

HIV-1 Infection in Patients Referred for Malaria Blood Smears at Government Health Clinics in Uganda

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Background: HIV is associated with an increased incidence of malaria in adult African populations. In children, the relationship between HIV and malaria is less clear. We investigated the relationship between malaria and HIV-1 infection among adults and children referred for malaria blood smears at government health clinics in Uganda.

Methods: This was a cross-sectional study in which 1000 consecutive patients referred for malaria blood smears over the course of 1 to 2 months at each of 7 government clinics (N = 7000) were tested for HIV-1 from dried blood spots using enzyme-linked immunosorbent assay (ELISA) screening and nucleic acid-based confirmatory testing. Risk factors for HIV-1 infection were identified using multivariate logistic regression.

Results: Among 4467 children aged 16 years or younger, 77 (1.7%) were HIV-1 infected. Of 2533 adults, 270 (10.7%) were HIV-1 infected. In children, having a negative malaria blood smear was associated with higher odds of HIV-1 infection (odds ratio [OR] = 1.90, 95% confidence interval [CI]: 1.18 to 3.06) after controlling for age and gender. In adults, having a positive malaria blood smear was moderately associated with higher odds of HIV-1 infection (OR = 1.41, 95% CI: 1.01 to 1.97) after controlling for age and gender.

Conclusions: In Ugandans evaluated for suspected malaria, associations between malaria smear results and HIV infection differed between children and adults. Although further operations research is needed, our results suggest that counseling and testing for HIV may be of particular importance in children suspected of malaria but with negative malaria smears and in adults with positive malaria smears.

Key Words: malaria, HIV, Africa, human immunodeficiency virus

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In sub-Saharan Africa, approximately 25 million people are infected with the human immunodeficiency virus (HIV), and there are over 300 million cases of malaria each year.^{1–3} Thus, any interaction between HIV and malaria could be of great public health importance. In adults, studies have demonstrated a clear association between HIV infection and increased frequency of clinical malaria and asymptomatic parasitemia, a relationship that becomes more pronounced with advancing immunosuppression.^{4–6} This association is thought to be mediated through impairment of acquired malaria immunity by progressive HIV disease.² In children, the relationship between HIV and malaria is less clear, with available studies reporting an increase,⁷ decrease,⁸ or no change^{9,10} in the risk of malaria in HIV-1-infected children compared to uninfected children.

Malaria is the most common diagnosis at community clinics in most of Africa, accounting for 30% to 50% of outpatient visits.^{11–13} An improved understanding about the relationship between malaria and HIV in the outpatient setting could lead to the identification of patient populations at increased risk for HIV who could be referred for counseling and testing. HIV testing is an important first step in preventing transmission of the virus and providing treatment to those in need; however, surveys estimate that only 10% to 12% of Africans know their HIV status.³ One promising strategy for expanding knowledge of HIV status is the introduction of routine counseling and testing into sites not specifically designated for HIV care.^{14,15} Thus, community clinics may be ideal locations to offer counseling and testing for HIV. However, it is unknown which populations presenting to community clinics have a high prevalence of HIV infection. In this study we investigated the relationship between HIV-1 infection and malaria smear results among adults and children referred for malaria blood smears at 7 government health clinics in Uganda, where there is significant geographic overlap between the 2 diseases. A population-based survey performed in 2004 to 2005 estimated the seroprevalence of HIV to be 6.3% in persons 15 to 49 years old and 0.5% in children under the age of 5 years.¹⁶ Malaria is endemic in 95% of Uganda, with the remaining 5% being epidemic-prone areas in the highlands of the southwest and east.^{17,18}

METHODS

Study Sites and Patient Population

This study was conducted at 7 government health clinics representing the diversity of malaria transmission intensity

in Uganda, ranging from highland areas with unstable transmission to highly endemic areas with perennial transmission (Fig. 1). Six of 7 sites were level 4 health clinics mainly serving the local community, where outpatients were primarily evaluated by nonphysician health workers and at least 1 laboratory technologist was available for microscopy. One site (Kabale) was a regional referral hospital with numerous physicians and laboratory technologists. Health care services were provided free of charge at all of the clinics.

We used a cross-sectional study design to prospectively evaluate 1000 consecutive patients at each site over the course of 1 to 2 months who were referred by local health care providers to existing on-site laboratories for malaria blood smears as part of routine care. Study staff did not interfere with patient referral, diagnosis, or management, preserving normal clinic practices. We collected data on patient age and gender at the time of presentation, and finger-prick blood was used to prepare blood smears for malaria parasites and saved on filter paper for HIV testing. For patients later determined to be HIV-1 infected, data regarding patients' diagnoses and treatments were extracted retrospectively from on-site clinic records by 2

independent study investigators and compared. Discrepancies were resolved by a third investigator.

Microscopy

Thick blood smears were stained with 10% Giemsa stain for 10 minutes and preserved. Smears were considered positive if any asexual parasites were detected. Smears were considered negative if examination of 100 high-power fields did not reveal asexual parasites. Initial readings were done by technicians at the study sites. Final microscopy results were based on a rigorous quality control system that included the rereading of all blood smears by an expert microscopist at a central facility and resolution of any discrepancies between the first and second readings by an additional expert microscopist.

HIV-1 Testing

Dried blood spots collected on qualitative grade plain filter paper were stored individually at room temperature and transported to a central laboratory for HIV testing. Screening for HIV infection was done using the Murex HIV 1.2.0 Enzyme Immunoassay (EIA) (Abbott Laboratories, Abbott

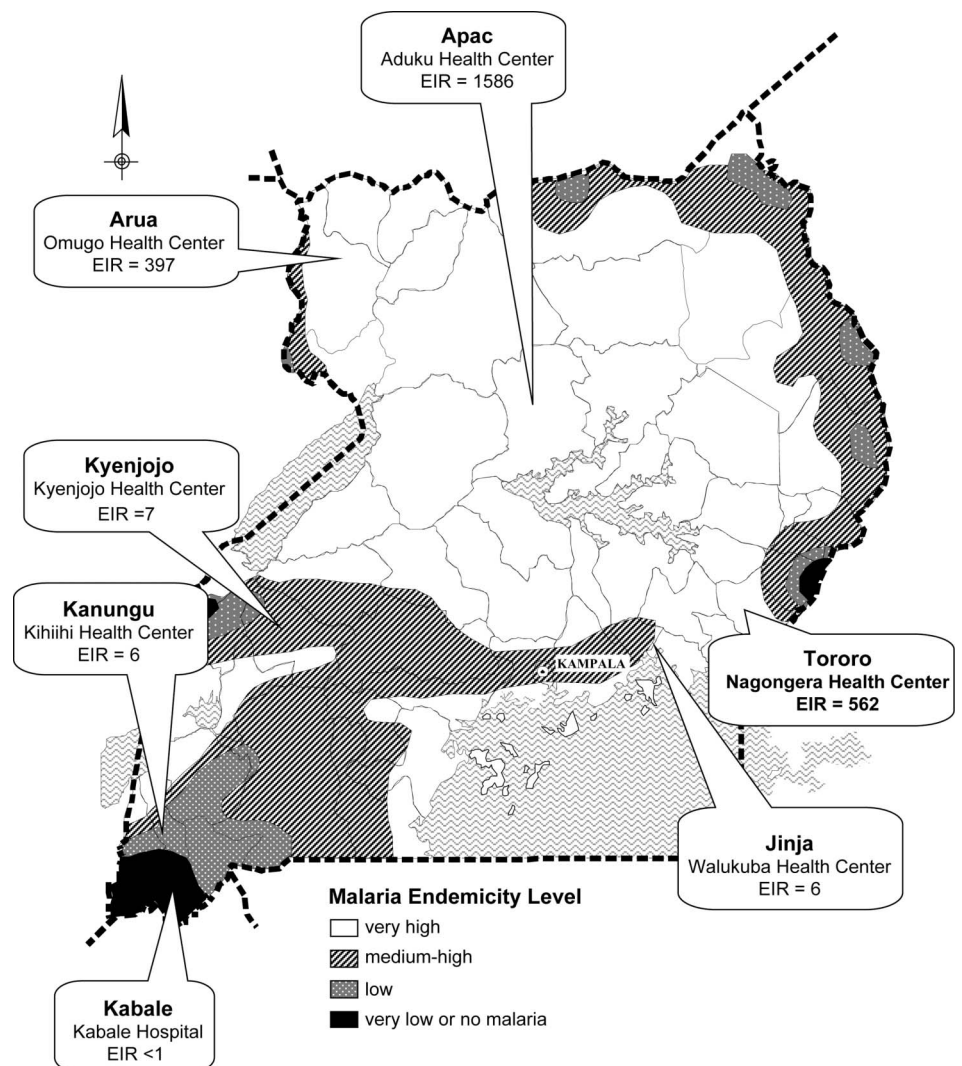


FIGURE 1. Map of malaria endemicity levels according to the Uganda Malaria Control Program, marked with sites where samples were collected. EIR indicates entomological inoculation rates.^{17,18}

Park, IL). Briefly, dried blood spots were eluted using 100 μ L of phosphate buffered saline (PBS) with 0.05% polysorbate 20 in 1.5 mL tubes at 4°C for at least 3 hours. The EIA was performed according to manufacturer's instructions, using 50 μ L of eluate. Based on the manufacturer's recommendations, samples with a corrected optical density at 450 nm of <0.2 were considered HIV-1 negative. Samples with a corrected optical density of \geq 0.2 received confirmatory testing for HIV-1 DNA using the Roche Amplicor HIV-1 DNA test (Roche Systems, Basel, Switzerland). HIV DNA was extracted from samples using chelex resin,¹⁹ and testing was performed according to the manufacturer's instructions as previously described.²⁰ Classification of HIV status was based on HIV-1 DNA results. A single EIA test was used for screening to reduce cost and minimize the quantity of blood needed. However, it is possible some patients were misclassified due to false-negative EIA test results. Results of HIV testing were delinked from patient identifiers.

Ethical Approval

Human subjects research approval was 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. The procedures followed were in accordance with the ethical standards of these committees on human experimentation, and with the Helsinki Declaration of 1975 as revised in 2000.

Data Management and Statistical Analysis

Data were double-entered into EpiInfo version 6.4 (Centers for Disease Control and Prevention, Atlanta, GA) and statistical analyses were performed using STATA version 8 (Stata, College Station, TX). Categorical variables were compared using the χ^2 test. Independent predictors of HIV-1 infection were identified using separate multivariate logistic regression models for children (age \leq 16 years) and adults. Multivariate models included all 3 covariates of interest: results of malaria blood smears, sex, and age categorized into quartiles. None of the possible 2-way interaction terms had a *P* value of <0.20 and therefore were not included in the multivariate models. Differences in associations between malaria smear results and HIV-1 infection across the 7 study sites were evaluated using the Mantel-Haenszel test for

heterogeneity of odds ratios. *P* values <0.05 were considered statistically significant.

RESULTS

Patient Characteristics

We evaluated 1000 consecutive patients referred at each of 7 clinics for malaria blood smears (Table 1). A total of 4467 patients (64%) were children aged 16 years or younger, with the proportions ranging from 41% at the lowest to 92% at the highest malaria transmission intensity site. A majority of the patients were female, with the proportions ranging from 50% to 61% at the 7 sites, with no relationship to malaria transmission intensity. A total of 2703 (38.6%) blood smears were positive for malaria parasites, with the proportion of positive smears varying greatly by site and age (Fig. 2A). Malaria prevalence was significantly higher in children than adults at all sites (*P* < 0.001) except the lowest transmission intensity site. Differences in the proportion of positive malaria smears between children and adults decreased with decreasing malaria transmission intensity (*P* < 0.001). A total of 347 patients (5.0%) were HIV-1 infected. HIV prevalence varied markedly between sites, from 0.8% in Arua to 10.1% in Kyenjojo, but was not related to malaria transmission intensity (Fig. 2B). HIV-1 prevalence was significantly higher in adults (10.5% overall) compared to children (1.6% overall) at all of the sites (*P* \leq 0.005).

Risk Factors for HIV-1 Infection

Among children aged \leq 16 years, 28 of 2312 (1.2%) patients with positive malaria smears were infected with HIV-1 compared to 49 of 2155 (2.3%) patients with negative smears. For these children, a negative malaria smear was associated with increased odds of HIV-1 infection (OR = 1.90, 95% CI: 1.18 to 3.06; *P* = 0.008) compared to a positive smear, after controlling for age and sex for all 7 sites combined (Table 2). Age and sex were not associated with HIV-1 infection in children. There was no evidence for heterogeneity of odds ratios for the association between malaria and HIV-1 infection among children across the 7 sites (*P* = 0.38).

Among adults aged >16 years, 50 of 391 (12.8%) patients with positive malaria smears were infected with HIV-1 compared to 220 of 2142 (10.3%) with negative smears. A

TABLE 1. Patient Characteristics According to Site of Sample Collection

	Site						
	Apac (n = 1000)	Tororo (n = 1000)	Arua (n = 1000)	Kyenjojo (n = 1000)	Kanungu (n = 1000)	Jinja (n = 1000)	Kabale (n = 1000)
Date samples collected	May 2006	Jan. to Feb. 2007	Nov. 2006	Dec. 2006 to Jan. 2007	Nov. 2006	Jan. 2006	Sept. to Oct. 2006
Median age, y (IQR)	2.0 (0.91 to 4.0)	3.3 (1.2 to 22.0)	16.5 (2.0 to 30.0)	13.0 (3.0 to 28.0)	4.5 (1.4 to 16.0)	10.0 (2.5 to 24)	20.0 (3.5 to 30.0)
Age \leq 16 y (%)	920 (92)	695 (70)	500 (50)	564 (56)	754 (75)	620 (62)	414 (41)
Female sex (%)	497 (50)	561 (56)	601 (60)	613 (61)	597 (60)	609 (61)	580 (58)
Malaria							
smear-positive (%)	774 (77)	382 (38)	428 (43)	446 (45)	363 (36)	271 (27)	39 (3.9)
HIV-1 infected (%)	33 (3.3)	29 (2.9)	8 (0.8)	101 (10.1)	28 (2.8)	71 (7.1)	77 (7.7)

IQR indicates interquartile range.

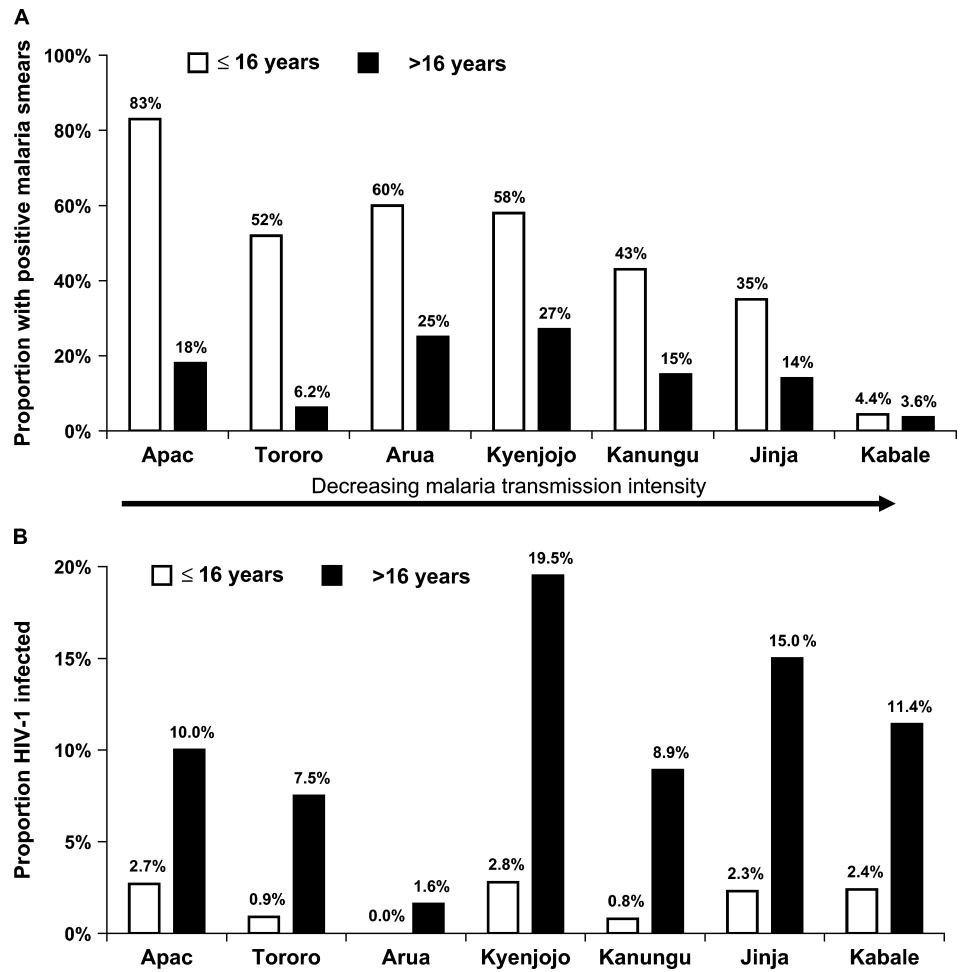


FIGURE 2. A, Proportion of malaria smears positive at each site, stratified by age group. B, Prevalence of HIV-1 at each site, stratified by age group.

positive blood smear was associated with a significantly higher odds of HIV-1 infection (OR = 1.41, 95% CI: 1.01 to 1.97; $P = 0.04$) after controlling for sex and age for all 7 sites combined (Table 2). Sex was not associated with HIV-1 infection in adults. HIV prevalence among adults differed significantly across age quartiles, with the highest prevalence among those aged 30 to 39 years (17.3%) and the lowest prevalence among those aged 17 to 22 years (6.1%, $P < 0.001$). There was no evidence for heterogeneity of odds ratios for the association between malaria and HIV-1 infection among adults across the 7 sites ($P = 0.44$).

Diagnosis and Treatment of HIV-1-Infected Patients

Because our study did not change routine patient care activities, clinic records of clinical diagnosis and treatment were frequently missing. However, information on clinical diagnosis and treatment was available for 187 (54%) HIV-1-infected patients (Table 3). HIV-1-infected patients with missing information were similar to those with available information with respect to malaria smear positivity (18% vs. 26%, $P = 0.07$), female sex (63% vs. 70%, $P = 0.17$), and median age (27 vs. 29 years, $P = 0.15$). Of note, HIV infection was not listed as a diagnosis and antiretrovirals were not prescribed for

any of the 187 HIV-1-infected patients with available records. However, it is possible that some patients received HIV care at other health facilities. Malaria was the most common stated diagnosis, made in 88 (47%) of all HIV-1-infected patients. Only 43 (49%) of those with a stated diagnosis of malaria had positive malaria blood smears. Other common diagnoses were respiratory tract infection (27%) and gastrointestinal illness (13%). Artemether-lumefantrine, the new standard therapy in Uganda for malaria, was the most commonly prescribed drug in HIV-1-infected patients (34%). Only 42 (45%) of those treated with an antimalarial drug had a positive malaria blood smear. An antimalarial drug, an antimicrobial drug, or both were prescribed to 87% of HIV-1-infected patients, including trimethoprim-sulfamethoxazole in 32 (17%) patients. Twenty-six of these 32 patients were being treated for gastrointestinal illness or respiratory tract infections, suggesting that the drug was not given as prophylaxis against opportunistic infections.

DISCUSSIONS

In this cross-sectional study of 7000 Ugandans presenting to government clinics and referred for malaria blood smears, we examined interactions between HIV and malaria. We identified significant but modest associations between

TABLE 2. Risk Categories and Relationship to HIV Status

Risk Category (n)	Proportion HIV-1 Infected (95% CI)	Unadjusted Odds Ratio (95% CI)	P	Adjusted Odds Ratio (95% CI)	P
Children (age ≤16 y)					
Malaria blood smear					
Positive (2155)	1.2 (0.8 to 1.7)	1.0 (reference)	NA	1.0 (reference)	NA
Negative (2312)	2.3 (1.7 to 3.0)	1.90 (1.19 to 3.03)	0.007	1.90 (1.18 to 3.06)	0.008
Sex					
Male (2204)	1.6 (1.1 to 2.2)	1.0 (reference)	NA	1.0 (reference)	NA
Female (2263)	1.9 (1.3 to 2.5)	1.17 (0.75 to 1.84)	0.49	1.15 (0.73 to 1.81)	0.55
Age quartiles, y					
0.1 to 0.9 (1054)	1.4 (0.8 to 2.3)	1.0 (reference)	NA	1.0 (reference)	NA
1.0 to 2.2 (1213)	1.5 (0.9 to 2.3)	1.04 (0.52 to 2.08)	0.90	1.13 (0.57 to 2.27)	0.73
2.3 to 5.3 (1078)	2.0 (1.3 to 3.1)	1.44 (0.74 to 2.80)	0.28	1.52 (0.78 to 2.96)	0.21
5.4 to 16 (1122)	2.0 (1.2 to 3.0)	1.39 (0.71 to 2.68)	0.33	1.28 (0.66 to 2.50)	0.46
Adults (age >16 y)					
Malaria blood smear					
Negative (2142)	10.3 (9.0 to 11.6)	1.0 (reference)	NA	1.0 (reference)	NA
Positive (391)	12.8 (9.6 to 16.5)	1.28 (0.92 to 1.78)	0.14	1.41 (1.01 to 1.97)	0.04
Sex					
Male (738)	11.1 (8.9 to 13.6)	1.0 (reference)	NA	1.0 (reference)	NA
Female (1795)	10.5 (9.1 to 12.0)	0.94 (0.71 to 1.23)	0.64	0.95 (0.72 to 1.25)	0.72
Age quartiles, y					
17 to 22 (672)	6.1 (4.4 to 8.2)	1.0 (reference)	NA	1.0 (reference)	NA
23 to 29 (609)	10.5 (8.2 to 13.2)	1.81 (1.20 to 2.72)	0.005	1.85 (1.23 to 2.79)	0.003
30 to 39 (620)	17.3 (14.4 to 20.5)	3.21 (2.20 to 4.69)	<0.001	3.32 (2.27 to 4.86)	<0.001
40 to 96 (632)	9.2 (7.0 to 11.7)	1.56 (1.03 to 2.36)	0.04	1.61 (1.06 to 2.45)	0.03

CI indicates confidence interval; NA, not applicable.

malaria blood smear results and risk of HIV-1 infection. Interestingly, these associations differed between age groups, with HIV-1 infection associated with a negative blood smear among children and a positive blood smear among adults. Among HIV-1–infected patients, none had a diagnosis of HIV or AIDS recorded, suggesting that HIV infection was unrecognized. Taken together, these findings suggest that current practices for identifying those at risk for HIV are inadequate and that results from routine malaria evaluation may help identify those who would benefit most from HIV testing.

In sub-Saharan Africa, assessment of febrile illnesses often begins with consideration of malaria. Indeed, malaria is the leading cause of attendance at outpatient clinics in Uganda, accounting for 25% to 40% of all outpatient diagnoses.^{11,18,21} In Africa, malaria is usually diagnosed on clinical grounds alone, and even when diagnostic tests are performed, patients with negative results are often diagnosed and treated for malaria.^{22,23} However, treating all fevers as malaria leads to overuse of antimalarials and failure to diagnose other important causes of fever, including HIV and related coinfections.²⁴

Our findings suggest that patients presenting to outpatient clinics with symptoms suggestive of malaria are more likely to have HIV-1 infection than the general Ugandan population. Among individuals in this study who were aged 15 to 49 years, the HIV prevalence was almost twice the population-based estimate for the same age group¹⁶ (10.5% vs.

6.3%). In children under 5 years of age, HIV prevalence was almost 3 times the national estimate¹⁶ (1.6% vs. 0.5%). However, the increased prevalence of HIV was modest compared to that seen with other HIV-related opportunistic infections, such as tuberculosis.²⁵ A higher prevalence (31% to 33%) of HIV in adults with malaria has been reported in other studies from Africa,^{5,26} but these studies did not include patients with negative malaria smears and therefore were unable to report on the association between malaria blood smear results and risk of HIV infection.

Interestingly, we observed opposite associations between malaria and HIV-1 infection in children and adults. In children, HIV was more common among those with negative malaria blood smears, whereas in adults HIV infection was more common among those with positive blood smears. All HIV-1–infected patients would be expected to be at increased risk of febrile illnesses, but associations between HIV and malaria may vary across age groups. Malaria immunity develops over years of repeated parasite exposure; therefore, children would be expected to have less acquired malarial immunity than adults at the time of HIV infection. The relative increase in HIV prevalence we observed in children with negative malaria smears may be the result of an increase in the risk of nonmalarial febrile illnesses among HIV-infected children.²⁵ It is also possible that HIV-infected children in this study were more recently exposed to antimalarials than HIV-uninfected children, reducing their probability of having

TABLE 3. Spectrum of Clinical Diagnoses and Treatments Among HIV-1–Infected Patients for Whom Records Were Available (n = 187)

Diagnoses, n (%)		Treatments, n (%)	
Malaria	88 (47)	Artemether-lumefantrine	64 (34)
Respiratory tract infection	50 (27)	Trimethoprim-sulfamethoxazole	32 (17)
Gastrointestinal illness	25 (13)	Penicillin or amoxicillin	21 (11)
Body aches NOS	11 (6)	Non-antimicrobials only	20 (11)
Sexually transmitted infection	10 (5)	Metronidazole	20 (11)
Urinary tract infection	4 (2)	Albendazole	19 (10)
Diagnosis illegible	3 (2)	Ciprofloxacin	17 (9)
No diagnosis listed	3 (2)	Chloroquine and sulfadoxine-pyrimethamine	13 (7)
Fever NOS	2 (1)	Quinine	13 (7)
Malnourishment	2 (1)	Cephalosporin	13 (7)
Allergies	1 (1)	Tetracycline or doxycycline	11 (6)
Early pregnancy	1 (1)	Fluconazole	11 (6)
Headache NOS	1 (1)	Sulfadoxine-pyrimethamine	4 (2)
Malaise NOS	1 (1)	Erythromycin or azithromycin	4 (2)
Mastitis	1 (1)	Treatment deferred	4 (2)
Neuropathy	1 (1)	Chloramphenicol	1 (1)
Pain NOS	1 (1)	Acyclovir	1 (1)
Skin infection	1 (1)	Artesunate	1 (1)
Stomatitis	1 (1)	Amodiaquine	1 (1)
		No medications given	1 (1)

Diagnoses and treatments are listed in order of decreasing frequency. Patient may have more than 1 diagnosis or treatment. NOS indicates not otherwise specified.

a positive malaria smear. In contrast, the relative increase in HIV prevalence we observed in adults with positive malaria smears is likely due to loss of acquired malaria-specific immunity.^{2,6,27,28}

Malaria was the most common diagnosis observed among HIV-1–infected patients in this study, though more than half of those diagnosed and treated for malaria had negative blood smears, a phenomenon reported in multiple studies from Africa.^{29,30} Although HIV-1–infected patients in this study were commonly diagnosed with malaria, HIV was not listed as a diagnosis in clinic records, nor was antiretroviral therapy recorded as treatment, suggesting the need to integrate routine HIV testing into outpatient clinics. If the results of this study are validated, knowledge of how malaria smear results predict HIV risk may help identify those who would benefit most from HIV testing. All of the government clinics included in this study offer HIV testing and 3 offer antiretroviral therapy. Thus, integrating HIV testing algorithms for high-risk outpatients referred for malaria smears should be feasible.

In conclusion, we found that associations between HIV and malaria operate differently in adults and children in Uganda. Presentation with malaria is a warning sign for HIV infection in adults, because HIV infection likely diminishes acquired antimalarial immunity. In children, malaria is very common, and a negative malaria smear suggests other causes of fever, including HIV-related infections. We support recommendations by other authors²⁸ that integrated HIV and malaria testing be utilized in the outpatient clinic setting. Further data would be particularly useful in refining such referral strategies.

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