episodes of malaria diagnosed among HIV-uninfected persons not exposed to household cotrimoxazole use, 163 (50%) filter paper specimens were randomly selected for molecular analysis and 146 (90%) were successfully assayed for all mutations tested. None of the specimens contained the *dhfr* Leu-164 mutation. The *dhfr* Asn-108 and Ile-51 mutations were virtually ubiquitous in

both groups, with 100% and 94% of samples containing pure mutants, respectively. The prevalence of the *dhfr* Arg-59 pure mutant was less common (41% overall), consistent with the stepwise progression of *dhfr* mutations, with Arg-59 occurring after Asn-108 and Ile-51. Considering only samples with pure mutants, the prevalence of the *dhfr* triple mutant (Asn-108 + Ile-51 + Arg-59) was similar in samples from HIV-uninfected patients with malaria living in households either unexposed or exposed to cotrimoxazole prophylaxis (45% versus 37%, $P = 0.18$) (Figure 2). The *dhps* Gly-437 and Glu-540 mutations were highly concordant, with 99% of samples having either both mutations or neither. The prevalence of the *dhps* double mutant (Gly-437 + Glu-540) was similar in samples from HIV-uninfected patients with malaria living in households either unexposed or exposed to cotrimoxazole prophylaxis (62% versus 64%, $P = 0.81$) (Figure 2). Considering all five mutations, the prevalence of the quintuple mutant was similar in samples from HIV-uninfected patients with malaria living in households unexposed and exposed to cotrimoxazole prophylaxis (32% versus 30%, $P = 0.74$) (Figure 2). Similar results were obtained when samples that were either mixed or pure mutants, rather than only pure mutants, were considered.

Incidence of malaria. The incidence of malaria during the study period was lower in HIV-uninfected household members staying in households with HIV-infected persons on cotrimoxazole prophylaxis, compared with those without cotrimoxazole exposure (incidence rate ratio [IRR] = 0.64, 95% confidence interval [CI] = 0.50–0.88). When we repeated the analysis considering only episodes of malaria that occurred more than 28 days apart (to reduce the effect of recrudescence clinical malaria due to treatment failure), similar results were obtained (IRR = 0.68, 95% CI = 0.54–0.85). Age was a strong independent predictor for clinical malaria, with HIV-uninfected children less than five years of age having a higher rate of malaria than those ≥ 5 years of age (IRR = 11.8, 95% CI = 9.3–15.0). However, the decreased incidence of malaria among HIV-uninfected household members exposed to cotrimoxazole was consistent across both age groups (Table 1).

To estimate the incidence of malaria due to SP-resistant parasites, we multiplied the prevalence of molecular markers

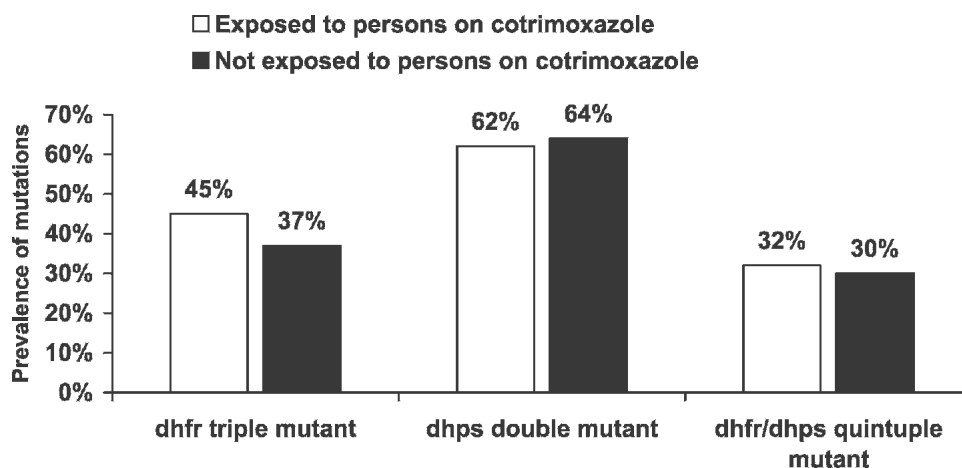


FIGURE 2. Proportion of specimens with molecular results containing dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) mutations from human immunodeficiency virus (HIV)-uninfected patients with *Plasmodium falciparum* malaria in households where HIV-infected household members were taking or not taking cotrimoxazole prophylaxis.

TABLE 1

Incidence of malaria in human immunodeficiency virus (HIV)-uninfected persons living in households with HIV-infected household members taking or not taking cotrimoxazole prophylaxis*

Clinical episode	Households with HIV-infected persons taking cotrimoxazole prophylaxis			Households with HIV-infected persons not taking cotrimoxazole prophylaxis			Incidence rate ratio (95% CI) Cotrimoxazole: no cotrimoxazole	P
	No.	Years of follow-up	Rate per 100 person-years	No.	Years of follow-up	Rate per 100 person-years		
Any episode of malaria	224	1,182.2	18.9	323	907.3	35.6	0.64 (0.50–0.83)†	0.001
< 5 years	163	190.9	85.4	222	149.6	148.4	0.68 (0.49–0.93)†	0.017
≥ 5 years	61	991.3	6.2	101	757.7	13.3	0.54 (0.37–0.80)†	0.002
Episodes of malaria due to mutant parasites (crude results)‡								
<i>dhfr</i> triple mutant	83	–	7.0	145	–	16.0	0.44 (0.33–0.58)	< 0.001
<i>dhps</i> double mutant	143	–	12.1	200	–	22.0	0.55 (0.44–0.68)	< 0.001
<i>dhps/dhfr</i> quintuple mutant	67	–	5.7	103	–	11.4	0.50 (0.36–0.69)	< 0.001
Episodes of malaria due to mutant parasites (imputed results)§								
<i>dhfr</i> triple mutant	84	–	7.1	133	–	14.7	0.49 (0.34–0.69)†	< 0.001
<i>dhps</i> double mutant	145	–	12.3	215	–	23.7	0.67 (0.49–0.92)†	0.014
<i>dhps/dhfr</i> quintuple mutant	75	–	6.3	97	–	10.7	0.61 (0.41–0.91)†	0.014

* *dhfr* = dihydrofolate reductase; *dhps* = dihydropteroate synthase.

† Analyses adjusted for age, sex, and season.

‡ Using proportions of each mutation type in specimens tested for resistance to estimate the number of resistant malaria episodes in the whole population.

§ Using the hotdeck imputation methods to assign mutation status for missing resistance data.

of SP resistance and the incidence of all episodes of malaria. The resulting crude IRR estimates for episodes due to the *dhfr* triple, *dhps* double, and *dhps/dhfr* quintuple mutants were 0.44, 0.55, and 0.50, respectively (Table 1). To account for missing data on molecular markers of SP resistance, we repeated the analysis using imputation to reconstruct a complete dataset based on a covariate structure that considered gender, age and calendar period. The HIV-uninfected household members in a household using cotrimoxazole prophylaxis had a decreased incidence of malaria due to parasites containing the *dhps* double mutant (IRR = 0.67, 95% CI = 0.49–0.92, $P = 0.014$), the *dhfr* triple mutant (IRR = 0.49, 95% CI = 0.34–0.69, $P < 0.001$), and the *dhfr/dhps* quintuple mutant (IRR = 0.61, 95% CI = 0.41–0.91, $P = 0.014$). Age and season were independent predictors for clinical malaria.

DISCUSSION

We compared episodes of malaria among HIV-uninfected individuals living with HIV-infected household members taking or not taking cotrimoxazole prophylaxis. There was no difference in the proportion of malaria episodes caused by SP-resistant parasites in these two groups. Furthermore, those exposed to a household member taking cotrimoxazole prophylaxis had a lower incidence of malaria and a lower overall incidence of infections with SP-resistant parasites compared with those who were not exposed. These findings allay previous concerns that widespread use of cotrimoxazole prophylaxis might contribute to the spread of malaria parasites resistant to SP in Africa.¹¹

The efficacy of cotrimoxazole prophylaxis in preventing morbidity and mortality among Africans with HIV infection has been well documented.^{1,2,19} There is concern, however, that malaria parasites will develop cross-resistance between cotrimoxazole and SP due to the similarities in the modes of action of these two drugs. Cotrimoxazole and SP are both

antifolates, with components that inhibit *dhfr* (trimethoprim and pyrimethamine, respectively) and *dhps* (sulfamethoxazole and sulfadoxine, respectively). An *in vitro* study demonstrated significant cross-resistance between trimethoprim and pyrimethamine in laboratory isolates containing the *dhfr* triple mutation commonly found in Africa.¹¹ However, it is not clear whether significant cross resistance between cotrimoxazole and SP occurs *in vivo*. In a clinical study from Uganda, children with uncomplicated malaria who failed treatment with cotrimoxazole paradoxically selected for the *dhfr* 108 wild-type allele.²⁰ In another study of children with malaria, the presence of SP-resistance conferring mutations was not linked to cotrimoxazole resistance in *Escherichia coli* stool isolates, although measures of resistance were high for both drugs.²¹

In our study, there was no evidence that cotrimoxazole prophylaxis lead to the spread of SP-resistant malaria parasites among household members not taking the drug. In the context of documented *in vitro* cross-resistance between cotrimoxazole and SP, why is there no *in vivo* selection of cross-resistant parasites by cotrimoxazole? It may be that cotrimoxazole does select for SP-resistant parasites, but that our period of observation was not long enough to capture this effect on the surrounding community. Alternatively, the spread of resistant parasites through the community may have occurred so rapidly that we were unable to detect a difference between households exposed and unexposed to cotrimoxazole. However, this explanation seems unlikely given the wide geographic dispersion of households in our rural setting. In addition, the possibility that SP-resistant malaria parasites arose in both the exposed and the unexposed groups due to contamination by mosquitoes crossing to unexposed households is very unlikely given that malaria incidence in general was less in the exposed group.

Despite the lack of evidence that cotrimoxazole prophylaxis contributes to the spread of malaria parasites resistant to

SP, the prevalence of molecular markers of SP resistance in our study population was very high. A recent study reported that SP-resistant parasites have spread rapidly across southern and eastern Africa from a few common ancestral clones.²² Our study was conducted during a period when Uganda switched from chloroquine to a combination of chloroquine plus SP as the recommended first-line treatment of uncomplicated malaria. It is unknown whether increasing use of SP in our community contributed to the high prevalence of SP-resistant parasites. From our results, one may infer that in a region with existing high-level resistance, such as Uganda, factors other than cotrimoxazole use in the community are primarily responsible for the selection and spread of SP-resistant parasites.

In addition to our observation that cotrimoxazole did not lead to resistance selection, we found that it offered the benefit of a reduced incidence of malaria among HIV-uninfected persons living in households exposed to cotrimoxazole. Considering high levels of SP resistance in this population, why might prophylaxis effectively prevent malaria? This effect may be seen because there is limited clinically relevant cross-resistance between the two medications, or because the antimicrobial effect needed to prevent an infection is less than that required to treat a clinical episode of malaria.²³

Cotrimoxazole prophylaxis by HIV-infected individuals was not associated with an increased prevalence of SP-resistant parasites among HIV-uninfected household members. Since cotrimoxazole prophylaxis reduces malaria and improves survival for HIV-infected persons in sub-Saharan Africa, the implementation of prophylaxis in this population should not be delayed because of theoretical concerns regarding the selection of SP-resistant malaria parasites.

Received February 13, 2006. Accepted for publication March 28, 2006.

Acknowledgments: We thank the staff and clients of TASO, the Uganda Virus Research Institute-CDC laboratory, and the staff of CDC-Uganda for participating in the study.

Financial support: This study was supported by the Fogarty AIDS International Training and Research Program/University of California, Berkeley (1-D43-TW00003), the Fogarty International Center/National Institutes of Health (TW00007), and the Centers for Disease Control and Prevention.

Authors' addresses: Samuel S. Malamba, Division of Epidemiology, School of Public Health, University of California, 1918 University Avenue, Fourth Floor, Berkeley, CA 94720 and CDC-Uganda, Global AIDS Program, National Center for HIV, STD and TB Prevention, c/o Uganda Virus Research Institute, PO Box 49, Entebbe, Uganda, Telephone: 256-41-320776, 256-752-790145, or 510-643-4922, Fax: 256-41-321457 or 510-643-4927, E-mails: zcq2@ug.cdc.gov and malambas@berkeley.edu. Jonathan Mermin, John R. Lule, Robert Downing, Ray Ransom, and Aminah Kigozi, Centers for Disease Control and Prevention-Uganda, Global AIDS Program, National Center for HIV, STD and TB Prevention, PO Box 49, Entebbe, Uganda. Arthur Reingold and Alan Hubbard, Division of Epidemiology, School of Public Health, University of California, Berkeley, CA 94720. Ben M. Hunt, Philip J. Rosenthal, and Grant Dorsey, Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA 94110.

REFERENCES

1. Wiktor SZ, Sassin-Morokro M, Grant AD, Abouya L, Karon JM, Maurice C, Djomand G, Ackah A, Domoua K, Kadio A, Yapi A, Combe P, Tossou O, Roels TH, Lackritz EM, Coulibaly D, De Cock KM, Coulibaly IM, Greenberg AE. 1999. Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1-infected patients with tuberculosis in Abidjan, Côte d'Ivoire: a randomised controlled trial. *Lancet* 353: 1469-1475.
2. Anglaret X, Chêne G, Attia A, Toure S, Lafont S, Combe P, Manlan K, 1999. N'Dri-Yoman T, Salamon R: Early chemoprophylaxis with trimethoprim-sulphamethoxazole for HIV-1-infected adults in Abidjan, Côte d'Ivoire: a randomised trial. *Lancet* 353: 1463-1468.
3. Mermin J, Lule J, Ekwaru JP, Downing R, Hughes P, Bunnell R, Malamba S, Ransom R, Kaharuza F, Coutinho A, Kizogi A, Quick R, 2005. Cotrimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-uninfected family members. *AIDS* 19: 1035-1042.
4. Chintu C, Bhat G, Walker A, Mulenga V, Sinyinza F, Farrelly L, Kaganson N, Zumla A, Gillespie SH, Nunn A, Gibb DM, CHAP Trial Team, 2004. Co-trimoxazole as prophylaxis against opportunistic infections as HIV-infected Zambian children (CHAP): a chap double-blind randomized placebo-controlled trial. *Lancet* 364: 1865-1871.
5. UNAIDS/WHO, 2000. *Provisional WHO/UNAIDS Secretariat Recommendations on the Use of Cotrimoxazole Prophylaxis in Adults and Children Living with HIV/AIDS in Africa*. Geneva: World Health Organization.
6. Boeree MJ, Harries AD, Zijlstra EE, Taylor TE, Molyneux ME, 1999. Co-trimoxazole in HIV-1 infection. *Lancet* 354: 334.
7. Whitty CJ, Jaffar S, 2002. *Plasmodium falciparum* cross resistance. *Lancet* 359: 80.
8. Greenwood BM, Bojang K, Whitty CJ, Targett GA, 2005. Malaria. *Lancet* 365: 1487-1498.
9. Eskild P, 1987. *In vitro* susceptibility of *Plasmodium falciparum* malaria to pyrimethamine, sulfadoxine, trimethoprim and sulfamethoxazole singly and in combination. *Trans R Soc Trop Med Hyg* 81: 238-241.
10. Dorsey G, Dokomajilar C, Kiggundu M, Staedke SG, Kanya MR, Rosenthal PJ, 2004. Principal role of dihydropteroate synthase mutations in mediating resistance to sulfadoxine-pyrimethamine in single-drug and combination therapy of uncomplicated malaria in Uganda. *Am J Trop Med Hyg* 71: 758-763.
11. Lyer JK, Milhous WK, Cortese JF, Kublin JG, Plowe CV, 2001. *Plasmodium falciparum* cross-resistance between trimethoprim and pyrimethamine. *Lancet* 358: 1066-1067.
12. Anglaret X, 2001. Trimethoprim-sulfamethoxazole prophylaxis in sub-Saharan Africa. *Lancet* 358: 1027-1028.
13. Sibley CH, Hyde JE, Sims PFG, Plowe CV, Kublin JG, Mberu EK, Cowman AF, Winstanley PA, Watkins WM, Nzila AM, 2001. Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? *Trends Parasitol* 17: 582-588.
14. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellemes T, 1995. Pyrimethamine and proguanil resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *Am J Trop Med Hyg* 52: 565-568.
15. Kyabayinze D, Cattamanchi A, Kanya M, Rosenthal PJ, Dorsey G, 2003. Validation of a simplified method for using molecular markers to predict sulfadoxine-pyrimethamine treatment failure in African children with falciparum malaria. *Am J Trop Med Hyg* 69: 247-252.
16. Duraisingh MT, Curtis J, Warhurst DC, 1998. *Plasmodium falciparum*: detection of polymorphisms in the dihydrofolate reductase and dihydropteroate synthetase genes by PCR and restriction digestion. *Exp Parasitol* 89: 1-8.
17. EpiInfo, 2005. *A Database and Statistics Program for Public Health Professionals*. Atlanta: Centers for Disease Control and Prevention.
18. Mander A, Clayton D, 1997. *Hotdeck Imputation Method*. College Station, TX: Stata Corporation.
19. Omar SA, Bakari A, Owiti A, Adugu IS, Warhurst DC, 2001. Co-trimoxazole compared with sulfadoxine-pyrimethamine in the treatment of uncomplicated malaria in Kenyan children. *Trans R Soc Trop Med Hyg* 95: 657-660.
20. Jelinek T, Kilian AH, Curtis J, Duraisingh MT, Kabagambe G, von Sonnenburg F, Warhurst DC, 1999. *Plasmodium falciparum*

- parum*: selection of serine 108 of dihydrofolate reductase during treatment of uncomplicated malaria with co-trimoxazole in Ugandan children. *Am J Trop Med Hyg* 61: 125–130.
21. Kofoed PE, Alfrangis M, Poulsen A, Rodrigues A, Gjedde SB, Ronn A, Rombo L, 2004. Genetic markers of resistance to pyrimethamine and sulfonamides in *Plasmodium falciparum* parasites compared with the resistance patterns in isolates of *Escherichia coli* from the same children in Guinea-Bissau. *Trop Med Int Health* 9: 171–177.
 22. Roper C, Pearce R, Bredenkamp B, Gumedde J, Drakeley C, Moshia F, Chandramohan D, Sharp B, 2003. Antifolate anti-malarial resistance in southeast Africa: a population-based analysis. *Lancet* 361: 1174–1181.
 23. Mermin J, Lule J, Ekwaru JP, Malamba S, Downing R, Ransom R, Kaharuza F, Culver D, Kizito F, Bunnell R, Kigozi A, Nakanjako D, Wafula W, Quick R, 2004. Effect of co-trimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. *Lancet* 364: 1428–1434.