Dynamics of the Human Infectious Reservoir for Malaria Determined by Mosquito Feeding Assays and Ultrasensitive Malaria Diagnosis in Burkina Faso

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Background. Plasmodium falciparum gametocytes are essential for malaria transmission. Malaria control measures that aim at reducing transmission require an accurate characterization of the human infectious reservoir.

Methods. We longitudinally determined human infectiousness to mosquitoes and P. falciparum carriage by an ultrasensitive RNA-based diagnostics in 130 randomly selected inhabitants of an endemic area.

Results. At least 1 mosquito was infected by 32.6% (100 of 307) of the blood samples; in total, 7.6% of mosquitoes (916 of 12 079) were infected. The proportion of infectious individuals and infected mosquitoes were negatively associated with age and positively with asexual parasites (P < .001). Human infectiousness was higher at the start of the wet season and subsequently declined at the peak of the wet season (adjusted odds ratio, 0.52; P = .06) and in the dry season (0.23; P < .001). Overall, microscopy-negative individuals were responsible for 28.7% of infectious individuals (25 of 87) and 17.0% of mosquito infections (145 of 855).

Conclusions. Our study reveals that the infectious reservoir peaks at the start of the wet season, with prominent roles for infections in children and submicroscopic infections. These findings have important consequences for strategies and the timing of interventions, which need to include submicroscopic infections and be implemented in the dry season.

Keywords. malaria; falciparum; gametocyte; reservoir; season; dynamics; QT-NASBA; mosquito; feeding; Burkina Faso.

Malaria is a life-threatening disease, with approximately 584 000 deaths and 198 million clinical cases in 2013, the great majority (90%) falling on poor rural communities of sub-Saharan Africa [1]. After the upscaling of efficacious malaria control efforts, the burden of malaria has declined in numerous African and non-African settings [2], renewing the global interest in local malaria elimination and eradication. For the majority of African settings where transmission continues to be intense [2], malaria elimination is unlikely to be achieved by further upsampling of conventional control measures [3]. Interventions that specifically aim to reduce malaria transmission may be needed, and for such interventions to succeed we must identify the human reservoir of malaria and understand temporal patterns in its infectiousness to optimize control interventions.

Human infectiousness to mosquitoes depends on the presence of mature gametocytes in the human peripheral blood. Although gametocyte density is a key determinant for malaria transmission, its association with mosquito infection rates is nonlinear [4]. High gametocyte densities may not always result in mosquito infection [5,6], and individuals without microscopically detectable gametocytes can be infectious [7]. In areas of intense transmission, >40% of individuals with microscopically detectable gametocytes are infectious to mosquitoes in either skin-feeding [8] or membrane-feeding [5,9] experiments, but successful mosquito infections are also commonly observed from individuals without microscopic evidence of gametocyte carriage. The observation of mosquito infections from individuals without microscopically detectable gametocytes has recently received a diagnostic foundation by the molecular detection of infectious submicroscopic gametocyte densities [5,6]. These submicroscopic infections are common in the long dry season [10,11] and may play an important role in launching the malaria epidemics at the onset of the transmission season.
Data from the malaria therapy studies suggest that malaria infections can persist and remain infectious to mosquitoes for several months in naive individuals [12, 13]. However, the contribution to transmission of semi-immune individuals with (sub)microscopic infections throughout the long dry season remains elusive [14, 15]. If these infections form an important source of infections for onward transmission, malaria control interventions must be adapted to target parasite carriers in the dry season and include submicroscopic malaria infections to have maximum impact. Here, we present the first longitudinal assessment of the human infectious reservoir for malaria by mosquito feeding assays in combination with ultrasensitive molecular malaria diagnostics in an area of intense seasonal malaria transmission in Burkina Faso.

MATERIALS AND METHODS

Ethics Statement
The study received ethical clearance from the Ethical Review Committee of the Ministry of Health of Burkina Faso (MS/MESSRS No. 2007-035). Study procedures, risks and benefits were explained to participants, and written informed consent obtained from adults and parents/guardians of children before enrollment.

Study Site and Population
Participants were recruited from the villages of Laye and Dapélogo in the Central Sudan savannah area of Burkina Faso. Malaria transmission is intense and seasonal, and Plasmodium falciparum is the major malaria species, accounting for 90% of all malaria infections. The study population is exclusively of the Mossi ethnicity. Individuals were randomly recruited from 4 age groups (<5, 5–14, 15–30, and >30 years) to be representative of the whole population in the study area. Individuals were invited to participate, and the first to arrive were enrolled until the sample size was reached.

Blood Sample Collection
Study participants were visited on 3 occasions coinciding with the start of the wet season, the peak of the wet season and in the subsequent dry season (Figure 1). Individuals presenting with serious acute disease, including clinical malaria, were not eligible for participation. These individuals were treated accordingly and referred to the nearest health facility for appropriate care. Approximately 10 participants were recruited per day and invited to participate in membrane feeding assays at enrollment and during subsequent surveys. Individuals who failed to attend follow-up surveys were systematically replaced by newly recruited participants and matched for age category and village of residence. During subsequent visits, participants with axillary temperature ≥37.5°C and a positive malaria rapid diagnostic test (Optimal-IT; DiaMed) were treated with antimalarial drugs according to current national malaria treatment guidelines.

P. falciparum Parasite Detection With Microscopy and Quantitative Nucleic Acid Sequence-Based Amplification
Densities of P. falciparum parasites were microscopically determined by assuming a standard leukocyte count of 8000/µL of blood. Both asexual and gametocyte parasite densities were simultaneously assessed by counting against 500 leukocytes in the thick smear, with a consequent limit of detection of 16 parasites per microliter. Each slide was considered negative if no parasites were detected in 100 microscopic fields. Slides were double read; a third reader was involved when the first 2 readers disagreed about the prevalence of parasite or when the estimated densities differed by ≥30%. In these cases, the mean density of the 2 closest readings was used.

Nucleic acids were extracted from 100 µL aliquots of venous blood samples using the guanidiumisothiocyanate/silica procedure [16]. Quantitative nucleic acid sequence–based amplification (QT-NASBA) was performed as described elsewhere for the detection of all parasite stages based on 18S ribosomal

Figure 1. Monthly rainfall data. Dashed arrows indicate the time points when the surveys were conducted at the start and peak of the rainy season and during the subsequent dry season.
RNA (rRNA) and for the specific detection of mature gametocytes based on Pfs25 messenger RNA (mRNA) [17]. The number of gametocytes was calculated in relation to a standard gametocyte stage V dilution series, using the time point of amplification at which the fluorescence detecting target amplicons exceeded the mean fluorescence of 3 negative controls plus 20 standard deviations. Negative controls were assayed in triplicate on each plate. The sensitivity of 18S rRNA and of Pfs25 mRNA is approximately 0.01–0.02 parasites per microliter, similar to that of a recently described ultrasensitive polymerase chain reaction based on multicopy subtelomeric targets [18]; this is considered the most sensitive diagnostic test currently available for gametocyte detection [14, 17, 19]. In the present study, samples with estimated parasite or gametocyte densities <0.01 parasite per microliter (ie, 1 parasite per 100-microliter blood sample) were considered parasite negative.

**Membrane Feeding Assays**

For each membrane feeding assay, 3 mL of venous blood sample was obtained from the participant. A maximum of 50 locally colony-reared 4–5-day-old female *Anopheles gambiae sensu stricto* mosquitoes [20] were prepared in a cup to feed on the blood sample. Blood was offered to the mosquitoes via an artificial membrane attached to a water-jacketed glass feeder maintained at 37°C. After 10–15 minutes, unfed and partially fed mosquitoes were removed and killed. Fully fed mosquitoes were kept and fed with glucose at 29°C for 7 days before dissection. After dissection, the mosquito midguts were stained with 1% mercurochrome before oocyst screening performed by an independent microscopist. The number of positive mosquitoes among all dissected mosquitoes that contained at least a single oocyst-positive mosquito were recorded by an independent microscopist. The number of positive mosquitoes and the oocyst count per mosquito were recorded for each feeding assay.

**Sample Size Considerations**

There are limited data on mosquito infection rates as a function of age and season on which to base formal sample size calculations. We therefore based our sample size calculations on gametocyte prevalence by Pfs25 QT-NASBA. We expected children <15 years of age to have a gametocyte prevalence of approximately 80%, compared with 50% in adults (≥15 years), as described elsewhere [21]. Including 50 children and 50 adults in each survey gave us 85% power to detect a significant difference in gametocyte prevalence between the 2 age groups at the 2-tailed 5% significance level.

**Data Analysis**

Data analyses were performed using SPSS (version 20.0; SPSS), R (version 3.1.1), and Stata (version 12; StataCorp) software. Study participants were categorized into groups by age (<5, 5–14, 15–30, and >30 years). Continuous variables were presented as medians and interquartile ranges (IQRs). Univariate analyses of discrete variables were based on $\chi^2$ or Fisher exact tests; for continuous variables, Mann–Whitney and Kruskal–Wallis tests were used. Binary logistic models using generalized estimating equations were used to determine the effect of age, season, asexual parasite prevalence, and gametocyte density on the proportions of infectious individuals and infected mosquitoes, adjusting for observations from the same individual. Exchangeable working correlation structure and a logit link function were used. Robust standard errors were estimated. Variables with $P$ values <.10 in the univariate analysis were kept for multivariate analysis.

The fractional contribution to the infectious reservoir of the $N_g$ individuals in population group $g$ (eg, gametocyte-detection state or age group) was calculated as follows:

$$F_{\text{reservoir}} = \frac{f_{\text{human}} f_{\text{mosquito}}}{\sum_g f_{\text{human}} f_{\text{mosquito}}},$$

where the fraction of infectious individuals in group $g$ in a given survey period is

$$f_{\text{human}} = \frac{N_g}{\sum_g N_g},$$

and the average fraction of mosquitoes infected by an individual $i$ in group $g$ is

$$f_{\text{mosquito}} = \frac{\sum_i f_{\text{mosquito}}}{N_g}$$

with $f_{\text{mosquito}} = \frac{n_{i,\text{oocyst-positive}}}{n_{i,\text{dissected}}}$ being the fraction of oocyst-positive mosquitoes among all dissected mosquitoes that fed on blood from individual $i$.

**RESULTS**

A total of 130 individuals of all ages (range, 1.1–55.3 years) were enrolled in this study. The median age at enrollment was 17.2 years (IQR, 8.4–29.6; Table 1). Participants provided 307 individual blood samples for membrane feeding, 292 blood slides, and 292 RNA samples over the 3 occasions. The majority of individuals (56.9%; 74 of 130) donated blood samples at 3 visits, 22.3% (29 of 130) at 2 visits, and 20.8% (27 of 130) at 1 visit. *P. falciparum* Parasite Carriage

A total of 42.1% of blood slides (123 of 292) were positive for asexual parasites at microscopy. As shown in Table 1, the prevalence of asexual parasites decreased with age ($P < .001$) and declined from the start (53.8%; 49 of 91) to the peak of the wet season (41.8%; 41 of 98; $P = .002$) and into the dry season (32.0%; 33 of 103; $P = .009$). Gametocytes were detected in 17.1% of blood slide (50 of 292) (Table 1). As for asexual parasites, the prevalence of gametocytes by microscopy was age dependent ($P < .001$) but did not vary significantly with season. The median density of gametocytes in gametocyte carriers by

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microscopy was 34/μL (IQR, 19–57/μL), and density did not vary with season or age (data not shown).

The 18S rRNA QT-NASBA demonstrated *P. falciparum* infection in 87.3% of all samples (255 of 292) and confirmed 99.1% of asexual parasite–positive slides (115 of 116; the 1 discordant sample showed amplification in the 18S rRNA QT-NASBA but with estimated densities below the predefined cutoff). There was no statistically significant association between age and 18S rRNA QT-NASBA parasite prevalence in individuals up to 30 years of age (P = .70) but the prevalence in older adults was significantly lower (P = .001). Pfs25 mRNA QT-NASBA demonstrated *P. falciparum* gametocytes in 65.1% of all samples (190 of 292) and confirmed 95.7% of gametocyte-positive slides (45 of 47; the remaining 2 samples were 18S rRNA QT-NASBA positive and showing amplification in the Pfs25 mRNA QT-NASBA but with estimated densities below the predefined cutoff). Gametocyte prevalence declined with increasing age (P < .001), as shown in Figure 2, but it was not associated with season (P = .40). Submicroscopic gametocytes were equally prevalent across age groups (P = .90), with prevalences of 51.4%, 54.7%, 51.2%, and 39.7% in individuals aged <5, 5–14, 15–30, and >30 years, respectively, with no seasonal association (P = .46, Table 1).

**Table 1. Demographic and Malariometric Indices in the Study Area**

<table>
<thead>
<tr>
<th>Index</th>
<th>Wet Season (Start) (n = 103)</th>
<th>Wet Season (Peak) (n = 100)</th>
<th>Dry Season (n = 104)</th>
<th>Total (n = 307)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>17.5 (8.9–29.3)</td>
<td>17.3 (9.8–29.2)</td>
<td>16.6 (7.9–29.2)</td>
<td>17.3 (8.3–29.2)</td>
</tr>
<tr>
<td>Male sex</td>
<td>55.3 (57/103)</td>
<td>55.0 (55/100)</td>
<td>56.3 (58/103)</td>
<td>55.6 (170/306)</td>
</tr>
<tr>
<td>Fever (temperature ≥37.5°C)</td>
<td>6.2 (8/61)</td>
<td>2.3 (2/86)</td>
<td>0 (0/98)</td>
<td>2.6 (7/265)</td>
</tr>
<tr>
<td>Asexual parasite prevalence by microscopy</td>
<td>53.9 (49/91)</td>
<td>41.8 (41/98)</td>
<td>32.0 (33/103)</td>
<td>42.1 (123/292)</td>
</tr>
<tr>
<td>Parasite prevalence by 18S rRNA QT-NASBA</td>
<td>85.0 (85/100)</td>
<td>93.6 (88/94)</td>
<td>83.7 (82/98)</td>
<td>87.3 (255/292)</td>
</tr>
<tr>
<td>Gametocyte prevalence by microscopy</td>
<td>19.8 (18/91)</td>
<td>21.4 (21/98)</td>
<td>10.7 (11/103)</td>
<td>17.1 (59/292)</td>
</tr>
<tr>
<td>Gametocyte prevalence by Pfs25 mRNA QT-NASBA</td>
<td>68.0 (68/100)</td>
<td>67.0 (63/94)</td>
<td>60.2 (59/98)</td>
<td>65.1 (190/292)</td>
</tr>
<tr>
<td>Submicroscopic gametocytes</td>
<td>50.6 (44/87)</td>
<td>48.9 (45/92)</td>
<td>50 (49/98)</td>
<td>49.8 (138/277)</td>
</tr>
<tr>
<td>Infectious individuals</td>
<td>48.1 (50/104)</td>
<td>34.0 (34/100)</td>
<td>15.5 (16/103)</td>
<td>32.6 (100/307)</td>
</tr>
<tr>
<td>Infected mosquitoes, % (No./total)</td>
<td>9.7 (417/4304)</td>
<td>9.5 (367/3853)</td>
<td>3.4 (132/3922)</td>
<td>7.6 (916/12 079)</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; mRNA, messenger RNA; QT-NASBA, quantitative nucleic acid sequence-based amplification; rRNA, ribosomal RNA.

* Unless otherwise specified, data reflect percentage (No/total sample) of individuals with blood samples.

Individuals with microscopic and Pfs25 mRNA QT-NASBA data in whom microscopy was negative for gametocytes but Pfs25 mRNA QT-NASBA was positive.

**P. falciparum** Infectiousness

A total of 307 membrane feeding experiments were performed, involving 12 079 dissected mosquitoes. On average, 39.3 fed mosquitoes (range, 14–65) were dissected per experiment. At least 1 mosquito was infected in 32.6% (100 of 307) of the feeding experiments (Table 1), with 7.7% of mosquitoes overall (916 of 12 079) becoming infected. The median percentage of infected mosquitoes per infectious feeding was 11.1% (IQR, 3.3%–33.7%), with a median of 2 oocysts (range, 1–97). Of individuals with microscopically detectable gametocytes, 70% (35 of 50) were infectious to 22.9% (426 of 1862) of fed mosquitoes. Of individuals with microscopically detected asexual parasites only, 40.5% (34 of 84) infected ≥1 mosquito; in total, 19.7% of mosquitoes (285 of 1444) became infected.

More than a third (36.2%; 92 of 254) of *P. falciparum* infections determined with 18S rRNA QT-NASBA were infectious to mosquitoes, compared with 43.70% (83 of 190) of gametocyte carriers by Pfs25 mRNA QT-NASBA. Of those individuals with submicroscopic gametocytes, 32.6% (45 of 138) infected ≥1 mosquito, and, in total, 7.7% (416 of 5424) of mosquitoes became infected. Of the 92 asexual parasite infections only (Table 2), 9.8% (9 of 92) were infectious.

Among all infectious individuals with complete data on parasite prevalence at microscopy and QT-NASBA, 37.9% (33 of 87) were carriers of microscopically detected gametocytes, 51.7% (45 of 87) were submicroscopic gametocyte carriers, and the remaining 10.3% (9 of 87) had submicroscopic infections with asexual parasites only. Truly submicroscopic infections, without gametocyte or asexual parasites at microscopy, were responsible for 28.7% of infectious individuals (25 of 87) and 17.0% of infected mosquitoes (145 of 855).

**Association of P. falciparum Infectiousness With Age and Season**

In Figure 3, the proportion of infected mosquitoes is presented in relation to Pfs25 mRNA QT-NASBA gametocyte density, age, and season. The proportions of infectious individuals were 60.0% (24 of 40), 46.1% (47 of 102), 21.0% (20 of 95), and 12.9% (9 of 70) in children aged <5 or 5–14 years and in adults aged 15–30 or >30 years, respectively. After adjustment for covariates, age, season, and Pfs25 mRNA QT-NASBA gametocyte density remained significant predictors of *P. falciparum* infectiousness. The proportion of infectious individuals (Table 1) declined over seasons, with significant changes at the peak of the wet season (P = .06; borderline significance) and in the dry
season \((P < .001)\) compared with the start of the wet season (Table 3). Similarly, a seasonally dependent decrease in the percentage of infected mosquitoes was observed from the start of the wet season toward the dry season \((P < .001)\), after correction for confounding factors (Table 3).

In Figure 4, the proportion of infected mosquito is presented in relation to gametocyte density at the season and age scales. The proportion of infected mosquitoes increases with gametocyte density in individuals aged <15 years at the start and peak of the wet season. In adults, the proportion of infected mosquitoes seemed comparatively lower, although we found no evidence of a significant interaction between age and gametocyte density in predicting mosquito infection rates.

Relative Contribution of Population Groups to Malaria Transmission

The relative contribution to transmission decreased with age and was estimated at 44.8%, 41.3%, 11.7%, and 2.2% for those aged <5, 5–14, 15–30, or >30 years, respectively (Table 2). The lower contribution of older age groups was consistent across seasons; in the dry season, only 1.5% of transmission was attributable to...
Table 2. Relative Contribution of Population Subsets (Age Group and Gametocyte Carriage Status) to the Infectious Reservoir at Different Seasons

<table>
<thead>
<tr>
<th>Population Subset</th>
<th>Overall % of Population</th>
<th>Infected Mosquitoes, % (No./Total)</th>
<th>Wet Season (Start)</th>
<th>Wet Season (Peak)</th>
<th>Dry Season</th>
<th>RCTa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group, yb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>20.0</td>
<td>18.5 (290/1565)</td>
<td>24.7 (149/603)</td>
<td>9.6 (45/471)</td>
<td>19.6 (96/491)</td>
<td>20.0</td>
</tr>
<tr>
<td>5–14</td>
<td>30.0</td>
<td>11.4 (463/4058)</td>
<td>13.6 (202/1490)</td>
<td>18.0 (228/1264)</td>
<td>2.5 (33/1304)</td>
<td>30.0</td>
</tr>
<tr>
<td>15–30</td>
<td>25.0</td>
<td>3.9 (143/3671)</td>
<td>4.7 (58/1247)</td>
<td>6.9 (84/1212)</td>
<td>0.1 (1/1212)</td>
<td>25.0</td>
</tr>
<tr>
<td>≥30</td>
<td>25.0</td>
<td>0.7 (20/2786)</td>
<td>0.8 (8/964)</td>
<td>1.1 (10/906)</td>
<td>0.2 (2/915)</td>
<td>25.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>7.6 (916/12 079)</td>
<td>9.7 (417/4304)</td>
<td>9.5 (367/3853)</td>
<td>3.4 (132/3922)</td>
<td>100</td>
</tr>
<tr>
<td>Gametocyte statusc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopic gametocytes</td>
<td>17.0 (47/277)</td>
<td>22.9 (426/1862)</td>
<td>27.7 (212/766)</td>
<td>20.7 (18/87)</td>
<td>20.8 (148/710)</td>
<td>17.1</td>
</tr>
<tr>
<td>Submicroscopic gametocytes</td>
<td>49.8 (138/277)</td>
<td>7.7 (416/5424)</td>
<td>8.8 (164/1855)</td>
<td>50.6 (44/87)</td>
<td>10.9 (187/1709)</td>
<td>3.5</td>
</tr>
<tr>
<td>Asexual parasite infection onlyd</td>
<td>33.2 (92/277)</td>
<td>0.4 (13/3577)</td>
<td>0.7 (7/989)</td>
<td>28.7 (25/87)</td>
<td>0.5 (5/1106)</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>7.9 (855/10 863)</td>
<td>10.6 (383/3610)</td>
<td>9.64 (340/3525)</td>
<td>3.5 (132/3728)</td>
<td>100</td>
</tr>
</tbody>
</table>

Abbreviation: RCT, relative contribution to transmission.

a RCT calculation is described in the section “Data analysis” and refers to the equation on fractional contribution of Ng individuals to the infectious reservoir.

b The proportion of individuals from each age group was provided by the National Institute for Demography (http://www.insd.bf/n/).

c Only individuals with complete data on parasite prevalence by microscopy and quantitative nucleic acid sequence-based amplification (QT-NASBA) were included.

d Infection detected with 18S ribosomal RNA QT-NASBA and/or microscopy but no gametocytes detected with microscopy or Pf35 messenger RNA QT-NASBA.
individuals >15 years (Table 2). There was also a significant decline in the infectious reservoir from the wet season (84.7%) toward the dry season (15.4%). Microscopically detectable gametocytes and submicroscopic gametocytes contributed to the infectious reservoir at 49.7% and 48.8%, respectively (Table 2).

**DISCUSSION**

In this study, we determined the human infectious reservoir for malaria and its dynamics in an area of seasonal malaria transmission in Burkina Faso. An important finding is that the infectious reservoir peaks at the onset of the transmission season. Children were more important for the infectious reservoir than adults, and submicroscopic infections form an important source for the onward malaria transmission.

We performed membrane feeding experiments in a cohort of individuals who were sampled at 3 time points during 1 full transmission cycle in Burkina Faso. Our longitudinal sampling showed that gametocytes are highly prevalent at the start of the transmission season [28] and that the human infectious reservoir is also at its largest at this time-point. The sharp increase in gametocyte carriage at the start of the transmission season has been observed before [29] and hypothesized to be associated with newly induced

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**Table 3. Adjusted Effect of Predictors on the Proportion of Infectious Individuals and the Proportion of Infected Mosquitoes**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Proportion of Infectious Individuals</th>
<th>Proportion of Infected Mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td><strong>Age group, y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>5–14</td>
<td>0.65 (0.22–1.93)</td>
<td>.44</td>
</tr>
<tr>
<td>15–30</td>
<td>0.28 (0.18–0.93)</td>
<td>.03</td>
</tr>
<tr>
<td>&gt;30</td>
<td>0.20 (0.05–0.79)</td>
<td>.02</td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet (start)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Wet (peak)</td>
<td>0.52 (0.26–1.03)</td>
<td>.06</td>
</tr>
<tr>
<td>Dry</td>
<td>0.23 (0.11–0.47)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Asexual parasites by microscopy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>2.0 (0.96–4.19)</td>
<td>.06</td>
</tr>
<tr>
<td><strong>Gametocytes by QT-NASBA, No./µL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>1.75 (1.63–4.29)</td>
<td>.28</td>
</tr>
<tr>
<td>10–50</td>
<td>1.55 (1.63–3.77)</td>
<td>.33</td>
</tr>
<tr>
<td>&gt;50</td>
<td>3.92 (1.73–8.89)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; QT-NASBA, quantitative nucleic acid sequence–based amplification.

* Generalized estimating equations were used to determine the effect of predictors on the proportions of infectious individuals and infected mosquitoes, allowing for within-subject correlation.
gametocyte production from chronic infections that are stimulated by uninfected mosquito bites at the onset of the transmission season [30–32]. The longevity of chronic infections throughout the dry season is thought to be driven by various factors, including the age of parasite carriers and superinfections [10,11,33,34]. The increased infectiousness of early-season gametocytes, after adjustment for their density, could plausibly be the result of a waning transmission-reducing immunity during the previous long dry season and/or transmission-enhancing immunity that has been associated with low antibody concentrations [35].

We observed that age and season were independent predictors of the human infectious reservoir for malaria. Previous studies in children found evidence that within a narrow age band—6 months to 10 years—there may be a positive association between age and the likelihood of transmission [4,6]. This has been associated with naturally acquired human immune responses against gametocyte antigens that may reduce or prevent the transmission of malaria parasites to mosquitoes [36,37] and may be highly prevalent in young age groups [36]. In our all-age study population, we found a contrasting association of declining P. falciparum infectiousness with age.

Although there is currently no evidence of transmission blocking immune responses in our study population, gametocyte commitment may increase with age [38,39] and be associated with transmission-reducing immune responses in semi-immune adults, resulting in a lowered infectiousness of malaria parasites. Alternatively, or in addition, the infectiousness of gametocytes may be related to the duration of infections. Human infectiousness to mosquitoes peaks shortly after incident infections [40]. This is not completely governed by gametocyte densities, and gametocytes may be detectable for several months after the peak of infectivity has passed and mosquito infection rates are much lower [40]. Infections are likely to be older in the dry season, resulting from inoculations during the preceding peak seasons [10] or possibly from inoculations in the preceding years [41], and likely to be present without
microscopically detectable asexual parasite densities [42]. Older infections that are less likely to be transmissible to mosquitoes may therefore explain our association of lower infectivity in the dry season and in the absence of microscopically detectable asexual parasites that dominate in older age groups. However, infections with submicroscopic densities were more likely to remain infectious throughout the long dry season. The long-lasting infectiosity of malarious individuals is consistent with reports of naive populations in the 1950s [13]. A long-lasting infectiosity, intriguingly enhanced at the onset of the wet transmission season, may explain the considerable contribution of children <15 years of age to the infectious reservoir. The remarkably high representation of this demographic group in the sub-Saharan African populations (approximately 50% in our setting) and a set of mechanisms underlying their increased infectiosity toward the start of the wet season make children <15 years old and the dry season duration essential parameters to consider for effective malaria control in seasonal transmission settings.

Despite the negative association between P. falciparum infectiosity and age, all age groups contributed to onward malaria transmission in our population. Previous studies have estimated the adult contribution as 22%–41% of the human infectious reservoir [25, 43, 44], and this relative contribution will increase if the increased attractiveness and availability to mosquitoes by adults is taken into account. In our study, adults contributed to 13%–21% of the human infectious reservoir during the wet season and <2% in the subsequent dry season. Because of their increased attractiveness to mosquitoes and larger body size [45–48], interventions aimed at reducing transmission would need to account for adults.

Our results demonstrate that submicroscopic gametocyte carriage is an important source of malaria transmission, being responsible for approximately half of the human infectious reservoir. Many of these submicroscopic gametocytes were accompanied by asexual parasites that were detectable by microscopy. A greater challenge lies in detecting infections that are truly submicroscopic, with densities of both asexual parasites and gametocytes being below the threshold for detection with microscopy [18]. In our setting, this group comprised 28.73% of all infections and was responsible for 16.95% of infected mosquitoes. Our finding that submicroscopic infections form an important source of mosquito infections through the seasons supports the hypothesis that malaria transmission from submicroscopic infections has contributed to the failure of mass screening and treatment campaigns in Burkina Faso and Zanzibar to reduce malaria transmission sustainably [49, 50].

To conclude, transmission of malaria parasites from human to Anopheles vectors takes advantage of the presence of high gametocyte densities. However, infections with submicroscopic densities are also efficiently transmitted. To the best of our knowledge, our findings are the first to demonstrate that the infectious reservoir is at its largest at the onset of the wet season in a setting of intense and seasonal malaria transmission. To achieve any reduction in malaria transmission in such endemic settings and significantly affect the epidemiology of the disease, effective interventions must account for submicroscopic infections and be implemented before the start of the wet season.

Notes
Acknowledgments. We thank the communities of the villages of Laye and Dapelogo for their cooperation, the Ministry of Health of Burkina Faso for providing with local staff support and help with operational logistics. We are also grateful to the director of the National Metrological Centre of Burkina Faso for providing the rainfall data.

Financial support. This work was supported by the Netherlands Organization for Higher Education in the Tropics (grant CF29132006 to A. L. O.), the Global Good Fund, Bellevue, Washington (A. L. O., E. A. W., and J. G.) and the European FP7 project REDMAL (grant 242079 to T. B.).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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