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Malaria Transmission, Infection, and Disease at Three Sites with Varied Transmission Intensity in Uganda: Implications for Malaria Control

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Abstract. The intensification of control interventions has led to marked reductions in malaria burden in some settings, but not others. To provide a comprehensive description of malaria epidemiology in Uganda, we conducted surveillance studies over 24 months in 100 houses randomly selected from each of three subcounties: Walukuba (peri-urban), Kihihi (rural), and Nagongera (rural). Annual entomological inoculation rate (aEIR) was estimated from monthly Centers for Disease Control and Prevention (CDC) light trap mosquito collections. Children aged 0.5–10 years were provided long-lasting insecticidal nets (LLINs) and followed for measures of parasite prevalence, anemia and malaria incidence. Estimates of aEIR were 2.8, 32.0, and 310 infectious bites per year, and estimates of parasite prevalence 7.4%, 9.3%, and 28.7% for Walukuba, Kihihi, and Nagongera, respectively. Over the 2-year study, malaria incidence per person-years decreased in Walukuba (0.51 versus 0.31, $P = 0.001$) and increased in Kihihi (0.97 versus 1.93, $P < 0.001$) and Nagongera (2.33 versus 3.30, $P < 0.001$). Of 2,582 episodes of malaria, only 8 (0.3%) met criteria for severe disease. The prevalence of anemia was low and not associated with transmission intensity. In our cohorts, where LLINs and prompt effective treatment were provided, the risk of complicated malaria and anemia was extremely low. However, malaria incidence was high and increased over time at the two rural sites, suggesting improved community-wide coverage of LLIN and additional malaria control interventions are needed in Uganda.

INTRODUCTION

The intensification of malaria control interventions, including long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) of insecticides, and prompt treatment with artemisinin-based combination therapies (ACTs) has been accompanied by marked reductions in transmission intensity, parasite prevalence, malaria incidence, malaria-associated hospitalizations, and malaria-associated deaths in some settings,[1–6] but not others.[7,8] To date, substantial successes in malaria control in sub-Saharan Africa have been mostly limited to relatively low-transmission settings. Comparatively, there have been fewer studies documenting the transitioning epidemiology in areas of high malaria transmission, such as Uganda, where the greatest burden of malaria in Africa remains focused.[9,10] A better understanding of the complex relationships between malaria transmission, infection, and disease is important to support the targeted use of control interventions, and maximize their impact.

Exposure of individuals to malaria parasites is typically estimated by the entomological inoculation rate (EIR), which is an estimate of the number of infective bites received per person per unit time, usually a transmission season or 1 year. In Uganda, the limited available data document significant heterogeneity of malaria transmission, ranging from under 10 to several hundred infective bites per person year.[11] Whether inoculation with sporozoites leads to patent infection and clinical disease depends on the ability of the host to control or clear parasites. Fortunately, in endemic areas most infections do not lead to symptomatic disease because of the development of clinical immunity. Immunity develops as a result of repeated exposure, first leading to protection against severe forms of disease, followed by protection against symptomatic illness.[12] Protection against illness is accompanied by a rise in the prevalence of asymptomatic parasitemia, in large part since asymptomatic individuals are less likely to receive antimalarial treatment.[13–15] In addition, there is an increasing appreciation of the role of sub-patent infections among asymptomatic individuals, which may also proportionally increase with increasing immunity.[16] Asymptomatic parasitemia provides a reservoir for transmission and may be associated with adverse health outcomes such as anemia.[17] An important metric for estimating malaria morbidity is malaria incidence, defined as the number of cases per person per time at risk. Incidence is best estimated using longitudinal studies of representative cohorts, in which all cases of suspected malaria are captured and accurately diagnosed.

To provide a comprehensive and contemporary description of malaria epidemiology in Uganda, we conducted entomologic surveys and prospective cohort studies in children from the same households, randomly selected from three sites with varied transmission intensity. Key metrics included measures of malaria transmission (EIR), infection (parasite prevalence), and disease (malaria incidence, severity of illness, and prevalence of anemia).

MATERIALS AND METHODS

Description of malaria in Uganda and study sites. The most recent national data on malaria epidemiology in Uganda come from a Malaria Indicator Survey (MIS) conducted in 2009.[18] Malaria was endemic in over 95% Uganda, with a parasite prevalence of 42% among children under 5 years of age. Over 98% of infections are caused by *Plasmodium falciparum*.

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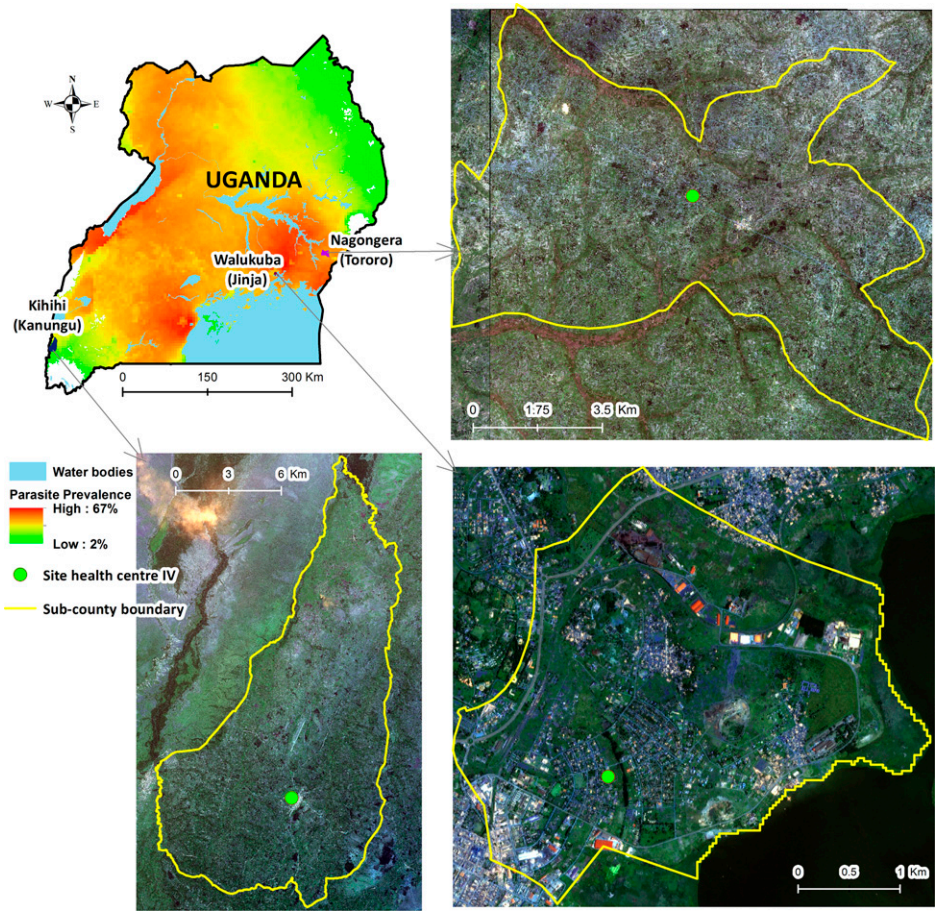

FIGURE 1. Map of Uganda showing study sites.

Detailed entomological surveys were last conducted in 2002; then the major mosquito vectors reported were *Anopheles gambiae* s.s. and to a lesser extent *An. funestus*.[11] Recent reports by our group show a decline of *An. funestus* and emergence of *An. arabiensis*.[19] Malaria transmission is perennial, with two annual peaks following the two rainy seasons (March–May and August–October).

For this report, comprehensive surveillance studies were conducted in three subcounties: Walukuba, Jinja District; Kihihi, Kanungu District, and Nagongera, Tororo District (Figure 1). These areas were purposively chosen to represent varied malaria transmission settings. Walukuba is a relatively low-transmission, peri-urban area near Lake Victoria in the south central part of the country. Kihihi is a rural area with moderate transmission intensity, which borders a national park in the southwestern part of the country. Nagongera is a rural area with high transmission intensity in the southeastern part of the country near the border with Kenya. Notable regional malaria control interventions included a single round of IRS using lambda-cyhalothrin in Kanungu District in 2007[20] and a mass, community-based distribution of free LLINs in Tororo District in January 2011. Additional descriptive characteristics of the study sites are available from an enumeration survey described below and a cross-sectional community survey conducted in 2012, which randomly selected households from each subcounty (Sarah G. Staedke, unpublished data).

Enumeration survey and selection of households for participation in surveillance studies. All households within each of the three subcounties were enumerated and mapped using handheld global positioning systems (Garmin e-Trex 10 GPS unit, Garmin International Inc., Olathe, KS). A household was defined as any single permanent or semipermanent dwelling acting as the primary residence for a person or group of people that generally cook and eat together. Using a computerized number generator, random samples of households from each subcounty were approached consecutively, and 100 households were enrolled per site into both the entomologic surveys and cohort studies if they met the following criteria: 1) at least one household resident 0.5–10 years of age and 2) at least one adult resident available for providing informed consent.

Entomologic surveys. Entomological surveys were conducted once a month from August 2011 to September 2013 in each household using miniature Centers for Disease Control and Prevention (CDC) light traps (Model 512; John W. Hock Company, Gainesville, FL) with the light positioned 1 m above the floor at the foot end of the bed where a cohort study participant slept. Traps were set at 7.00 PM and collected at 07.00 AM the following morning by field workers. Methods used for the processing of mosquito specimens and identification of sporozoites were as described previously (processing of mosquito specimens in methods section).[19]

Enrollment and follow-up of cohort study participants. All children from each household were enrolled into the cohort

study if they met the following eligibility criteria: 1) documented age between 6 months and less than 10 years, 2) full-time resident of the household, 3) no intention to move out of the subcounty for the next 2 years, 4) agreement to come to a dedicated study clinic located within the subcounty for any febrile illness, 5) agreement to avoid antimalarial medications administered outside the study, and 6) provision of written informed consent from parent or guardian. The cohorts were followed from August 2011 to September 2013, and they were dynamic, such that all newly eligible children from participating households were enrolled and study participants who reached 11 years of age were excluded from further follow-up. At enrollment, study participants and their parents/guardians were given a LLIN and underwent a standardized evaluation including a history, physical examination, and collection of blood for hemoglobin (Hb) measurement and thick/thin blood smear.

Cohort study participants received all medical care free of charge at a designated study clinic open every day. Parents/guardians were encouraged to bring their children to the clinic any time they were ill and were reimbursed for transport costs. Participants who required inpatient care were referred to the local district hospital. Children who presented with a documented fever (tympanic temperature $\geq 38.0°C$) or history of fever in the previous 24 hours had blood obtained by finger prick for a thick blood smear. If the smear was positive, the patient was diagnosed with malaria, and an Hb measurement and thin blood smear for parasite species identification were performed. Episodes of uncomplicated malaria were treated with artemether-lumefantrine (AL), the recommended first-line treatment in Uganda, administered at home twice a day for 3 days. Episodes of complicated malaria (severe malaria or danger signs)[21] or recurrent malaria occurring within 14 days of prior therapy were treated with quinine. For patients with anemia (Hb < 10 gm/dL), Integrated Management of Childhood Illness guidelines were followed, with administration of iron sulfate (100 mg daily for 2 weeks) and mebendazole (only children > 1 year of age; 250 mg age 1–2 years; 500 mg > 2 years age; no more than every 6 months). Routine evaluations, including thick blood smears, Hb measurements, and assessment of adherence with LLINs by self-report (did the child sleep under an LLIN the prior evening), were done every 3 months. Study participants were withdrawn from the study for 1) permanent movement out of the subcounty, 2) inability to be located for > 4 months, 3) withdrawal of informed consent, 4) inability to comply with the study schedule and procedures, or 5) reaching 11 years of age.

Laboratory procedures. Thick and thin blood smears were stained with 2% Giemsa for 30 minutes and read by laboratory technologists at the field sites who were not involved in direct patient care. Parasite densities was calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if the count was < 10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μL. A blood smear was considered negative when the examination of 100 high-power fields did not reveal asexual parasites. Thin smears were used for parasite species identification. For quality control, all slides were read by a second microscopist, and discrepancies resolved by a third reviewer at the field sites. In addition, all positive blood smears with a parasite densities $\leq$ 20,000/μL based on the field readings were reread by an expert microscopist based in Kampala, Uganda, and had to be confirmed to be considered positive in the final analyses. Hb measurements were performed using a portable spectrophotometer (HemoCue, Ängelholm, Sweden).

Statistical analysis. All data were collected using standardized case record forms and double entered using Microsoft Access (Microsoft Corporation, Redmond, WA). Analyses were performed using Stata, version 12 (Stata Corporation, College Station, TX), and R version 2.15 (R Core Team (2013), Vienna, Austria). Estimates of monthly rainfall were obtained from the NASA Tropical Rainfall Measuring Mission Project.[22] Data for this report covers the period from October 2011 (the first full month following enrollment of study households) through September 2013. Descriptive statistics included proportions and incidence measures using standard techniques for estimating 95% confidence intervals. The annual human biting rate was calculated as the total number of female *Anopheles* mosquitoes captured/number of house nights of collection × 365 days/year. The sporozoite rate was calculated as the number of mosquitoes testing positive for sporozoites/the number of mosquitoes tested. The annual EIR (aEIR) was the product of the annual human biting rate and the sporozoite rate. Routine blood smears were done every 3 months. Parasite prevalence was calculated as the number of routine blood smears positive for asexual parasites/total number of routine blood smears done. The incidence of malaria was calculated as the number of episodes of malaria/person-years of observation. Comparisons of changes in incidence measures over time were made using negative binomial regression models. Comparisons of change in prevalence measures over time were made using generalized estimating equations with adjustment for repeated measures in the same study participant. A P value < 0.05 was considered statistically significant.

Ethics statement. Ethical approval was obtained from the Makerere University School of Medicine Research and Ethics Committee, the Uganda National Council for Science and Technology, the London School of Hygiene and Tropical Medicine Ethics Committee, the School of Biological and Biomedical Sciences Ethics Committee, Durham University and the University of California, San Francisco Committee on Human Research.

RESULTS

Characteristics of the study sites. Characteristics of the three subcounties are presented in Table 1. Population-level coverage estimates of key malaria control interventions were from separate cross-sectional studies done from January–June 2012 in 200 households from each subcounty (Sarah G. Staedke, unpublished data). The proportion of households reporting ownership of at least one LLIN ranged from 51.0% in Kihihi to 78.5% in Nagongera. The proportion of households with at least one LLIN per two residents was significantly lower, ranging from 17.0% in Kihihi to 35.5% in Nagongera. The higher level of LLIN coverage in Nagongera is likely explained by a mass distribution campaign conducted in Tororo District in 2011. In the cohort studies described below, all children and their primary care givers were given a LLIN at enrollment, and over 99% of study participants reported sleeping under a LLIN the prior night at the time of routine assessments done every 3 months. None of the study sites were part of a government IRS program, and only 2.5% of the houses in Walukuba

TABLE 1
Study site characteristics

Characteristic	Study site		
	Walukuba	Kihihi	Nagongera
Altitude above sea level, range	1,102–1,500 m	886–1,329 m	695–1,443 m
Setting	Peri-urban	Rural	Rural
Population estimate (UBOS)	31,900	55,700	37,500
Total number of households	9,881	12,774	6,992
Median number of household residents (range)	3 (1–13)	4 (1–11)	5 (1–15)
Households with at least one LLIN	57.5%	51.0%	78.5%
Households with at least one LLIN per 2 residents	28.5%	17.0%	35.5%
Households receiving IRS in prior 12 months	2.5%	0	0
ACT use in children with fever treated with an antimalarial	75.0%	82.1%	77.8%

ACT = artemisinin-based combination therapies; LLIN = long-lasting insecticidal nets; IRS = indoor residual spraying, UBOS = Uganda Bureau of Statistics.

reported IRS in the previous 12 months. Reported ACT coverage among children with recent fever treated with an antimalarial ranged from 75.0% to 82.1% at the three sites using population-level estimates. With initiation of the cohort studies, all children presenting to our study clinics with a recent fever and laboratory confirmed malaria were treated with AL, and over 94% were treated within 3 days of onset of fever.

Study profile. Details of screening, enrollment, and follow-up of households and study participants are presented in Figure 2. To enroll 300 households (100 per subcounty), 466 were screened. Of the 166 households excluded, 159 (96%) were due to having no children within the target age range or the house being unoccupied. Of 765 children initially screened, 755 (99%) were enrolled, ranging from 213 in Walukuba to 298 in Kihihi. An additional 123 children were enrolled during dynamic recruitment and 175 were excluded over the 2-year observation period, primarily due to reaching 11 years of age or moving out of the study subcounties. During follow-up, the number of routine visits done every 3 months ranged from 1,826 in Walukuba to 2,611 in Kihihi. The mean age of study participants was similar across the three sites, ranging from 5.2 to 5.6 years (Table 2). Of a total of 13,811 visits to the study clinics after enrollment, in only 12 (0.1%) instances was outside medical care reported, including seven times when antimalarial

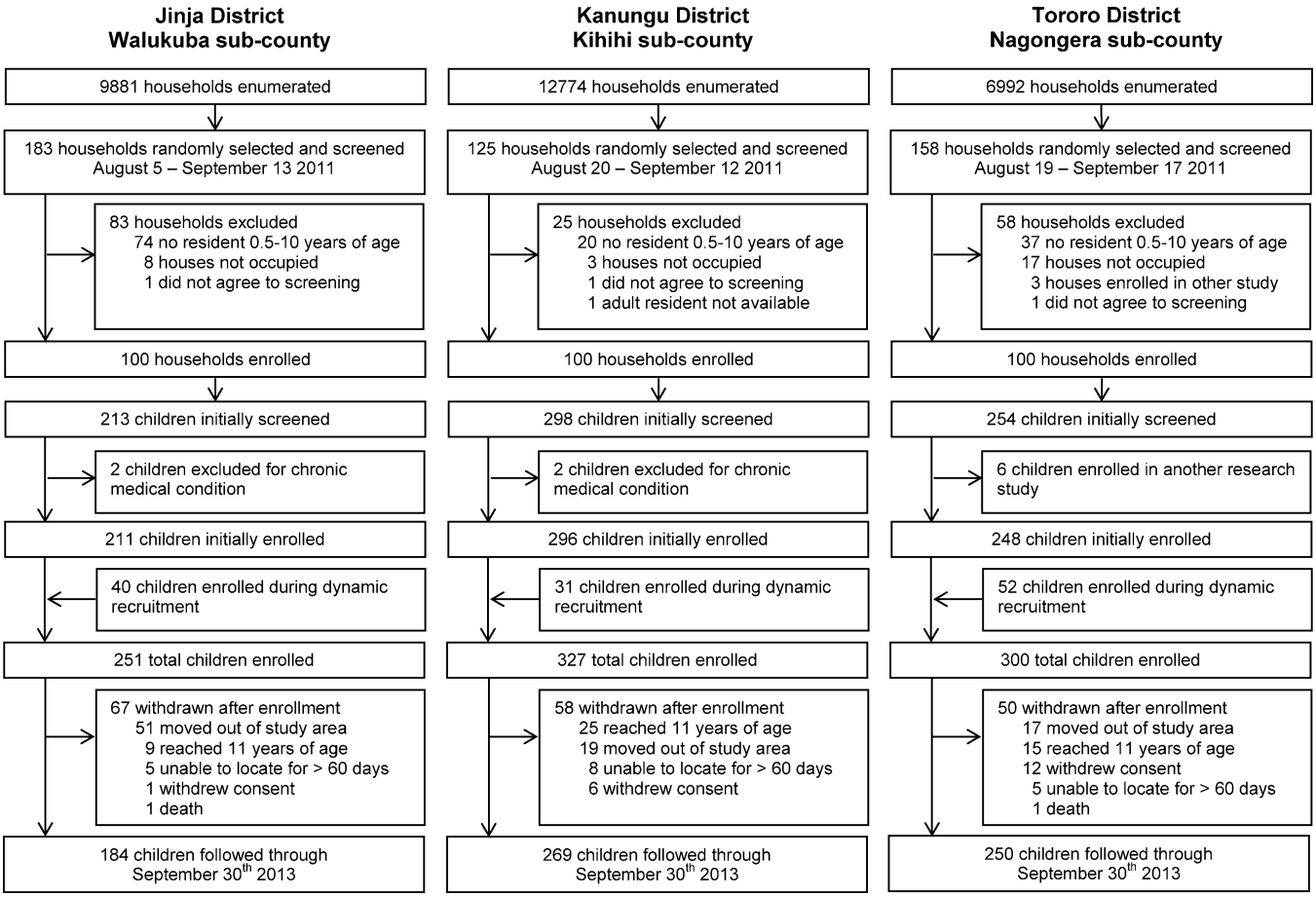

FIGURE 2. Enrolment and follow-up of study participant at the three study sites.

TABLE 2
Comparison of measures of transmission, infection, and disease across the three study sites

	Study site		
	Walukuba	Kihihi	Nagongera
Characteristics of study subjects			
Number of households enrolled	100	100	100
Number of children enrolled	251	327	300
Person years of observation	423	597	550
Number of routine visits	1,826	2,611	2,380
Number of febrile visits*	1,140	1,903	3,425
Mean age in years during follow-up	5.2	5.6	5.6
Measures of transmission			
Annual human biting rate (95% CI)	394 (378–410)	1,681 (1649–1713)	15,811 (15714–15909)
Sporozoite rate (95% CI)	0.71% (0.41–1.15%)	1.91% (1.59–2.27%)	1.96% (1.84–2.09%)
aEIR (95% CI)	2.8 (1.6–4.5)	32.0 (26.7–38.1)	310 (291–330)
Measures of infection			
Parasite prevalence (95% CI)†	7.4% (6.2–8.7%)	9.3% (8.2–10.5%)	28.7% (26.8–30.5%)
Measures of disease			
Episodes of malaria	182	854	1,546
Uncomplicated malaria	180	841	1,534
Danger signs	1	9	9
Severe malaria	1	4	3
Slide positivity rate (95% CI)‡	16.0% (13.9–18.2%)	44.9% (42.6–47.1%)	45.1% (43.5–46.8%)
Incidence of malaria PPY (95% CI)	0.43 (0.37–0.50)	1.43 (1.34–1.53)	2.81 (2.67–2.95)
Prevalence of anemia§ (95% CI)†	25.3% (23.3–27.4%)	15.4% (14.1–16.9%)	29.0% (27.1–30.8%)

aEIR = annual entomological inoculation rate; CI = confidence interval; PPY = per person-year.
*Tympanic temperature ≥ 38.0 °C or history of fever in the previous 24 hours.
†Measured at the time of three monthly routine visits.
‡Proportion of febrile visits where malaria diagnosed.
§Hemoglobin < 11 g/dL.

drugs were prescribed (all without laboratory confirmation and not included as episodes of malaria in our analyses).

Measures of transmission. Monthly mosquito collections from each household resulted in 2,358, 10,370, and 100,890 female anopheles and annual human biting rates of 394, 1,681, and 15,811 from Walukuba, Kihihi, and Nagongera, respectively ($P < 0.001$ for all pairwise comparisons). The sporozoite rate was similar in Kihihi (1.91%) and Nagongera (1.96%), but significantly lower in Walukuba (0.71%; $P < 0.001$ for both pairwise comparisons). The aEIR was estimated to be 2.8 in Walukuba, 32.0 in Kihihi, and 310 in Nagongera ($P < 0.001$ for all pairwise comparisons) (Table 2).

Measures of infection. The overall parasite prevalence was similar in Walukuba and Kihihi (7.4% versus 9.3%, $P = 0.23$), but significantly higher in Nagongera (28.7%) compared with the other 2 sites ($P < 0.001$ for both pairwise comparisons) (Table 2). The parasite prevalence peaked at approximately 8% around 3 years of age in Walukuba and at approximately 11% around 7 years of age in Kihihi, and then remained relatively constant. In Nagongera, the parasite prevalence increased with increasing age, reaching approximately 34% at 10 years of age (Figure 3).

Measures of disease. The incidence of malaria was 0.43 episodes per person-years (PPY) in Walukuba, 1.43 episodes PPY in Kihihi, and 2.81 episodes PPY in Nagongera ($P < 0.001$ for all pairwise comparisons) (Table 2). In two of the three sites (Kihihi and Nagongera), the incidence of malaria increased from age 6 months to approximately 3 years of age. However, the age and rate at which the incidence of malaria declined (a sign of the development of clinical immunity) varied across the sites. In Walukuba, the incidence of malaria was ~0.4 episodes PPY, with little variation from 2 to 8 years of age, followed by a gradual decline (Figure 4). In Kihihi, the incidence peaked at ~1.8 episodes PPY, with little variation from 3 to 6 years of age, followed by a gradual decline to ~1.0 episodes PPY. In Nagongera, the incidence peaked at ~4.3 episodes PPY, with a sharp decline from 3 to 10 years of age to ~1.3 episodes PPY. Of the 2,582 treatments given for malaria, 2,555 (99.0%) were for uncomplicated disease, 19 (0.7%) were for danger signs, and only 8 (0.3%) were for episodes that met criteria for severe malaria (5 with multiple convulsions, 2 with severe anemia (Hb < 5 g/dL), and 1 with respiratory distress). There were no deaths due to malaria, although two children died of diarrheal illnesses with negative blood smears. 99.7% of malaria episodes were due to *P. falciparum*. Response to treatment with AL was excellent, with only three episodes

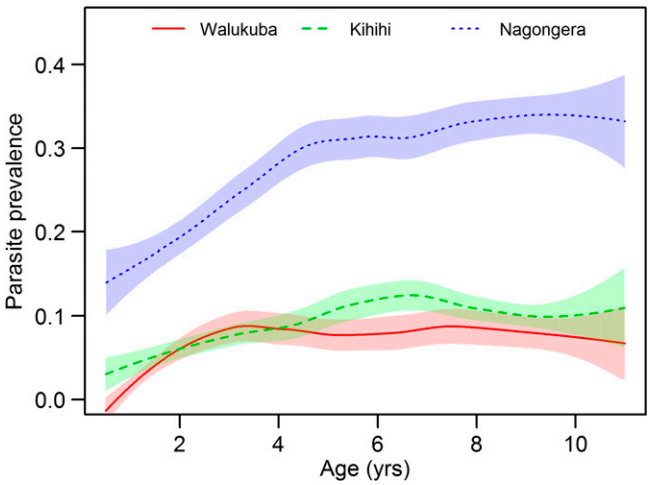

FIGURE 3. Parasite prevalence by age at the three study sites with varying malaria transmission intensity. Curves for each site were fitted using locally weighted least squares (lowess) regression, with standard errors (shaded region) estimated with 1,000 bootstrapping replicates, with resampling at the level of the subject.

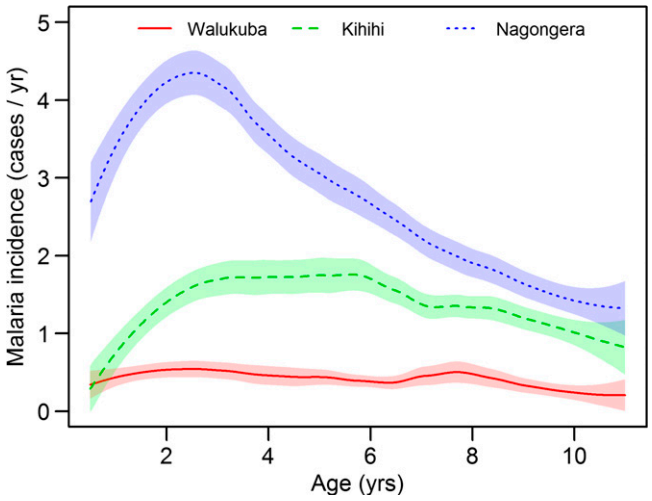

FIGURE 4. Malaria incidence by age at the three study sites with varying malaria transmission intensity. Curves for each site were fitted using locally weighted least squares (lowess) regression, with standard errors (shaded region) estimated with 1,000 bootstrapping replicates, with resampling at the level of the subject.

requiring rescue therapy within 3 days of initiation of treatment and 16 requiring repeat therapy within 14 days.

The prevalence of anemia (Hb < 11 g/dL) at routine visits did not follow the same pattern as transmission intensity and was 15.4% in Kihihi, 25.3% in Walukuba, and 29.0% in Nagongera (Table 2). To provide a historical comparison, combined anemia data were compared with national estimates for children 6–59 months of age from the Uganda 2009 MIS[18] and 2011 Demographic and Health Survey (DHS).[23] The prevalence of anemia decreased dramatically among the 2009 MIS, the 2011 DHS, and the cohort studies (Table 3). In addition, the prevalence of various degrees of anemia in cohort subjects declined significantly when comparing values at enrollment and the first routine visit with values during the last 18 months of follow-up: Hb < 11 g/dL (26.3% versus 21.6%, $P = 0.001$), Hb < 10 g/dL (11.1% versus 7.7%, $P < 0.001$), and Hb < 8 g/dL (1.6% versus 0.9%, $P = 0.03$).

Temporal changes in malaria metrics. Temporal trends in measures of transmission, infection, and disease for the three study sites are presented in Figure 5. At all three sites, monthly estimates of aEIR were characterized by two annual peaks from October to January and April to July. The timing of the peaks varied somewhat and the amplitude of the peaks varied greatly between sites and year to year. Monthly estimates of malaria incidence followed similar temporal trends as estimates of transmission, with two annual peaks and appreciable changes between years. However, the relative difference in malaria incidence between the high- and low-transmission seasons was less pronounced in Nagongera, the site with the highest transmission intensity. Parasite prevalence was estimated every 3 months and did not reflect the same degree of temporal variation as measures of transmission and disease. Only in Kanungu, there were appreciable temporal changes in parasite prevalence. In addition, there were no significant changes in parasite densities over time at any of the three sites (data not shown). Comparing malaria metrics between the first (October 2011–September 2012) and second (October 2012–September 2013) years of observation yielded interesting differences between sites. In Walukuba, there were significant decreases in the aEIR (4.0 versus 1.8, $P = 0.007$) and malaria incidence (0.51 PPY versus 0.31 PPY, $P = 0.001$), with a modest decrease in parasite prevalence that did not reach statistical significance (9.0% versus 5.7%, $P = 0.11$). In Kihihi, there were significant increases in aEIR (18.9 versus 46.6, $P = 0.001$), malaria incidence (0.97 PPY versus 1.93 PPY, $P < 0.001$), and parasite prevalence (7.6% versus 11.6%, $P < 0.001$). In Nagongera, there was a significant decrease in aEIR (348 versus 267, $P = 0.008$), a significant increase in malaria incidence (2.33 PPY versus 3.30 PPY, $P < 0.001$), and no significant change in parasite prevalence (27.4% versus 28.7%, $P = 0.50$). At all three sites, there were no significant differences in average monthly rainfall between the 2 years of observation (data not shown).

TABLE 3
Comparison of prevalence of anemia between national surveys and cohort studies

Measurement	2009 MIS[17]	2011 DHS[21]	2011–2013 Cohort studies†
Number of observations*	3529	2142	6816
Hb < 11 g/dL	62.3%	49.3%	22.8%
Hb < 10 g/dL	40.6%	N/A	8.5%
Hb < 8 g/dL	9.7%	4.7%	1.0%

DHS = Demographic and Health Survey; Hb = hemoglobin; MIS = Malaria Indicator Survey.
*Includes children 6–59 months of age.
†Data presented in this paper.

DISCUSSION

We investigated the relationship between malaria transmission, infection, and disease at three sites in Uganda with varied transmission intensity. The study benefited from detailed knowledge of the study sites, dynamic cohorts in which all participants received LLINs and prompt treatment of malaria with an ACT, and entomologic data collected from the households occupied by the cohort members. We found 1) highly seasonal transmission and varied relationships between measures of transmission, infection, and disease; 2) decreasing incidence in the peri-urban site, but high and increasing incidence in the two rural sites; 3) very low incidence of severe disease; and 4) low and decreasing prevalence of anemia at all sites. These results highlight the current complexity of malaria dynamics in Uganda.

Malaria surveillance, monitoring, and evaluation are critical for estimating disease burden and assessing the level of coverage and impact of control interventions. In highly endemic areas, such as Uganda, malaria indicators are largely derived from nationally representative household surveys and routine health information systems.[24] Household surveys are useful for estimating the coverage level of key control interventions such as LLINs, IRS, and ACT use as well as some indicators of disease burden such as parasite prevalence and anemia. However, these surveys are cross-sectional in nature and infrequently done in resource-limited countries, limiting their ability to monitor temporal trends and provide detailed information on disease epidemiology. Data from routine health information systems, generally health facility–based reporting of malaria cases, are used to estimate malaria morbidity and mortality and monitor temporal trends. However, in resource-limited countries where the malaria burden is highest, the quality of these data is often poor because of lack of laboratory confirmation, incomplete reporting, and failure to capture cases outside the public health

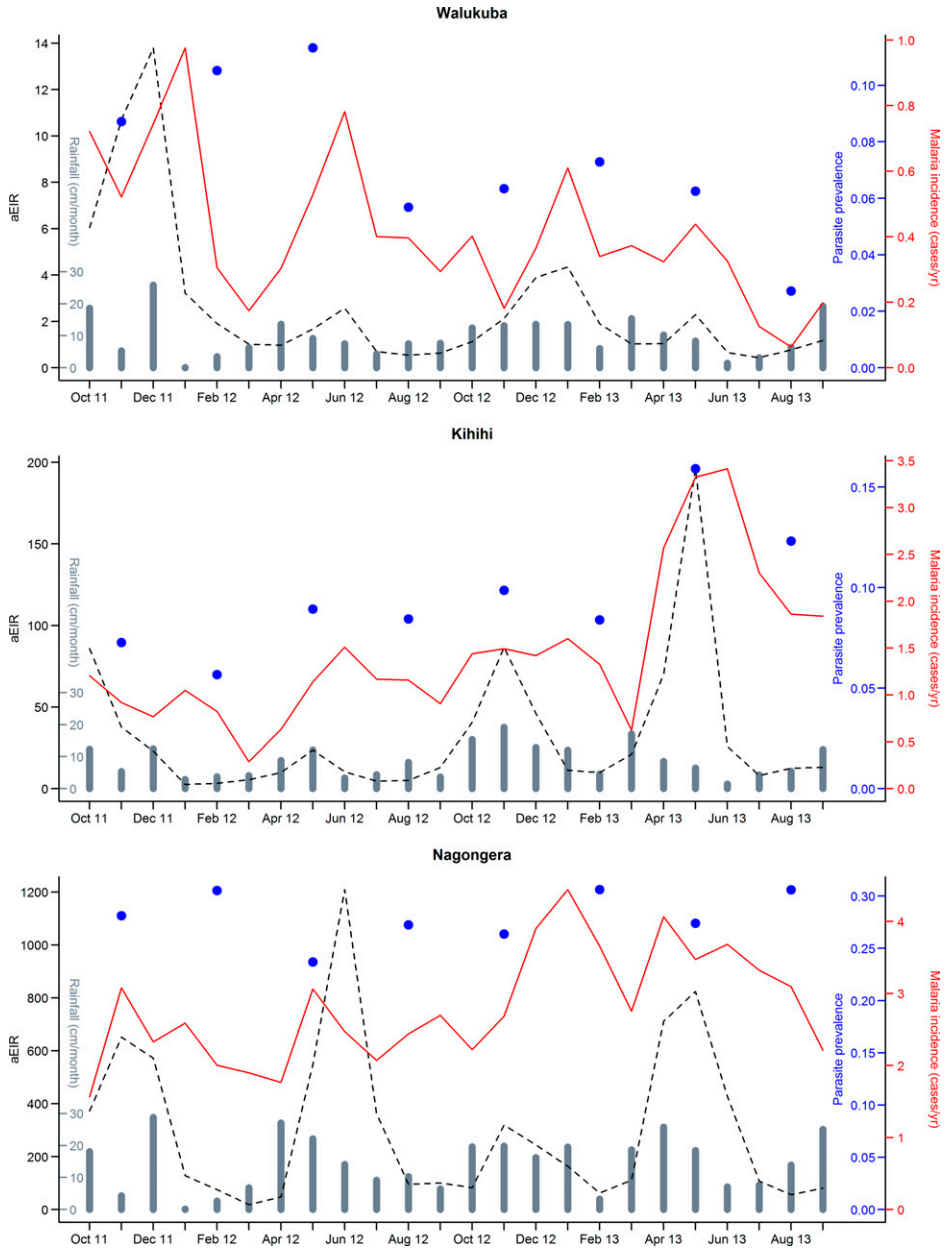

FIGURE 5. Temporal trends in measures of malaria infection, transmission, and disease at the three study sites. Entomological inoculation rates (dashed lines) for each site were estimated using the product of human biting rates (averaged monthly) and sporozoite rates (averaged yearly). Malaria incidence (solid lines) for each site was estimated using mean monthly incidence; prevalence (dots) for each site was estimated from active surveillance occurring every 3 months. Estimates of average monthly rainfall were obtained from the NASA Tropical Rainfall Measuring Mission Project.

sector. There has been a strong push for improving surveillance in countries approaching malaria elimination.[25] However, there is also a great need to improve malaria surveillance in highly endemic countries where the focus remains disease control. In this report, we describe the findings of a comprehensive malaria surveillance program conducted in three areas of Uganda with differing disease epidemiology.

To provide context for the data presented in this report it is useful to review recent trends in the coverage of key control interventions in Uganda.[18,23,26] Considering use of bed nets, nationally representative estimates of LLIN coverage, defined as the proportion of households with at least one LLIN, increased from 16% in 2006, to 47% in 2009, to 60% in 2011. In the three subcounties included in this report, estimates ranged from 51 to 79%. However, the proportion of households with at least one LLIN per two persons was much lower (17–36%), suggesting low LLIN coverage and a considerable intra-household ownership gap. Considering IRS, in 2006, Uganda implemented the first large-scale program since the 1960s. This program has focused principally on 10 districts in northern Uganda, and on a national scale only 6–7% of households reported receiving IRS between 2006 and 2011. Considering malaria therapy, the proportion of children under 5 years with recent fever treated for malaria who were given an ACT dramatically increased in Uganda from 5% in 2006, to 39% in 2009, to 69% in 2011. Indeed this trend appears to have

continued, as in 2012 estimates for this indicator ranged from 75–82% for the subcounties included in this report (Sarah G. Staedke, unpublished data).

Comparison of the relationships between measures of transmission, infection, and disease within and between the three sites provided a picture of the contrasting epidemiology of malaria in Uganda. Transmission (defined by EIR) was highly seasonal, with two annual peaks at all sites, but between sites there was marked variation in the magnitude of these peaks and in changes from year to year. Compared with Walukuba, the EIR for the full observation period was approximately 10-fold higher in Kihihi and 100-fold higher in Nagongera. In Walukuba and Nagongera, there were significant decreases in EIR from the first year to the next, while in Kihihi, there was a significant increase in EIR. Of note, differences in EIR between the sites and over time were primarily driven by differences in the numbers of mosquitoes collected (human biting rate) and not by the proportion of infected mosquitoes (sporozoite rate). Although EIR is considered the gold standard metric of malaria transmission, the large uncertainty inherent in measuring the human biting rate and the sporozoite rate limit the precision and accuracy of EIR across small temporal and spatial scales.[27] In contrast to transmission, our measure of infection (defined as the parasite prevalence in cohort children 0.5–10 years of age) showed less variation over time. Although parasite prevalence is a useful tool for rapid estimates of endemicity, it is not a direct measure of transmission intensity, and can become saturated at higher intensities.[28,29] Indeed, large changes in EIR can lead to relatively small changes in parasite prevalence, as prevalence is influenced by heterogeneous biting, multiple infections, acquired immunity, and antimalarial therapy.[27] Our most direct measure of disease burden was childhood malaria incidence. Considering temporal and geographic variation, incidence correlated well with EIR. In our two lower transmission sites, changes in malaria incidence from the first to second year correlated well with changes in EIR. However, in our highest transmission intensity site, malaria incidence increased significantly from the first to second year despite a decrease in EIR, suggesting that associations between estimates of transmission intensity and disease incidence are complex, especially in high transmission intensity settings.

Although this study was not designed to make causal inferences about factors responsible for changes in malaria over time, observed differences between our low- and high-transmission cohorts, all of whom received LLINs and prompt effective treatment of malaria, allow some conclusions. Our lowest transmission intensity site, a peri-urban setting, was the only site that exhibited a significant decline in measures of transmission and disease. A similar decline in malaria incidence was reported from 2004 to 2008 in a cohort of children living in the urban center of Kampala.[30] Many reports from Africa have demonstrated significant declines in malaria morbidity and mortality following the scale-up of available malaria control interventions such as LLINs and ACTs, although most of these reports come from areas with low to moderate baseline levels of transmission intensity.[1,2,4–6,31,32] In contrast, recent reports from sites in Malawi with intermediate to high transmission intensity have documented no change or increased malaria burden, despite a modest scale-up of control interventions.[7,33] In a high transmission site from Senegal, scale-up of LLINs was initially associated with a decline in malaria incidence but subsequently malaria incidence rose to almost pre-intervention levels, corresponding with increasing pyrethroid resistance of mosquito vectors.[8] Data from our two highest transmission sites also showed temporal increases in malaria incidence. Of note, several reports from Uganda have shown a dramatic increase in the prevalence of pyrethroid resistance among mosquito vectors over the last 5 years.[34–36] Taken together, our findings suggest that the impacts of scale-up of control interventions are site specific and depend on the underlying epidemiology of malaria. For example, mathematical models have predicted bigger impacts over a shorter time frame following scale-up of LLINs when baseline levels of transmission intensity are lower.[37] Indeed, in high-transmission settings that are characteristic much of Uganda, reductions in malaria incidence may require higher coverage levels, longer durations, and possibly additional interventions. This "race" to reduce the burden of malaria must be carried out under the constant threat of the emergence and spread of insecticide and drug resistance.

An encouraging finding from this study was the remarkably low incidence of severe malaria and malaria-associated mortality. Historically it has been estimated that each clinical attack of malaria among African children is associated with a 2% risk of severe disease and a mortality rate of 0.5%.[38] In this study, with over 2,500 treatments for malaria, the risk of severe malaria was < 0.3%, and there were no episodes of cerebral malaria or malaria-associated death. In addition, the prevalence of anemia was surprisingly low across all sites and declined over the period of observation. Indeed, nationally representative surveys in Uganda suggest that there has been a marked reduction in the prevalence of anemia and under five mortality in recent years.[18,23,26] Similarly, low rates of severe malaria and mortality have been found elsewhere whenever malaria studies provide high-quality health care.[30] We hypothesize that the encouraging findings from this study and possibly for Uganda as a whole can be largely attributed to prompt and effective therapy with AL, which results in rapid parasite clearance, resolution of symptoms, and Hb recovery, thus reducing the risk of progression to severe disease.[39,40] Another encouraging finding was the relatively low prevalence of anemia in our cohorts, which appeared to be decreasing over time, consistent with recent trends from national surveys. We believe that decreasing anemia may also have been due to prompt and effective treatment with AL, along with other routine measures such as deworming and iron supplementation.

There were several limitations to this study. First, estimates of parasite prevalence were based on microscopy and may have significantly underestimated the true prevalence of infection. Indeed, there is an increasing appreciation of the role of asymptomatic carriage in transmission and more sensitive methods, such as polymerase chain reaction (PCR), have revealed that the proportion of infections due to sub-patent parasitemia (i.e., not detected by microscopy) is inversely proportional to transmission intensity.[16] Second, the assessment of LLIN use in both the cohort studies and complimentary community surveys was by self-report and was not based on directly observed LLIN usage, which may have overestimated the utilization of LLINs.[41] Finally, the descriptive nature of the study limits our capacity to make causal inference in terms of the potential impact of control interventions on our outcome measures.

In summary, despite reports of decreasing malaria burden across many parts of sub-Saharan Africa, the burden continues

to be very high in Uganda. Indeed, the incidence of malaria rose in our two rural cohorts from 2011 to 2013. The use of both LLIN and ACTs may be sufficient for minimizing the severity of disease, improving child health, and reducing childhood mortality at the level of an individual. However, in areas with high transmission intensity, reducing morbidity will likely require higher community-wide coverage of these interventions and consideration of additional interventions such as expansion of IRS, larval source management, and other novel vector control strategies, chemoprevention at the individual and/or community level, mass drug administration, and/or an effective vaccine. Indeed, the true prevalence of parasitemia in our study sites was likely higher than our estimates based on microscopy, highlighting the challenges of malaria control and elimination when the reservoir of infection is so large. High-transmission countries such as Uganda already bear a disproportionate burden of this terrible disease and will likely require further scaling up of widely accepted existing interventions and possibly novel approaches to realize the successes now being celebrated in other parts of the world.

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REFERENCES

1. Aregawi MW, Ali AS, Al-mafazy AW, Molteni F, Katikiti S, Warsame M, Njau RJ, Komatsu R, Korenromp E, Hosseini M, Low-Beer D, Bjorkman A, D'Alessandro U, Coosemans M, Otten M, 2011. Reductions in malaria and anaemia case and death burden at hospitals following scale-up of malaria control in Zanzibar, 1999–2008. *Malar J 10:* 46.
2. Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJ, Sesay SS, Abubakar I, Dunyo S, Sey O, Palmer A, Fofana M, Corrah T, Bojang KA, Whittle HC, Greenwood BM, Conway DJ, 2008. Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet 372:* 1545–1554.
3. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD, 2012. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet 379:* 413–431.
4. Nyarango PM, Gebremeskel T, Mebrahtu G, Mufunda J, Abdulmumini U, Ogbamariam A, Kosia A, Gebremichael A, Gunawardena D, Ghebrat Y, Okbaldet Y, 2006. A steep decline of malaria morbidity and mortality trends in Eritrea between 2000 and 2004: the effect of combination of control methods. *Malar J 5:* 33.
5. O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, Newton CR, Marsh K, 2008. Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. *Lancet 372:* 1555–1562.
6. Otten M, Aregawi M, Were W, Karema C, Medin A, Bekele W, Jima D, Gausi K, Komatsu R, Korenromp E, Low-Beer D, Grabowsky M, 2009. Initial evidence of reduction of malaria cases and deaths in Rwanda and Ethiopia due to rapid scale-up of malaria prevention and treatment. *Malar J 8:* 14.
7. Roca-Feltrer A, Kwizombe CJ, Sanjoaquin MA, Sesay SS, Faragher B, Harrison J, Geukers K, Kabuluzi S, Mathanga DP, Molyneux E, Chagomera M, Taylor T, Molyneux M, Heyderman RS, 2012. Lack of decline in childhood malaria, Malawi, 2001–2010. *Emerg Infect Dis 18:* 272–278.
8. Trape JF, Tall A, Diagne N, Ndiath O, Ly AB, Faye J, Dieye-Ba F, Roucher C, Bouganali C, Badiane A, Sarr FD, Mazenot C, Toure-Balde A, Raoult D, Druilhe P, Mercereau-Puijalon O, Rogier C, Sokhna C, 2011. Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. *Lancet Infect Dis 11:* 925–932.
9. Overgaard HJ, Reddy VP, Abaga S, Matias A, Reddy MR, Kulkarni V, Schwabe C, Segura L, Kleinschmidt I, Slotman MA, 2012. Malaria transmission after five years of vector control on Bioko Island, Equatorial Guinea. *Parasit Vectors 5:* 253.
10. Trape JF, Tall A, Sokhna C, Ly AB, Diagne N, Ndiath O, Mazenot C, Richard V, Badiane A, Dieye-Ba F, Faye J, Ndiaye G, Diene Sarr F, Roucher C, Bouganali C, Bassene H, Toure-Balde A, Roussilhon C, Perraut R, Spiegel A, Sarthou JL, da Silva LP, Mercereau-Puijalon O, Druilhe P, Rogier C, 2014. The rise and fall of malaria in a west African rural community, Dielmo, Senegal, from 1990 to 2012: a 22 year longitudinal study. *Lancet Infect Dis 14:* 476–488.
11. Okello PE, Van Bortel W, Byaruhanga AM, Correwyn A, Roelants P, Talisuna A, D'Alessandro U, Coosemans M, 2006. Variation in malaria transmission intensity in seven sites throughout Uganda. *Am J Trop Med Hyg 75:* 219–225.
12. Rogier C, 2000. Natural history of *Plasmodium falciparum* malaria and determining factors of the acquisition of antimalaria immunity in two endemic areas, Dielmo and Ndiop (Senegal). *Bull Mem Acad R Med Belg 155:* 218–226.
13. Aponte JJ, Menendez C, Schellenberg D, Kahigwa E, Mshinda H, Vountasou P, Tanner M, Alonso PL, 2007. Age interactions in the development of naturally acquired immunity to *Plasmodium falciparum* and its clinical presentation. *PLoS Med 4:* e242.
14. Rogier C, Tall A, Diagne N, Fontenille D, Spiegel A, Trape JF, 1999. *Plasmodium falciparum* clinical malaria: lessons from longitudinal studies in Senegal. *Parassitologia 41:* 255–259.
15. Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Ndiaye P, Brahimi K, Faye O, Druilhe P, da Silva PL, 1994. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am J Trop Med Hyg 51:* 123–137.
16. Okell LC, Bousema T, Griffin JT, Ouedraogo AL, Ghani AC, Drakeley CJ, 2012. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. *Nat Commun 3:* 1237.
17. Kurtzhals JA, Addae MM, Akanmori BD, Dunyo S, Koram KA, Appawu MA, Nkrumah FK, Hviid L, 1999. Anaemia caused by asymptomatic *Plasmodium falciparum* infection in semi-immune African schoolchildren. *Trans R Soc Trop Med Hyg 93:* 623–627.

18. Uganda Bureau of Statistics and ICF Macro, 2010. *Uganda Malaria Indicator Survey 2009*. Available at: http://www.measuredhs.com/pubs/pdf/MIS6/MIS6.pdf. Accessed April 27, 2014.
19. Kilama M, Smith DL, Hutchinson R, Kigozi R, Yeka A, Lavoy G, Kamya MR, Staedke SG, Donnelly MJ, Drakeley C, Greenhouse B, Dorsey G, Lindsay SW, 2014. Estimating the annual entomological inoculation rate for *Plasmodium falciparum* transmitted by *Anopheles gambiae* s.l. using three sampling methods in three sites in Uganda. *Malar J 13:* 111.
20. Bukirwa H, Yau V, Kigozi R, Filler S, Quick L, Lugemwa M, Dissanayake G, Kamya M, Wabwire-Mangen F, Dorsey G, 2009. Assessing the impact of indoor residual spraying on malaria morbidity using a sentinel site surveillance system in western Uganda. *Am J Trop Med Hyg 81:* 611–614.
21. World Health Organization, 2010. *Who Guidelines for the Treatment of Malaria*. Available at: http://www.who.int/malaria/publications/atoz/9789241547925/en/. Accessed April 10, 2014.
22. National Aeronautics and Space Administration. Available at: http://mirador.gsfc.nasa.gov/collections/TRMM_3B43__006.shtml. Accessed April 27, 2014.
23. Uganda Bureau of Statistics and ICF Macro, 2011. *Uganda Demographic and Health Survey 2011*. Available at: http://www.measuredhs.com/pubs/pdf/PR18/PR18.pdf. Accessed April 27, 2014.
24. World Health Organization, 2013. *World Malaria Report, 2013*. Available at: http://www.who.int/malaria/world_malaria_report_2012/en/. Accessed April 27, 2014.
25. The malERA Consultative Group on Monitoring, Evaluation, and Surveillance, 2011. A research agenda for malaria eradication: monitoring, evaluation, and surveillance. *PLoS Med 8:* e1000400.
26. Uganda Bureau of Statistics and ICF Macro, 2006. *Uganda Demographic and Health Survey 2006*. Available at: http://www.measuredhs.com/pubs/pdf/FR194/FR194.pdf. Accessed April 27, 2014.
27. Tusting LS, Bousema T, Smith DL, Drakeley C, 2014. Measuring changes in *Plasmodium falciparum* transmission: precision, accuracy and costs of metrics. *Adv Parasitol 84:* 151–208.
28. Gething PW, Patil AP, Smith DL, Guerra CA, Elyazar IR, Johnston GL, Tatem AJ, Hay SI, 2011. A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malar J 10:* 378.
29. Smith DL, Dushoff J, Snow RW, Hay SI, 2005. The entomological inoculation rate and *Plasmodium falciparum* infection in African children. *Nature 438:* 492–495.
30. Clark TD, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Greenhouse B, Staedke SG, Kamya MR, Dorsey G, Rosenthal PJ, 2010. Incidence of malaria and efficacy of combination antimalarial therapies over 4 years in an urban cohort of Ugandan children. *PLoS ONE 5:* e11759.
31. Bhattarai A, Ali AS, Kachur SP, Martensson A, Abbas AK, Khatib R, Al-Mafazy AW, Ramsan M, Rotllant G, Gerstenmaier JF, Molteni F, Abdulla S, Montgomery SM, Kaneko A, Bjorkman A, 2007. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Med 4:* e309.
32. Okiro EA, Hay SI, Gikandi PW, Sharif SK, Noor AM, Peshu N, Marsh K, Snow RW, 2007. The decline in paediatric malaria admissions on the coast of Kenya. *Malar J 6:* 151.
33. Okiro EA, Kazembe LN, Kabaria CW, Ligomeka J, Noor AM, Ali D, Snow RW, 2013. Childhood malaria admission rates to four hospitals in Malawi between 2000 and 2010. *PLoS ONE 8:* e62214.
34. Mawejje HD, Wilding CS, Rippon EJ, Hughes A, Weetman D, Donnelly MJ, 2013. Insecticide resistance monitoring of field-collected *Anopheles gambiae* s.l. populations from Jinja, eastern Uganda, identifies high levels of pyrethroid resistance. *Med Vet Entomol 27:* 276–283.
35. Ramphul U, Boase T, Bass C, Okedi LM, Donnelly MJ, Muller P, 2009. Insecticide resistance and its association with target-site mutations in natural populations of *Anopheles gambiae* from eastern Uganda. *Trans R Soc Trop Med Hyg 103:* 1121–1126.
36. Verhaeghen K, Van Bortel W, Trung HD, Sochantha T, Keokenchanh K, Coosemans M, 2010. Knockdown resistance in *Anopheles vagus*, *An. sinensis*, *An. paraliae* and *An. peditaeniatus* populations of the Mekong region. *Parasit Vectors 3:* 59.
37. Smith DL, Hay SI, Noor AM, Snow RW, 2009. Predicting changing malaria risk after expanded insecticide-treated net coverage in Africa. *Trends Parasitol 25:* 511–516.
38. Greenwood B, Marsh K, Snow R, 1991. Why do some African children develop severe malaria? *Parasitol Today 7:* 277–281.
39. Dorsey G, Staedke S, Clark TD, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Dokomajilar C, Kamya MR, Rosenthal PJ, 2007. Combination therapy for uncomplicated falciparum malaria in Ugandan children: a randomized trial. *JAMA 297:* 2210–2219.
40. Muhindo MK, Kakuru A, Jagannathan P, Talisuna A, Osilo E, Orukan F, Arinaitwe E, Tappero JW, Kaharuza F, Kamya MR, Dorsey G, 2014. Early parasite clearance following artemisinin-based combination therapy among Ugandan children with uncomplicated *Plasmodium falciparum* malaria. *Malar J 13:* 32.
41. Frey C, Traore C, De Allegri M, Kouyate B, Muller O, 2006. Compliance of young children with ITN protection in rural Burkina Faso. *Malar J 5:* 70.