TITLE: A randomized, controlled, dose-adaptive trial of primaquine to reduce transmission of *Plasmodium falciparum* malaria in Mali

Authors: Alassane Dicko MD*, Joelle Brown PhD*, Halimatou Diawara MD, Ibrahima Baber PhD , Almahamoudou Mahamar PharmD, Harouna M Soumare PharmD, Koualy Sanogo MD, Fanta Koita PharmD, Sekouba Keita MS, Sekou F Traore PhD, Ingrid Chen PhD, Eugenie Poirot MPH, Jimee Hwang MD, Charles McCulloch PhD, Kjerstin Lanke, Helmi Pett MS, Mikko Niemi MD, François Nosten MD, Teun Bousema PhD ¶, Roly Gosling MD ¶
*The first two authors contributed equally to this work
¶The last two authors contributed equally to this work

Institutions:

Malaria Research and Training Centre, Faculty of Pharmacy and Faculty of Medicine and Dentistry, University of Science, Techniques and Technologies of Bamako, Bamako, Mali (Prof A Dicko, MD; H Diawara, MD; I Baber, PhD; A Mahamar, H Soumare, PharmD; PharmD; K Sanogo, MD; F Koita, PharmD; S Keita, MS; S Traore, PhD)

Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA (J M Brown, PhD; E Poirot, MPH; Prof C McCulloch, PhD, R Gosling, MD)

Global Health Group, Malaria Elimination Initiative, University of California, San Francisco, San Francisco, CA (R Gosling, MD; I Chen, PhD; E Poirot, MPH; J Hwang, MD)

Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA (J Hwang, MD)

President's Malaria Initiative, USA (J Hwang, MD)

Department of Medical Microbiology, Radboud university medical center, Nijmegen, the Netherlands (T Bousema, PhD; H Pett, MSc; K Lanke)

Department of Immunology & Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom (Bousema, PhD)

Department of Clinical Pharmacology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland (M Niemi, MD; H Pett, MSc)

Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, UK (Prof F Nosten, MD)

Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand (Prof F Nosten, MD)

Corresponding author:
Dr. Roly Gosling
Department of Epidemiology and Biostatistics
University of California, San Francisco
San Francisco, California 94158
Roly.Gosling@ucsf.edu

Co-corresponding authors:
Prof. Alassane Dicko
Malaria Research and Training Centre, Faculty of Pharmacy and Faculty of Medicine and Dentistry,
University of Science, Techniques and Technologies of Bamako
BP 1805, Point G
Bamako, Mali

ADicko@icermali.org

Dr. Teun Bousema
Department of Medical Microbiology
Radboud university medical center
PO Box 9101, 6500HB
Nijmegen, the Netherlands
Teun.Bousema@radboudumc.nl

SUMMARY

Background: Single low dose primaquine (SLD-PQ), when added to artemisinin-based combination therapy (ACT), may prevent *Plasmodium falciparum* malaria transmission to mosquitoes. We determined the efficacy and safety of four low doses of primaquine combined with dihydroartemisinin-piperaquine (DP).

Methods: We conducted a phase 2, randomized, controlled, dose-adaptive study of SLD-PQ among glucose-6-phosphate dehydrogenase (G6PD) non-deficient males in Mali with uncomplicated *P. falciparum* malaria. Microscopy positive gametocyte carriers were treated with DP and 0.5, 0.25, 0.125, 0.0625 or 0 mg/kg (control) groups of primaquine. The primary efficacy outcome was the average within-person percentage change in mosquito infectivity 2 days following primaquine treatment. Infectivity was assessed through membrane-feeding assays. The safety endpoint was the average within-person change in hemoglobin concentration during 28 days of study follow-up. (ClinicalTrials.gov: NCT01743820)

Findings: We enrolled 81 participants. . Among the subgroup of participants that infected mosquitoes pre-treatment, primaquine doses of 0.125, 0.25 and 0.5 mg/kg resulted in large and significant reductions in infectivity compared to the control group of 95% (95%CI: 87,100%, *P*=0.04), 99% (95%CI: 98,100%, *P*=0.008) and 88% (95%CI: 60,100%, *P*=0.03), respectively. There were no clinically meaningful or statistically significant drops in hemoglobin in any group during follow-up. No serious adverse events were reported and adverse events were not associated with the treatment group (*P*=0.48).

Interpretation: SLD-PQ at the recommended dose of 0.25 mg/kg, given in conjunction with DP, appears to be safe and efficacious for the prevention of *P. falciparum* malaria transmission in G6PD non-deficient populations.

Funding: Bill & Melinda Gates Foundation

Word Count
Abstract: 247; Manuscript: 5162

BACKGROUND

Over the past decade, great progress has been made in the control of malaria following the widespread access to and use of artemisinin-based combination therapy (ACT) and insecticide treated bed nets (1). Although malaria transmission has been significantly reduced (2), malaria elimination is unlikely to be achieved with the further scale-up of conventional tools in the majority of African settings (3). New tools and strategies to reduce malaria transmission are necessary to maintain the gains of malaria control and push towards malaria elimination (4).

One such tool recommended by the World Health Organization (WHO) is single low-dose (0.25 mg/kg) primaquine (SLD-PQ) as an addition to standard ACT to prevent onward transmission of *Plasmodium falciparum* infections to anopheline mosquitoes, in settings of low transmission and drug resistance (5). Primaquine, an 8-aminoquinoline, has a unique drug action against mature *P. falciparum* gametocytes, the sexual stages of the parasite responsible for onward transmission (5). However, the wide-scale use of primaquine is hindered by safety concerns of dose dependent hemolysis in people who are deficient in glucose-6-phosphate dehydrogenase (G6PD), the most common inherited enzyme deficiency prevalent throughout malaria-endemic areas (6). Although recommendations for the use of SLD-PQ in conjunction with an ACT now exist, no formal dose finding experiments using an ACT and standardized infectivity assay have been done. The current recommendation is based on a review of historical infectivity trials and there were no formal assessments of the efficacy of SLD-PQ in reducing mosquito infection rates when added to the currently used ACTs (7). Recently, the first formal primaquine dose-finding study concluded that low densities of *P. falciparum* gametocytes detected by molecular methods persist after SLD-PQ treatment. That study did not test the new WHO recommended low dose but concluded the inferiority of the 0.10 mg/kg compared to the standard 0.75 mg/kg primaquine dose when given in combination with artemether-lumefantrine (8). Importantly, the infectivity of low density gametocytes after primaquine in combination with an ACT has never been determined. Molecular gametocyte detection tools may lead to false conclusions on residual transmission potential if these tools detect non-transmissible gametocytes (9).

Determination of the lowest efficacious dose of SLD-PQ is of great importance because of the risk of dose dependent hemolysis (10). The main aim of this study was to establish the lowest efficacious dose of SLD-PQ by repeated assessments of human infectivity to mosquitoes alongside sensitive molecular gametocyte detection and quantification.

METHODS

Study Design

This study was a randomized, controlled, dose-adaptive study of SLD-PQ among males infected with *P. falciparum* malaria. This study was conducted in Ouelessebougou, Mali at the Clinical Research Center of the Malaria Research and Training Centre (MRTC) of the University of Bamako, Mali. Malaria transmission is high and seasonal in this setting; an incidence rate of ~ 2 episodes per year in children under 5 years of age (11) illustrates the high level of malaria study subjects were exposed to.

This study was conducted in two phases (Figure 1). In the first phase, following optimization of the membrane feeding assay (details in Supplemental section 1), 50 participants were block randomized into three primaquine dose groups: 0 mg/kg primaquine, 0.125 mg/kg primaquine, and 0.5 mg/kg primaquine. The Data Safety Monitoring Committee (DSMC) with the study team reviewed the infectivity and safety results from the first phase and selected two additional primaquine dose groups: 0.25 mg/kg primaquine and 0.0625 mg/kg primaquine to be tested sequentially in the second phase. The 0.25mg/kg arm was included to confirm the efficacy of the WHO-recommended dose; the inclusion of the ultra-low dose of 0.0625mg/kg was decided upon to explore the lowest dose with transmission-blocking properties after efficacy of the 0.125mg/kg dose was established in the first phase of the study.

Participants

Participants were recruited from the community. Inclusion criteria included male gender, ages 5-50 years (inclusive), having ≥2 *P. falciparum* gametocytes per 500 white blood cells on thick film microscopy

(corresponding to ≥32 gametocytes/μL assuming 8000 white blood cells/μL blood), a hemoglobin level of ≥8 g/dL, and a normal G6PD test result using colorimetric quantification (OSMMR2000-DG-6-PD, R&D Diagnostics Limited, Papagos, Greece). Participants who had taken malaria medications within the past 7 days, those with known allergies to the study medications, serious or chronic illness, and those with cardiac arrhythmias were excluded from the study. All participants provided written informed consent prior to screening and enrollment. Male subjects were selected to reduce the risk of SLD-PQ induced hemolysis through incorrect classification of G6PD status that more commonly occurs in heterozygote females.

Randomization and masking

Eligible participants were assigned sequentially numbered study IDs based on a pre-printed Study ID List created by a study investigator based at UCSF. The study pharmacist in Mali opened the corresponding sealed, opaque envelopes and provided the intervention to study participants. Study staff assessing study outcomes were masked to participant treatment groups. Participants were not blinded to treatment group, as they were allowed to ask the study physician which treatment they received, and may have recognized the presence of primaquine through the bitter taste of the active pharmaceutical ingredient.

One participant was inadvertently given 0.375mg/kg PQ rather than 0.125 mg/kg PQ. This error was discovered at the day 0 visit, and the participant was retained in follow-up through day 28 and included in the hemoglobin and adverse event (AE) analysis but excluded from the change in infectivity analyses.

Procedures

At the day 0 visit, after collection of day 0 samples, the study pharmacist provided study treatment, primaquine (Primaquine®, Sanofi, Canada Inc, Laval Quebec), using 15 mg primaquine tablets crushed and dissolved in 15mL of drinking water and primaquine administered to the nearest mL, as described elsewhere (8). Each participant received an oral dose of primaquine according to their group assignment (0.5, 0.25, 0.125, 0.0625 and 0 (control) mg PQ/kg) after a fatty snack (biscuits) to prevent gastrointestinal complaints. In addition, all participants were given the first tablet of a 3-day course of Dihydroartemisinin-Piperaquine (DP) (Eurartesim®, Sigma-Tau, Italy). DP (320 mg/40 mg per tablet) was administered as per manufacturer's guidelines (13 - □<□24 kg: 1 tablet; 24 -□<□36 kg: 2 tablets; 36 -□<□75 kg: 3 tablets; and 75-100 kg: 4 tablets once daily for three days). All treatment doses were directly observed by the study pharmacist and his assistant.

Participants were evaluated at the study clinic to assess study outcomes at hours 2, 6, 12, and days 1, 2, 3, 7, 14, and 28 following treatment.

Outcomes

The primary efficacy outcome was the average within-person percent change in mosquito infectivity two days post primaquine administration. Mosquito infectivity from the human to the mosquito was defined as the proportion of dissected mosquitoes with oocysts. In addition, participants were classified as infectious (yes/no) if at least one dissected mosquito had at least one oocyst. Secondary efficacy endpoints included mosquito infectivity one day and seven days following administration of primaquine. Mosquito infectivity was measured at day 0 (pre-treatment), day 1, day 2, and day 7. Venous blood was collected in heparin tubes kept at 37°C and placed in a membrane feeding system within a minute of being collected. Three cups of 30 *Anopheles gambiae* mosquitoes each (90 mosquitoes in total except at day 0 when 180 mosquitoes were used) were fed on the participant's blood for 15-20 minutes using previously described protocols (12). Blood fed mosquitoes were then transported the next day to the insectary in Bamako, Mali, where they were kept until dissection on day 7 post feeding and examination for oocysts in 1% mercurochrome. The presence of oocysts was confirmed by a second microscopist.

Safety outcomes were hemolysis and AEs. Hemoglobin was measured before treatment (day 0) and at each follow-up visit (day 1, 2, 3, 7, 14, 28) using HemoCue (AB Leo Diagnostics, Helsingborg, Sweden). We calculated the average within-person change in hemoglobin, the proportion of participants with a 25% or greater drop in hemoglobin, and the proportion of participants with a hemoglobin level ≤8 g/dL during follow-up.

AEs were assessed actively during follow-up visits (day 1, 2, 3, 7, 14, 28) and passively through the availability of study clinicians 24 hours/day, 7 days/week, free of charge during the follow-up period, including free transportation if needed.. AEs were categorized by severity (mild, moderate, or severe in intensity, and

whether they were considered serious) and their relationship with the study drugs. In addition, the duration of AE, actions taken, and outcome of AEs were recorded.

Gametocyte density measurements were conducted at day 0 (pre-treatment), at hours 2, 6, and 12, and on days 1, 2, 3, 7, 14, and 28. Blood slides stained with Giemsa were double read over 500 fields for quantification of gametocytes and asexual stages. 100µL of whole blood was collected in L6 buffer (Severn Biotech Ltd, Kidderminster, United Kingdom), total NA was extracted using a MagNAPure LC automatic extractor (Total Nucleic Acid Isolation Kit – High Performance, Roche Applied Science) followed by RQ1 DNaseI digest (Promega Inc, Sunnyvale, CA, USA), cDNA synthesis (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems Inc, Foster City, CA, USA) and molecular quantification of gametocytes using Pfs25 mRNA qRT-PCR as described elsewhere (13). Gametocyte concentration was estimated using a reference trendline of cultured mature stage V gametocytes (NF54 strain(14)) that was serially diluted using tenfold dilutions from 10^6-10^1 gametocytes/mL.

Genomic DNA available from total nucleic acids extracted for gametocyte quantification were used for genotyping Cytochrome P450 2D6 (*CYP2D6*). Samples from all participants were tested, and participants with a sufficient amount and quality of DNA were genotyped for *CYP2D6 *2*, **3*, **4*, **6*, **7*, **8*, **9*, **10*, **11*, **15*, **17*, **18*, **19*, **20*, **29*, **40*, and **41* alleles using Openarray technology on a QuantStudio 12K Flex real-time PCR system (Life Technologies, Carlsbad, CA, USA). The *CYP2D6* copy number was determined with a TaqMan copy number assay targeting intron 2 on the QuantStudio 12K Flex system, and *CYP2D6* metabolizer status was inferred from the genotypes using both classical and activity score methods (15, 16).

Statistical analysis and sample size considerations

We performed a sample size calculation to detect a 90% or greater reduction with 80% power and a significance level of 0.05. To allow for 10% loss to follow-up, sample size estimates were increased by 10%. Sample size calculations assumed that the proportion of people that would infect at least one mosquito was 0.80, and that on average the proportion of mosquitoes that would become infected was 0.25. Using a standard deviation of 0.24 for the change in the proportion of infected mosquitoes before and after treatment with primaquine, estimated from preliminary data, we required 15 individuals per group.

We calculated the average within-person percent change in infectivity and accompanying 95% confidence interval for each treatment group. In order to make pairwise comparisons of the change in infectivity and change in hemoglobin after treatment (e.g., control vs. 0.125 mg/kg primaquine) we used the one-sided non-parametric Wilcoxon ranksum test. In addition, we used the one-sided exact probability test to compare the proportion of infectious individuals in each treatment group to the control group at each visit, and the one-sided non-parametric Wilcoxon ranksum test to compare the proportion of mosquitoes infected in each treatment group to the proportion of mosquitoes infected in the control group at each visit.

qRT-PCR gametocyte density and prevalence at individual treatment days were compared between individual SLD-PQ arms and the control arm, using linear (log10 density) and logistic (prevalence) regression models with treatment arm and baseline qRT-PCR gametocyte density as independent variables. qRT-PCR gametocyte density was used to generate the area under the curve (AUC) of gametocyte density versus time that was compared between SLD-PQ arms and the control arm as described previously (17), using log10 transformed AUC as dependent variable in a linear regression model with treatment arm and baseline qRT-PCR gametocyte density as independent variables. The association between the proportion of infected mosquitoes and qRT-PCR gametocyte density was determined by Spearman correlation coefficient before and after treatment.

The group size did not allow for estimating the occurrence of AEs in different study arms. Rather, AEs were summarized by type, frequency, severity, and relatedness to study treatment.

Analysis groups

Analyses of gametocytes were conducted among all enrolled participants excluding protocol violations. Analyses of AEs and the change in hemoglobin following treatment were restricted to participants with a pre-treatment measurement and at least one follow-up visit (‘total enrolled sample with a follow-up visit ’). Analysis of the change in infectivity was carried out among participants who completed the study after optimization of

the infectivity assay, who had a pre-treatment infectivity measurement and at least one follow-up infectivity measurement, and who were given the correct primaquine dose ('primary infectivity analysis sample'). In addition, we conducted a change in infectivity analysis among a subset of the 'primary analysis sample' including only those participants who infected at least one mosquito pre-treatment ('subgroup infectivity analysis sample'). Restricting our analyses to individuals who were infectious prior to treatment allowed us to have a positive measure from which to measure the proportion reduction in infectivity. Analyses were conducted in Stata v 12.1 (StataCorp, College Station, TX).

Ethical considerations

The study was approved by the Ethics Committee of the Malaria Research and Training Centre Faculty of Medicine, Pharmacy and Dentistry of the University of Science, Techniques and Technologies of Bamako, and the Committee on Human Research at the University of California, San Francisco (UCSF). A Data Safety Monitoring Committee (DSMC) oversaw the study, and the study was monitored by an external clinical trials monitoring group, *Agence Africaine de Recherche en Santé Humaine*, Dakar Senegal (http://www.aarsh.com/). The study registration was ClinicalTrials.gov: NCT01743820.

Role of funding source

The funder of the study had no role in the data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit the publication.

RESULTS

We screened 1,485 individuals, enrolling 81 participants into the study between 19 January 2013-20 December 2014. Of those screened, the majority were not eligible due to the absence of gametocytes assessed by microscopy (n=1159). Additionally, 147 individuals had gametocyte densities below the minimum density of 2/500 WBC, 18 individuals had mixed species infections, and 17 individuals who met all other enrollment criteria were G6PD-deficient. Sixteen, 16, 17, 15, and 17 participants were enrolled into the control, 0.0625 mg/kg, 0.125 mg/kg, 0.25 mg/kg, and the 0.5 mg/kg primaquine group, respectively (Figure 1, Table 1). The median age was 12 years (IQR 7-25; Range 5-49) (Table 1). All participants were male, screened negative for G6PD deficiency, and had a screening hemoglobin level of ≥ 8 g/dL. Seventy-seven participants (95%) were retained through the day 28 follow-up visit; in total, four participants withdrew consent, two after completing the day 0 visit, one after day 1, and one after day 2. No participants were lost to follow-up.

Analyses of AEs, and the change in hemoglobin following treatment were restricted to 79 participants with a follow-up visit. The primary analysis of the change in infectivity was restricted to 71 participants after assay optimization ('primary analysis sample'; two participants were excluded because one had no follow-up visits, and one received an erroneous dose of primaquine). The subgroup analysis of the change in infectivity was further restricted to 57 participants who infected at least one mosquito pre-treatment ('subgroup sample'). (See Figure 1). Analyses of gametocyte densities were restricted to all 80 enrolled participants who received the correct dose of primaquine.

Infectivity

Following optimization of the infectivity assay, 79% (58/73) of pre-treatment membrane feeding experiments resulted in ≥1 infected mosquito, and 24.1% (1763/7301) of mosquitoes became infected. Overall, we used 32,040 mosquitoes in feeding experiments of which 24,009 (74.9%) survived to be dissected on day 7, resulting in 2,043 infections (Supplemental Table S1). Analysis of the primary sample showed statistically significant reductions in infectivity on day 2 post treatment in the 0.25mg/kg and 0.5mg/kg doses compared to controls. Among individuals who were infectious prior to treatment (infected ≥1 mosquito; subgroup sample), we found a large, clinically important, and in most cases, statistically significant within-person reduction in infectivity following treatment on: day 1 with 0.125, 0.25 and 0.5 mg/kg doses of primaquine of 69.4%, 87.8% and 91.5% respectively compared to reduction of infectivity in controls of 22.6%; P=0.06, P=0.01 and P=0.006 respectively; day 2 with 0.125, 0.25 and 0.5 mg/kg doses of primaquine of 94.9%, 99.2% and 87.6% compared to reduction of infectivity in controls of 27.5%; P=0.04, P=0.008 and P=0.03 respectively; and at day 7 with 0.0625, 0.125, 0.25 and 0.5 mg/kg doses of primaquine of 97.4%, 100%, 100% and 95.1% compared to reduction of infectivity in controls of 66.1%; P=0.04, P=0.05, P=0.05, and P=0.11 respectively (Table 2). The reduction in mosquito infectivity was also evident when the analyses were conducted on the primary analysis sample, which included non-infectious individuals (Table 2).

These findings were supported by examining the proportion of participants in each dose group that infected at least one mosquito at each visit. We found a statistically significant reduction in the proportion of individuals that infected at least one mosquito 2 days following treatment with 0.125 mg/kg, 0.25 mg/kg, and 0.5 mg/kg doses of primaquine in the primary sample for analysis (Figure 2a, table S1). Analysis of the median number of mosquitoes infected at each time point by dose group showed significantly fewer mosquitoes becoming infected on day 2 in dose groups ≥0.125mg/kg compared to control (Figure 2B, table S1). No participants in either the 0.125 mg/kg group or the 0.25 mg/kg group infected mosquitoes at day 7, and one participant in the 0.5mg/kg primaquine arm infected a single mosquito on day 7 following treatment.

Gametocyte carriage

All study participants were gametocyte positive by Pfs25 mRNA qRT-PCR at enrollment with a statistically significant higher enrollment gametocyte density in the 0.25 mg/kg primaquine arm compared to the DP only arm (Supplemental Table S3; p=0.013). After adjustment for baseline gametocyte density, gametocyte prevalence was significantly lower in the 0.5 mg/kg primaquine arm on day 7, day 14 and day 28. On day 28, gametocyte prevalence was lower in all PQ arms compared to the DP comparator arm (Figure 3A). Gametocyte density declined during follow-up for all treatment arms (Figure 4B); the density of gametocytes in gametocyte positive individuals was significantly lower in SLD-PQ arms ≥0.125 mg/kg from day 3 onwards, after adjusting for baseline density (Figure 3B; Table S3). The area under the curve of gametocyte density over time was significantly lower in all PQ arms compared to the control only arm, after adjustment for baseline density (Table S3; p ≤ 0.027). Prior to treatment, Pfs25 mRNA qRT-PCR gametocyte density was strongly associated with mosquito infection rates (Figure 4A; n=146; Spearman correlation coefficient 0.56, 95% CI 0.44 – 0.66, *P*<0.0001); this association was no longer apparent on day 1 (n=71; r=0.07, 95% CI -0.17 – 0.30, p=0.57); day 2 (n=71; r=0.17, 95% CI -0.07 – 0.39, p=0.16) or day 7 (n=46; r=0.15,95% CI -0.15 – 0.42, p=0.34) after initiation of treatment (Figure 4B).

Safety

The overall median hemoglobin concentration at days 0, 7, and 28 was 12.4 g/dL (range 8.6, 16.2), 11.6 g/dL (range 8.5, 17.9), and 12.3 g/dL (range 10.2, 17.3), respectively. We did not find a meaningful or statistically significant within-person change in hemoglobin in any of the treatment groups compared to controls at any time point (Supplemental Figures S1a and S1b). During follow-up, no participant had a hemoglobin result of <8 g/dL, and no participant experienced a 25% or greater drop in hemoglobin after treatment.

Overall, 53% (42/79) of participants experienced a total of 79 AEs during follow-up (Supplemental Table S4). There was no statistically significant difference between treatment groups in the number of participants that experienced an AE (Fisher's exact *P-value*=0.48). The majority of AEs (87% (69/79)) were mild (grade 1), and 13% (10/79) were moderate (grade 2). No AEs resulted in stopping study treatment or study participation. One participant in the 0.5 mg/kg PQ group reported transitory dark urine (mild/grade 1) on day 1, which was considered unrelated to study drugs as this participant's hemoglobin concentration was 14.2 g/dL on day 0 and remained above this level until day 28. One participant in the control group reported shortness of breath (mild/grade 1), which was resolved and considered unrelated to study treatment. No participants experienced cyanosis or jaundice. There were no severe AEs or serious AEs detected during the study. All AEs resolved during study follow-up.

Cytochrome P450 2D6 genotyping

We successfully genotyped 53% (43/81) of the participants for *CYP2D6*, the enzyme responsible for the activation of primaquine to its active metabolite for the radical cure of vivax malaria (18). Only two of these were poor metabolizers (one in the control group and one in the 0.0625 mg/kg group), four were intermediate metabolizers (IM) by both conventional phenotype inference. When using activity score (AS), five were IM by the conventional method, but extensive metabolizers (EM) by AS, 30 were EM by both the conventional method and AS, and two were ultra-rapid metabolizers (one in the 0.0625 mg/kg group and one in the 0.25 mg/kg group). *CYP2D6* typing failed for the participant who received 0.5mg/kg primaquine and was infectious on day 7. *CYP2D6* genotypes did not appear to differ significantly between treatment groups (Fisher's exact *P*=0.35).

DISCUSSION

In this study, we describe for the first time the dose response of SLD-PQ in conjunction with an ACT on the transmissibility of *P. falciparum* gametocyte carriers. We observe that whilst gametocytes persist by molecular qRT-PCR, malaria infectivity is reduced by greater than 90% on days 2 and 7 after 0.25mg/kg primaquine. Among participants that infected mosquitoes pre-treatment, we saw > 90% reductions in infectivity with primaquine doses ≥0.125mg/kg by day 2, and in all SLD-PQ arms by day 7. We observed no serious AEs in our study population of G6PD normal male participants and no evidence of hemolysis resulting in anemia.

Treatment with ACTs results in a rapid reduction in *P. falciparum* gametocyte carriage and transmissibility. In our study, 79% of all participants with microscopically detectable gametocytes infected at least one mosquito prior to treatment, similar to findings from a meta-analysis on membrane feeding assays (19). With DP treatment, this proportion was reduced to 54% by day 2 and 23% by day 7, representing reductions in infectivity of 27% and 66% respectively. The residual transmission following DP treatment has been observed before (20) and is partially explained by the limited effect of the artemisinin component against mature (stage V) gametocytes that were present prior to treatment (21). Doses of SLD-PQ greater than 0.125mg/kg potentiate the transmission-reducing effect of DP. Only one individual was infectious on day 7 after primaquine treatment among the 0.125mg/kg, 0.25mg/kg and 0.5mg/kg arms and this person infected only one mosquito. Even the 0.0625mg/kg dose was associated with borderline statistically significant reductions in the proportions of mosquitoes infected by day 7 and the number of participants infecting ≥ 1 mosquito. Our findings of near complete prevention of transmission in all individuals treated with ≥0.125mg/kg primaquine when given in combination with DP is consistent with historical data where primaquine was administered in conjunction with other antimalarial drugs (7). Future studies should explore whether the transmission-blocking effects of SLD-PQ depends on the type of ACT it is co-administered with.

Gametocytes were detected in all treatment arms in samples post treatment. Of particular note, presence of gametocytes and gametocyte density were not associated with infectivity post treatment. Recent studies examining efficacy of SLD-PQ frequently used gametocyte prevalence as the primary endpoint as a surrogate for infectivity (22, 23). In our study population the majority of individuals in the control arm, who did not receive primaquine, retained low densities of gametocytes measured by Pfs25 mRNA qRT-PCR until the end of follow-up on day 28. While SLD-PQ shows a dose-dependent effect on gametocyte prevalence, there was a clear discordance between gametocyte prevalence and density estimates and the likelihood of mosquito infection after SLD-PQ. Prior to treatment, gametocyte densities were strongly associated with the proportion of infected mosquitoes, but this association was lost during post-treatment days. Older studies had similar findings with gametocytes being detectable by microscopy for several days after primaquine but failed to infect mosquitoes (21). Our results support the view that currently available tests for assessing gametocyte density cannot be used to estimate post treatment infectivity.

Gametocyte density and infectivity mismatch may be explained by preferential measurement of female gametocytes. Pfs25 mRNA transcripts, used to measure gametocytes in this study, may be female specific (9, 24). Recently, it was hypothesized that primaquine may disproportionally kill male gametocytes (9) and thereby prevent transmission that depends on the presence of both male and female gametocytes in permissive concentrations. Methylene blue, another anti-gametocyte drug, is more potent against male gametocytes than against female gametocytes (25). It is conceivable that our quantification of gametocytes is female biased and becomes progressively more so after treatment. This may explain the absence of mosquito infectivity at later time-points after primaquine when male gametocytes may have been effectively cleared. We tested samples with a new qRT-PCR that detects male Pfs230p transcripts (24), however the transcript levels of Pfs230p are much lower than those for Pfs25 (14) and we were unable to detect male gametocytes in the majority of baseline samples (data not shown). Future studies should determine if primaquine has a higher potency against male gametocytes and is a potential explanation for our findings of gametocytes being present yet non-infective. The present study leaves the hypothesis of a differential effect of antimalarial treatment on male and female gametocytes unaddressed. Until better indicators of gametocyte infectivity become available, which will probably comprise of sensitive, sex-specific, quantitative molecular gametocyte detection methods, the transmission-blocking properties of antimalarial drugs can only be assessed by mosquito feeding assays (5, 7).

The labor intensiveness of membrane feeding assays limits the number of sites at which trials with transmission endpoint can be conducted, and limits the study size that can be achieved. Although our study had a relatively limited sample size, it more than doubles the available evidence on SLD-PQ. Current recommendations are based on retrospective analysis of nine studies and a total of 65 individuals who received SLD-PQ in combination with an ACT (7). The current study includes 24,009 mosquito observations from 284 experiments before and at three time-points after SLD-PQ. Infectivity in our study was maximized through enrollment of high density gametocyte carriers that are highly infectious compared to average malaria-infected individuals who have much lower gametocyte densities and are considerably less infectious (26) . In these individuals with low gametocyte densities, who comprise a considerable proportion of the human

infectious reservoir for malaria (27), SLD-PQ is likely to be even more efficacious than we reported for our study population. It is commonly observed that a fraction of gametocyte carriers are non-infectious to mosquitoes (19). In our study, 14/71 participants were not measured to have infectivity pretreatment and 11/14 never infected mosquitoes during the whole study period. This failure to infect mosquitoes despite relatively high gametocyte densities may be a consequence of gametocyte fitness or host factors such as naturally acquired immune responses to gametocyte antigens that may reduce transmission efficiency as well as limitations of the membrane feeding assay (19). These factors are unlikely to have affected our comparisons of within-person changes in infectivity or comparisons of SLD-PQ arms.

Our adaptive study design resulted in two phases of enrollment. In the first phase subjects were randomly allocated to SLD-PQ dose group, in the second phase recruitment was sequential, initially into the 0.25mg/kg group followed by 0.0635mg/kg SLD-PQ. In the second phase, we enrolled participants with a younger average age and higher mean gametocyte density prior to treatment. We consider it unlikely that this shortcoming has affected our conclusions on the transmission blocking properties of the different SLD-PQ arms since our main analysis was based on within-person changes in infectivity; other comparisons between treatment arms were adjusted for baseline infectivity or baseline gametocyte density, where appropriate.

Residual transmission after SLD-PQ is of particular importance considering possible inter-individual variation in primaquine metabolism (18). In our study, we successfully genotyped 53% of participants for cytochrome P450 2D6 (*CYP2D6*). This low success rate is a result of the availability of small volume blood samples that were collected for gametocyte detection and not for detailed human genetic analyses. There was a single participant who received the highest dose of SLD-PQ tested (0.5mg/kg) yet remained infectious, infecting 1 mosquito on day 7. *CYP2D6* typing failed for this individual; whether this participant was a *CYP2D6* poor metabolizer is therefore unknown.

This study was carried out in a relatively small population of G6PD normal Malian males. Thus, we are unable to generalize the findings to young children, G6PD deficient people, females and other African populations or those residing outside sub-Saharan Africa where G6PD variants that are associated with more severe G6PD enzyme deficiency may occur. G6PD normal males with hemoglobin concentrations >8g/dL prior to treatment were chosen because their risk of hemolysis after exposure to SLD-PQ would be low. A previous study in Uganda showed a transient reduction in hemoglobin concentrations in individuals who were genotypically G6PD deficient but were screened G6PD normal by fluorescence spot test (28). We observed no significant decreases in hemoglobin or increases in AEs in SLD-PQ groups compared to the control group. Future studies should address the safety profile of SLD-PQ in larger populations that include G6PD deficient participants to inform the highest tolerable dose of SLD-PQ without prior testing for G6PD deficiency. The WHO currently recommends the use of 0.25mg/kg SLD-PQ without prior G6PD testing in areas of low transmission or areas threatened by artemisinin resistance (7). However, most antimalarials are dispensed based on age-based categories, removing the need to weigh patients before treatment. If SLD-PQ is to be rolled out, similar categories for SLD-PQ dosing will be needed requiring a defined range of dosing that is both safe and efficacious (10). A second more challenging safety issue is around the safety of SLD-PQ in pregnancy (29). Safety of SLD-PQ in pregnancy may better be assessed through active pharmacovigilance of the use of SLD-PQ in program use (29) or safety assessments of mass drug administration studies where early stage pregnancies may be inadvertently treated and later followed up to observe pregnancy outcomes (30).

Interpretation

The current WHO recommendation for preventing *P. falciparum* transmission with the addition of SLD-PQ at a dose of 0.25mg/kg is supported by this study when used in combination with the ACT dihydroartemisin-piperaquine. In community treatment campaigns, a simplified delivery of SLD-PQ may involve age-based dosing. This may result in relative over-dosing and under-dosing of fractions of the population. In that context, our study provides valuable information that the dose of 0.125mg/kg is efficacious at preventing transmission by day 2. Further studies to assess the highest tolerable SLD-PQ dose in G6PD deficient individuals are needed in order to define the therapeutic range for simplified delivery of the intervention. Community studies can then be undertaken to demonstrate the impact of SLD-PQ on malaria transmission in populations that may benefit from this intervention, including in areas of low malaria transmission intensity.

Acknowledgements

The study team wishes to thank the DSMC (Professor Phillip Rosenthal, Dr Kevin Baird and Dr Diadier Diallo), the hard work of the insectary, laboratory and field staff, and the participants, the population, the members of community health association, and the staff of the health centers in Ouelessebougou for their cooperation throughout the study. We thank Sanofi for their kind donation of primaquine. We are also very grateful to the study participants.

Declaration of Interests/Funding statements

The trial was funded by the Bill & Melinda Gates Foundation (OPP1013179). Support for AD come from the government of the Mali and different grants including from Bill & Melinda Gates Foundation (OPP1013179); the UK Medical Research Council (MRC grant MR/K0073191/1 to London School of Hygiene and Tropical Medicine) and the Partnerships for Enhanced Engagement in Research (PEER) Health (under grant award no AID-OAA-A-11-00012. TB is supported by grants of the Bill & Melinda Gates Foundation (AFIRM OPP1034789 and a fellowship from the European Research Council (ERC-2014-StG 639776).JH receives salary support from the President's Malaria Initiative. All other authors were supported by the Bill and Melinda Gates Foundation (OPP1013179). CM is supported through grants from the National Institutes of Health USA.

Disclaimer: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

Table 1. Baseline characteristics of participants overall and stratified by primaquine dose group

	Primaquine Dose group (mg/kg)					
	Overall N=81	Control N=16	0.0625 N=16	0.125 N=17	0.25 N=15	0.5 N=17
Age (years)	12 (7-25)	22 (7-30)	10 (8-11)	22 (7-32)	10 (7-16)	18 (8-30)
Hemoglobin (g/dL)	12.4 (11.2 – 13.8)	13.0 (11.0-13.9)	12.0 (10.8-12.9)	13.5 (11.1-14.4)	11.9 (11.2-13.2)	12.2 (11.3-14.2)
Gametocyte prevalence by microscopy	100.0 (81/81)	100.0 (16/16)	100.0 (16/16)	100.0 (17/17)	100.0 (15/15)	100.0 (17/17)
Gametocyte density by microscopy (per µL)	64 (32-720)	64 (32-144)	96 (48-576)	80 (32-320)	144 (48-720)	48 (32-192)
Asexual parasite prevalence by microscopy	66.7 (54/81)	50.0 (8/16)	68.8 (11/16)	82.4 (14/17)	53.3 (8/15)	76.5 (13/17)
Asexual parasite density by microscopy (per µL)	376 (64-2,128)	176 (40-3,840)	1,088 (144-2,832)	216 (48-1264)	1,656 (432-9,808)	144 (32-1,792)
Symptomatic malaria (temperature ≥37.5 °C and parasitemic)	7.4 (6/81)	0.0 (0/16)	18.8 (3/16)	5.9 (1/17)	0.0 (0/15)	11.8 (2/17)

Numbers in table represent median (interquartile range) or proportion (n/N). Median parasite densities were calculated for parasite positive individuals only.

Table 2. Average within-person percent reduction in mosquito infectivity at Day 1, 2, and 7 following treatment

		Total population assessed for infectivity (primary sample, N=71)							
	Reduction at Day 1			Reduction at Day 2			Reduction at Day 3		
	%	95% CI	*P**	%	95% CI	*P**	%	95% CI	*P**
Primaquine dose (mg/kg)									
Control	16.2	14.7 –46.9	-	11.3	-27.4 – 50.0	-	45.8	11.9 – 82.2	-
0.0625	38.2	7.4 – 68.9	0.17	41.9	1.4 – 82.5	0.16	77.9	55.1 – 100	0.05
0.125	37.9	-6.7 – 82.6	0.19	54.9	13.4 – 96.3	0.09	66.7	35.4 – 97.9	0.25
0.25	80.9	59.4 – 100	0.004	92.6	78.3 – 100	0.001	93.3	79.0 – 100	0.01
0.5	78.5	54.0 – 100	0.004	75.0	45.7 – 100	0.01	81.5	59.6 - 100	0.04
	Subgroup of the total population who was infectious prior to treatment (Subgroup Sample, N=57)								
	Reduction at Day 1			Reduction at Day 2			Reduction at Day 3		
	%	95% CI	*P**	%	95% CI	*P**	%	95% CI	*P**
Primaquine dose (mg/kg)									
Control	22.6	-22.4 – 67.6	-	27.5	-22.7 – 77.7	-	66.1	22.7 - 100	-
0.0625	47.0	10.2 – 83.6	0.18	59.3	16.9 – 100		97.4	91.7 - 100	0.04
0.125	69.4	21.4 – 100	0.06	94.9	84.6 – 100		100	100 - 100	0.05
0.25	87.8	70.4 – 100	0.01	99.2	97.6 - 100		100	100 - 100	0.05
0.5	91.5	73.4 – 100	0.006	87.6	60.2 - 100		95.1	84.2 - 100	0.11

Figure Legends:

Figure 1. Trial profile

Figure 2: Proportion (95%CI) of individuals who infected ≥1 mosquito, by visit and treatment group (2A). Proportion (median, IQR) of mosquitoes infected among participants who infected ≥1 mosquito (2B)

Figure 2A:
* $P<0.05$, ** $P<0.01$ (one sided exact probability test comparing proportion of individuals that infected at least one mosquito in each treatment group to the proportion of individuals that infected at least one mosquito in the control group.)

Figure 2B:
Error bars show IQR- Interquartile range:
* $P<0.05$, ** $P<0.01$ (one sided non-parametric Wilcoxon ranksum test comparing median proportion of mosquitoes infected in each treatment group to the median proportion of mosquitoes infected in the control group.)

Figure 3. Gametocyte prevalence (3A) and density (3B) by Pfs25mRNA qRT-PCR by visit and treatment group. Gametocyte density was determined for gametocyte-positive individuals only.

Figure 4: The association between gametocyte density and infectivity pre-treatment (4A) and post-treatment (4B).

References

1. WHO. World Malaria Report. Geneva: World Health Organization. 2014.
2. Noor AM, Kinyoki DK, Mundia CW, Kabaria CW, Mutua JW, Alegana VA, et al. The changing risk of Plasmodium falciparum malaria infection in Africa: 2000-10: a spatial and temporal analysis of transmission intensity. Lancet. 2014 May 17;383(9930):1739-47. PubMed PMID: 24559537. Pubmed Central PMCID: 4030588.
3. Griffin JT, Hollingsworth TD, Okell LC, Churcher TS, White M, Hinsley W, et al. Reducing Plasmodium falciparum malaria transmission in Africa: a model-based evaluation of intervention strategies. PLoS Med. 2010;7(8). PubMed PMID: 20711482. Pubmed Central PMCID: 2919425.
4. Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: new strategies for new challenges. Lancet. 2013 Sep 7;382(9895):900-11. PubMed PMID: 23594387.
5. WHO. Evidence Review Group on the Safety and Efficacy of Gametocytocidal Doses of Primaquine for Plasmodium falciparum malaria. 2012.
6. Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, Gupta S, et al. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature. 1995 Jul 20;376(6537):246-9. PubMed PMID: 7617034.
7. White NJ, Qiao LG, Qi G, Luzzatto L. Rationale for recommending a lower dose of primaquine as a Plasmodium falciparum gametocytocide in populations where G6PD deficiency is common. Malar J. 2012;11:418. PubMed PMID: 23237606. Pubmed Central PMCID: 3546849.
8. Eziefula AC BT, Yeung S, Kamya M, Owaraganise A, Gabagaya G, Bradley J, Grignard L, Lanke KH, Wanzira H, Mpimbaza A, Nsobya S, White N J, Webb EL, Staedke SG, Drakeley C. Single dose primaquine for clearance of Plasmodium falciparum gametocytes in children with uncomplicated malaria in Uganda: a randomised, controlled, double-blind, dose-ranging trial. Lancet Infect Dis. 2014 Feb;14(2):130-9. PubMed PMID: 24239324.
9. White NJ, Ashley EA, Recht J, Delves MJ, Ruecker A, Smithuis FM, et al. Assessment of therapeutic responses to gametocytocidal drugs in Plasmodium falciparum malaria. Malar J. 2014;13:483. PubMed PMID: 25486998. Pubmed Central PMCID: 4295364.
10. Chen I, Poirot E, Newman M, Kandula D, Shah R, Hwang J, et al. An assessment of the supply, programmatic use, and regulatory issues of single low-dose primaquine as a Plasmodium falciparum gametocytocide for sub-Saharan Africa. Malar J. 2015;14(1):204.
11. Dicko A, Diallo AI, Tembine I, Dicko Y, Dara N, Sidibe Y, et al. Intermittent Preventive Treatment of Malaria Provides Substantial Protection against Malaria in Children Already Protected by an Insecticide-Treated Bednet in
Mali: A Randomised, Double-Blind, Placebo-Controlled Trial. PLoS Medicine. 2011;8(2).
12. Ouedraogo AL, Guelbeogo WM, Cohuet A, Morlais I, King JG, Gonçalves BP, et al. A protocol for membrane feeding assays to determine the infectiousness of P. falciparum naturally infected individuals to Anopheles gambiae. Malaria World Journal. 2013 (4):16.
13. Wampfler R, Mwingira F, Javati S, Robinson L, Betuela I, Siba P, et al. Strategies for detection of Plasmodium species gametocytes. PLoS One. 2013;8(9):e76316. PubMed PMID: 24312682. Pubmed Central PMCID: 3848260.
14. Schneider P, Wolters L, Schoone G, Schallig H, Sillekens P, Hermsen R, et al. Real-Time Nucleic Acid Sequence-Based Amplification Is More Convenient than Real-Time PCR for Quantification of Plasmodium falciparum. J Clin Microbio. 2005;43:402-5.
15. Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. Naunyn Schmiedebergs Arch Pharmacol. 2004 Jan;369(1):23-37. PubMed PMID: 14618296.
16. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther. 2008;83(2):234-42.

17. Shekalaghe S, Drakeley C, Gosling R, Ndaro A, van Meegeren M, Enevold A, et al. Primaquine Clears Submicroscopic Plasmodium falciparum Gametocytes that Persist after Treatment with Sulphadoxine-Pyrimethamine and Artesunate. PLoS ONE. 2007 (10):e1023.
18. Bennett JW, Pybus BS, Anjali Y, Tosh D, Sousa JC. Primaquine Failure and Cytochrome P-450 2D6 in Plasmodium vivax Malaria. New England Journal of Medicine. 2013;269(14):1381-82.
19. Bousema T, Dinglasan RR, Morlais I, Gouagna LC, van Warmerdam T, Awono-Ambene PH, et al. Mosquito feeding assays to determine the infectiousness of naturally infected Plasmodium falciparum gametocyte carriers. PLoS One. 2012;7(8):e42821. PubMed PMID: 22936993. Pubmed Central PMCID: 3425579.
20. Sawa P, Shekalaghe SA, Drakeley CJ, Sutherland CJ, Mweresa CK, Baidjoe AY, et al. Malaria transmission after artemether-lumefantrine and dihydroartemisinin-piperaquine: a randomized trial. The Journal of infectious diseases. 2013 Jun 1;207(11):1637-45. PubMed PMID: 23468056.
21. White NJ. Primaquine to prevent transmission of falciparum malaria. Lancet. 2013;13:175-81.
22. Sutanto I, Suprijanto S, Kosasih A, Dahlan MS, Syafruddin D, Kusriastuti R, et al. The effect of primaquine on gametocyte development and clearance in the treatment of uncomplicated falciparum malaria with dihydroartemisinin-piperaquine in South sumatra, Western indonesia: an open-label, randomized, controlled trial. Clin Infect Dis. 2013 Mar;56(5):685-93. PubMed PMID: 23175563.
23. Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo APP, et al. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. Lancet Infectious Diseases. 2010 Oct;10(10):673-81. PubMed PMID: WOS:000282911200015. English.
24. Schneider P, Reece SE, van Schaijk BC, Bousema T, Lanke KH, Meaden CS, et al. Quantification of female and male Plasmodium falciparum gametocytes by reverse transcriptase quantitative PCR. Mol Biochem Parasitol. 2015 Jan-Feb;199(1-2):29-33. PubMed PMID: 25827756.
25. Delves MJ, Ruecker A, Straschil U, Lelievre J, Marques S, Lopez-Barragan MJ, et al. Male and Female Plasmodium falciparum Mature Gametocytes Show Different Responses to Antimalarial Drugs. Antimicrobial Agents and Chemotherapy. 2013 Jul;57(7):3268-74. PubMed PMID: WOS:000320229600045. English.
26. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nature reviews Microbiology. 2014 Dec;12(12):833-40. PubMed PMID: 25329408.
27. Ouedraogo AL, Gonçalves BP, Gneme A, Wenger EA, Guelbeogo MW, Ouedraogo A, et al. Dynamics of the Human Infectious Reservoir for Malaria Determined by Mosquito Feeding Assays and Ultrasensitive Malaria Diagnosis in Burkina Faso J Infect Dis. 2015;Advanced access.
28. Eziefula AC, Pett H, Grignard L, Opus S, Kiggundu M, Kamya MR, et al. Glucose-6-phosphate dehydrogenase status and risk of hemolysis in Plasmodium falciparum-infected African children receiving single-dose primaquine. Antimicrob Agents Chemother. 2014 Aug;58(8):4971-3. PubMed PMID: 24913169. Pubmed Central PMCID: 4136063.
29. Chen IT, Gosling RD. Targeting Plasmodium falciparum with primaquine: same efficacy, improved safety with a lower dose? Expert Rev Clin Pharm. 2014;7(6):681-6.
30. Deen JL, von Seidlein L, Pinder M, Walraven GE, Greenwood BM. The safety of the combination artesunate and pyrimethamine-sulfadoxine given during pregnancy. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2001 Jul-Aug;95(4):424-8. PubMed PMID: 11579889.

Figure 1

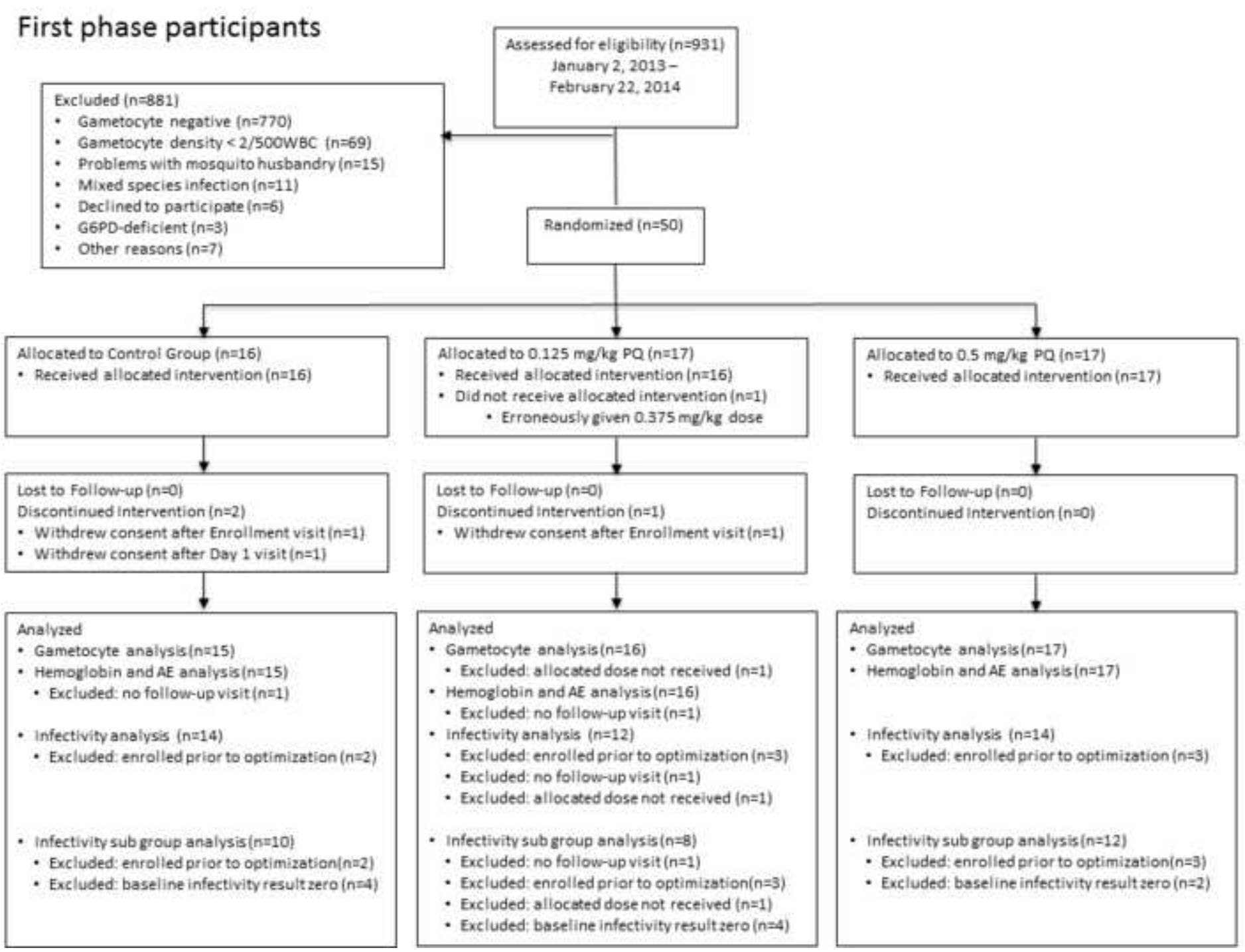

First phase participants
Assessed for eligibility (n=931)
January 2, 2013 –
February 22, 2014
Excluded (n=881)
• Gametocyte negative (n=770)
• Gametocyte density < 2/500WBC (n=69)
• Problems with mosquito husbandry (n=15)
• Mixed species infection (n=11)
• Declined to participate (n=6)
• G6PD-deficient (n=3)
• Other reasons (n=7)
Randomized (n=50)
Allocated to Control Group (n=16)
• Received allocated intervention (n=16)
Allocated to 0.125 mg/kg PQ (n=17)
• Received allocated intervention (n=16)
• Did not receive allocated intervention (n=1)
• Erroneously given 0.375 mg/kg dose
Allocated to 0.5 mg/kg PQ (n=17)
• Received allocated intervention (n=17)
Lost to Follow-up (n=0)
Discontinued Intervention (n=2)
• Withdrew consent after Enrollment visit (n=1)
• Withdrew consent after Day 1 visit (n=1)
Lost to Follow-up (n=0)
Discontinued Intervention (n=1)
• Withdrew consent after Enrollment visit (n=1)
Lost to Follow-up (n=0)
Discontinued Intervention (n=0)
Analyzed
• Gametocyte analysis (n=15)
• Hemoglobin and AE analysis (n=15)
• Excluded: no follow-up visit (n=1)
• Infectivity analysis (n=14)
• Excluded: enrolled prior to optimization (n=2)
• Infectivity sub group analysis (n=10)
• Excluded: enrolled prior to optimization(n=2)
• Excluded: baseline infectivity result zero (n=4)
Analyzed
• Gametocyte analysis (n=16)
• Excluded: allocated dose not received (n=1)
• Hemoglobin and AE analysis (n=16)
• Excluded: no follow-up visit (n=1)
• Infectivity analysis (n=12)
• Excluded: enrolled prior to optimization (n=3)
• Excluded: no follow-up visit (n=1)
• Excluded: allocated dose not received (n=1)
• Infectivity sub group analysis (n=8)
• Excluded: no follow-up visit (n=1)
• Excluded: enrolled prior to optimization(n=3)
• Excluded: allocated dose not received (n=1)
• Excluded: baseline infectivity result zero (n=4)
Analyzed
• Gametocyte analysis (n=17)
• Hemoglobin and AE analysis (n=17)
• Infectivity analysis (n=14)
• Excluded: enrolled prior to optimization (n=3)
• Infectivity sub group analysis (n=12)
• Excluded: enrolled prior to optimization(n=3)
• Excluded: baseline infectivity result zero (n=2)

Figure 1

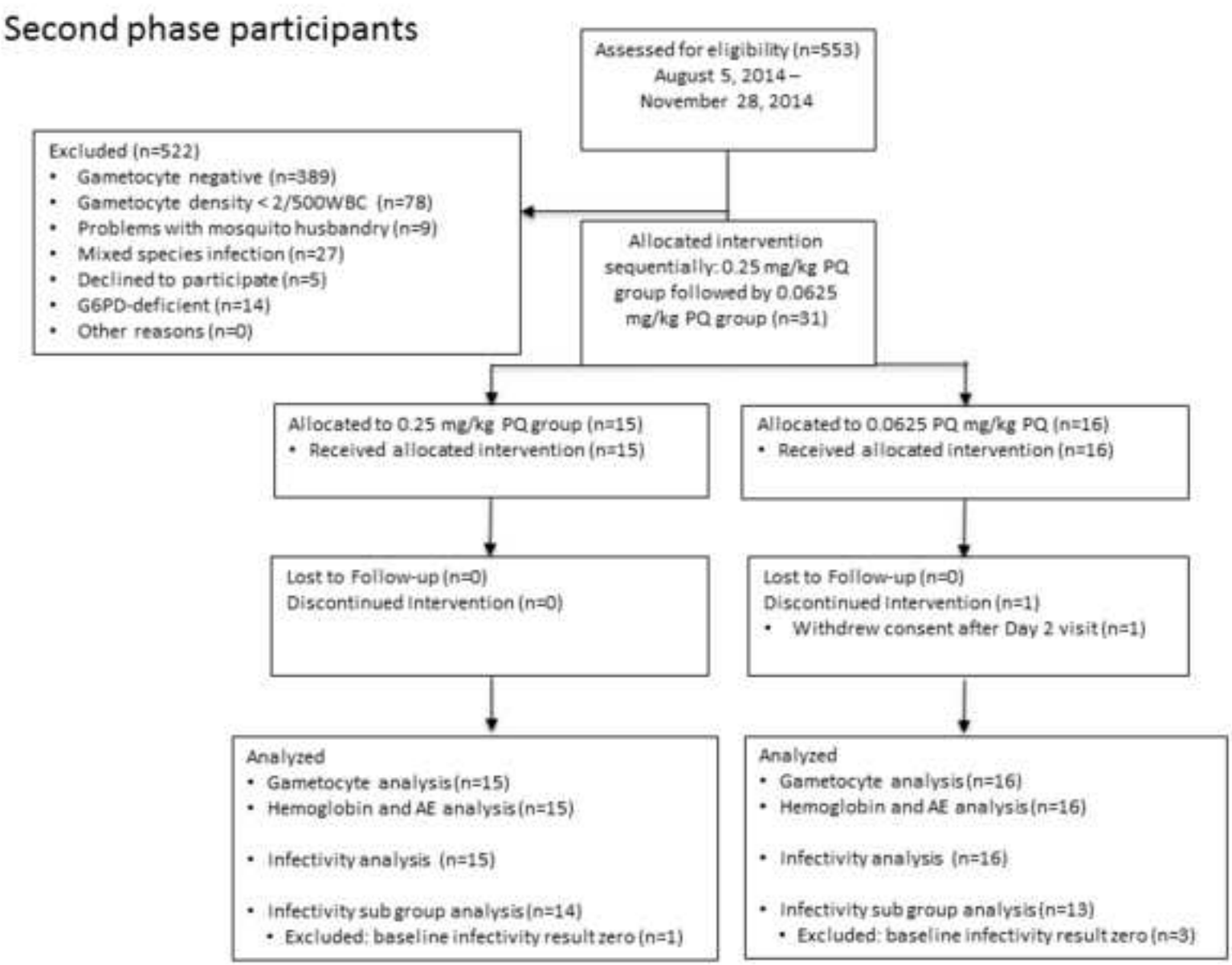

Second phase participants
Assessed for eligibility (n=553)
August 5, 2014 –
November 28, 2014
Excluded (n=522)
• Gametocyte negative (n=389)
• Gametocyte density < 2/500WBC (n=78)
• Problems with mosquito husbandry (n=9)
• Mixed species infection (n=27)
• Declined to participate (n=5)
• G6PD-deficient (n=14)
• Other reasons (n=0)
Allocated intervention sequentially: 0.25 mg/kg PQ group followed by 0.0625 mg/kg PQ group (n=31)
Allocated to 0.25 mg/kg PQ group (n=15)
• Received allocated intervention (n=15)
Allocated to 0.0625 PQ mg/kg PQ (n=16)
• Received allocated intervention (n=16)
Lost to Follow-up (n=0)
Discontinued Intervention (n=0)
Lost to Follow-up (n=0)
Discontinued Intervention (n=1)
• Withdrew consent after Day 2 visit (n=1)
Analyzed
• Gametocyte analysis (n=15)
• Hemoglobin and AE analysis (n=15)
• Infectivity analysis (n=15)
• Infectivity sub group analysis (n=14)
• Excluded: baseline infectivity result zero (n=1)
Analyzed
• Gametocyte analysis (n=16)
• Hemoglobin and AE analysis (n=16)
• Infectivity analysis (n=16)
• Infectivity sub group analysis (n=13)
• Excluded: baseline infectivity result zero (n=3)

Figure 2

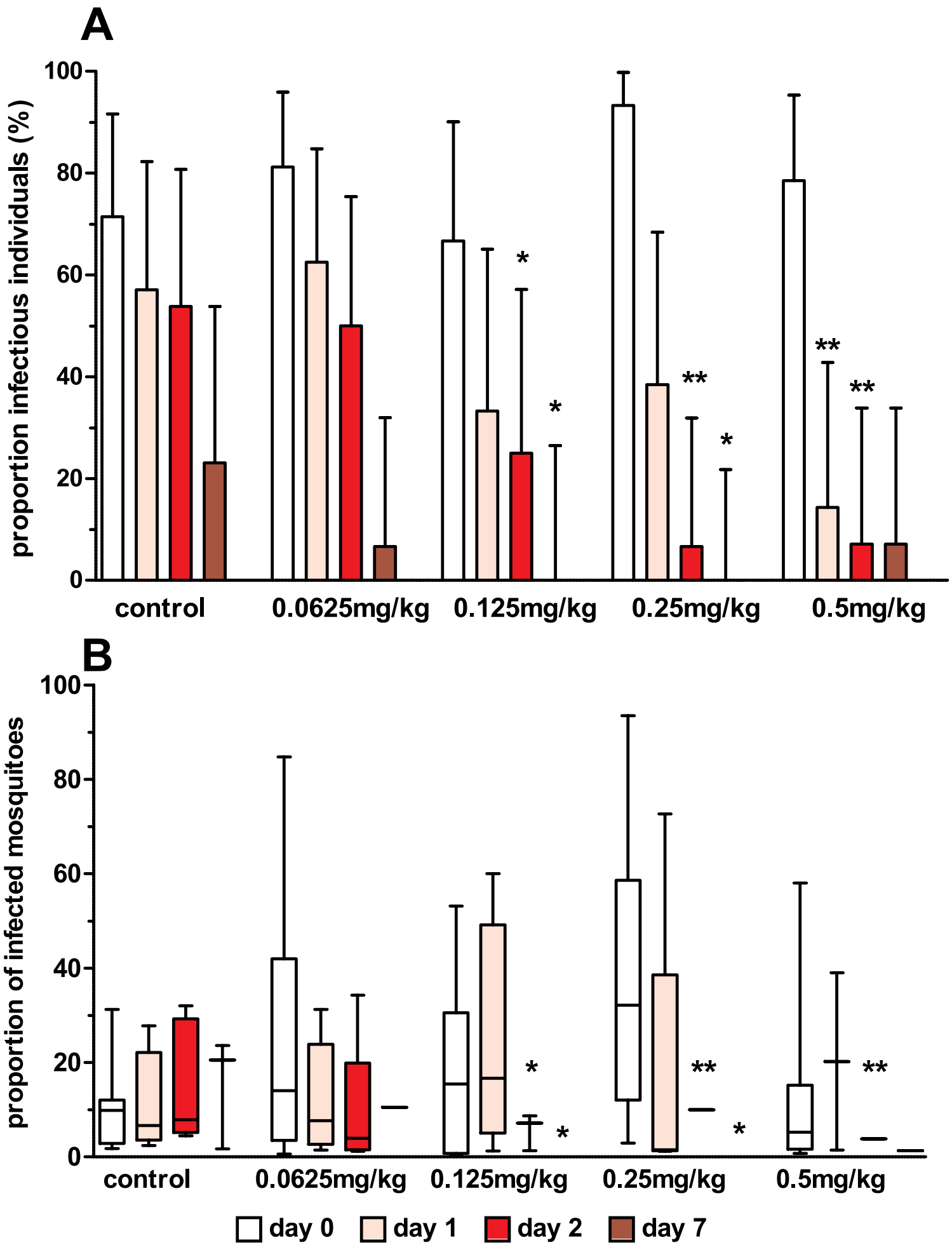

A
proportion infectious individuals (%)
100
80
60
40
20
0
control
0.0625mg/kg
0.125mg/kg
0.25mg/kg
0.5mg/kg
B
proportion of infected mosquitoes
day 0
day 1
day 2
day 7

Figure 3

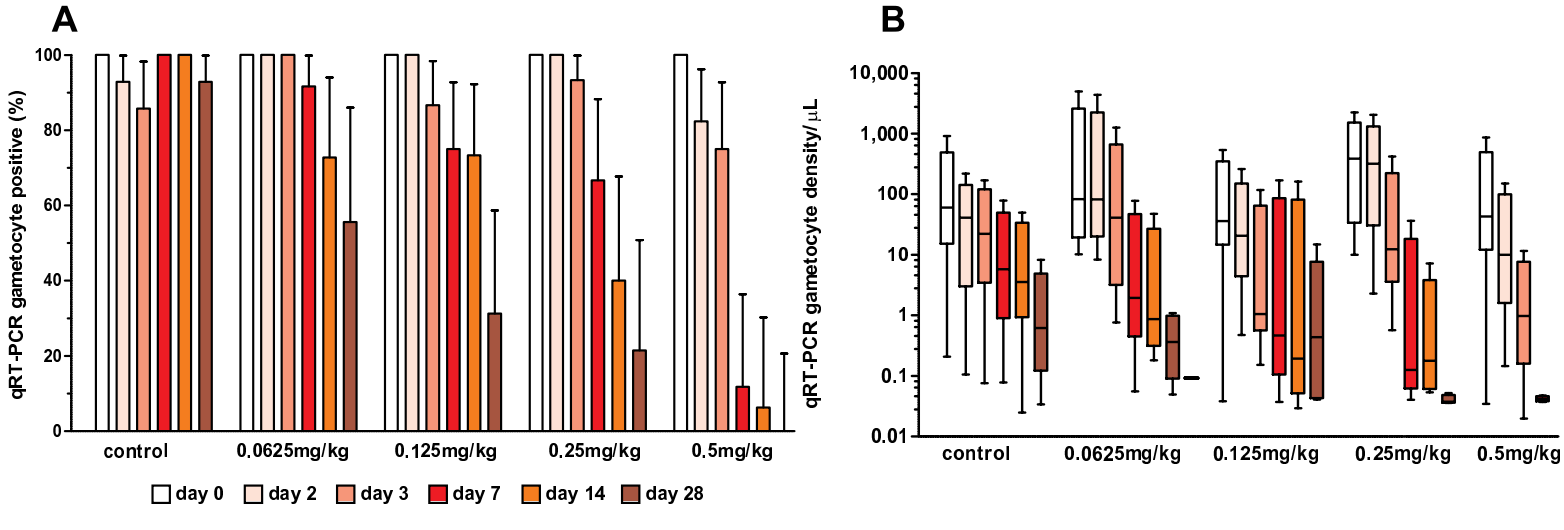
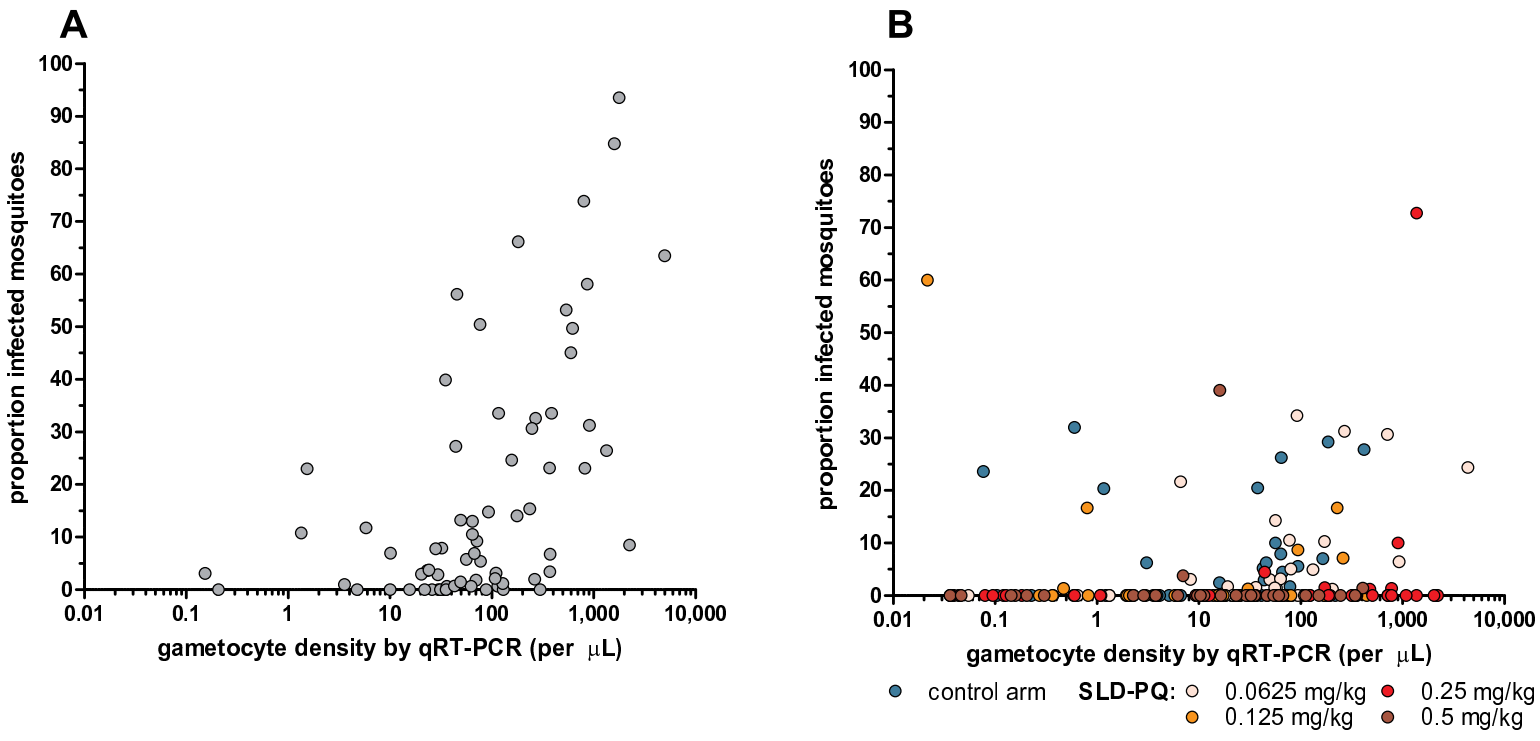

A
qRT-PCR gametocyte positive (%)
100
80
60
40
20
0
control
0.0625mg/kg
0.125mg/kg
0.25mg/kg
0.5mg/kg
day 0
day 2
day 3
day 7
day 14
day 28
B
qRT-PCR gametocyte density/µL
10,000
1,000
100
10
1
0.1
0.01
control
0.0625mg/kg
0.125mg/kg
0.25mg/kg
0.5mg/kg

Figure 4


A
B
proportion infected mosquitoes
gametocyte density by qRT-PCR (per μL)
0
10
20
30
40
50
60
70
80
90
100
0.01
0.1
1
10
100
1,000
10,000
control arm
SLD-PQ:
0.0625 mg/kg
0.125 mg/kg
0.25 mg/kg
0.5 mg/kg