Residual *Plasmodium falciparum* Parasitemia in Kenyan Children After Artemisinin-Combination Therapy Is Associated With Increased Transmission to Mosquitoes and Parasite Recurrence

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**Background.** Parasite clearance time after artemisinin-based combination therapy (ACT) may be increasing in Asian and African settings. The association between parasite clearance following ACT and transmissibility is currently unknown.

**Methods.** We determined parasite clearance dynamics by duplex quantitative polymerase chain reaction (qPCR) in samples collected in the first 3 days after treatment of uncomplicated malaria with ACT. Gametocyte carriage was determined by PfS25 quantitative nucleic acid sequence–based amplification assays; infectiousness to mosquitoes by membrane-feeding assays on day 7 after treatment.

**Results.** Residual parasitemia was detected by qPCR in 31.8% (95% confidence interval [CI], 24.6–39.8) of the children on day 3 after initiation of treatment. Residual parasitemia was associated with a 2-fold longer duration of gametocyte carriage \( (P = .0007) \), a higher likelihood of infecting mosquitoes \( (\text{relative risk}, 1.95; 95\% \text{ CI}, 1.17–3.24; P = .015) \), and a higher parasite burden in mosquitoes \( (\text{incidence rate ratio}, 2.92; 95\% \text{ CI}, 1.61–5.31; P < .001) \). Children with residual parasitemia were also significantly more likely to experience microscopically detectable parasitemia during follow-up \( (\text{relative risk}, 11.25; 95\% \text{ CI}, 4.08–31.01; P < .001) \).

**Conclusions.** Residual submicroscopic parasitemia is common after ACT and is associated with a higher transmission potential. Residual parasitemia may also have consequences for individual patients because of its higher risk of recurrent parasitemia.

**Keywords.** artemisinin; anopheles; infectivity; transmission; resistance; submicroscopic; PCR.

Reduced susceptibility of *Plasmodium falciparum* to artesunate monotherapy has been reported in western Cambodia [1, 2] and was recently shown to have emerged in or spread westward to the Thailand–Myanmar border [3]. The time to clearance of parasites after mefloquine-artesunate combination therapy is also becoming longer along the Thailand–Cambodia border [4], and treatment failure rates of mefloquine-artesunate or artemether-lumefantrine (AL) often exceed 10% [5–8]. Reduced susceptibility to artemisinin-combination therapy (ACT) may also affect the
African continent, where the majority of countries have adopted ACT as first-line antimalarial treatment.

In the first such report from Africa, a measurable increase in parasite clearance time after AL and dihydroartemisinin-piperaquine (DP) was reported in coastal Kenya during 2005–2009 [9]. Parasite clearance time is influenced by parasite drug susceptibility, parasite density before initiation of treatment, and individual differences in antimalarial pharmacokinetics and immunity [10, 11]. With the decline in transmission intensity in various regions in Africa [12, 13] and consequent changes in the rate at which immunity is acquired, time trends in parasite clearance times are difficult to interpret [9]. Detailed monitoring of parasite clearance dynamics after ACT is needed to determine whether parasite responsiveness to ACT is changing. Clearance dynamics in vivo can be analyzed as the parasite half-life if blood samples are obtained more than once per day after treatment [2, 11] or as the simple presence or absence of parasites at a single time point after initiation of treatment [14]. A simple duplex quantitative polymerase chain reaction (qPCR) method has been proposed for the analysis of sequentially collected daily filter paper blood samples to sensitively detect and quantify parasites below the microscopic threshold for detection [15].

A less conventional indicator of reduced susceptibility of malaria parasites to antimalarial drugs is the malaria transmission potential after treatment [16]. Transmission potential is frequently reported as the prevalence or density of malarial transmission stages (ie, gametocytes) and can be directly quantified by determining the infectivity of naturally infected individuals in mosquito-feeding assays [17]. For chloroquine (CQ) and sulfadoxine-pyrimethamine (SP), gametocyte carriage is increased or prolonged in resistant or partially resistant parasites [18–20], and enhanced transmission to Anopheles mosquitoes has been shown for CQ-resistant parasite isolates [21]. Parasites with mutations conferring SP resistance exhibit enhanced transmissibility even when circulating asexual parasites are effectively cleared by treatment [22], suggesting that, as antimalarial resistance develops in a population, changes in transmission potential may precede changes in treatment response [16, 22, 23]. Understanding the phenotype of enhanced parasite transmissibility is important for predicting the spread of resistant parasites.

Treatment with ACT is associated with rapid reductions in asexual parasite densities, lower prevalence of gametocyte carriage, and reduced but not completely abrogated posttreatment malaria transmission [24, 25]. The association between parasite clearance following ACT and transmissibility is unknown and regarded as one of the key questions for successful containment of artemisinin resistance [26, 27]. We determined the association between parasite clearance dynamics after ACT treatment of uncomplicated malaria in Kenyan children and posttreatment gametocyte carriage, malaria transmission to mosquitoes, and recurrent asexual parasitemia during follow-up.

METHODS

Study Design, Site, and Enrollment

Between April and June 2009, a randomized, open-label trial was conducted in Mbita Point, western Kenya, and has been described in detail elsewhere [28]. Briefly, children aged 6 months–10 years with uncomplicated malaria who were attending the health facility of the International Center of Insect Physiology and Ecology were included in the study if they had a microscopically confirmed P. falciparum mono infection with an asexual parasite density of 1000–200 000 parasites/μL and a hemoglobin level of >5 g/dL. The protocol received approval from the Kenya Medical Research Institute Ethical Review Committee and the London School of Hygiene and Tropical Medicine Ethics Committee (reference 5455). Written informed consent was obtained from a parent or guardian of each participating child.

Treatment, Follow-up, and Clinical and Laboratory Procedures

Eligible children were randomly allocated to receive supervised treatment with AL (Coartem; Novartis Pharma), administered as half a tablet (20 mg of arte mether and 120 mg of lumefantrine) per 5 kg of body weight in a 6-dose regimen, or with DP (Duocotexin, Holley Pharm, 40 mg dihydroartemisinin/320 mg piperaquine tablets), administered with targeted total doses of 6.4 and 51.2 mg/kg of dihydroartemisinin and piperaquine, respectively, given in 3 equally divided daily doses to the nearest quarter tablet. New participants were enrolled into the trial on a daily basis between 10 AM and 12 PM (noon). Follow-up sampling was performed on days 1, 2, 3, 7, 14, 28, and 42 between 8 AM and 12 PM. Participants who did not attend the clinic on any of the follow-up days were visited at home before noon. On treatment days, sampling was done before treatment: blood smears, dried filter paper blood spots, and 50–μL whole blood samples were collected for microscopy, qPCR, and quantitative nucleic acid sequence-based amplification (QT-NASBA), respectively. QT-NASBA was done for a random selection of individuals per treatment arm, as described previously [28].

MSP-1 and MSP-2 genotyping in cases of treatment failure was performed in accordance with World Health Organization (WHO) criteria [28, 29] but with additional time points; samples collected on days 0, 1, 2, and 3 and at the visit before recurrent patent parasitemia were included in each case. The original trial report was restricted to a conventional presentation of microscopically defined treatment outcomes and transmission potential [28]. For the current study, a duplex qPCR method was used to determine parasite clearance dynamics for all patients for whom a complete set of day 0, 1, 2, and 3 filter papers were available. qPCR was used as previously described [15], using 6-mm punches from Whatman 3MM filter paper (Whatman, Maidstone, United Kingdom). The qPCR generates distinct fluorescent signals for human (β-tubulin) and parasite
(methionine transfer RNA gene [pgm]) DNA targets. In the course of validating the modified protocol used here, the assay displayed a sensitivity comparable with that of conventional nested PCR [30] and reliably detected parasite densities of 1 parasite/μL; lower densities were frequently detected but with a stochastic element that is also observed for nested PCR [31]. The qPCR has a coefficient of variation between 0.03% and 0.68% at densities of ≥5 parasites/μL and between 4.0% and 6.8% at lower parasite densities. The WHO international standard for \textit{P. falciparum} DNA was used as positive control [15].

The following modified amplification cycling conditions were used in the current study: 95°C for 6 minutes and then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. qPCR failed initially in 3 samples (ie, no β-tubulin signal was detected); repeating the samples using the same DNA material gave interpretable results. Pf25 QT-NASBA gametocyte carriage, and infectiousness to mosquitoes at day 7 of follow up were determined as reported elsewhere [26].

**Sample Size Considerations**

The current study aims to determine parasite clearance by qPCR, a newly developed assay for which no baseline data were available, and test for association of clearance time with treatment outcome, gametocyte carriage, and malaria transmission. Parasite clearance was evaluated in 154 individuals by qPCR. As an indicative estimate of study power, if it is assumed a priori that 20% of individuals might display discernibly slower parasite clearance dynamics in the qPCR analysis and if both treatment arms are analyzed together, this sample size could detect, with 86% power at the 5% significance level, a 2-fold increase in the proportion of individuals who are infectious to mosquitoes on day 7 after treatment, based on an average of 30% of individuals infecting at least one mosquito [28].

**Data Analysis**

The main outcome of this study was parasite clearance by qPCR. Parasite clearance dynamics were described by generating a log-linear line of best fit for parasite density plotted across the 4 time points—days 0, 1, 2, and 3. From these data, 4 parameters of interest were determined: parasite clearance time (in hours), parasite reduction rate 48 hours after treatment, time to 95% reduction in parasite density (in hours), and a binary variable denoting whether parasite DNA was detectable at day 3. The parasite reduction rate 48 hours after treatment was arbitrarily set at 10^5 for isolates negative by qPCR at 48 hours. Gametocyte carriage was compared between groups as microscopic and Pf25 QT-NASBA gametocyte prevalence at enrollment and day of feeding; the mean duration of gametocyte carriage after treatment was estimated using a previously published mathematical model for repeated QT-NASBA measurements [32]. Continuous data were compared between groups, using the \textit{t} test or nonparametric tests. The proportion of parasitemic or gametocytemic individuals and the proportion of infectious individuals (ie, subjects who infected at least 1 mosquito) were compared between groups by the χ^2 test and logistic regression models. The proportion of infected mosquitoes and the oocyst burden in mosquitoes were compared between groups by logistic or negative binomial regression models, using generalized estimating equations to adjust for clustering between observations from the same individual.

**RESULTS**

**Residual Parasitemia Detected by qPCR**

As reported elsewhere, conventional microscopic assessment of parasitological efficacy found both regimens very effective at initial parasite clearance, with only a single child in the DP arm found to harbor detectable asexual parasitemia 3 days after treatment [28]. Prevalence and relative density of parasitemia determined by qPCR over the first 72 hours following treatment are plotted in Figure 1 for 154 children with a full set of 4 daily samples available. Residual parasitemia on day 3 after initiation of treatment was observed in 33.3% (28/84) of the children treated with AL and 30.0% (21/70) of the children treated with DP (\(P = .66\)). The median estimated density of these residual parasites was 0.17% (interquartile range, 0.0001–1.54) of the enrollment parasite density and was not different

![Figure 1. \textit{Plasmodium falciparum} parasite prevalence and relative density by duplex quantitative polymerase chain reaction (qPCR), by treatment arm. Left \(y\)-axis, qPCR parasite prevalence (bars) for children treated with artemether-lumefantrine (AL; \(n = 84\)) or dihydroartemisinin-piperaquine (DP; \(n = 70\)). Error bars indicate the upper limit of the 95% confidence interval. Right \(y\)-axis, median relative parasite density relative to starting infection is plotted (line) for PCR-positive individuals treated with AL (solid line) or DP (dashed line). All samples were collected between 10 AM and 12 PM (day 0) or between 8 AM and 12 PM (days 1, 2, and 3).](http://jid.oxfordjournals.org/DownloadedFrom)
between treatment arms ($P = .26$; Figure 1). qPCR positivity on day 3 was strongly associated with the parasite reduction rate 48 hours after treatment ($P < .001$) and with the time to 95% reduction in parasite density ($P < .001$; Table 1). We retained the binary variable residual parasitemia by qPCR on day 3 for exploring associations with other trial outcomes.

Children with residual parasitemia on day 3 were significantly younger ($P < .001$; Table 2), had a higher multiplicity of infection at enrollment ($P = .047$), but did not have a statistically significant different enrollment asexual parasite density ($P = .91$) or mean total drug dose ($P = .34$). Microscopic gametocyte carriage at enrollment was significantly more common for children with residual parasitemia on day 3 ($P = .006$), whereas Pfs25 QT-NASBA–detected gametocyte carriage was not significantly different ($P = .34$). There was limited agreement between the qPCR parasite detection and the Pfs25 QT-NASBA gametocyte detection. On days 1, 2, and 3 after initiation of treatment, 45.0% (18/40), 53.4% (15/28), and 50.0% (15/30), respectively, of the qPCR-positive samples were QT-NASBA gametocyte negative. The scatter plot of qPCR signal on day 3 and QT-NASBA gametocyte prevalence is given in Supplementary Figure 1. The $k$ values for agreement were $-0.09$, $0.15$, and $0.25$ on days 1, 2, and 3, respectively, after initiation of treatment. The estimated parasite density determined by qPCR was not associated with the Pfs25 QT-NASBA gametocyte prevalence on the same day ($P \geq .50$).

### Table 2. Characteristics of Individuals Whose *Plasmodium falciparum* Infections Were Cleared or Who Had Residual Parasitemia Detected by Quantitative Polymerase Chain Reaction (qPCR) on Day 3 After Initiation of Treatment With Artemether-Lumefantrine (AL) or Dihydroartemisinin-Piperaquine (DP)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Parasite Free (n = 105)</th>
<th>Residual Parasitemia (n = 49)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated with AL</td>
<td>53.3 (56/105)</td>
<td>57.1 (28/49)</td>
<td>.66</td>
</tr>
<tr>
<td>Total dose of lumefantrine in AL arm, mg/kg body weight</td>
<td>59.8 ± 10.1</td>
<td>60.1 ± 8.6</td>
<td>.93</td>
</tr>
<tr>
<td>Total dose of piperaquine in DP arm, mg/kg body weight</td>
<td>65.6 ± 7.3</td>
<td>67.5 ± 10.5</td>
<td>.34</td>
</tr>
<tr>
<td>Age, y</td>
<td>6 (5–8)</td>
<td>4 (3–6)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Temperature ≥37.5°C</td>
<td>36.2 (38/105)</td>
<td>49.0 (24/49)</td>
<td>.13</td>
</tr>
<tr>
<td>Hemoglobin level, mmol/dL, mean (95% CI)</td>
<td>6.8 (6.6–7.0)</td>
<td>6.6 (6.3–6.9)</td>
<td>.28</td>
</tr>
<tr>
<td>Multiplicity of infection, mean (95% CI)</td>
<td>3.6 (3.2–3.9)</td>
<td>4.3 (3.7–5.0)</td>
<td>.047</td>
</tr>
<tr>
<td>Microscopy finding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asexual parasite density, geometric mean (95% CI)</td>
<td>18 560 (14 989–22 981)</td>
<td>13 720 (10 411–18 081)</td>
<td>.91</td>
</tr>
<tr>
<td>Gametocyte prevalence</td>
<td>4.2 (4/95)</td>
<td>18.2 (8/44)</td>
<td>.006</td>
</tr>
<tr>
<td>Pfs25 QT-NASBA finding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gametocyte prevalence</td>
<td>71.7 (38/53)</td>
<td>81.5 (22/27)</td>
<td>.34</td>
</tr>
</tbody>
</table>

Data are % (proportion) of participants, mean ±SD, or median (interquartile range), unless otherwise indicated.

Abbreviations: CI, confidence interval; qPCR, duplex quantitative polymerase chain reaction; QT-NASBA, quantitative nucleic acid sequence–based amplification.
on day 3, which infected 1.6% (49/2998) of the mosquitoes with 1–4 oocysts. The proportion of infected mosquitoes (odds ratio [OR], 2.41; 95% CI, 1.18–4.92) and the oocyst burden in mosquitoes (incidence rate ratio [IRR], 2.92; 95% CI, 1.61–5.31; \( P < .001 \)) were significantly higher in experiments in children with residual parasitemia on day 3, after adjustment for correlations between observations from the same individual. These associations were not confounded by enrollment parasite density, multiplicity of infection, treatment arm, or age.

We repeated our analyses after exclusion of children who had microscopically detectable gametocytes at enrollment. Gametocyte carriage determined by microscopy on day 7, which was strongly associated with microscopically detectable gametocyte carriage at enrollment (\( P < .001 \)), was no longer elevated in children with residual parasitemia on day 3 (\( P = .93 \)). Residual parasitemia was still significantly associated with a longer duration of Pf25 NASBA gametocyte carriage (\( P = .021 \)), a higher likelihood of infecting at least 1 mosquito (OR, 3.71; 95% CI, 1.01–13.66; \( P = .049 \)), and a higher oocyst burden in mosquitoes (IRR, 1.85; 95% CI, 1.02–3.35; \( P = .040 \)). Because 75.0% (60/80) of children with qPCR results harbored gametocytes as determined by QT-NASBA at enrollment and because de novo gametocyte production was rare, we did not restrict our analyses to children who were gametocyte free at enrollment according to QT-NASBA.

### Table 3. Association Between Residual Parasitemia on Day 3 After Initiation of Treatment and Malaria Transmission Potential

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>qPCR Result on Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parasite Free</td>
</tr>
<tr>
<td>Duration of gametocyte carriage, d, mean (95% CI)</td>
<td>6.6 (4.7–9.3)</td>
</tr>
<tr>
<td>Microscopy finding on feeding day</td>
<td></td>
</tr>
<tr>
<td>Gametocyte prevalence</td>
<td>5.0 (5/100)</td>
</tr>
<tr>
<td>Gametocyte density, gametocytes/μL, median (IQR)(^{a})</td>
<td>48.0 (16.0–48.0)</td>
</tr>
<tr>
<td>Pf25 QT-NASBA finding on feeding day</td>
<td></td>
</tr>
<tr>
<td>Gametocyte prevalence</td>
<td>30.2 (13/43)</td>
</tr>
<tr>
<td>Individuals participating in membrane feedings, no.</td>
<td>101</td>
</tr>
<tr>
<td>Infected ( \geq 1 ) mosquito</td>
<td>24.8 (25/101)</td>
</tr>
<tr>
<td>Infected mosquitoes, no. (proportion)</td>
<td>1.6 (49/2998)</td>
</tr>
<tr>
<td>Oocysts in infected mosquitoes, no., mean (range)</td>
<td>1.3 (1–4)</td>
</tr>
</tbody>
</table>

Data are \% (proportion) of participants, unless otherwise indicated.

Abbreviations: CI, confidence interval; IQR, interquartile range; qPCR, duplex quantitative polymerase chain reaction; QT-NASBA, quantitative nucleic acid sequence–based amplification.

\(^{a}\) Data are for gametocyte carriers only.

\(^{b}\) Adjusted for correlations between observations from the same individual.

\(^{c}\) Determined using a negative binomial regression model that incorporated both prevalence and intensity of infection among mosquitoes.
parasitemia during follow-up remained statistically significant after adjustment for multiplicity of infection \((P < .001)\). For all 25 children, we performed merozoite antigen genotyping analysis on days 0, 1, 2, the scheduled follow-up day preceding failure, and the day of failure. PCR was successful for 24 of 25 samples from day 0, 23 of 24 from day 1, 18 of 25 from day 2, 17 of 24 from the day before failure, and 24 of 25 from day of failure. As a result, 96.0\% (24/25) of the individuals had \(\geq 2\) observations from the parasite population within 48 hours of treatment, and 64.0\% (16/25) had 2 observations from the parasite population at or shortly before microscopy-detected parasitological treatment failure. Reworking WHO criteria to suit this approach [29], recrudescent infections were defined as the presence of \(\geq 1\) allele on the day of failure or the day preceding failure that were also present on \(\geq 1\) of days 0–2. By using these criteria, 19 infections were now classified as recrudescent and 6 as new infections. Residual parasitemia on day 3 remained significantly associated with both recrudescent infections (RR, 11.43; 95\% CI, 3.49–37.40; \(P < .001\)) and new infections (RR, 10.71; 95\% CI, 1.29–89.27; \(P = .006\)).

**DISCUSSION**

In this study, we provide the first evidence from Africa that low-density residual parasitemia after treatment with ACT is common and has consequences for onward malaria transmission to mosquitoes. Children with residual parasitemia detected by qPCR on day 3 after initiation of treatment had a longer duration of gametocyte carriage, were more likely to infect mosquitoes, and infected a higher proportion of mosquitoes. In addition, they were \(>10\)-fold more likely to have recurrent microscopic asexual parasite carriage during follow-up.

Despite excellent parasite clearance rates by microscopy [28], we observed that residual parasitemia detected by PCR persisted in approximately one third of ACT-treated children until day 3 after initiation of treatment. Submicroscopic parasite carriage persisting after ACT has recently been described in Sudan and Ghana [33, 34], but because PCR is not routinely used to monitor parasite clearance dynamics, it is currently unknown how often parasites remain PCR detectable after successful ACT treatment. Our study is the first to directly relate parasite clearance dynamics after ACT to malaria transmissibility. We show that children who had residual parasitemia on day 3 after treatment initiation had a longer duration of gametocyte carriage, were more likely to be infectious to mosquitoes, and infected more mosquitoes with a higher oocyst burden. These associations remained apparent if children with microscopically detectable gametocytes at enrollment were excluded from the analyses.

The association between slow parasite clearance and gametocyte carriage is at least partly the result of ongoing gametocyte production by persisting asexual parasites [19]. Because ACT rapidly clears the pool of asexual parasites from which gametocytes are derived [8] and because the artemisinin-component of ACT is given over 3 days, during which it efficiently clears developing gametocytes [8, 35], it is striking that parasite-clearance dynamics shortly after initiation of ACT determine malaria transmission potential 1 week later. Our results confirm previous suggestions that increased gametocyte production is a valuable marker of a slow parasite-clearance phenotype after ACT [27, 36, 37] and are in line with findings from the Thailand–Myanmar border, where slow microscopic parasite clearance was associated with a longer duration of microscopic gametocyte carriage following mefloquine-artsunate treatment [4]. We confirm this trend in Africa and add 2 relevant lines of evidence: we show that this association is also apparent if residual parasitemia after treatment and gametocytes are present only at submicroscopic levels, and we provide direct evidence that this longer duration of gametocyte carriage results in higher infectivity to mosquitoes.

Similar to previous studies, we found that gametocyte carriage at enrollment may be an indicator of subsequent treatment responses [3, 4, 38]. One may hypothesize that this indicates that slow clearing infections are have an intrinsic higher commitment to gametocyte production [39] or that slow clearing infections have been present for a longer period than fast clearing infections and therefore have a higher likelihood of gametocyte development during the course of infection [40]. It was recently suggested that long-term chronic infections in equilibrium may elicit weak immune responses and thus leave drug clearance unassisted [34], which may result in slower parasite clearance and longer gametocyte production. Whatever the reason, careful monitoring of transmission potential after ACT is justified by earlier demonstrations that high gametocyte production and infectivity were linked with the development of resistance to CQ and SP [18, 19, 21].

In our setting, we observed a strong association between residual submicroscopic parasitemia and PCR-unadjusted treatment failure during follow-up. This association was independent of starting parasite density, treatment arm, multiplicity of infection, and age and was not explained by differences in the administered dose of AL or DP. Using standard methods, we previously determined that the vast majority of recurrent infections on day 28 and day 42 were new infections [28]. It was therefore surprising that residual parasitemia on day 3 was strongly associated with recurrent infections. We see 3 possible explanations for the association between early residual parasitemia and later recurrent infections that appear to be mostly “new” infections. First, higher multiplicity of infection, a characteristic of slow-clearing infections in this setting, may be associated with a higher malaria exposure [41] that may be stable over time [42] and may thereby predict a higher risk of new malaria infections during follow-up [43]. Second, immunity is an important factor in determining parasite-clearance time [10,
14], and slow parasite clearance may be indicative of a less efficient immune response in clearing parasites that may also be associated with a higher chance of new infections becoming detectable by microscopy. Third, we may have misclassified recrudescent infections as new infections.

We have provided evidence that the third explanation is plausible for a proportion of the infections by extending our parasite genotyping to multiple days. The risk of overestimating the number of recrudescent infections because some new infections may have the same genetic profile as original infections is reasonably well appreciated [44, 45]; the risk of underestimating the number of recrudescent infections as a consequence of incomplete detection of all parasite clones that are present at enrollment and failure has received less attention [46, 47]. Clones may go undetected if genotyping is done on single days as a consequence of sequestration of parasites, differences in clonal densities and a stochastic element in PCR detection [31, 48].

When we analyzed sample series from the first 3 days after enrollment and the day of failure combined with the day preceding failure, we observed that parasites were often detectable on all of the examined days. Based on MSP-1 and MSP-2 typing in these extended sample sets, 15 infections that were initially classified as new infections [28] were now classified as recrudescent infections. This is in line with a study that used a similar approach in Tanzania, where genotypes that were subsequently found to be recrudescent were undetectable at enrollment but detected as late as day 3 after initiation of treatment [49]. Our data illustrate the difficulty of distinguishing between recrudescent and new infections in areas with complex infections and suggest that the original genotyping approach may have underestimated the true number of recrudescent infections [47].

In summary, we demonstrated that 24-hour DNA sampling followed by qPCR analysis identifies submicroscopic, slow-clearing *P. falciparum* in approximately one third of Kenyan children treated with ACT. Children harboring such parasites were significantly more likely to be infectious to mosquitoes and are more likely to experience recurrent asexual parasitemia on day 28 or day 42.

### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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