

1 Randomized non-inferiority trial of dihydroartemisinin-piperaquine compared with sulfadoxine-
2 pyrimethamine plus amodiaquine for SMC in Burkina Faso

3 Running title: DHAPQ for Seasonal Malaria Chemoprevention

4 Authors and affiliations

5 Issaka Zongo^{1,2}, Paul Milligan², Yves Daniel Compaore¹, A Fabrice Some¹, Brian Greenwood², Joel
6 Tarning^{3,5}, Philip J. Rosenthal⁶, Colin Sutherland², Francois Nosten^{3,4}, Jean-Bosco Ouedraogo¹

7
8 ¹ Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo-Dioulasso,
9 Burkina Faso

10 ² London School of Hygiene & Tropical Medicine, London, UK

11 ³ Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University,
12 Bangkok, Thailand

13 ⁴ Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical
14 Medicine, Mahidol University, Mae Sod, Thailand

15 ⁵ Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine,
16 University of Oxford, Oxford, UK

17 ⁶ Department of Medicine, University of California, San Francisco General Hospital, San Francisco,
18 California, USA

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21 **Abstract**

22 **Background:** WHO recommends that children living in areas of highly seasonal malaria transmission
23 in the Sahel subregion should receive Seasonal Malaria Chemoprevention (SMC) with sulfadoxine-
24 pyrimethamine plus amodiaquine (SPAQ). We evaluated the use of dihydroartemisinin- piperaquine
25 (DHAPQ), as an alternative drug that could be used if SPAQ starts to lose efficacy.

26 **Methods:** 1499 children aged 3-59 months were randomized to receive SMC with SPAQ or DHAPQ
27 over three months. The primary outcome measure was the risk of clinical malaria (fever or a history
28 of fever with a parasite density of at least 3000/ μ L). A cohort of 250 children outside the trial was
29 followed up as a control group. Molecular markers of drug resistance were assessed.

30 **Results:** The risk of a malaria attack was 0.19 in the DHAPQ group and 0.15 in the SPAQ group, an
31 odds ratio of 1.33 (95%CI 1.02,1.72). Efficacy of SMC compared to the control group was 77% (67%,
32 84%) for DHAPQ and 83% (74%,89%) for SPAQ. *pfdhfr* and *pfdhps* mutations associated with
33 antifolate resistance were more prevalent in parasites from children who received SPAQ than in
34 children who received DHAPQ.

35 **Conclusions:** Both regimens were highly efficacious and well tolerated. DHAPQ is a potential
36 alternative drug for SMC.

37 [200 words]

38 Keywords: Seasonal Malaria Chemoprevention; dihydroartemisin-piperaquine; Burkina Faso; non-
39 inferiority trial

40 This trial is registered at www.clinicaltrials.gov, NCT00941785

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42 **Footnotes:**

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44 **Conflict of interest:** none declared.

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50 **Corresponding author:**

51 Issaka Zongo, Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo-
52 Dioulasso, Burkina Faso

53 Email: zongo_issaka@yahoo.fr

54 Paul Milligan, London School of Hygiene&Tropical Medicine, Keppel Street, London WC1E 7HT, UK

55 Email: paul.milligan@lshtm.ac.uk

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58 Introduction

59 Substantial progress has been made in the control of malaria [1], but in most of Sub-Saharan Africa
60 the disease remains a major public health problem. In Burkina Faso, malaria is still a leading cause of
61 severe illness and mortality, accounting for 63% of hospital admissions and 71% of all deaths in
62 hospital among children under five years of age in 2011 [2]. Results from research studies indicate
63 that the burden remains very high. In Bousse, in the Sahelian zone of the country, in 2011, 1232
64 episodes of malaria were recorded over one transmission season in 1500 children under 5 years of
65 age who were using an insecticide treated net [3]. In such areas new strategies for malaria control
66 are needed. WHO now recommends Seasonal Malaria Chemoprevention (SMC) with sulfadoxine
67 pyrimethamine plus amodiaquine (SPAQ) as a new strategy for malaria control in children in areas of
68 highly seasonal transmission [4,5], defined as areas where at least 60% of malaria cases occur during
69 4 months of the year and where SP and AQ retain good efficacy. Most parts of Burkina Faso fit these
70 criteria, and implementation of SMC in Burkina Faso started in 2014 in seven districts.

71 Resistance to both SP and AQ is common in much of Africa, but in most areas of seasonal
72 transmission in the Sahel these drugs retain their antimalarial efficacy [5]. However, alternative drug
73 regimens may be needed in these areas in the future, and are needed now if SMC is to be deployed
74 in areas of eastern or southern Africa where anti-folate resistance makes SP an unsuitable drug for
75 SMC. Dihydroartemisinin-piperaquine (DHAPQ) is a potential alternative. Piperaquine (PQ) is a long
76 acting antimalarial, administration daily for 3 days results (in adults) in a 3- to 7-fold accumulation
77 and a long terminal half-life [6,7], making it suitable for chemoprevention. PQ has been used
78 extensively in the past for chemoprophylaxis in China, and is now available in a fixed combination
79 with dihydroartemisinin. Two studies have investigated the use of DHAPQ for SMC [8,9]. When
80 DHAPQ and SPPQ (sulfadoxine-pyrimethamine plus piperaquine) were compared with SPAQ, efficacy
81 was similar for all three regimens, but the incidence of malaria was low, limiting the power to
82 differentiate between regimens. DHAPQ and SPPQ were better tolerated than SPAQ, and DHAPQ
83 was associated with lower selection of *dhfr* and *dhps* mutations, strongly associated with anti-folate
84 resistance in *P. falciparum*, compared to the SP-containing drug combinations [9]. The haemoglobin
85 concentration at the end of the transmission season was slightly lower in children who had received
86 DHAPQ than in the other groups. In Uganda, Nankabirwa *et al.* [10] compared the efficacy of single
87 preventive treatments with SP alone, SPAQ or DHAPQ in school children, and found DHAPQ to be
88 the most effective with a substantially reduced prevalence of parasitemia assessed 42 days after
89 treatment. In adults in Thailand, the protective efficacy of DHAPQ was 98% when administered
90 monthly and 86% when administered bimonthly [11]. Despite extensive clinical evaluation and use

91 of DHAPQ in Southeast Asia [12,13] and Africa [14,15,16] few studies have addressed
92 pharmacokinetics of PQ in children [17-19]. However, one of these studies suggested that children
93 are under-dosed with current regimens [17], which was also supported by a meta-analysis recently
94 [20]. The primary objective of this study was to determine whether DHAPQ is as effective as SPAQ
95 for SMC in an area where SPAQ is highly efficacious, and to compare the tolerability and safety of
96 two regimens when used for SMC in children.

97 **Methods**

98 *Study site*

99 The study was conducted between August 2009 and January 2010 in a rural area served by three
100 health centres (Satiri, Kadomba and Balla) in the district of Lena, approximately 30 miles from the
101 city of Bobo-Dioulasso in western Burkina Faso. The climate of the area is typical of the Sudan
102 savannah with a long dry season and a shorter rainy season (July to October). Transmission of
103 malaria is highly seasonal.

105 *Recruitment of participants*

106 Before the trial started, meetings were held in the community to explain the study aims. A
107 population census was done in July 2009. Households with a child under 5 years of age were then
108 visited to explain the procedures of the study and to seek signed consent from parents. If parents
109 were unable to read, a witness signed to indicate that the study details had been explained
110 correctly. The inclusion criteria were: age between 3 and 59 months, the family expected to remain
111 in the study area over the study period, no history of allergy to the study medications, no chronic
112 condition requiring hospitalisation (for example severe malnutrition), and parental consent. The
113 presence of malaria at enrolment was not an exclusion criterion, if malaria was diagnosed, the
114 patient was enrolled and treated with artemether-lumefantrine (AL; Coartem®), SMC was not given
115 but the child was eligible to receive subsequent monthly doses of SMC.

117 *Enrolment and randomization procedures*

118 On the day of enrolment, a clinical assessment was made including the measurement of weight,
119 height and axillary temperature. A physical examination was done and questions asked about the
120 use of insecticide-treated bednets (ITNs) and past medical history. After a further check of eligibility
121 children were assigned a randomization envelope bearing a randomization number. Allocations
122 (generated using permuted blocks of 10 in Stata version 10) were sealed in opaque envelopes which
123 were assigned in a strict numerical sequence. A finger-prick blood sample was taken for preparation

of thick and thin blood smears and for blood spots on filter paper (Whatman No 3) for molecular analyses. Participants were then referred to the study nurse who opened the envelope to determine the treatment allocation, and administered the first dose of medication. This was an open trial, blinding was not feasible due to the difference in the appearance of the study drugs, but steps were taken to ensure concealed randomization, and staff who performed laboratory analyses were not aware of the child's treatment group.

Study drugs and SMC administration

Children in the SPAQ arm received one dose of SP (Fansidar® Roche 500mg/25 mg tablets) in a dosage of 25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine, and three doses of AQ (Camoquin®, Parke-Davis syrup 60 ml, 50mg/5ml) in a dosage of 10 mg/kg each day for three consecutive days. Children in the DHAPQ group received Duocotexin tablets (DHA 40 mg and PQ-phosphate 320 mg (Duocotexin, Holley Cotec, China) in a dosage of 4mg/kg DHA and 18mg/kg PQ daily for three consecutive days. Children were weighed each month to determine dosage which was rounded to the nearest ¼ tablet or the nearest 5 ml of AQ syrup. Three rounds of preventive treatment were given (August, September and October, 2009). The single dose of SP and the first dose of AQ or DHAPQ was given at the study clinic observed by the study nurse. A field worker visited the child at home on each of the next two days to administer the two remaining doses of AQ or DHAPQ, and to ask about adverse events. Children were observed for 30 minutes after each dose, and a repeat dose was given if the child vomited.

Follow up visits and malaria diagnosis and treatment

Parents were asked to bring their child to the clinic whenever the child was unwell. A fieldworker visited each family two weeks after each SMC round to check the child was well and to refer any children who were unwell to the clinic where a study physician was available. Children who presented with a history of fever had a rapid diagnostic test for malaria (SD Bioline, Standard Diagnostics, Korea) performed and if this was positive they were treated with AL. A blood smear was taken to be read later. If a child was diagnosed with malaria on the day SMC was scheduled to be given, SMC was withheld that month and they were treated with AL. Medications commonly used to treat other illnesses included antibiotics (amoxicillin, oxacillin tablets or syrup), and paracetamol.

Control group

To estimate the incidence of malaria in untreated children, a separate cohort was enrolled in part of the study area (one of the three areas used for the main trial), with the same inclusion criteria as

those used for the main trial cohorts, but one month later, at the time of the second round of SMC administration. These children were followed up in a similar way as the other study children.

Cross-sectional surveys

At the end of the malaria transmission season, a survey of all study children was undertaken (one month after the last administration of SMC in the randomized groups and one month later in the control group) to determine the prevalence of parasitemia and gametocytemia and the concentration of haemoglobin using a Haemocue (Angelholm, Sweden).

Laboratory methods

Thick and thin blood smears were stained with 2% Giemsa for 30 minutes and double read by experienced laboratory technicians. For parasite isolates sampled during the first episode of malaria following the initiation of SMC, mutations in the *pfmdr1* (N86Y, F184Y, D1246Y), *pfdhfr* (N51I, C59R, S108N), and *pf dhps* (A436S, A436F, A437G, K540E, A613S) genes were detected by dideoxy sequencing and mutations in the *pf crt* gene (K76T) by qPCR as described previously [21,22]. A subset of 45 children was identified at randomization for assessment of biochemical and haematological parameters, 15 to be sampled each month, and a subset of 210 children in the DHAPQ group (70 each month) gave additional blood samples for evaluation of the pharmacokinetic properties of piperazine (full details of these results will be published separately; here we present only the day 7 concentrations).

Statistical methods

The primary outcome measure of the trial was the risk of clinical malaria, (axillary temperature $\geq 37.5^{\circ}\text{C}$ or history of fever in the last 24 hours and *P.falciparum* density of at least 3000 parasites/ μL). Secondary outcome measures included the incidence of clinical malaria with any parasitaemia, the prevalence of asexual parasitaemia and gametocyte carriage and the presence of anaemia at the end of the malaria transmission season; the presence of molecular markers of resistance to study drugs among patients diagnosed with malaria during the trial or with parasitaemia at the end of the transmission season; and the pharmacokinetics of PQ. Sample size was chosen to give adequate power to demonstrate that SMC with DHAPQ was non-inferior to SMC with SPAQ with respect to the risk of malaria with a parasite density of 3000/ μL or more. The non-inferiority margin was specified as an odds ratio of 1.64, equivalent to a risk difference of 4% if the risk in the comparator group was 7%. A sample size of 1500 children was needed to give a study with 80% power using a one-sided 2.5% significance level, allowing for up to 10% of subjects being

excluded from the according-to-protocol analysis due to loss to follow-up or non-adherence to the protocol. The intention to treat (ITT) analysis (considered primary) included all randomized children, in the group they were assigned at randomization. For according to protocol (ATP) analysis, we excluded children who did not attend for an SMC treatment round but children who attended, but did not receive SMC because they had malaria and were treated with AL were included in the ATP analysis. Analysis of non-inferiority was based on the 95% confidence interval on the odds ratio for malaria, obtained from the Kaplan-Meier estimate of the risk and its standard error.

Further details of methods are given in the supplement.

Ethics

The study protocol was approved by the ethics committee of Centre Muraz (Comité d’Ethique Institutionnel du Centre Muraz) and by the ethics committee of the London School of Hygiene & Tropical Medicine. A Data Safety Monitoring Board was appointed to oversee the trial and an independent monitor provided oversight of the conduct of the trial.

Results

Characteristics of study children

The trial profile is shown in Figure 1. 1499 children were randomized, 750 to DHAPQ and 749 to SPAQ. The randomized groups were similar in terms of baseline characteristics. The cohort of 250 children who did not receive SMC, recruited at the time the main cohorts received their second round of SMC, was similar to the trial cohorts in terms of age but were more malnourished with a higher prevalence of being underweight, and higher prevalence of parasitaemia (Table 1).

Adherence to daily doses

All daily doses of SMC were supervised. Three children in the SPAQ group did not complete the course in August, two children in the DHAPQ group did not complete the course in August and one child in the DHAPQ group did not complete the course in October. Between 7% and 9% of children missed SMC doses each month because they required treatment for malaria (Figure 1).

Efficacy of SPAQ and DHAPQ against clinical malaria

The incidence of malaria in the SMC treatment groups and in the untreated group was compared during the two months following the second round of SMC. There were 229 episodes of malaria, defined as fever with a parasite density $\geq 3000/\mu\text{L}$, in the untreated cohort, a mean of 0.92 episodes per child, compared with 108 episodes (mean 0.14 per child) in the DHAPQ group and 78 (mean 0.11 per child) in the SPAQ group giving an efficacy against malaria (adjusted for covariates site, age and ITN use) of 79% (95%CI 70%,85%) and 84% (76%,90%), respectively. Efficacy against malaria defined as fever with any parasitaemia, adjusted for the same covariates was 74% (65%, 81%) for DHAPQ and 80% (72%, 86%) for SPAQ (Figure 2). To estimate the duration of protection provided by SMC, the incidence of clinical malaria after the last round of SMC in each treatment group was compared with the incidence in controls over the same period, adjusted for covariates. In both groups protection persisted at a high level for 3-4 weeks and decreased rapidly thereafter (Figure 3).

Analysis of the non-inferiority of DHAPQ to SPAQ

Over the three months of the trial there were 281 episodes of malaria (fever with parasite density $\geq 3000/\mu\text{L}$), 122 in children in the SPAQ group and 159 in children in the DHAPQ group. The Kaplan-Meier estimate of the risk of malaria during the 3 months was 0.15 for the SPAQ group and 0.19 for the DHAPQ group (odds ratio 1.33, 95%CI 1.02,1.72) (Figure 4). This confidence interval is above 1, indicating superiority of SPAQ, the upper limit just exceeding the margin for non-inferiority. The cumulative hazard at 3 months was 0.16 (SPAQ) and 0.21 (DHAPQ), hazard ratio 1.29 (95%CI 0.97,1.71) (Table 2). Similar results were obtained by ATP analysis (Table 3).

Concentration of piperaquine on Day 7 and its relationship to efficacy against malaria

Piperaquine plasma concentration was measured in capillary samples in 159 children on day 7 after treatment with DHAPQ. The mean concentration was 48 ng/ml (SD 21) in August, 52 ng/ml (SD 26) in September and 60 ng/ml (SD 31) in October. To assess the association between piperaquine concentration measured on day 7 and protection against clinical malaria during the month that the concentration was measured, these children were divided into three equal groups according to the tertiles of the day 7 concentration. The incidence of malaria that month decreased with increasing concentration, (logrank test for trend, stratified by month, $\chi^2 = 5.10$ (1df), $P=0.024$) (Table 4).

In these children, the mean estimated dose of piperaquine administered was 50 mg/kg (SD 7.95, range 28.6-67.9). In linear regression analysis, a 10 mg/kg increase in dose of PQ administered was associated with an increase of 4.7 ng/ml (95%CI -1.2,11) in the day-7 plasma concentration of PQ in August, 5.7 ng/ml (0.3,11) in September and 7.7 ng/ml (2.0,13) in October (Fig 5).

To illustrate the relationship between the dose of piperaquine administered and the incidence of malaria in the subsequent month, children who received DHAPQ were divided into three groups according to the tertiles of the dose administered (<45mg/kg, 45-55 mg/kg, and >55mg/kg), and the timing of malaria episodes in each of these groups that month was shown in a plot of the cumulative hazards (Fig 6). In Cox regression analysis, an increase in piperaquine dose administered was associated with a reduction in the incidence of malaria with a hazard ratio of 0.62 (95%CI 0.43,0.90) for a 10 mg/kg increase in dose administered in August, 0.52 (0.31,0.89) in September and 0.85 (0.43,1.7) in October.

Efficacy against parasite and gametocyte prevalence and anaemia at the end of the malaria transmission season

At the end of transmission season, the prevalence of parasitaemia by microscopy was 12% in each group of treated children (88/731 and 88/722 in the SPAQ and DHAPQ groups, respectively) and 36% (88/247) in the control group (efficacy for each group compared with control 34% (95%CI 26%,44%). The prevalence of gametocytaemia measured by microscopy was 0.8% in each treatment group (6/727 and 6/721 in the SPAQ and DHAPQ groups respectively) and 1.6% (4/243) in the control group (efficacy for the combined SMC groups compared to controls was 50%, 95%CI -55%,84%). The prevalence of anaemia (Hb<8g/dL) was 14% (35/243) in the control group, 15% (108/719) in the DHAPQ group and 16% (117/713) in the SPAQ group. The difference in prevalence between the two SMC groups was 1.4% (95%CI 2.3%,5.2%), and the difference from controls was 0.6% (-4.5%,5.7%) (DHAPQ) and 2.0% (-3.2%,7.2%) (SPAQ) (Tables S3 and S4).

Adverse events

The most commonly reported mild adverse events were cough, diarrhoea, vomiting, and fever. The incidence of these adverse events was higher after the first round of SMC than in subsequent rounds, and in each round was similar in both treatment groups (Figure 7). Four cases of severe anaemia were recorded (one in the SPAQ, two in the DHAPQ and one in the untreated group) and 7 deaths occurred (two in the SPAQ, four in the DHAPQ and one in the untreated group), three of these deaths (one in each group) occurred at home. None of these deaths was considered related to SMC. In the untreated cohort, a girl aged 3 years died in November, with malnutrition. Further details of these adverse events are provided in the Supplement. Biochemical and haematological parameters were similar in the two treated groups on day 7, apart from the haemoglobin concentration which was slightly lower in children who received DHAPQ (10.4 g/dl compared to 11.3 g/dl in those who received SPAQ), adjusted difference between groups 1.03 (0.51, 1.55). A small

number of children had values outside the normal range (see Supplement) but these were not associated with clinical symptoms.

Drug resistance markers

The *pfdhfr* 51I, 59R and *dhps*437 mutations, and the *pfdhfr* (I51/R59/S108) and *pfdhfr*(I51/R59/S108) plus *pfdhps* G437 haplotypes were more common in samples from children who had received SPAQ than in samples from children who had received DHAPQ, and those in the untreated children (Table 5). The prevalence of these mutations in samples from children who had received DHAPQ was similar to the prevalence in samples from untreated children. The *pfdhps* S613 mutation, was detected in the study area for the first time, but its prevalence was similar among the study groups.

Discussion

There is increasing recognition of the potential importance of drugs for malaria prevention in malaria endemic countries, but the choice of drug regimens remains limited. SPAQ, the regimen used for SMC, remains highly effective in the areas of seasonal transmission where its use is recommended, but resistance to SP is likely to spread, so alternative regimens will be needed. We have shown that DHAPQ is highly effective for SMC, and similar in efficacy to SPAQ, in an area where *P.falciparum* is still sensitive to SP and AQ. Both regimens had an efficacy over 70%. The duration of protection was similar with both regimens, with a high level of protection for about 4 weeks followed by a rapid decrease, highlighting the importance of strict timing in SMC programmes to ensure that children receive treatment at monthly intervals. These results are consistent with those of previous studies in children [8,9] and a study in adults in Thailand [11] which showed that DHAPQ was well tolerated and highly effective when used for monthly prophylaxis and with a study in schoolchildren in Uganda, which showed that monthly DHPAQ was well tolerated and reduced malaria incidence by 96% and the prevalence of anaemia by 40% [23]. In another study in Uganda in younger children, efficacy of monthly DHAPQ was only 58%, possibly due to poor adherence and under-dosing [24]. In our study, the efficacy of DHAPQ was related to the circulating concentration of piperaquine; there was a steady reduction in incidence of malaria with increasing day 7 concentration. This is consistent with results from two previous studies of recurrence of malaria after treatment, that of Price *et al.* [25] who found that patients with day 7 concentrations of piperaquine less than 30 ng/ml had an increased risk of recurrence of malaria, and Creek *et al.* [26] who found a similar figure (≤ 27.3 ng/mL). In our study children in the upper third of concentrations had a substantially lower risk of malaria than children in the lower third (rate ratio 0.32), highlighting

the importance of choosing dosing schemes carefully, balancing efficacy with tolerability, in order to maximise protection.

Both treatment regimens were well tolerated. As seen in other studies the incidence of mild adverse events decreased in successive rounds of treatment. There was a slight drop in haemoglobin after treatment with DHAPQ, that was not seen after SPAQ, but the prevalence of anaemia at the end of the transmission season was similar in all groups. In a previous SMC study [8], anaemia was more common in children who received DHAPQ compared with other treatments, but other studies have not reported anaemia associated with DHAPQ, although artemisinins may reduce reticulocyte count [27].

When isolates from the first incident malaria cases were typed, the triple dhfr mutation and the dhps g437 mutation were more frequent in children who received SPAQ than in the malaria cases in the DHAPQ group and in the control group, but the frequencies of pfcr1 CVIET T76, pfmdr1 Y86 and pfmdr1 Y184 associated with resistance to AQ, were similar in all three groups. Analysis of molecular markers of resistance in a subset of samples from children in this study taken at enrolment and at the end of the transmission season, have been reported separately [28]. Among children who received SPAQ who had parasitaemia at the end of the transmission season, the frequency of pfcr1 76T, pfdhfr 59R and pfdhfr 108N was greater than at baseline. In children who received DHAPQ, there was no evidence of an increase in frequency of the markers that were investigated, but it is possible other factors may be involved in resistance to PQ [29].

SPAQ has the disadvantage that being a loose combination, tablets could be used separately. DHAPQ is a fixed dose combination, but the rapid elimination of DHA means that parasites are exposed to PQ monotherapy. This is a concern, with potential selection of parasites with decreased sensitivity to PQ. However, selection for resistance to artemisinins, which is a growing concern [30], is less likely as the combination ensures parasites are exposed to DHA only when the concentration of PQ is high [31]. However, there is a need for new long-acting antimalarial combinations to be developed to be used for prevention. Monitoring of the emergence of resistance to these drugs where SMC is used will require continued clinical and molecular surveillance.

A limitation of this study was the use of a non-randomized control group. As SMC with SPAQ had been shown to be highly effective when used for SMC at the time the trial was planned, it was considered unethical to randomize children to a placebo. An untreated control group outside the

trial but living under similar circumstances to the study children, was recruited. Adjusted analyses were used for comparisons to the untreated group, to control for potential confounding, but some residual confounding may have remained. A second limitation is that collection of data on the use of ITNs relied on carer givers' affirmation which may not reflect real use.

This study has confirmed a continued high burden of malaria in Burkina Faso, with 338 episodes of malaria in 250 control children over two months. New malaria control tools are needed urgently and the potential of SMC with SPAQ to reduce the burden in countries such as Burkina Faso needs to be fully realised by scaling-up access to this intervention. In situations where SPAQ cannot be used, we have shown that DHAPQ appears to offer an effective alternative.

[3572 words]

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496 Table 1: Characteristics of children in the randomized groups and the untreated cohort at enrolment

	SMC randomized groups		
Variable	SPAQ (n=742)	DHAPQ (n=757)	Untreated cohort (n=250)
Date enrolled	11-20 Aug	11-20 Aug	17-19 Sep
Study site: Kadomba	323 (44%)	325 (43%)	0
Balla	151 (20%)	150 (20%)	250 (100%)
Satiri	268 (36%)	282 (37%)	0
Gender %Male:%Female	49%:51%	50%:50%	49%:51%
Age group: <12months	129 (17%)	153 (20%)	47 (19%)
12-23months	155 (21%)	158 (22%)	63 (25%)
24-35months	155 (21%)	152 (20%)	56 (22%)
36-47months	147 (20%)	138 (18%)	45 (18%)
48-59months	156 (21%)	156 (21%)	39 (16%)
Mean weight kg (SD)	10.9 (3.22)	10.7 (3.13)	10.1 (2.72)
Underweight	193 (26%)	189 (25%)	95 (38%)
Stunting	186 (25%)	182 (24%)	35 (14%)
Wasting	163 (22%)	167 (22%)	105 (42%)
Use of bed nets (%)	267 (36%)	273 (36%)	79 (32%)
Slept under ITN the night before (%)	204 (27%)	186 (25%)	77 (31%)
Number with fever*(%)	213 (29%)	216 (29%)	179 (72%)
Number with malaria**(%)	72 (9.5%)	70 (9.4%)	160 (64%)
Geometric mean parasite density (/μL) (range)	2655 (16,185000)	2216 (12,180000)	2950 (12,111000)
Prevalence of parasitaemia (%)	336 (45%)	323 (43%)	152 (61%)
Prevalence of gametocyte carriage (%)	80 (11%)	80 (11%)	75 (30%)

497 * Axillary temperature >37.5°C or history of fever in the past 24 hours and ** Fever with any parasitaemia,
 498 measured in August for the randomized groups and a month later in September, for the untreated cohort.

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500 Table 2: ITT analysis* of malaria incidence during a three month period from the time of the first
 501 round of SMC.

	N	Cases	Person months	Rate /1000 /month	proportion with malaria (K-M estimate)	se	OR (95%CI)	Cum. hazard	se	HR (95%CI)
Fever with parasitaemia $\geq 3000/\mu\text{L}$										
SPAQ	749	122	2202.5	56.1	0.151	0.0126	1	0.163	0.0148	1
DHAPQ	750	159	2216.6	71.3	0.191	0.0137	1.33 (1.02,1.72)	0.210	0.0168	1.29 (0.97,1.71)
P=0.075										
Fever with any parasitaemia										
SPAQ	749	161	2202.5	73.1	0.195	0.0138	1	0.215	0.017	1
DHAPQ	750	199	2216.6	89.8	0.234	0.0146	1.26 (1.00,1.59)	0.264	0.0188	1.22 (0.95,1.58)
P=0.122										

502 *Analysis of non-inferiority was based on the 95% confidence interval on the odds ratio for malaria, obtained from the
 503 Kaplan-Meier estimate of the risk and its standard error, using the delta method. The cumulative hazard function, (an
 504 estimate of the average number of malaria episodes per child), was estimated using the Nelson-Aalen method. The hazard
 505 ratio was obtained using Cox regression with confidence intervals calculated using a robust standard error to account for
 506 repeated malaria episodes in the same child.

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509 Table 3: ATP analysis of malaria incidence during a three-month period from the time of the first
 510 round of SMC.

	N	Cases	Person months	Rate /1000 /month	proportion with malaria (K-M estimate)	se	OR (95%CI)	Cum. hazard	se	HR (95%CI)
Fever with parasitaemia $\geq 3000/\mu\text{L}$										
SPAQ	740	119	2175.4	54.7	0.149	0.0127	1	0.161	0.0148	1
DHAPQ	754	161	2228.9	72.2	0.192	0.0137	1.36(1.04,1.76)	0.212	0.0168	1.31(0.99,1.74)
P=0.072										
Fever with any parasitaemia										
SPAQ	740	156	2175.4	71.7	0.193	0.0139	1	0.213	0.017	1
DHAPQ	754	200	2228.9	89.7	0.236	0.0146	1.29(1.02,1.64)	0.266	0.0188	1.25(0.97,1.62)
P=0.090										

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516 Table 4: Incidence of malaria cases in children whose piperaquine concentration in plasma was

517 measured in capillary samples on Day 7.

Piperaquine concentration		No. of Children	No. of malaria cases	Person months at risk	Rate/ month	Rate
ng/ml mean (SD)	Range					Ratio(95%CI)
27.7 (8.40)	7.4-40.5	53	10	78.81	0.127	1
50.4 (6.14)	40.6-63.0	53	8	94.55	0.085	0.67 (0.23,1.9)
85.2 (21.8)	63.1-163	53	4	99.11	0.040	0.32 (0.07,1.1)

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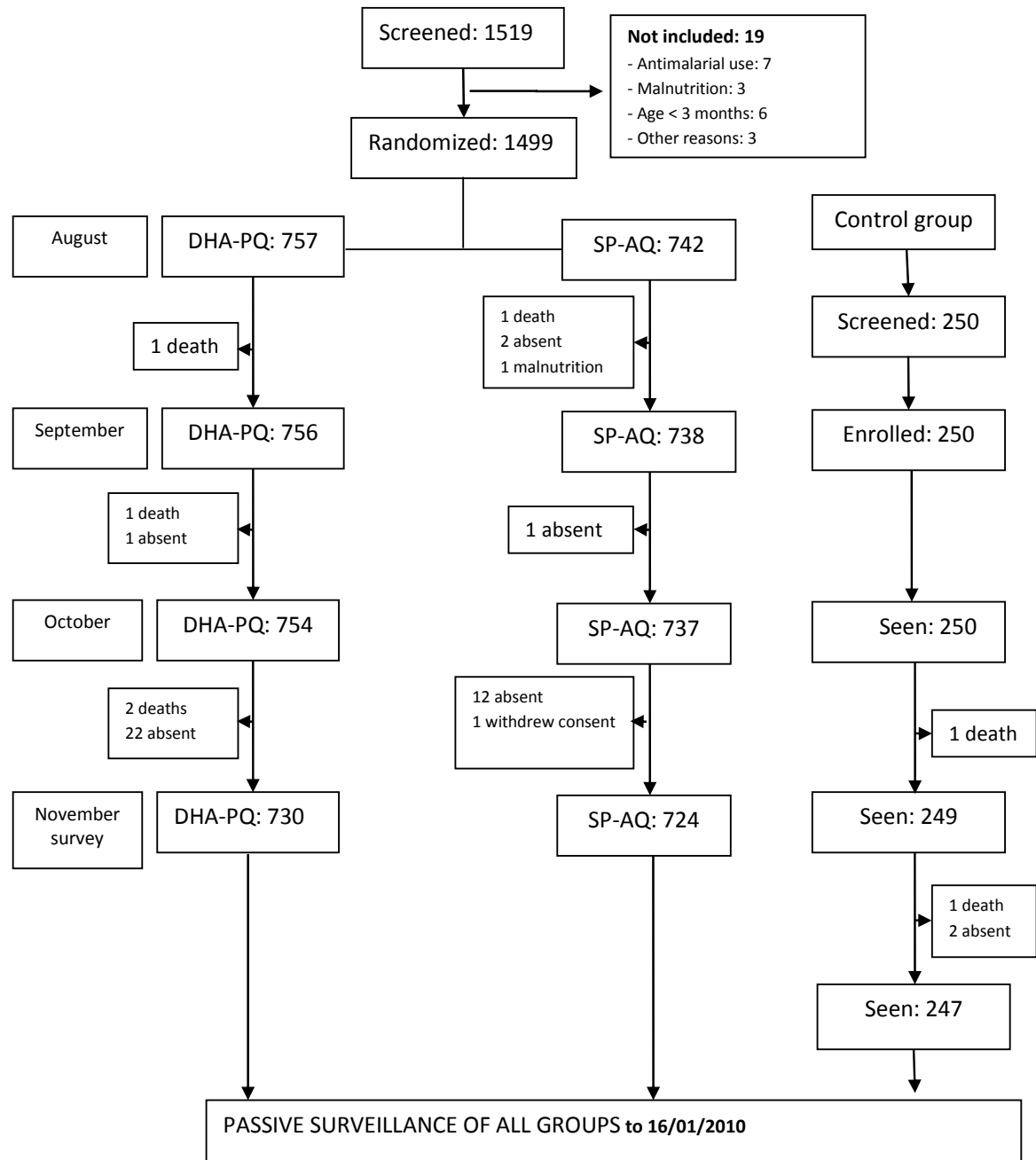
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521 Table 5. Prevalence of molecular markers of resistance among samples from malaria cases.

Genotype	DHAPQ	SPAQ	No SMC	Odds ratio SPAQ:DHAPQ	Odds ratio DHAPQ:NoSMC	Odds ratio SPAQ :NoSMC
<i>pfcr</i> t CVIET T76	62.9% (66/105)	61% (50/82)	61.5% (24/39)	0.9 (0.5 to 1.7), p=0.79	1.1 (0.5 to 2.4), p=0.88	0.98 (0.4 to 2.3), p=0.97
<i>pfmdr</i> 1 Y86	33% (30/91)	44.4% (28/63)	30.4% (24/79)	1.6 (0.8 to 3.3), p=0.14	0.7 (0.3 to 1.5), p=0.33	1.8 (0.9 to 3.9), p=0.08
<i>pfmdr</i> 1 Y184	36.4% (32/88)	32.3% (20/62)	39.2% (31/79)	1.2 (0.6 to 2.5), p=0.6	0.9 (0.5 to 1.7), p=0.7	0.7 (0.3 to 1.6), p=0.3
<i>pfdhfr</i> i51	40.6% (54/133)	64% (64/100)	51.3% (41/80)	2.6 (1.5 to 4.6), p<0.001	0.7 (0.4 to 1.2), p=0.1	1.6 (0.9 to 3.2), p=0.08
<i>pfdhfr</i> s108	58.3% (77/132)	27% (27/100)	70% (56/80)	0.3 (0.1 to 1.5), p<0.001	0.6 (0.3 to 1.1), p=0.08	0.2 (0.1 to 0.3), p<0.001
<i>pfdhfr</i> r59	43.6% (58/133)	71% (71/100)	53.75% (43/80)	3.2 (1.8 to 5.7), p<0.001	0.7 (0.4 to 1.2), p=0.15	2.1 (1.1 to 4.1), p=0.01
<i>pfdhps</i> g437	63.4% (85/134)	84% (84/100)	75% (48/64)	3 (1.5 to 6.1), p<0.001	0.6 (0.3 to 1.2), p=0.10	1.8 (0.7 to 4.1), p=0.15
<i>pfdhps</i> s613	6.5% (19/138)	6.9% (7/101)	13.6% (9/66)	1.1 (0.3 to 3.3), p=0.9	0.4 (0.1 to 1.3), p=0.09	0.5 (0.4 to 1.5), p=0.1
<i>pfmdr</i> 1 Y86 + <i>pfcr</i> t T76 (CVIET)	3.3% (5/149)	6% (7/116)	1.2% (1/81)	1.8 (0.5 to 7.6), p=0.29	2.8 (0.3 to 133), p=0.33	5.3 (0.6 to 234), p=0.09
<i>pfmdr</i> 1 Y184 + <i>pfcr</i> t T76 (CVIET)	3.3% (5/149)	3.4% (4/116)	6.2% (5/81)	1 (0.2 to 4.9), p=0.96	0.5 (0.1 to 2.4), p=0.31	0.5 (0.1 to 2.6), p=0.36
<i>pfdhfr</i> (I51/R59/S108)	30.9% (38/123)	53.2% (50/94)	33.9% (20/59)	2.5 (1.4 to 4.6), p=0.001	1.1 (0.6 to 2.4), p=0.68	2.1 (1.1 to 4.6), p=0.01
<i>pfdhfr</i> (I51/R59/S108) + <i>Pfdhps</i> G437	19.5% (24/123)	41.5% (39/94)	23.7% (14/59)	2.9 (1.5 to 5.6), p<0.001	0.8 (0.3 to 1.8), p=0.5	2.3 (1.1 to 5.1), p=0.02

Figure 1: Trial profile*.



*Twelve allocation errors occurred, 7 children randomized to SPAQ received DHAPQ in error, 2 randomized to SPAQ received mixed treatments, and 3 randomized to DHAPQ received mixed treatments, leaving 754 who received DHAPQ and 740 who received SPAQ in the ATP analysis. At enrolment, 9.4% (70/742) and 9.5% (72/757) of children in the SPAQ and DHAPQ and groups respectively did not receive SMC because they had clinical malaria. These proportions were 7.5% (55/738) and 8.1% (61/756) in September and 8.3% (61/737) and 8.8% (66/754) and in October. Ninety-seven percent (1454/1499) of randomized children were seen at the survey at the end of the transmission season.

Figure 2. Cumulative hazard of malaria (fever or history of fever with any parasitaemia) in children who received SMC with DHA-PQ or SP-AQ on three occasions in August, September and October. A cohort of untreated children were recruited as a control group, at the time the main cohorts received the second round of SMC. The y-axis shows the mean number of episodes per child since the start of surveillance. Malaria episodes were detected by passive detection, and at cross-sectional surveys performed just before each round of SMC.

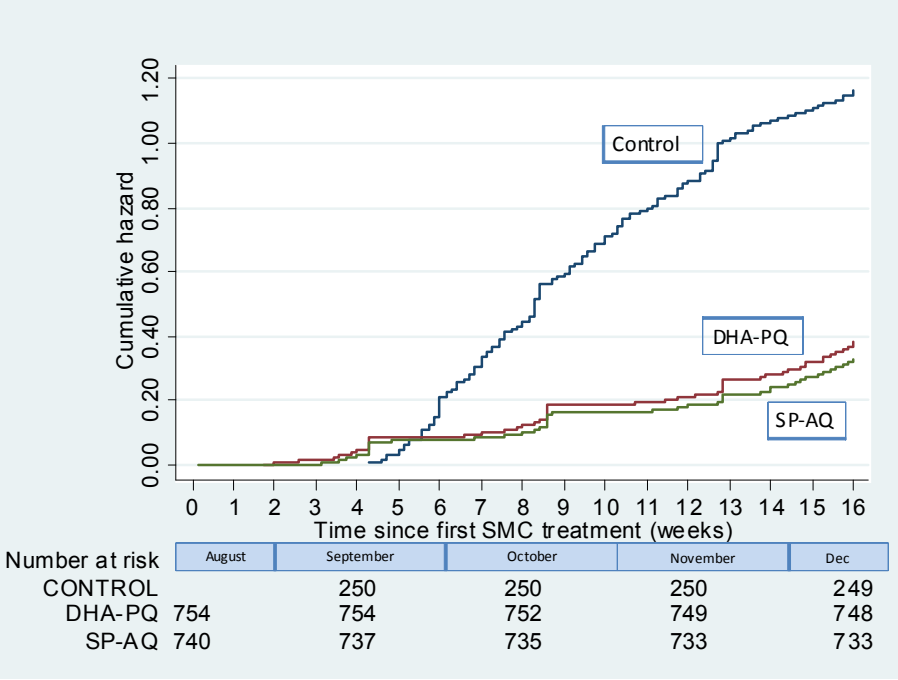


Fig 3. Duration of protection (malaria with any parasitaemia). Smoothed estimate of the hazard ratio was obtained using regression splines using the method of Lambert and Royston [19], the efficacy (1-hazard ratio) with 95% confidence band is plotted against time since the final round of SMC.

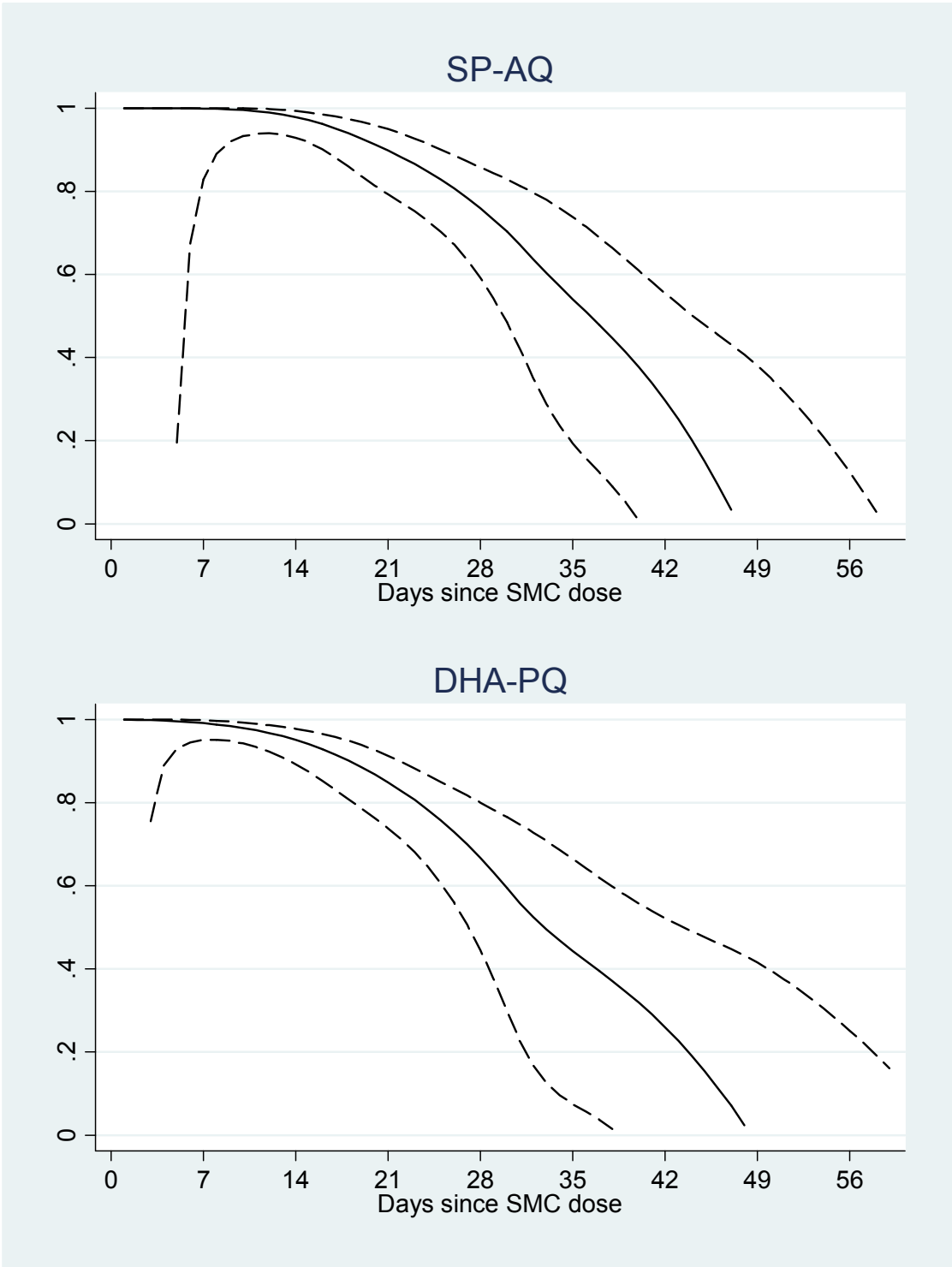


Figure 4a. Kaplan-Meier estimates of the proportion of children with an episode of malaria

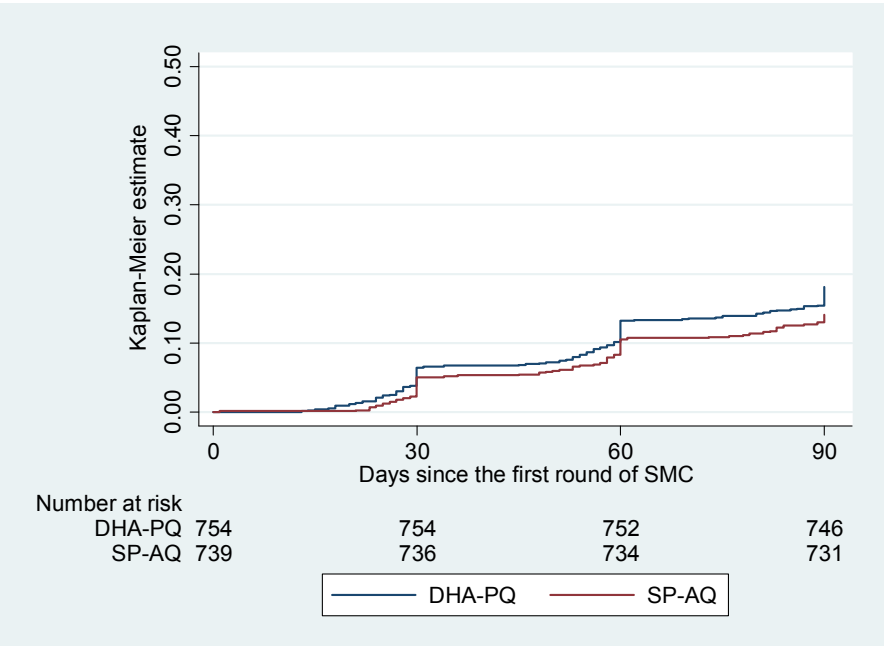
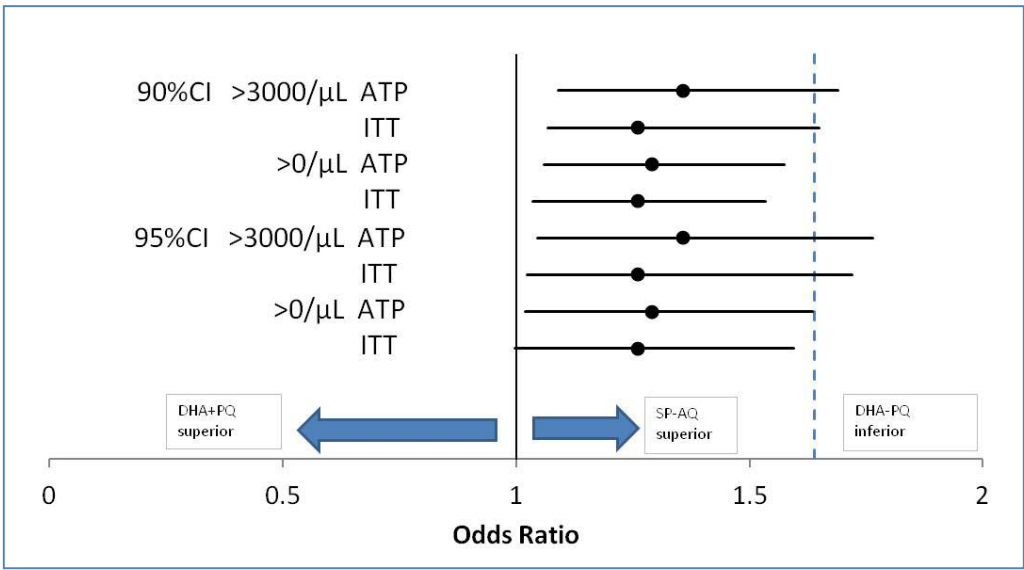
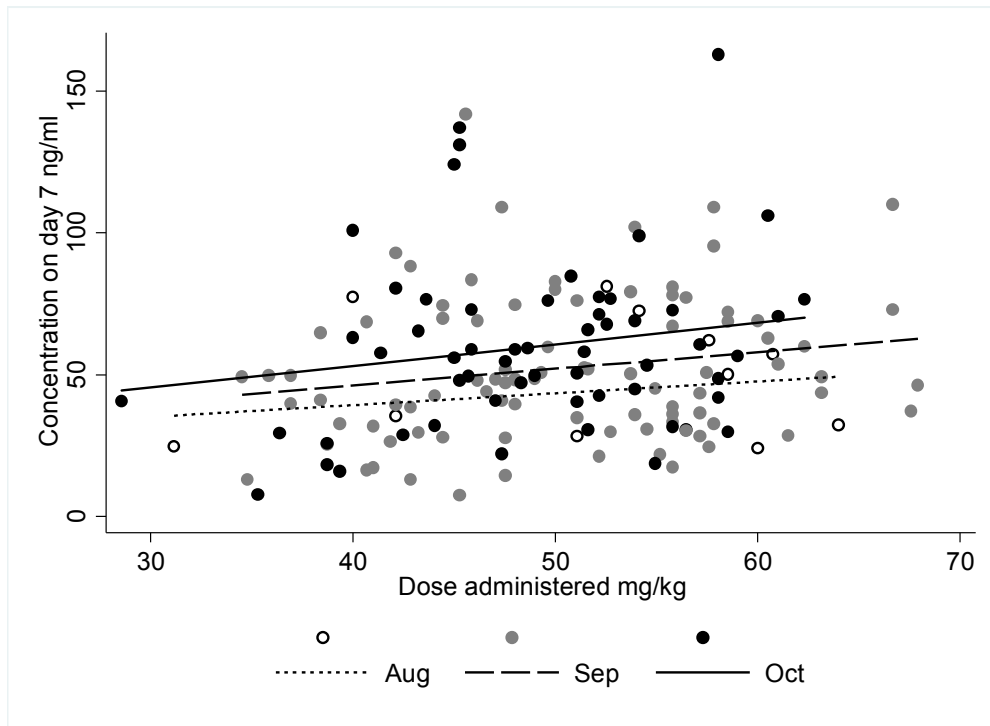


Figure 4b: The diagram shows the 90% and 95% confidence intervals for the odds ratios for ATP and ITT analyses for the primary endpoint (malaria with parasitaemia above 3000/ μ L) and for the secondary endpoint (malaria with parasitaemia at any density). An odds ratio of 1.64 was specified as the non-inferiority margin. The 90% and 95%CI's cross this margin for some analyses, but are entirely above 1, so we are confident SPAQ is superior to DHAPQ, and are somewhat less confident in our conclusion that the DHAPQ is not inferior to SPAQ.



583 Fig 5. Relationship between dose of piperazine administered and plasma concentration on day 7.



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Figure 6. The cumulative hazard of malaria in children who received DHAPQ, according to the dose of piperavaquine administered.

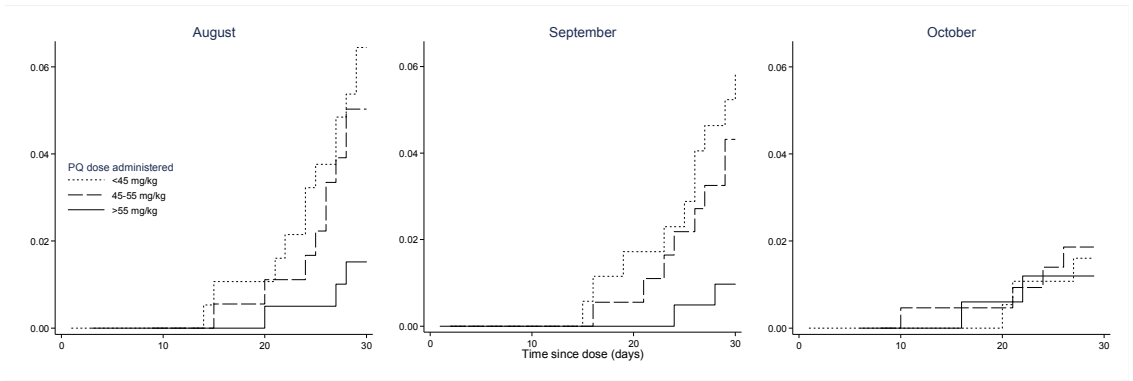
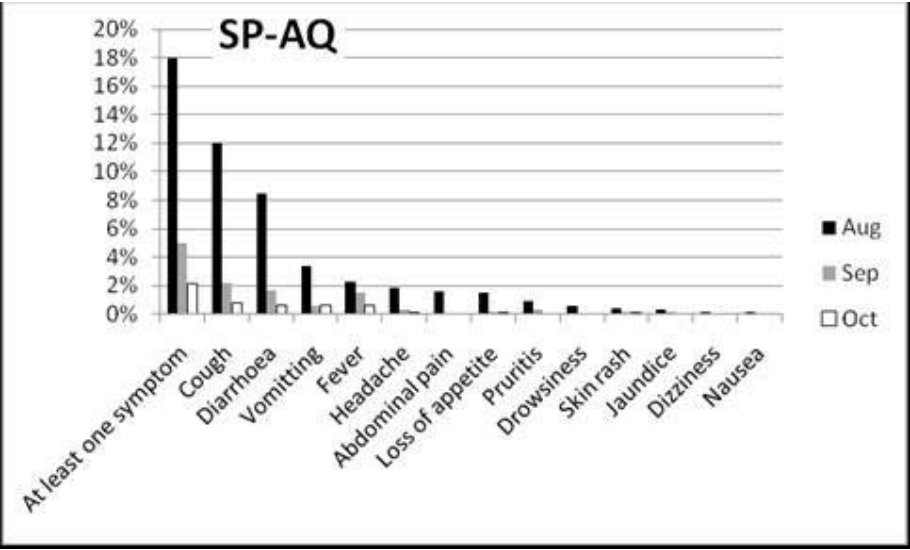
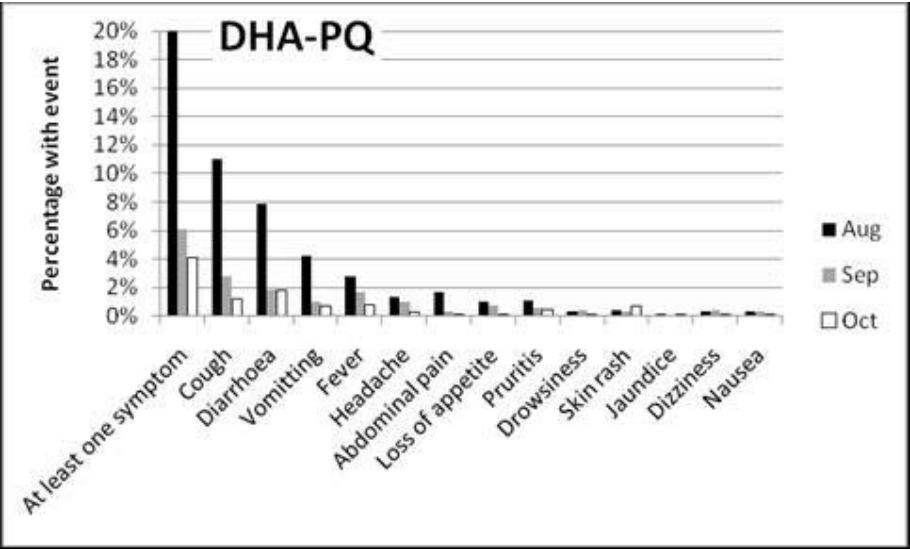


Figure 7: Incidence of mild adverse events during each SMC round.



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