**Author’s response to reviews**

**Title:** Single low-dose primaquine for blocking transmission of Plasmodium falciparum malaria - a proposed model-derived age-based regimen for sub-Saharan Africa

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Reviewer reports:

Reviewer #1: The manuscript by Taylor et al. is timely and clinically relevant for malaria elimination in light of the increasing interest of transmission-blocking interventions for falciparum malaria, particularly in the absence of clinically approved vaccines. The adoption of age-based G6PD deficiency-independent regimens of LSDPQ from anthropometric databases is further interesting. However, I have some minor comments that may contribute to the improvement of the manuscript:

- The abbreviation "ACT" should be "artemisinin-based combination therapy".

Corrected

- The authors used two spelling styles: "organization" and "organisation".

Corrected to organization

- Greater Mekong Subregion was abbreviated though it had been mentioned once. The authors may need to revise all abbreviations, and for terms mentioned only once, there is no justification for their abbreviation. Please check all abbreviations for the terms that have been mentioned once throughout the manuscript.

GMS removed and several other abbreviations

- The paragraph "One of us (WRJ Taylor) approached individuals explaining the purpose of this project and requesting relevant, anonymised anthropometric, demographic, geographical, and haematological data for this project. All those who freely gave data were expected to provide feedback on the study and co-author the paper." Is unclear to me, and may need to be clarified further.

Amended as follows:
The first author approached individuals to explain the purpose of this project and requested anonymised anthropometric data. Later haematological data were sought. All those who freely gave data would be coauthors and were expected to provide feedback on this paper.

- Can authors explain why they used Fisher’s exact test not Pearson’s chi-square test for male-female differences? Is it more accurate?

Although one is more familiar with the use of Fisher’s exact test for small sample sizes, it is also used for large sample sizes and is more accurate compared to the chi squared test.

- The authors used the term "age-dosing SLDPQ regimen". I think it would be better written as "age-based SLDOQ regimen" to be consistent with the title.

We have changed this to:

(iv) how well a given dosing band fitted in with the next dosing band

- What is the opinion of the authors about modeling the regimen based on body mass index or weight instead of age because doses are calculated per kg weight? Can this issue be discussed in the paper?

We have not discussed this because we propose an age based regimen for malaria control programmes, which could be used if weighing scales were not available. It is clear that weight based dosing is more accurate in terms of complying with recommended mg/kg doses. Therefore, if weighing scales are available, then we would recommended dosing by weight. As for body mass index, this is probably more relevant in settings where there are large rates of obesity.

Having said that our understanding of PQ PK is increasing and a paper by Gonclaves et al. shows that age, weight and CYP2D6 status are important factors in children aged 2 to 14 y (paper published after our submission). We have added this paragraph at the end of the Introduction:

These aspects have been investigated elsewhere [16] but a recent study of PQ PK in falciparum infected children aged 2 to 14 years from Burkina Faso illustrates the independent effects of age, weight and cytochrome 2D6 metaboliser status on PQ and carboxyPQ exposure [26]; younger children and children with lower body weight had lower PQ and carboxyPQ exposures and poor PQ metabolisers had increased PQ but reduced carboxyPQ exposures.
Reviewer #4: Taylor and colleagues report on an anticipated primaquine dosing schedule for their planned trial of primaquine for transmission blocking in Pf in sub-Saharan Africa. This is very similar to a paper by many of the same authors published in BMC Medicine last year, based on Cambodian demographic data. They analyse the expected dosing range based on five age-dosing groups, and demographic data on weight for age. The article provides a detailed background discussion of the risks and benefits of low-dose primaquine, and suggests optimal dose ranges and maximal levels. They then divide dose by observed weight distribution (from the demographic data) to see how their proposed dosing regime performs in staying within these limits. However, other than reporting the proportion of subjects expected to have under-dosing and overdosing, it provides little information to help us understand whether these doses / groups are optimal, or what the expected risk / benefit tradeoffs are.

1) This manuscript is very similar to one recently published in BMC Med by many of the same authors (ref 16). However, it uses slightly different age classes (for <1 yr). The reasons for this are not well explained. Is this because of the different weight for age in the SSA data, different Hb, or different perceived risk of G6PDd (frequency / severity of defect)?

Response:

This reviewer is right to compare and contrast the African dosing scheme with that of Cambodia but we do not wish to give the same emphasis in our paper because there are several important differences with Cambodia.

The age weight distribution in Africa is different to that in Cambodia. African children are heavier and this will affect the age cuts off for each dosing band.

Secondly, the most common G6PD variant in Africa is the A- variant which is milder compared to the variants found in SE Asia like G6PD Mahidol and Viangchan. Therefore, the upper limit for PQ is higher in Africa compared to Cambodia.

However, there are cautions. Anaemia is much more common in African children (thanks in substantial part to malaria). Younger children suffer a greater fall in Hb post treatment but the data in children aged 6m to < 1y are limited compared to older children. This explains why we are being particularly cautious in this age group.

In the 'explanation' for the dosing regime (p9, 117-21), the authors state the upper limits they choose for the older age groups. However, for children 6-11 months they just state the dose. Given that the maximal tolerated levels they suggest in SSA is higher than in Cambodia for all
older ages, it seems slightly unexplained why the dose for <1yr is half that recommended in Cambodia. Is this now also the preferred regime for Cambodia? The authors mention low Hb in children, but children <1yr have higher Hb than older children (table 3). Some explanation would be helpful.

Response:

The maximum doses for all ages (except 6m-<1y) is higher compared to Cambodia because of the milder G6PD form in Africa. The Hb value in Table 3 is in healthy individuals from the community and the 0.3 g/dL difference in the median Hb value is not clinically significant and could have occurred by chance, given the small sample size.

We have not given a lengthy explanation for the chosen dose in the children aged 6-11m because published data are few and we have adopted a common sense approach and been cautious. We are not privy to the unpublished data from the World Wide Antimalarial Drug Resistance Network because a paper is in preparation and there was reluctance for us to show data in our paper. However, we have amended the paragraph on page 9 which we think is an improvement:

**Summary of SLDPQ risk and background anaemia**

There are two main reasons to be cautious in setting the upper dose limit of SLDPQ. Firstly, all the PQ studies that have recruited G6PDd patients have had high baseline Hb concentrations; therefore, we cannot extrapolate with confidence these findings to young, symptomatic, anaemic P. falciparum-infected children who have lower Hb concentrations (Table 4) and, secondly, the initial reduction in Hb level in PQ-treated asymptomatic P. falciparum carriers is similar to uncomplicated falciparum malaria patients, with some individuals experiencing Hb drops > 2 g/dL [36, 37].

Predicting the fall in Hb in young PQ-treated malaria patients is challenging, but based on (i) the published data cited above, (ii) unpublished, summarised, post-treatment, Hb dynamics data in African patients aged < 5 years (kindly provided by the WorldWide Antimalarial Resistance Network), (iii) the inverse relationship between baseline Hb and post-treatment Hb decline, and (iv) avoiding the tendency to underplay the toxicity of SLDPQ, we hypothesise that an ‘average’ population of P. falciparum-infected African children aged < 5 years may experience Hb declines of (i) 1 g/dL (median), (ii) 1.5 g/dL (lower interquartile range), and (iii) 3 g/dL (lower 5%).

On the other hand, Shekalaghe et al. provide some reassurance that toxic doses of PQ were well tolerated in apparently healthy Tanzanian children aged 1–12 years with Hb concentrations ≥ 8 g/dL [40]. Moreover, in areas of low malaria transmission, the rates of background anaemia are less, so the risk of SLDPQ-induced AHA should be less compared to areas of high transmission and high background rates of anaemia.
We adopted a ‘risk-stratified’ approach to setting the dose of SLDPQ. Given the uncertainty of the haemolytic potential of PQ in very young P. falciparum-infected children aged 6–11 months and the need to be cautious, we decided to under dose them and arbitrarily set a dose of 1.25 mg PQ base, for a median PQ dose of 0.16 mg/kg (i.e. ~60% of the WHO recommendation). For children aged 1–5 years, we set an upper limit of 0.4 mg/kg of PQ base but increased this upper limit to 0.5 mg/kg for older children because they have less post-treatment declines in Hb and appear to tolerate SLDPQ well.

2) Although this is a primarily quantitative study, it does not provide a quantitative basis for its starting points. That is, the rationale for choosing the particular dosing regime studied appears to rely on qualitative arguments. The authors state (p11, line 17) "Setting the upper therapeutic range of PQ is crucial for safety". However, all of the subsequent arguments for dosing are qualitative. There is a good discussion of the potential risks of primaquine for G6PDd individuals in the introduction. However, there seems no calculation of the clinical effects of the expected dosing regime. The authors report the predicted extent of under-dosing (proportion of patients with low dose). They also report the upper limits they are trying to avoid (p9, l 17-21) - but simply as a threshold to keep under - and the proportion exceeding this, but do not estimate the potential impact of normal or overdosing.

Data exist on the predicted Hb losses in G6PDd and G6PDn individuals, for different primaquine doses. For example, Eziefula et al (ref 38), Goncalves et al (ref 35 - on supple table 4 &5), and Shekalaghe (ref 39) provide information on expected hemoglobin drops for G6PDd and G6PD normal individuals for different primaquine doses. This could be used to predict the proportion of patients with hemoglobin drops of different levels, given the proposed dosing regime and predicted proportion of individuals receiving a given mg/kg dose. This then allows a quantitative estimate of the (for example) total Hb drop across the population for a given dosing regime. As it stands, there is no measure of the ‘trade-off’ against higher primaquine doses, as only the proportion over-dosed is reported. The threshold for ‘overdose’ is 0.5 for children >6 years, and some estimate of the Hb drop for 0.4 mg / kg is provided in the references above (even if in some cases it is not significantly different from placebo). Predicting the ‘penalties’ (in terms of Hb loss) of increasing the dose, and therefore better justifying the choice of dose would be helpful.

Response

We agree with these comments. At the time of the first submission, we did not have access to additional Hb data but we have been sent summary Hb data from WWARN which has allowed us to predict what we think the declines in Hb will be with SLDPQ for children aged ≤ 5 years. However, we have not been able to put a price of the additional decline in Hb to keep the data we recieved confidential.
We have also summarised better studies of PQ in Africa and quoted Hb drops e.g. mean of 2.5 g/dL in the study by Shekaghe. Mwaiswelo et al. report that four G6PDd heterozygous females given AL alone had a mean absolute decline in Hb of ~1.7 g/dL whilst the G6PDd hemizygous males (n=9) and homozygous females (n=5) in the AL+SLDPQ arm had the greatest fall in Hb (~1.5 g/dL). So we have provided some quantitative data based on what has been published.

These data are useful but it must be remembered that we do not yet have a robust PK PD model of PQ vs. Hb decline. Therefore, when deciding PQ therapeutic limits, we have to make reasonable assumptions that we then need to test in the field. The age based some regimen we propose is being tested as we write. From this study, we will have a good quantity of PQ PK data to make greater inferences compared to the data we have currently.

3) The children who receive the highest doses are the youngest in the age bracket, and those on the lowest centile for body weight. There is likely a link between weight for age and haemoglobin, which might affect the impact of any subsequent (primaquine-dose-induced) hemolysis. Exploring the relationship between weight, dose, starting hemoglobin, and expected Hb drop may indicate children at particular risk.

This would be great if we had a large database of 1000s of treated patients who also received primaquine. Sadly, we do not. The best PQ PK paper to date has 38 patients all of whom were G6PD normal and all were asymptomatic falciparum carriers with baseline Hbs of 8 and above g/dL.

4) A side-by-side table of the SSA and Cambodian dosing regimes would be helpful, as well as proportion under / over dosed in each.

Response
We prefer to keep the paper Africa focused. The Cambodian paper is an access free paper. However, we would agree that with more data to underpin our proposed age based regimens, we could then offer a more robust comparison of the two regimens.

5) In figure 4 and 5, a horizontal bar indicating the therapeutic range and upper limits proposed for each age group (p9, l 17-21) would be helpful.

Response
This is a bit tricky because we do not have a therapeutic range for children aged 6-11m and have tow ranges for older children and adults. We prefer to add a foot note (to support what is written...
in Table 5) as we have three proposed therapeutic limits; the one in the children aged 6m-<1y is arbitrary.

As it presently stands, the manuscript reproduces (for SSA) the approach recently applied in Cambodia. However, the justification for the regime change from the Cambodian regime seems largely qualitative. A more quantitative assessment of the risk / benefit tradeoff for different regimes (which would seem to be the major issue that is being optimized here) would greatly strengthen the manuscript.

We certainly appreciate the comments from reviewer 4 which have made us be more critical in our thinking. We now believe we have answered well his or her comments.

Reviewer #5: This paper is a well-written review and analysis of considerations for age-dependent primaquine dosing.

Much of the value of this work comes from the comprehensive review of the existing literature of safety and efficacy in different contexts.

Additionally, the very large anthropometric database allows careful consideration of rare over- and under-dosing frequencies.

I have a few mostly minor comments on the text:

P5/L11 (and throughout): "et al" -> "et al."
Corrected

P5/L44: "PQ is generally a well-tolerated and very safe drug." This sentence is somewhat subjective depending on what one thinks "generally" means.
We have deleted generally: PQ is a well-tolerated and very safe drug.

P6/L6 (and similar pattern throughout): "~6 (%110) and ~5.5 (6/110)" -> "6.4% (7/110) and 5.5% (6/110)" (i.e. move percent symbols after numbers that are percentages)
Done
P6/L28-31: The logic could be clearer here. Is metHb concentration not a clinical concern because post-treatment values are typically in the range of mean levels?

Sentence amended to be clearer:

Data from Cambodia show that and 0.25 mg/kg of PQ (L. Desoley, unpublished) in falciparum-infected patients and 0.75 mg/kg in vivax-infected patients resulted in a maximum metHb concentration of 3.6 and 4.9% [46], respectively. Thus, methaemoglobinaemia is not considered a clinical concern in SLDPQ-treated African children.

P8/L45: spacing is inconsistent between "4 g/dL" and "5g/dL"

Sorted.

P9/L14: "...should be less compared [to] areas..."

Corrected

P10/L41: The difference between 27% and 3.3% under-dosing depending on expected threshold of impact is an important point.

Setting the upper therapeutic range of PQ is crucial for safety and challenging in the absence of a robust dose-response relationship of PQ PK and Hb decline. In contrast to our approach for Cambodia, where malaria is mostly a disease of adults and where background rates of anaemia and malaria-related anaemia are lower [18], we placed much emphasis on the risk of developing CSA in young paediatric patients following ACT+PQ because they have lower pre-treatment Hb concentrations and greater falls in Hb, and so a higher risk of CSA compared to older children. In addition, two studies support high parasite biomass as another risk factor for CSA [42, 80].

Therefore, we decided to be cautious and deliberately under dose infants with 1.25 mg of PQ base, resulting in a mg/kg dose range of 0.1–0.3 (median of 0.16); in this scenario, ~60% infants would receive less than the WHO-recommended PQ dose of 0.25 mg/kg. What effect this would have on mosquito infectivity is unknown and more research is needed to establish the dose response (i.e. PQ PK–infectivity) relationship and refine the minimum PQ dose.

We set a minimum PQ dose of 0.15 mg/kg, based on data from the field experience of MDA in Cambodia [81], which is higher than 0.125 mg/kg suggested by Dicko et al. in their mosquito infectivity experiments [82]; which of the two is the true value is unknown. Our threshold
predicts ~25% under dose rate in infants compared to 3% if we use the threshold of Dicko et al. This emphasizes the need to define accurately the minimum transmission blocking dose and to consider how much of a transmission reservoir such young children represent and, therefore, the need to give them SLDPQ.

P10/L45-57: The wording in these paragraphs is pretty awkward in describing the 1-year age bins with significant under dosing. Consider rephrasing.

Done:

Primaquine dose breakdown by individual ages and sex

The proportions of females and males predicted to receive subtherapeutic, supratherapeutic, and therapeutic doses are shown in Figures 2 and 3, respectively. Rates of supratherapeutic dosing are low, a maximum of 1.3% in females aged 1 year. Age groups at risk of underdosing exceeding 5% are females and males aged 6–< 12m, 4 and 5 years, and females aged 14 years. 73.5 to 100% of females (Fig. 2) and 67.5 to 100% for males (Fig. 3) would receive a therapeutic dose.

P11/L37-40: Consider rephrasing this long and awkward sentence.

Done:

Much emphasis and anxiety has been placed on the potential dangers of PQ-induced AHA in treated P. falciparum-infected G6PDd individuals but there are many other factors that may play an important role in post-treatment Hb dynamics and the risk of post-treatment CSA such as sickle-cell trait/disease, alpha-thalassaemia, HIV infection, hookworm infection, vitamin A deficiency, poor nutritional state, schistosomiasis, baseline parasitaemia and Hb, and PQ PK [42] [67] [73] [80] [83].

P11/L46: Consider breaking up this long and difficult-to-digest sentence.

Done:

Insights will be forthcoming from our safety trial of SLDPQ and from the large multicentre TRACT study (Transfusion and treatment of severe anaemia in African children trial, [84]) which is designed to address optimal blood transfusion strategies in children admitted to hospital with a Hb ≤ 6 g/dL.
P11/L61: "...is very small: <1.4%..."

Done

P12/L14: This statement of "potentially massive" usage in vivax-elimination scenarios could use more quantification and context.

Modified to:

This SLDPQ regimen shares the same tablet strengths (aside from the 6–11 months age band) as the one designed for Cambodia [16]. This overlap in regimens between Africa and Cambodia (and by extension the Greater Mekong Subregion) may allay the anxieties of the pharmaceutical industry regarding the ‘market’ for SLDPQ; indeed, the 2012 WHO recommendation was global. Moreover, if similar table strengths could be used in regimens for P. vivax radical cure, this should stimulate the pharmaceutical industry to produce 2.5- and 5-mg tablet strengths and paediatric formulations.

P12/L29: A bit more discussion would be appreciated on how problematic the non-overlapping ACT dosing categories might be, depending on the distribution context: clinic, community, campaign, etc.

Done:

DHAPP has eight weight-based dosing bands with an emphasis on children who weigh < 25 kg to ensure they receive sufficient dihydroartemisinin. AL, currently the most widely used ACT in Africa, has four weight-based dosing bands and ASAQ has four age-based bands (2–11 months, 1–5, 6–13, ≥ 14 years) that share two of the five age-based bands of our SLDPQ regimen. Suitable training would be essential before SLDPQ is implemented by programmes.

Figure 1: Please move legend entries (e.g. "1-survey data") to above each facet title replacing the numbers.

Done

Figure 2/3: Relabel y-axis "Proportion (%)" or "Percentage"

Done