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High Levels of Asymptomatic and Subpatent *Plasmodium falciparum* Parasite Carriage at Health Facilities in an Area of Heterogeneous Malaria Transmission Intensity in the Kenyan Highlands

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Abstract. In endemic settings, health facility surveys provide a convenient approach to estimating malaria transmission intensity. Typically, testing for malaria at facilities is performed on symptomatic attendees, but asymptomatic infections comprise a considerable proportion of the parasite reservoir. We sampled individuals attending five health facilities in the western Kenyan highlands. Malaria prevalence by rapid diagnostic test (RDT) was 8.6–32.9% in the health facilities. Of all polymerase chain reaction-positive participants, 46.4% (95% confidence interval [95% CI] = 42.6–50.2%) of participants had infections that were RDT-negative and asymptomatic, and 55.9% of those infections consisted of multiple parasite clones as assessed by merozoite surface protein-2 genotyping. Subpatent infections were more common in individuals reporting the use of non-artemisinin–based antimalarials in the 2 weeks preceding the survey (odds ratio = 2.49, 95% CI = 1.04–5.92) compared with individuals not reporting previous use of antimalarials. We observed a large and genetically complex pool of subpatent parasitemia in the Kenya highlands that must be considered in malaria interventions.

INTRODUCTION

To allow national programs to effectively tailor malaria control strategies to local transmission dynamics, it is essential that existing surveillance systems are capable of providing accurate, spatially specific measures of malaria transmission intensity.[1,2] Most malaria surveillance systems, including the system in Kenya, are predicated on passive detection of cases at health facilities using either clinical diagnosis alone or clinical diagnosis with parasitological confirmation by microscopy or rapid diagnostic tests (RDTs).[3–6] However, estimates of malaria burden from passive case detection data are subject to a number of potential biases that can vary considerably between health facilities, including the occurrence of non-malarial fevers, variations in accessibility of health services, willingness to pay any ancillary costs, and diagnostic test used. In addition, the experience of the laboratory and clinical personnel, quality of microscopy, particular brand or availability of RDTs, and time dedicated to malaria testing are also important potential sources of bias, making results difficult to compare.[6,7]

Health facility-based cross-sectional surveys that sample from all individuals presenting at the facility as well as any accompanying individuals (as distinct from sampling only among individuals with suspected malaria) have been shown to be a useful tool for measuring malaria transmission intensity.[8,9] Health facility surveys provide an operationally attractive method to estimate malaria prevalence in the wider catchment population, because the inclusion of all health facility attendees mitigates against some of the biases associated with passive case detection.[7,10] However, most health facility malaria surveys have relied on diagnosis by microscopy or RDT, both of which have a limited ability to detect parasitemia at low parasite densities.[8,11,12] The number of malaria infections detected through these surveys is, therefore, likely to have been substantially lower than would have been achieved using a more sensitive diagnostic approach, such as polymerase chain reaction (PCR).[11,13,14] The potentially large proportion of infections that is undetected poses a significant challenge for malaria surveillance, control, and elimination strategies: transmission is likely underestimated, and reservoirs of infection missed. As a result, control programs may only target a subset of the actual parasite population, or campaigns may be implemented before the parasite reservoir is at or below the threshold where elimination is feasible.[13,15–17]

In this study, two cross-sectional surveys were carried out in five rural health facilities in the highlands of western Kenya to (1) assess the use of this type of survey approach for measuring malaria transmission, (2) identify the prevalence and complexity of asymptomatic and subpatent infections, and (3) evaluate factors associated with having asymptomatic and subpatent infections.

METHODS

Study site and population. This study was conducted in health facilities in a highland fringe area covering a region of approximately 200 km^2 in Rachuonyo South, Nyanza Province in the western Kenyan highlands. The area is situated between 1,400 and 1,600 m above sea level, and the landscape is characterized by rolling terrain intersected with rivers and streams. The population is predominantly people from the Luo ethnic group, with subsistence farming being the main occupation.[18] Malaria in the area is spatially heterogeneous,

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with prevalence estimates in primary schools ranging between 0% and 71%, and transmission follows a bimodal seasonal pattern associated with the long and short rainy seasons typically occurring between April and June and between October and December, respectively.[19,20] The predominant malaria vectors in the area are *Anopheles funestus* and *An. arabiensis*, and *Plasmodium falciparum* is the principal malaria parasite species present.[21] Two surveys were conducted in five rural health facilities representing all government facilities in the area in collaboration with the District Ministry of Health. Sampling took place in Agawo, Ober, Omiro, and Tala health facilities in both surveys. In the second survey, Othoro Health Center was replaced with Wire Dispensary, a faith-based facility, to achieve maximum overlap with the ongoing community work (Figure 1). The surveys were conducted in October of 2011 and July of 2012 to correspond with periods of low and high transmission, respectively, and we examine the sensitivity of these surveys to changes in transmission intensity.[18]

Consenting and sample collection. All consenting patients and those accompanying them who attended the outpatient department during the 4-week survey period were eligible for inclusion. At each facility, maximums of 150 people from each of three age categories (0.5–5, 6–15, and > 15 years old) were included. Recruitment within an age category was stopped after the target had been reached. Individuals were excluded if they were extremely ill and required immediate medical attention, were < 6 months of age, were attending a scheduled clinic or other ward of the health facility, were unwilling or unable to provide consent (e.g., under 18 years old without being accompanied by a suitable guardian), or had been previously sampled at that same facility during this study.

Two field workers were stationed at each facility, and data collection activities were integrated into the normal day-to-day operations as far as possible. A field worker would approach each potential eligible participant and explain the study while he/she was waiting to visit the clinician. After the consenting process, a short questionnaire was administered on participant demographics, malaria history, control behaviors, whether he/she was a patient or accompanying person, current and recent symptoms, recent drug use, and travel history. Each participant was screened by RDT to determine the presence of current patent infections; three blood spots were collected on filter paper (3MM Whatman, Maidstone, United Kingdom) for subsequent molecular and serological analysis. Filter papers were dried and stored with desiccant at −80 °C. In the first year of the survey, axillary temperature was measured using a digital thermometer, and those with temperature > 37.2°C were considered febrile.[18] In the second year, tympanic thermometers were used because of the increased accuracy and shorter time to result. For those tested with the tympanic thermometers, only those with temperatures > 37.5 °C were considered febrile. In the second survey, the RDT was changed from Paracheck (Orchid Biomedical Systems, Goa, India) to the more sensitive First Response Kit (Premier Medical Corporation Ltd., Nani Daman, India).[22] All diagnostic information was made available to the clinician for clinical decision-making. The final diagnosis and any drugs prescribed by the clinician to study participants were also recorded.

Research ethics. The ethical committees of the London School of Hygiene and Tropical Medicine (LSHTM 5956) and the Kenya Medical Research Institute (SSC 1589) approved this study. Individual informed consent was obtained from all participants by signature or thumbprint accompanied by the signature of an independent witness. Consent for children under the age of 18 years old was provided by a parent/guardian, and children between 14 and 17 years old also provided written assent by signature or thumbprint accompanied by the signature of an independent witness. As defined in the

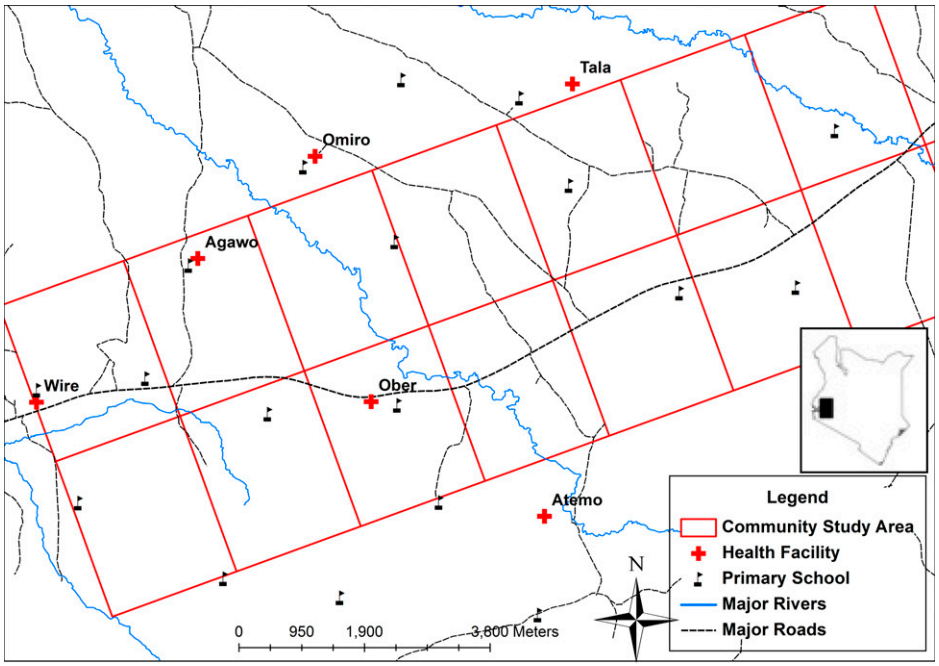

FIGURE 1. Health facility survey study area. Locations of rural health facilities included in the study as well as government primary schools and boundaries of the community survey. Note that Othoro Health Center is located along the main road approximately 20 km to the west of this area.

Kenya national guidelines, participants below 18 years of age who were pregnant, married, or had a child were considered mature minors and consented for themselves.[23]

Laboratory analysis. Filter paper blood spots were used to test for antibodies to malaria to ascertain malaria exposure and transmission intensity. Antibodies to *P. falciparum* Apical Membrane Antigen-1 (AMA1) and Merozoite Surface Protein-1 (MSP1-19) were detected by enzyme-linked immunosorbent assay (ELISA). Briefly, two blood spot sections per sample were punched, and antibodies were eluted according to work by Baidjoe and others.[24] Antibody prevalence for each antigen was determined after defining a cutoff optical density (OD) based on a standard curve of known antibody concentration using the mixture model and normalized across plates.[20,25] A person was considered to be seropositive if they had normalized OD values above the cutoff for at least one of the antigens tested. Age-adjusted seroconversion rates (SCRs) were calculated.[25]

Nested PCR (nPCR) was used to test for the presence of parasite DNA to provide a gold standard measure for current infection. A Chelex-saponin approach was used to extract DNA as described by Baidjoe and others,[24] and the nPCR assay targeting the 18S ribosomal subunit of *P. falciparum* was used as previously described.[26] Samples that were positive by nPCR were then selected for subsequent analysis to identify allelic diversity using the polymorphic MSP2 region to provide an alternate measure of transmission intensity.[24,25,27] An additional nPCR reaction was conducted to amplify the block-3 region of the MSP2 domain targeting the FC27 and IC3D7 allelic variants.[28] The product of the MSP2 PCR was viewed on 1.5% agarose gel to determine the dilution factor necessary to prepare samples for capillary electrophoresis: intense bands were diluted at 1:100, moderate bands were diluted at 1:40, and faint bands were diluted at 1:10. Electropherograms were viewed using Peak Scanner (version 1.0), and all discrete peaks > 500 florescent units were considered to be distinct allelic types.[29]

Case definitions. Subpatent malaria infections were infections in individuals who tested positive for malaria by nPCR but negative for malaria by RDT; patent infections were defined as infections in individuals who were positive by both nPCR and RDT. Individuals who were positive by RDT but negative by PCR ($N = 267$) were considered to be false positives (likely attributable to residual HRP2 antigen) and not included in the analysis exploring subpatent infections (however, they were included in estimates of RDT prevalence).[30] Asymptomatic infections were infections in individuals who tested positive for malaria by nPCR but were afebrile at the time of sampling and did not report history of fever in the 24 hours before sampling.[14]

Statistical analysis. Statistical analysis was conducted using Stata 12.1 (STATACorp LP) and R, version 3.02. Comparisons of parasite prevalence estimates between facilities, between years, and between age categories were performed using a two-sided test for proportions and the corresponding exact binomial 95% confidence intervals (95% CIs). To assess the ability of health facility surveys to provide reasonable estimates of the community, data from a large community cross-sectional survey conducted in July of 2011 in the same study area were used.[18] Data were restricted to those sampled as part of the community survey who resided within the health facility catchment areas as defined by cost–distance analysis, and SCR was calculated as described above.[31] The health facility samples were restricted to those collected in July of 2012 to minimize any potential seasonal bias. Multiplicity of infection (MOI) was calculated for all PCR positive samples, and 95% CIs were calculated assuming a zero truncated Poisson distribution to account for all samples containing a minimum of one clone. Allelic richness (Rs), a metric for allelic diversity, was calculated using FSTAT, version 2.9.3.2, software as previously described.[32]

Random effects logistic regression was used to assess factors associated with having subpatent as well as asymptomatic malaria infection. Explanatory variables tested included year, sex, age, whether the individual was a patient or an accompanying person, reported taking an antimalarial drug in the past 2 weeks, reported taking an antipyretic drug, reported using a bed net the previous night, reported living in a household where indoor residual spraying had taken place in the previous 6 months, and number of infecting parasite clones. Because of the non-specificity of malaria symptoms, it was not possible to further stratify patients by reason for attending the facility. The final adjusted models were generated by retaining all variables that were significant at the 0.05 level in a backward fashion, and akaike information criteria (AIC) values were used to confirm the optimum model fit.

RESULTS

Population demographics. In total, 1,598 and 1,444 people were sampled in the first and second surveys, respectively, and most were patients (Table 1). There were similar proportions of males and females sampled in the < 5 and 6–15 years age categories, but significantly more females than males were sampled in the > 15 years age group ($P < 0.0001$). Most of the accompanying people were > 15 years of age. Also, the majority of individuals reported that they had slept under a bed net the previous night, although in both surveys, participants ages 6–15 years were less likely to have reported using a net than younger children ($P < 0.0001$) or adults ($P < 0.0001$) (Table 1). The majority of patients (63.4%; 95% CI = 61.4–65.3%; facility range [range] = 25.5–79.0%) reported having a fever in the previous 24 hours compared with 19.0% of accompanying people (95% CI = 15.9–22.4%; range = 0–37.7%), but only 23.2% (95% CI = 21.5–24.9%; range = 18.4–37.0%) and 7.5% (95% CI = 5.4–9.7%; range = 0–19.7%) of patients and accompanying people, respectively, had a current fever at the time of their health visit. Overall, 30.6% (95% CI = 28.9–23.2%; range = 15.6–39.6%) of participants reported having taken antipyretic drugs, and 13.7% (95% CI = 12.5–15.0%; range = 8.8–21.9%) of participants reported taking an antimalarial drug in the past 2 weeks.

Malaria transmission intensity. All metrics tested were able to detect a change in malaria burden between the two surveys. Seroprevalence estimates increased from 37.6% (95% CI = 35.2–40.0; range = 24.5–53.0%) during the first survey to 46.8% (95% CI = 44.2–49.4%; range = 34.4–62.0%) in the second survey ($P < 0.0001$). Similarly, malaria parasite prevalence by RDT increased from 16.9% (95% CI = 15.1–18.8%; range = 8.6–30.1%) to 22.4% (95% CI = 20.3–24.6%; range = 9.5–32.9%) and by PCR from 20.4% (95% CI= 18.4–22.4%; range = 9.5–40.3%) to 25.5% (95% CI = 23.2–27.7%; range = 8.7–51.5%) during the first and second surveys, respectively (Table 2). Prevalence within age categories also increased

TABLE 1

Demographics of the study population in health facility surveys in five rural health facilities carried out during the short and long malaria transmission seasons

	Low-transmission season (October of 2011)			High-transmission season (July of 2012)		
	Mean	95% CI	Range	Mean	95% CI	Range
N						
All	1,598	–	284–388	1,444	–	203–379
6 months to 5 years	537	–	76–147	514	–	52–150
6–15 years	304	–	32–90	249	–	28–79
> 15 years	767	–	149–150	681	–	104–150
Sex (% male)						
All	37.5	35.2–40.0	33.8–38.9	38.7	36.2–41.3	34.6–40.1
6 months to 5 years	49.0	44.7–53.3	43.7–53.9	52.3	47.9–56.7	44.4–58.0
6–15 years	47.0	41.3–52.8	42.9–54.2	46.6	40.3–53.0	39.7–54.4
> 15 years	25.6	22.5–28.9	20.6–31.8	25.5	22.3–29.0	22.4–31.5
Patient/accompanying status (% patient)						
All	81.4	79.4–83.3	66.9–93.0	79.5	77.3–81.5	53.7–90.5
6 months to 5 years	96.5	94.5–97.8	91.6–91.7	93.8	91.3–95.7	88.5–98.0
6–15 years	96.0	93.2–97.9	90.6–100	97.2	94.3–98.9	92.9–100
> 15 years	64.9	61.4–68.3	43.9–85.3	62.3	58.5–65.9	30.4–80.8
Bed net (% reported sleeping under net previous night)						
All	87.2	85.5–88.9	82.2–94.0	90.4	88.8–91.9	89.0–91.8
6 months to 5 years	86.8	83.6–89.6	82.6–92.1	94.0	91.5–95.9	88.7–97.5
6–15 years	82.1	77.3–86.3	69.6–92.3	81.1	75.7–85.8	76.0–84.8
> 15 years	89.6	87.2–91.7	84.1–96.7	91.2	88.8–93.2	89.6–93.3
Recent IRS (% reported having IRS in past 12 months)						
All	77.8	75.4–80.4	70.1–87.4	76.9	74.6–79.0	70.6–81.0
Recent travel (% reporting having traveled in past 3 months)						
All	32.5	30.0–35.1	26.7–39.9	20.1	18.1–22.3	10.7–29.8
6 months to 5 years	27.9	23.8–32.4	17.3–50.0	16.1	13.1–19.6	6.0–25.9
6–15 years	21.9	17.1–24.4	0–32.6	6.8	4.0–10.7	2.0–10.3
> 15 years	40.7	36.7–44.8	22.2–49.0	28.0	24.7–31.6	14.4–39.3

IRS = indoor residual spraying.

between surveys, with the highest estimates in the 6–15 years age category and the lowest estimates in adults ($P < 0.001$) (Supplemental Table 1).

Similarly, SCR indicated a range of transmission intensity between facilities and an increase in transmission intensity between the two surveys (Figure 2A). Also, based on this small sample of five facilities, SCR estimates from the health facility survey during the high-transmission season were strongly correlated ($r = 0.96$) with estimates obtained from a community cross-sectional survey in the same area conducted the previous year (Figure 3). With the exception of allelic diversity ($P = 0.62$), the malaria metrics tested were able to consistently rank health facilities according to transmission intensity, which was quantified by SCR. The intensity of malaria transmission (indicated by SCR) experienced by individuals attending the selected health facilities during the first survey was associated with health facility-level parasite prevalence by both RDT ($P = 0.04$) and PCR ($P = 0.05$) as well as MOI ($P = 0.04$). Despite the association of RDT and transmission intensity, it is worth noting that one facility (Agawo) would have been misclassified as being in a high-transmission setting based on RDT results in symptomatic patients alone (Figure 2B). SCR during the first survey was also strongly associated with SCR in the second survey ($P < 0.001$), and ranks between transmission intensity and all malaria metrics showed similar trends (data not shown).

Subpatent and asymptomatic infections. Overall, 586 infections were detected by RDT, and 54.4% of them were confirmed by PCR. PCR identified an additional 358 infections (12.0% of the total study population). In total, 52.9%

TABLE 2

Prevalence of malaria per facility for all malaria metrics, including seroprevalence (Sero), and RDT prevalence, MOI, and Rs ordered from highest to lowest transmission intensity

	SCR	95% CI	Sero (%)	95% CI	PCR (%)	95% CI	RDT (%)	95% CI	MOI	95% CI	Rs
Low-transmission season (October of 2011)											
Tala	0.076	0.06–0.10	53.0	47.1–58.8	35.0	29.4–40.6	29.4	24.7–35.5	2.33	2.07–2.65	30.9
Omiro	0.069	0.05–0.09	49.1	43.1–55.0	40.3	34.4–46.1	16.9	13.7–23.2	1.99	1.79–2.24	24.1
Agawo	0.054	0.04–0.07	42.8	37.0–48.5	14.8	10.7–18.9	19.8	15.3–24.5	1.97	1.68–2.40	29.2
Ober	0.028	0.02–0.04	25.8	21.4–30.2	9.5	6.6–12.5	11.6	8.4–14.8	1.72	1.44–2.19	27.0
Othoro	0.025	0.02–0.03	24.5	19.9–29.0	9.5	6.4–12.6	8.6	5.7–11.6	1.84	1.56–2.28	29.0
High-transmission season (July of 2012)											
Tala	0.114	0.09–0.14	62.1	56.3–67.7	51.6	45.8–57.3	32.9	27.4–38.3	2.29	2.09–2.52	37.1
Omiro	0.113	0.09–0.15	55.6	48.9–62.2	31.3	25.1–37.5	27.6	21.6–33.6	1.85	1.63–2.15	28.0
Wire	0.069	0.05–0.09	52.2	45.3–59.1	28.8	22.5–35.0	18.0	12.7–23.6	1.5	1.34–1.75	20.5
Agawo	0.061	0.05–0.07	39.5	34.2–44.5	16.2	12.4–20.1	27.1	22.4–31.7	2.12	1.84–2.50	39.5
Ober	0.048	0.04–0.06	34.2	29.4–39.0	8.7	5.8–11.5	9.5	6.6–12.5	1.95	1.60–2.53	18.0

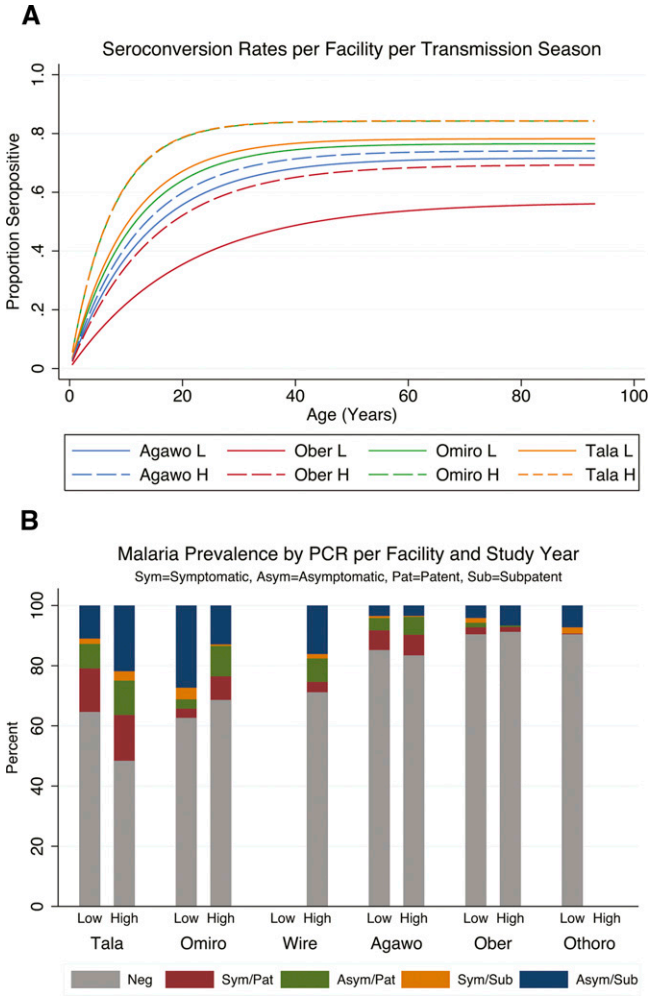

FIGURE 2. Malaria results per facility. (**A**) Seroconversion rates per health facility and transmission season (low [L] = October of 2011, high [H] = July of 2012) for facilities sampled in both surveys. Note that OmiroH and TalaH curves overlap. (**B**) PCR prevalence ordered according to transmission intensity including subpatent and asymptomatically infected individuals per health facility and transmission season. Bars are stacked in the order of the legend, with negative on the bottom and Asym/Sub on the top.

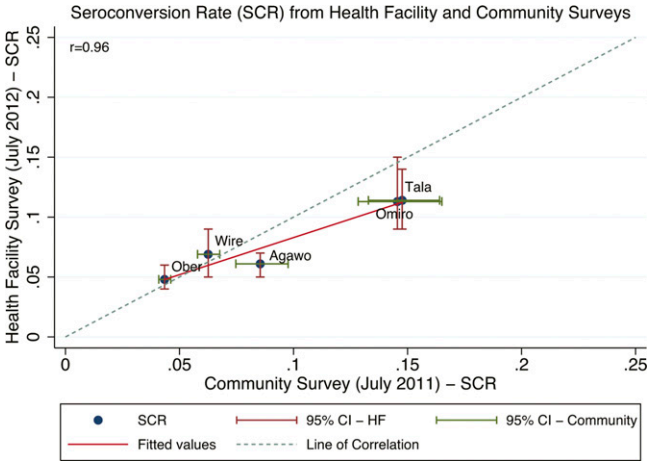

FIGURE 3. Comparison of health facility (HF) and community. Comparison of transmission intensity estimates based on SCR from HF and community surveys and the corresponding correlation coefficient (r). HF estimates were restricted to sampling that occurred in the high-transmission season, and community estimates were restricted to those residing in the health facility catchment area to minimize spatial or seasonal biases as much as was possible.

(range = 24.7–97.0%) and 67.5% (range = 27.3–81.4%) of the PCR-positive individuals had subpatent and asymptomatic infections, respectively; the majority was found in adults ($P < 0.0001$) (Supplemental Table 2). Based on the clinical records, most subpatent infections (83.8%; 95% CI = 79.6–87.5%) were not provided treatment, whereas 95.1% (95% CI = 93.0–96.7%) of RDT-positive individuals were prescribed an antimalarial drug.

Of all PCR-positive participants, 26.0% (range = 3.0–42.6%) were patent and symptomatic; 21.1% (range = 0–32.7%) had patent and asymptomatic infections, whereas 46.4% (range = 21.8–75.7%) were subpatent and asymptomatic for malaria. In total, 6.5% (range = 3.0–21.2%) of PCR-positive individuals were subpatent and symptomatic; 38.6% (17 of 44) of these individuals were diagnosed with malaria, whereas 10 of 17 participants as well as 27 participants not treated for malaria were diagnosed with another fever-inducing illness, such as flu or typhoid (Figure 2B).

Most infected individuals had one (43.2%) or two (29.4%) allelic types, with the most diverse samples showing evidence of seven different parasite clones. The FC27 subtype was most prevalent, with 57 distinct allelic types identified compared with 31 unique types from the 3D7 family. The MOI in the study population was low, with a mean of 2.05 (95% CI = 1.92–2.19; range = 1.7–2.3) and 2.02 (95% CI = 1.91–2.15; range = 1.5–2.3) clones per person in the first and second surveys, respectively. Estimates of MOI were slightly higher in the 6–15 years population, but no difference was observed between patent and subpatent and symptomatic and asymptomatic infections (Tables 3).

Factors associated with subpatent/asymptomatic infections. In adjusted models, individuals > 15 years had 2.55 (95% CI = 1.50–4.30) times the odds of having an asymptomatic infection compared with those < 5 years. The odds of asymptomatic infections also being subpatent compared with patent were 7.53 (95% CI = 4.88–11.62). If a person was attending the health facility seeking care or sampled during the first survey, they were more likely to be symptomatic (Table 4).

Similarly, those > 15 years had over three times the odds of having a subpatent infection (odds ratio [OR] = 3.53; 95%

TABLE 3

Unadjusted MOI and range per facility, number of distinct alleles (As), and allelic diversity (Rs) for PCR-positive samples (combined results for both health facility surveys)

	MOI	95% CI	Range	A	R_s
Age					
6 months to 5 years	1.98	1.85–2.13	1.46–2.36	70	67.59
6–15 years	2.23	2.03–2.46	1.75–2.45	67	67.0
> 15 years	1.97	1.84–2.13	1.39–2.5	58	56.77
Malaria drugs					
No drug	2.02	1.93–2.13	1.56–2.31	80	47.45
ACT	2.02	1.74–2.42	1.33–2.5	37	36.39
Non-ACT	2.26	1.89–2.78	1.96–2.75	32	32.0
Detectable parasites					
Patent	2.06	1.93–2.21	1.67–2.31	78	78.0
Subpatent	2.01	1.89–2.14	1.32–2.79	62	62.85
Symptoms					
Symptomatic	2.03	1.92–2.16	1.40–2.34	78	76.14
Asymptomatic	2.03	1.89–2.18	1.52–2.51	62	62.0

TABLE 4

Unadjusted and adjusted results for fixed effects of mixed effects logistic regression using health facility as random effect for variables associated with having an asymptomatic malaria infection compared with a symptomatic infection

	Unadjusted		Adjusted	
Outcome: asymptomatic infection	OR	95% CI	OR	95% CI
Study year	1.3	0.92–1.83	1.67	1.13–2.47
Age category				
6 months to 5 years	1.00	1.00	1.00	1.00
6–15 years	1.64	1.09–2.47	1.98	1.26–3.11
> 15 years	6.14	3.89–9.71	2.55	1.50–4.30
Patient (versus accompanying person)	0.11	0.05–0.25	0.26	0.10–0.67
Subpatent (versus patent)	8.64	5.81–12.83	7.53	4.88–11.62

CI = 2.23–5.59) compared with the youngest age group, and older children were one-half as likely to be asymptomatic (OR = 0.54; 95% CI = 0.33–0.90). Those who had reported taking antimalarial drugs in the past 2 weeks had greater odds of having a subpatent infection: participants reporting having taken non-artemesinin–based antimalarial drugs (i.e., quinine or sulphadoxine-pyramethanime) had a 2.49 greater odds of being subpatent (95% CI = 1.04–5.92), and those reported having used artemesinin combination therapy (ACT) had almost two times the odds of being subpatent, although this finding was not significant (Table 5).

DISCUSSION

This study is one of the few studies and the first study in Kenya to assess the use of surveys in health facilities as a means of measuring malaria transmission intensity in an area where transmission varies over a small geographical area.[9,10,33] The results of this study indicate that health facility-derived serological, parasitological, and molecular measures can detect differences in transmission intensity at a small geographical scale and are sensitive to seasonal changes. These findings suggest that health facility surveys are able to provide a reasonable measure of community-level transmission, are capable of delineating areas of high or low malaria transmission and that the use of serology and PCR added useful information to assessment of transmission levels in the sampled populations that would have been missed if sampling focused solely on those cases suspected of having malaria.[8,9,20]

TABLE 5

Unadjusted and adjusted results for fixed effects of mixed effects logistic regression using health facility as random effect for variables associated with having a subpatent malaria infection compared with a patent infection

	Unadjusted		Adjusted	
Outcome: subpatent infection	OR	95% CI	OR	95% CI
Age category				
6 months to 5 years	1.0	1.0	1.00	31.00
6–15 years	0.79	0.51–1.23	0.55	0.33–0.90
> 15 years	6.00	3.91–9.20	3.53	2.23–5.59
Asymptomatic	9.08	5.97–13.80	7.65	4.86–12.04
Antimalarial drug (2 weeks)				
No drug	1.0	1.0	1.0	1.0
ACT	1.58	0.83–3.01	1.81	0.84–3.89
Non-ACT	1.64	0.81–3.29	2.49	1.04–5.92

Similar to other studies, subpatent and asymptomatically infections were detected in this setting. It is likely that over one-half of malaria infections would have been missed had testing been restricted to use of RDTs for symptomatic cases.[11–13] The proportion of asymptomatic and subpatent infections differed by health facility, the main implication of which is that variations in transmission intensity will affect the proportion of infections missed using RDTs. The underestimation of malaria burden can have significant implications for malaria surveillance or development of control or elimination strategies based on clinical data.[16,30,34] For surveillance programs to capture the complete burden of malaria in a region, the proportion of infections missed should be taken into account. More robust data could be collected through use of more sensitive diagnostic tools, such as PCR, or a high-quality surveillance system targeting sentinel populations to get a more comprehensive picture of malaria transmission.[34–36] Alternatively, the limited sensitivity of RDT/microscopy can be acknowledged and adjusted for to estimate true prevalence or modify policy guidelines on an expectation of missed infections.[11,37]

Obtaining a better understanding of subpatent and asymptomatic infections is key to identifying which individuals are most likely to be missed by the current malaria surveillance practices. Similar to other studies,[14] our results suggest increased odds of having subpatent and asymptomatic infections in older age groups. These findings align with the current theory that, in areas with stable transmission, older individuals will have sufficient immunity to tolerate infections and maintain parasite densities below the limit of detection of RDTs.[30,38] Also, reporting taking malaria drugs in the 2 weeks before the survey was associated with having a subpatent malaria infection. The increased odds of being subpatent in those reporting that they took antimalarial drugs may be associated with residual parasitemia shortly after treatment or the detection of DNA from persisting gametocytes.[39,40] An alternative explanation for our finding is drug resistance: resistance to sulphadoxime-pyrametamine is highly prevalent in western Kenya, and although the use of this drug is officially limited to intermittent treatment of pregnant women, it is widely available in most private retailers.[41,42] Another possible explanation includes suboptimal or self-dosing with malaria drugs. Compliance to drug regimens in this area has not been studied to our knowledge, but it is possible that, if people are not completing their regimen properly, the drugs may only reduce parasite densities to subpatent levels without completely clearing the infection. Bias in recalling when or if they took that specific drug is also a possibility.

We also explored the complexity of malaria infections to gain additional insight into the molecular epidemiology of this study population. MOI has been shown to be a marker of transmission intensity that may have advantages in relatively high-transmission settings, where parasite prevalence may saturate.[3] Although MOI has proven to be a useful metric of malaria transmission intensity in certain settings,[27,32] no significant difference was found between facilities. This finding may be because of the spatial overlap of the health facility catchment areas, confounding factors not accounted for in the unadjusted analysis, such as age, or the small sample sizes. However, lower allelic diversities were observed in subpatent and asympatomic infections as well as older individuals and those who reported taking antimalarial drugs. The lower allelic richness observed in facilities experiencing lower

transmission intensity could be related to lower parasite densities expected in these populations or could indicate that certain low-density allelic forms were missed because of the PCR process.

The study design had some important limitations. The introduction of more sensitive diagnostic tools during the second survey may have reduced the proportion of subpatent and asymptomatic infections in that season. This was, however, incorporated in the statistical analysis and had little impact on the model results. Also, because of the cross-sectional nature of this survey, misclassification of participants by asymptomatic/subpatent status could have occurred.[14] It is possible that some individuals may have developed fever in subsequent days, which may have impacted our estimates of asymptomatic malaria. Similarly, the few studies that have looked at misclassification of patent/subpatent over time suggest that a small proportion of infections will shift between states, but the overall proportion detected does not shift dramatically, suggesting that it is unlikely that following these individuals over time would have a significant impact on these findings.[28,43] Finally, to obtain a specific understanding of how well health facilities are able to gauge transmission intensity in the surrounding community, health facility estimates need to be explicitly compared with those of the community population that they are supposed to represent. In this study, we have made use of an existing community sample from the same area collected the year before. Despite the temporal difference, the results indicate a strong correlation in SCR between the convenience and community sampling strategies, suggesting that the health facility provides a reasonable proxy for transmission intensity in the surrounding community.

Ultimately, health facility surveys provide an attractive tool to measure and detect heterogeneity in malaria transmission. In terms of sampling, they include a broader sample of the healthcare-seeking population instead of being restricted to those suspected of having malaria, while at the same time, they are more operationally attractive compared with community-based surveys in terms of the time and cost required to collect samples.[9,20] However, more work is required to determine how these estimates compare with the surrounding community. Estimates based on routinely used diagnostic tools, such as RDTs, are likely to underestimate malaria prevalence because of the presence of subpatent and asymptomatic infections, but in our study, RDTs correctly identified those health facilities with the highest transmission intensity in their catchment area. More research is needed to further explore the molecular epidemiology of malaria infections and develop strategies that can easily identify these populations to ensure that malaria control decisions are based on a complete picture of malaria transmission.

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