Asymptomatic *Plasmodium* Infections in Children in Low Malaria Transmission Setting, Southwestern Uganda[1]

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A survey of asymptomatic children in Uganda showed *Plasmodium malariae* and *P. falciparum* parasites in 45% and 55% of microscopy-positive samples, respectively. Although 36% of microscopy-positive samples were negative by rapid diagnostic test, 75% showed *P. malariae* or *P. ovale* parasites by PCR, indicating that routine diagnostic testing misses many non–*P. falciparum* malarial infections.

Since 2000, substantial progress has been made in reducing malaria worldwide. In Uganda, malaria transmission is heterogeneous, yet 97% of all cases are attributed to *P. falciparum* (*1*). Accordingly, detection and treatment algorithms have targeted *P. falciparum* over less virulent species. Inadequate attention to non–*P. falciparum* infections has several implications for malaria transmission. For example, gametocytemia can occur earlier (e.g., *P. vivax* and perhaps *P. ovale*) and remain undetected for longer periods because of milder clinical symptoms (e.g., *P. malariae* and *P. ovale*) than for *P. falciparum* infections, enabling persistent transmission of non–*P. falciparum* infections (*2*).

In the southwestern region of Uganda, *Plasmodium* transmission is low and unstable. In 2004 and 2010, we conducted surveys that showed progress in decreasing *P. falciparum* infections in this region, although comparatively little is known about the prevalence of other species in this region (*3*). To determine the comparative species prevalence by multispecies rapid diagnostic test (RDT) and blood-smear microscopy, we conducted a cross-sectional survey of 631 children <5 years of age during the low transmission season of 2014 in 3 districts in southwestern Uganda (Mbarara, Bushenyi, and Isingiro) (Figure). These 3 districts represent a range of transmission intensities from low to high, respectively (*4*).

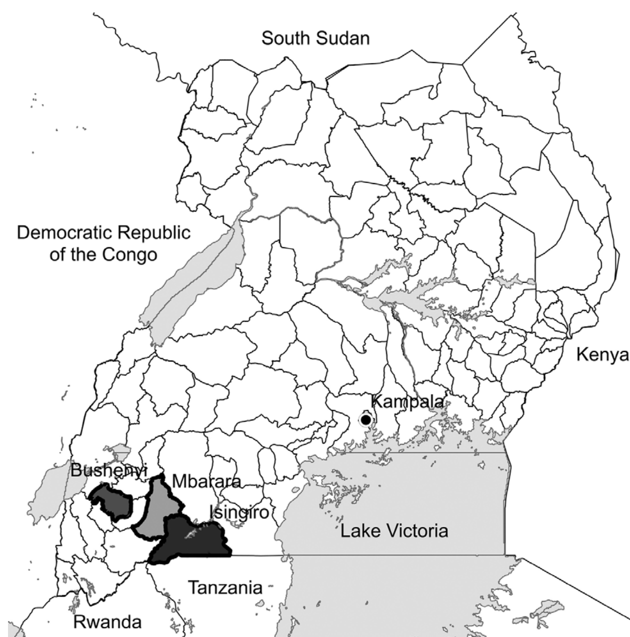

Figure. Districts where surveys of asymptomatic children were conducted to determine *Plasmodium* infections, southwestern Uganda.

The Study

Stratified, 2-stage cluster sampling was used to select study participants. We administered questionnaires to gather information about standard knowledge, attitudes, and practices related to malaria and collected blood for testing with microscopy, RDT, and PCR (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/8/16-0619-Techapp1.pdf). RDT was a combined *P. falciparum*–specific, histidine-rich protein-2 (HRP-2)/pan-*Plasmodium* lactate dehydrogenase (pLDH) RDT (SD Bioline Malaria Ag P.f/Pan [*P. falciparum* or other *Plasmodium* species]; Standard Diagnostics, Gyeonggi-do, South Korea). Nested PCR was performed on all RDT- or microscopy-positive

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Table 1. Characteristics of asymptomatic children surveyed for *Plasmodium* infections, by district, southwestern Uganda*

Characteristic	District†				p value‡
	Mbarara, n = 242	Bushenyi, n = 157	Isingiro, n = 232	Total, N = 631†	
Mean age, y ± SD	2.4 ± 1.2	2.3 ± 1.2	2.4 ± 1.3	2.4 ± 1.3	0.850
Sex					
M	122 (50.4)	80 (51.0)	120 (51.7)	322 (51.0)	0.958
F	120 (49.6)	77 (49.0)	112 (48.3)	309 (49.0)	
Rural	177 (73.1)	133 (84.7)	202 (87.1)	512 (81.1)	0.472
Wealth quartile					<0.001
1st	42 (17.4)	45 (28.7)	97 (41.8)	184 (29.2)	
2nd	27 (11.2)	58 (36.9)	55 (23.7)	140 (22.2)	
3rd	83 (34.3)	32 (20.4)	56 (24.1)	171 (27.1)	
4th	90 (37.2)	22 (14.0)	24 (10.3)	136 (21.6)	
Roofing material					<0.001
Corrugated metal	237 (97.9)	152 (96.8)	206 (88.8)	595 (94.3)	
Thatch or leaf	3 (1.2)	5 (3.2)	17 (7.3)	25 (4.0)	
Other	2 (0.8)	0	9 (3.9)	11 (1.7)	
Household crowding§					0.239
1–2	68 (28.1)	52 (33.1)	61 (26.3)	181 (28.7)	
3	123 (50.8)	71 (45.2)	134 (57.8)	328 (52.0)	
≥4	51 (21.1)	34 (21.7)	37 (16.0)	122 (19.3)	
Consistent bed net use	228 (94.2)	151 (96.2)	198 (85.7)	577 (91.6)	0.003
Indoor residual spraying	4 (1.7)	1 (0.6)	0	5 (0.8)	0.122
Malaria prevalence					
By RDT	4 (1.7)	8 (5.1)	30 (13.0)	42 (6.7)	<0.001
Pf+	1	3	7	11	
Pan+	2	0	1	3	
Pf/Pan+	1	5	22	28	
By microscopy	4 (1.7)	5 (3.2)	13 (5.6)	22 (3.5)	0.067
P. falciparum	2	3	4	9	
P. malariae	0	1	6	7	
P. ovale	1	1	1	3	
Mixed *Pf/Pm*	1	0	2	3	

*Values are no. (%) children surveyed except as indicated. Pan+, positive for non–*P. falciparum* infection only; Pf+, positive for *P. falciparum* monoinfection only; Pf/Pan+, positive for *P. falciparum* monoinfection or *P. falciparum* mixed infection; RDT, rapid diagnostic test; mixed *Pf/Pm*, positive for *P. falciparum* monoinfection or *P. malariae* mixed infection.
†Totals in columns may not add up to total because of missing data.
‡Determined by Fisher exact test or χ^2 test, as appropriate.
§Defined as number of persons who sleep in the same room.

samples. Predictors of malaria were selected a priori (online Technical Appendix).

We surveyed 631 children with a mean age of 2.4 years (Table 1). Bed net coverage was high (91.6%) and met targets for 2014 (*5*). Only 5 households (0.8% of children surveyed) reported use of indoor residual spraying. Of the 3 districts, Isingiro had the highest proportion of children living in the lowest wealth quartile (41.8%) and in households with thatched or leaf roofing (7.3%); this district also had the lowest consistent bed net use (85.7%) (Table 1).

Overall prevalence of parasitemia by microscopy was 3.5% (95% CI 1.9%–5.1%). Speciation by microscopy revealed a higher proportion of non–*P. falciparum* infections than *P. falciparum* monoinfections. Of 22 microscopy-positive samples, 9 (40.9%) were *P. falciparum* monoinfections, 7 (31.8%) were *P. malariae* monoinfections, 3 (13.6%) were *P. ovale* monoinfections, and 3 (13.6%) were *P. falciparum*/*P. malariae* mixed infections. Most *P. malariae* monoinfections occurred in Isingiro district. Sixteen (72.7%) of 22 blood-smear readings correlated directly with PCR results (online Technical Appendix Table 2).

Malaria parasite prevalence was 2-fold higher by RDT than by microscopy (6.7% vs. 3.5%; Table 1). RDT correctly identified 9 of 12 *P. falciparum* monoinfections and mixed infections. A comparison of the diagnostic performance of RDT and microscopy (uncorrected by PCR) indicates that agreement of results from these methods was high (>94%); however, agreement was poor in detecting non–*P. falciparum* infections (k = 0.15) compared with detecting overall infection (k = 0.41) and *P. falciparum* infection (k = 0.33) (Table 2). PCR detected parasite DNA in 53.7% (22/41) of RDT-positive samples; of these, 55% (12/22) correlated with the correct RDT band pattern interpretation (online Technical Appendix Table 2).

Approximately one third (8/22) of children with microscopy-positive cultures had negative RDT results (online Technical Appendix Table 3). Of these 8 discordant cases, 5 harbored PCR-confirmed *P. malariae* or *P. ovale* monoinfections; all had parasite densities <1,060/µL (online Technical Appendix Table 2). Conversely, two thirds (28/42) of RDT-positive samples were negative by microscopy. Of the 28 children with RDT-positive and microscopy-negative samples, 8 (28.6%) had a malaria

Table 2. Diagnostic performance of RDT and microscopy for *Plasmodium* infections in children in 3 districts, southwestern Uganda*

Diagnostic accuracy of RDT†	*Plasmodium* infection	*P. falciparum* infection	Non–*P. falciparum* infection‡
Sensitivity (95% CI)	63.6 (40.7–82.8)	75.0 (42.8–94.5)	10.0 (0.3–44.5)
Specificity (95% CI)	95.4 (93.4–96.9)	95.1 (93.1–96.7)	99.8 (99.1–100.0)
PPV (95% CI)	33.3 (19.6–49.5)	23.1 (11.1–39.3)	50.0 (1.3–98.7)
NPV (95% CI)	98.6 (97.3–99.4)	99.5 (98.5–99.9)	98.6 (97.3–99.3)
Agreement, %	94.3	94.8	98.3
κ	0.41	0.33	0.15

*NPV, negative predictive value; PPV, positive predictive value; RDT, rapid diagnostic test.
†SD Bioline Malaria Ag Pf/Pan (*P. falciparum* or other *Plasmodium* species; Standard Diagnostics, Gyeonggi-do, South Korea).
‡Children with mixed infections were excluded from analysis.

infection within the previous month; 4 of those had detectable parasite DNA.

Conclusions

Our findings indicate that strides in the control of *P. falciparum* malaria have continued in Uganda. Compared with data from 2010, *P. falciparum* prevalence by microscopy had a 4- and 5-fold decrease in urban and rural villages, respectively (*3*). Our estimates are consistent with prevalence estimates from 2009 (12%) and 2014–2015 (4%) (*1*,*6*).

In our study, 46% of asymptomatically infected children harbored non–*P. falciparum* species, particularly *P. malariae*, in contrast to the 1.2% non–*P. falciparum* species prevalence in 2009 (*6*). In addition, 1 *P. vivax* monoinfection was detected by PCR in Isingiro, confirming the continued presence of all 4 major species in Uganda (online Technical Appendix Table 2) (*1*). Furthermore, although most *P. malariae* cases were from Isingiro, recent studies in other regions of Uganda (i.e., northern and eastern) have also reported a rise in non–*P. falciparum* infections, particularly *P. malariae* (*7*–*9*).

One possible reason for the nearly equivalent prevalence of asymptomatic *P. falciparum* and non–*P. falciparum* infections is the influence of seasonal fluctuations in species prevalence; for example, *P. malariae* prevalence has been higher during the dry season in West Africa (*10*). Another possibility is that the use of *P. falciparum*–based RDTs, which are advantageous because of low infrastructure costs and high prevalence of this species in Uganda, has enabled non–*P. falciparum* prevalence to go undetected. Alternatively, our results may represent a true shift in species prevalence. What is apparent is that pLDH/HRP-2–based RDTs may not be the most sensitive diagnostic method to determine true prevalence in the future. In our study, RDT was negative in all 3 microscopy-identified *P. ovale* and in 3 of 7 *P. malariae* monoinfections, a finding that may be in part attributable to these species' low parasite densities (range 39–1,057/µL).

Identifying *P. malariae* and *P. ovale* infections is critical because *P. malariae* has been associated with chronic infections that can persist for years, including a chronic nephrotic syndrome that, once established, is unresponsive to treatment (*10*). Because these species have milder symptoms and lower parasite prevalence than *P. falciparum*, infections can remain undetected for extended periods, enabling persons to serve as reservoirs for ongoing transmission (*2*). These species may transmit gametocytes more efficiently at low parasite densities; a recent study found increased *P. falciparum* gametocyte production in the setting of mixed *P. malariae* infections (*11*). In our study, 30% of non–*P. falciparum* monoinfections harbored gametocytes. Finally, on the therapeutic side, studies have shown failure of parasite clearance after artemisinin-based combination therapy in non–*P. falciparum* infections, including *P. malariae* (*2*,*9*,*12*). *P. ovale* and *P. vivax* also form hypnozoites in the liver, and safe treatment with a 14-day course of primaquine is necessary to clear parasitemia. Six children in our study harbored *P. ovale* or *P. vivax* infections.

From a malaria control perspective, the performance of the pLDH/HRP-2–based RDT was suboptimal in our data, indicating a need for accurate diagnostic methods aimed at detecting *Plasmodium* infections in this region. A diagnostic method that has been effective in resource-constrained settings is loop-mediated isothermal amplification (LAMP), which affords higher sensitivity in detecting low-level parasitemia, especially *P. malariae* infections that tend to exhibit lower parasite densities than *P. falciparum* and *P. vivax* (*13*). In a 2013 rural Uganda study, the sensitivity of LAMP was ≈1.8-fold greater than microscopy, comparable to PCR (*14*). Wide-scale application of a field-friendly technique such as LAMP might be possible in southwestern Uganda, where asymptomatic persons might have low-density malaria infections that persist during the low malaria transmission season, enabling these persons to serve as reservoirs for ongoing transmission and disease (*15*). Effective methods for detecting and treating these infections are essential for controlling and eliminating malaria.

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References

1. Uganda Bureau of Statistics (UBOS) and ICF International. Uganda malaria indicator survey 2014–15: key indicators. 2015 Apr [cited 2016 Mar 24]. http://pdf.usaid.gov/pdf_docs/PBAAC390.pdf
2. White NJ. The role of anti-malarial drugs in eliminating malaria. Malar J. 2008;7(Suppl 1):S1–S8. http://dx.doi.org/10.1186/1475-2875-7-S1-S8
3. De Beaudrap P, Nabasumba C, Grandesso F, Turyakira E, Schramm B, Boum Y II, et al. Heterogeneous decrease in malaria prevalence in children over a six-year period in south-western Uganda. Malar J. 2011;10:132. http://dx.doi.org/10.1186/1475-2875-10-132
4. Uganda Ministry of Health. National Malaria Control Program. Uganda malaria quarterly bulletin. Issue 10. 2015 Apr–June [cited 2016 Apr 11]. http://www.health.go.ug/sites/default/files/Ugand%20Malaria%20Bulletin%20Issue%2010.pdf
5. President's Malaria Initiative. President's Malaria Initiative Uganda Malaria Operational Plan FY 2014. 2014 [cited 2016 Apr 05]. http://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy14/uganda_mop_fy14.pdf?sfvrsn=14
6. Uganda Bureau of Statistics (UBOS) and ICF Macro. Uganda malaria indicator survey 2009. 2010 Aug [cited 2016 Mar 24]. http://dhsprogram.com/pubs/pdf/MIS6/MIS6.pdf.
7. Proietti C, Pettinato DD, Kanoi BN, Ntege E, Crisanti A, Riley EM, et al. Continuing intense malaria transmission in northern Uganda. Am J Trop Med Hyg. 2011;84:830–7. http://dx.doi.org/10.4269/ajtmh.2011.10-0498
8. Pullan RL, Bukirwa H, Staedke SG, Snow RW, Brooker S. *Plasmodium* infection and its risk factors in eastern Uganda. Malar J. 2010;9:2. http://dx.doi.org/10.1186/1475-2875-9-2
9. Betson M, Sousa-Figueiredo JC, Atuhaire A, Arinaitwe M, Adriko M, Mwesigwa G, et al. Detection of persistent *Plasmodium* spp. infections in Ugandan children after artemether-lumefantrine treatment. Parasitology. 2014;141:1880–90. http://dx.doi.org/10.1017/S003118201400033X
10. Mueller I, Zimmerman PA, Reeder JC. *Plasmodium malariae* and *Plasmodium ovale*—the "bashful" malaria parasites. Trends Parasitol. 2007;23:278–83. http://dx.doi.org/10.1016/j.pt.2007.04.009
11. Bousema JT, Drakeley CJ, Mens PF, Arens T, Houben R, Omar SA, et al. Increased *Plasmodium falciparum* gametocyte production in mixed infections with *P. malariae*. Am J Trop Med Hyg. 2008;78:442–8.
12. Dinko B, Oguike MC, Larbi JA, Bousema T, Sutherland CJ. Persistent detection of *Plasmodium falciparum*, *P. malariae*, *P. ovale curtisi* and *P. ovale wallikeri* after ACT treatment of asymptomatic Ghanaian school-children. Int J Parasitol Drugs Drug Resist. 2013;3:45–50. http://dx.doi.org/10.1016/j.ijpddr.2013.01.001
13. Lau YL, Lai MY, Fong MY, Jelip J, Mahmud R. Loop-mediated isothermal amplification assay for identification of five human *Plasmodium* species in Malaysia. Am J Trop Med Hyg. 2016;94:336–9. http://dx.doi.org/10.4269/ajtmh.15-0569
14. Hopkins H, González IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, et al. Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda. J Infect Dis. 2013;208:645–52. http://dx.doi.org/10.1093/infdis/jit184
15. Bousema JT, Gouagna LC, Drakeley CJ, Meutstege AM, Okech BA, Akim IN, et al. *Plasmodium falciparum* gametocyte carriage in asymptomatic children in western Kenya. Malar J. 2004;3:18. http://dx.doi.org/10.1186/1475-2875-3-18

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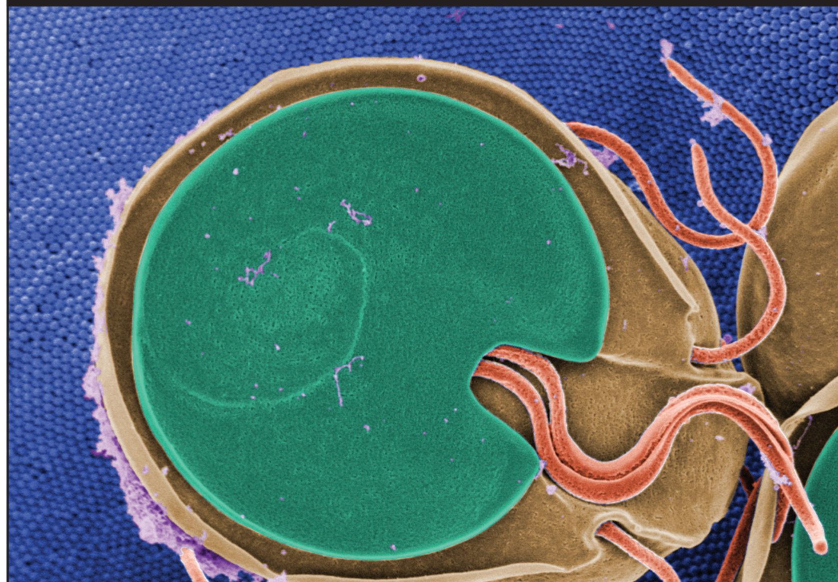

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Asymptomatic *Plasmodium* Infections in Children in Low Malaria Transmission Setting, Southwestern Uganda

Technical Appendix

Methods

The study was conducted in southwestern Uganda, in an area with an annual entomologic inoculation rate of 2–4 infective bites per year, and biannual rainy seasons. The survey occurred during August–October 2014, corresponding to the low transmission season. A stratified, two-stage cluster sampling design was used to select participants. In the first sampling stage, 60 villages were sampled (20 from each of 3 districts) and stratified on the basis of their urban or rural status. Villages were sampled by using probability-proportionate-to-size sampling. Within each village, households were randomly selected by using the World Health Organization's Expanded Program on Immunization methodology. The number of households selected in each village was weighted on the basis of the population size of each district. In each household, only 1 child <5 years of age who met the eligibility criteria was randomly selected to participate in the survey. If no children were in the household, the field team would move on to the next house until the total number of households required for each village was met. Allowing for a 10% nonresponse rate and a design effect of 1.5, a sample size of 601 children had 80% power to detect 5% difference in malaria prevalence from that determined in the 2009 Uganda Malaria Indicator Survey (12% in southwestern Uganda) (*1*).

Field Procedures and Sample Collection

After informed parental consent was obtained, standardized questionnaires were administered to the head of household or parent or guardian as face-to-face interviews to collect information about their demographics and malaria control measures. Information included consistent bed net use, household wealth, household construction, household crowding, district, and rural or urban status of residence. We collected ≈250 µL of blood by fingerprick into an

EDTA-coated microtainer (BD Diagnostics, Franklin Lakes, NJ, USA). Rapid diagnostic tests (RDTs) were performed, and thick and thin smears for microscopy were prepared on-site by trained laboratory technicians. In sets of 3 from participants, 50 µL of blood was spotted onto filter paper (Whatman 903 Protein Saver Card, Sigma-Aldrich, St. Louis, MO, USA) and stored at –80°C until shipment to the Yale School of Public Health (New Haven, CT, USA). QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) was used to extract DNA from dried blood spots. A total of 100 µL was eluted from each sample.

Parasite Detection

Parasite detection was assessed by using a combined *P. falciparum*–specific histidine-rich protein-2 (HRP-2)/pan-*Plasmodium* lactate dehydrogenase (pLDH) RDT (SD Bioline Malaria Ag Pf/Pan, Standard Diagnostics, Gyeonggi-do, Republic of Korea) and microscopy. RDT was performed according to manufacturer's instructions. If the control line of the RDT did not appear, the test was considered invalid and was repeated. A positive Pf-HRP-2 band indicated a *P. falciparum* infection, whereas a positive pan-pLDH band alone represented an infection with ≥1 non–*P. falciparum* species. A result with both the Pf-HRP-2 and pan-pLDH bands indicated a *P. falciparum* monoinfection or a mixed *P. falciparum*/non–*P. falciparum* infection. For microscopy, smears stained with 10% Giemsa (pH 7.2) were read independently by 2 trained microscopists, with discordant results resolved by a third reader. Parasite and gametocyte densities, expressed as number of parasites/µL, were calculated by counting parasite numbers against 200 leukocytes and multiplied by an assumed standard leukocyte count of 8,000 leukocytes/µL, as recommended by the World Health Organization (*2*).

For all patients with either a positive microscopy or RDT result, species confirmation by nested PCR was performed for *Plasmodium falciparum, P. vivax, P. ovale,* and *P. malariae*. Primers were complementary to the *Plasmodium* small subunit ribosomal DNA gene as previously described by Singh et al (*3*). For both the primary and nested rounds of amplification, PCR was performed by using 5X GoTaq Flexi Buffer (Promega, Madison, WI, USA), 1.5 mmol/L magnesium chloride, 0.2 µmol/L deoxyribonucleotide triphosphate, 0.2 µmol/L of forward and reverse primers, 1 U GoTaq polymerase (Promega), and 2 µL template DNA. Thermocycling conditions were as follows: 94°C for 1 min, followed by 35 and 30 cycles of 94°C for 1 min, 58°C for 2 min, 72°C for 5 min for the primary and nested rounds, respectively, and a final extension of 72°C for 5 min. Positive controls were acquired from Malaria Research

and Reference Reagent Resource Center (MR4, Biodefense and Emerging Infections Resources Repository, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Manassas, VA, USA). Products of nested PCR were analyzed by gel electrophoresis.

Statistical Analysis

Data was analyzed by using Stata 14.0 (StataCorp, College Station, TX, USA). Categorical variables were compared by using the χ^2 test or Fisher exact test, as appropriate. T-tests were performed when comparing 2 groups. Wilcoxon rank-sum tests were used when continuous variables were nonparametric. If the variables contained >2 independent groups, 1-way ANOVA was performed to calculate the difference between means. All statistical tests accounted for our clustered sampling design.

To assess the diagnostic performance of the SD Bioline Malaria Ag P.f/Pan (*P. falciparum* and other *Plasmodium* species) RDT (Standard Diagnostics, Gyeonggi-do, South Korea), results of RDT and microscopy were treated as binary (i.e., positive or negative) outcomes, and microscopy was considered the gold standard. Sensitivity, specificity, and negative and positive predictive values were calculated to assess RDT performance in diagnosing infection with any species, *P. falciparum* infection, and non–*P. falciparum* infection. Mixed infections with *P. falciparum* were excluded from the analysis only when assessing RDT performance in diagnosing non–*P. falciparum* infections. The kappa statistic was calculated to measure agreement between RDT and microscopy results.

Predictor variables were selected *a priori* based on their individual, household, or environmental risk for malaria infection. Predictors of interest were consistent bed net use, household wealth, household building materials, household crowding, district of residence, and rural or urban status of the village in which the participant resided. Consistent bed net use (a binary variable) was defined as a participant who reported always sleeping under a bed net. Household wealth quartiles were constructed by using a multicomponent analysis of household possessions, household roof and flooring material, and source of drinking water. For assessing predictors of positive microscopy cases, Poisson regression with robust variance was used to calculate bivariate and multivariate prevalence ratios. Predictors that were statistically significant at the 0.10 level in the univariate analysis were included in the multivariate model.

Ethics Approval

Written informed consent for study participation was obtained from the children's parent or guardian. Ethical approval was obtained from the Uganda National Council of Science and Technology, the Mbarara University of Science and Technology Institutional Review Council, and the Yale University Human Investigation Committee.

References

1. Uganda Bureau of Statistics (UBOS) and ICF Macro. Uganda malaria indicator survey 2009. 2010 Aug [cited 2014 March 24]. http://dhsprogram.com/pubs/pdf/MIS6/MIS6.pdf.

2. World Health Organization. Basic malaria microscopy: part II. Tutor's guide. 2010 [cited 2016 Jun 10]. http://apps.who.int/iris/bitstream/10665/44208/2/9789241547918_eng.pdf?ua=1&ua=1

3. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. Am J Trop Med Hyg. 1999;60:687–92.

Technical Appendix Table 1. Factors associated with asymptomatic parasitemia detectable by microscopy*

Variable	Unadjusted PR	(95% CI)	p value†	Adjusted PR	(95% CI)	p value†
Location of residence						
Urban	1.00	Ref				
Rural	2.33	(0.58-9.37)	0.229			
District of residence						
Mbarara or Bushenyi	1.00	Ref		1.00	Ref	
Isingiro	2.48	(0.97-6.34)	0.058	1.81	(0.68-4.83)	0.229
Wealth quartile						
1st–3rd	1.00	Ref		1.00	Ref	
4th	0.17	(0.24-1.23)	0.079	0.33	(0.05-2.35)	0.266
HH roofing material						
Corrugated metal/other	1.00	Ref		1.00	Ref	
Thatch/leaf	7.12	(2.56-19.76)	<0.001	5.13	(1.72-15.31)	0.004
HH flooring material						
Cement	1.00	Ref		1.00	Ref	
Earth/dirt	2.96	(0.91-9.67)	0.071	1.38	(0.49-3.97)	0.533
HH crowding						
<3 person/room	1.00	Ref		1.00	Ref	
≥3 person/room	1.94	(0.92-4.11)	0.081	1.76	(0.78-3.96)	0.168
Bed net use						
Inconsistent	1.00	Ref		1.00	Ref	
Consistent	0.31	(0.13-0.74)	0.009	0.40	(0.19-0.83)	0.016

*HH, household; PR, prevalence ratio; Ref, reference.
†p values determined χ^2 test or Fisher exact test, as appropriate.

Technical Appendix Table 2. PCR confirmation of microscopy-positive and RDT-positive results*

Test type	Type of infection	Nested PCR results						
		Negative	*P. falciparum*	*P. malariae*	*P. ovale*	*P. vivax*	Mixed	Total
Microscopy, n = 22	*P. falciparum*	0	6	0	2	0	1†	9
	P. malariae	0	0	6	0	0	1‡	7
	P. ovale	1	0	0	2	0	0	3
	Mixed§	0	0	1	0	0	2§	3
	Total	1	6	7	4	0	4	22
RDT¶, n = 41	Pf-band+	6	3	0	0	1	1§	11
	Pan-band+	0	0	2	0	0	1#	3
	Pf/Pan-bands+	13	6	3	3	0	2‡,	27
	Total	19	9	5	3	1	4	41

*Pan-band+, positive for non–*P. falciparum* infection only; Pf-band+, positive for *P. falciparum* monoinfection only; Pf/Pan-bands+, positive for *P. falciparum* monoinfection **or** *P. falciparum* mixed infection; RDT, rapid diagnostic test.
†Mixed infection of *P. falciparum, P. malariae, P. ovale*, and *P. vivax*.
‡Mixed infection of *P. malariae, P. ovale*, and *P. vivax.*
§Mixed infection of *P. falciparum* and *P. malariae*.
¶1 RDT result did not have an available blood sample.
#Mixed infection of *P. ovale* and *P. vivax.*

Technical Appendix Table 3. Results of RDT and microscopy for malaria species infections in 631 children in 3 districts in southwestern Uganda*

Rapid diagnostic test results	Light microscopy results					
	Pf+	Pm+	Po+	Pf/Pm+	Negative	Total
Pf+	1	0	0	1	9	11
Pan+	0	1	0	1	1	3
Pf/Pan+	6	3	0	1	18	28
Negative	2	3	3	0	579	587
Total	9	7	3	3	607	629†

*Pan+, positive for non–*P. falciparum* infection only; Pf+, positive for *P. falciparum* monoinfection only; Pf/Pan+, positive for *P. falciparum* monoinfection or *P. falciparum* mixed infection; Pf/Pm+, positive for *P. falciparum* and *P. malariae* mixed infection; Pm, positive for *P. malariae* infection; Po, positive for *P. ovale* infection; RDT, rapid diagnostic test.
†RDT data is missing for 1 child; microscopy data is missing for 1 child.