Supplementary information

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

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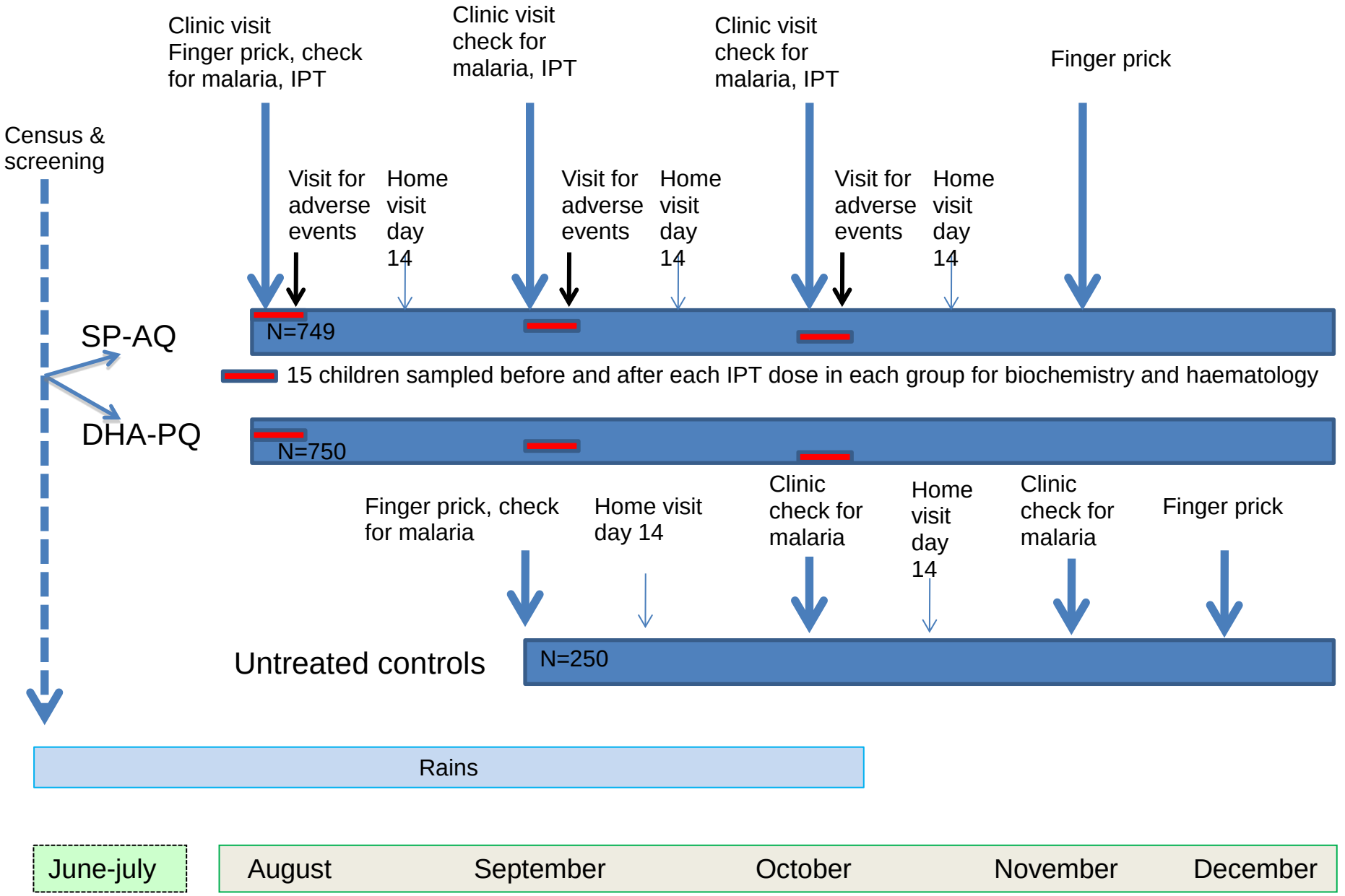
Figure S1: Study plan



Methods:

Measurement of parasitaemia and molecular markers: Parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes, or per 500 leukocytes if the count was <10 asexual parasites/200 leukocytes, assuming a leukocyte count of 8,000/μl. One hundred high power fields were read before a blood smear was considered negative for asexual parasites. Gametocytemia was also determined from thick smears counting against 2000 leucocytes. During follow-up, 2-3 drops of whole blood from finger-prick samples were collected from children who presented to the study clinic with fever, saved onto filter paper, air dried and stored with desiccant. DNA was extracted from these samples using the chelex method (Plowe and Wellems 1995). Mutations in the *pfmdr1* (N86Y, F184Y, D1246Y), *pfdhfr* (N51I, C59R, S108N), and *pfdhps* (A436S, A436F, A437G, K540E, A613S) genes were detected by dideoxy sequencing, and mutations in the *pfcrt* gene (K76T) were detected using RT PCR (Gadalla, Elzaki et al. 2010) in samples obtained during the first episode of malaria following the initiation of SMC.

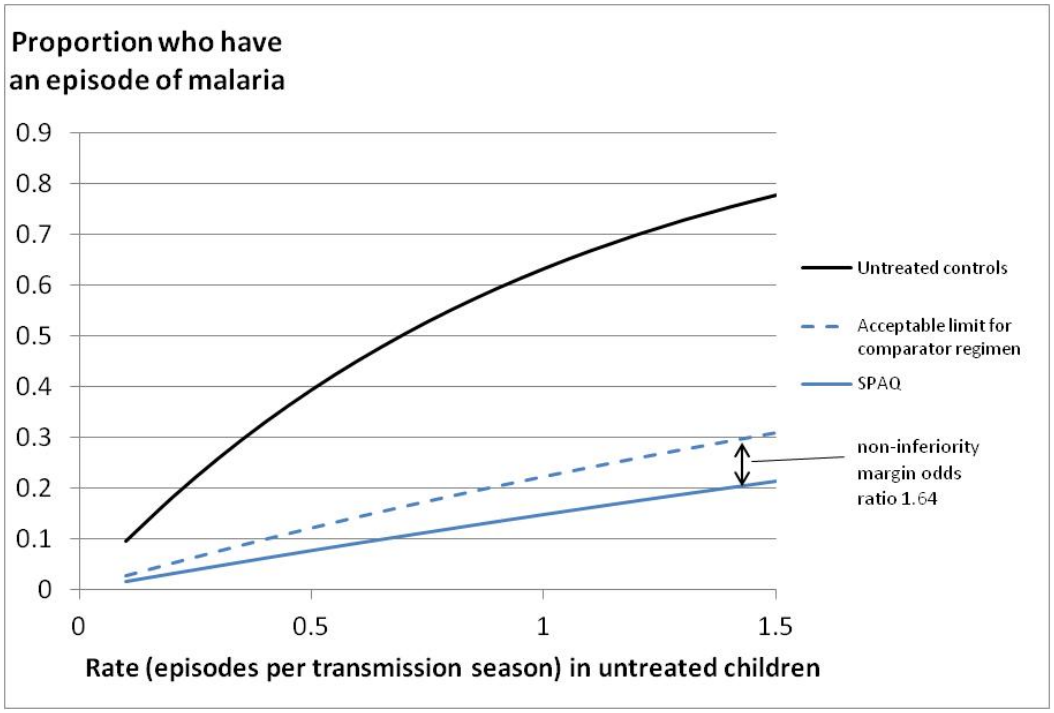
Haematology and biochemistry: A subset of 45 children was identified at randomization for assessment of biochemical and haematological parameters; 15 children were assessed after the SMC rounds in August, September, and October. A venous blood sample of 2.5ml was taken on day 0 and again on day 7, and samples were transported to the biomedical laboratory, Centre Muraz, for processing. Haematology assays were performed using a SYSMEX analyser (Sysmex Corporation, Japan) and biochemical assays using a Lisa 300 Plus biochemistry analyser (Diamond Diagnostics, USA).

Measurement of PQ plasma concentrations: A subset of 210 children in the DHAPQ group was identified at randomization to give additional blood samples for evaluation of the pharmacokinetic properties of piperaquine; 70 children were sampled in August, 70 in September, and 70 in October. PQ concentration was measured on day 0 (immediately before SMC), at a random time between day 0 and day 6, on day 7, and between day 8 and day 30. Samples were collected by finger prick; 200 μl of blood was saved in three capillary tubes, and then centrifuged, and plasma collected in 0.5 ml cryo-tubes, stored at -20 °C before shipment on dry-ice to the Department of Clinical Pharmacology, Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand. PQ concentrations were determined using solid-phase extraction and liquid chromatography coupled with mass spectroscopy detection (Lindegardh et al. 2008).

Statistical methods:

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. We considered the risk, rather than the incidence, since SMC aims to completely protect children from malaria. If the risk was 7% in the SPAQ group, (as observed in the study by Cisse et al. 2009), it was considered that any difference should not exceed 4% to demonstrate non-inferiority of DHAPQ. This is equivalent to an odds ratio of 1.64. It was preferable to specify the margin in terms of the odds ratio, rather than the risk difference, so that the relative difference and hence the power would be approximately constant whatever the risk in the comparison group. To meet this requirement, 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 figure below shows the size of the margin in relation to the risks in untreated and treated children.

Figure S2: Illustration of size of the margin in relation to the risks in untreated and treated children. The expected proportion with malaria (y-axis) corresponding to a given incidence rate of malaria (x-axis)is shown. For a rate λ per child per season, the proportion in the untreated group was calculated assuming a Poisson distribution, as 1-exp(-λ), and the proportion in the SPAQ group calculated assuming an efficacy of 84% (the efficacy of SPAQ in this study), as 1-exp(-λx0.16).



The purpose of the untreated cohort was to estimate the incidence of the primary endpoint in untreated persons. So the sample size was based on the degree of precision we need on this estimate. The 95% confidence interval for the rate was (r/n)xEF where EF is the error factor exp(1.96xsqrt(1/r)), r was the number of events and n the number of child years. We estimated that with a rate of 1 episode per child year at risk, we needed 250 children to have a 95% CI [0.88 to 1.13].

Data were entered using Access 2000 and analysed with Stata 11.0, following an analysis plan written before the end of the trial. For efficacy endpoints, both intention-to-treat (ITT) and according-to-protocol (ATP) analyses were done. The ITT population included all randomized children, analysed in the group they were assigned at randomization. For the ATP analysis, we excluded children who did not attend for an SMC treatment round. Six children who attended and received SMC but did not complete all the daily doses were included in the ATP. Children who attended, but did not receive SMC because they had malaria and were treated with AL were

included in the ATP analysis – excluding them would lead to a bias, these children followed the protocol but received AL in place of SMC drugs. The WHO 2006 growth standard was used to determine the nutritional status of the children. Two episodes of malaria occurring seven or more days apart were considered two different episodes. For the primary analysis, time at risk was assumed to start at randomization (when the child received their first dose of SMC), and end one month after the last SMC round. For children who died or were known to have left the study areas, observations were censored at the date of death or emigration. 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 proportion of children who had malaria and its standard error, using the delta method. This gives the standard error of the log odds ratio as s=v{V(p1)/[p1(1-p1)]2+V(p0)/[p0(1-p0)]2} where p1 and p0 are the Kaplan meier failure estimates and V(p1), V(p0) their variances estimated by Greenwood's formula, and the odds ratio is OR=[p1/(1-p1)]/[p0/(1-p0)] and confidence limits OR.exp(1.96xs) to OR/exp(1.96s).

For comparisons with the control group, time at risk started on the date of SMC round 2, or the date of enrolment for the untreated cohort, and ended one month after SMC round 3, and efficacy of SMC (the percentage reduction in the number of episodes) was estimated as 100x(1-R) where R is the hazard ratio from Cox regression, with confidence intervals calculated using a robust standard error to account for repeated malaria episodes in the same child. Adjustment for covariates was critical because this was a non-randomized comparison, the effects of age (age at entry in whole years), ITN use at baseline, village, and nutritional status at entry, were assessed by including these variables in the regression model, variables which did not contribute (no evidence of confounding and not associated with outcome) were removed from the model. adjusted for effects of age, ITN use and nutritional status at baseline by including these variables in the regression model. Follow-up was continued until 16th January 2010 and in a further analysis of duration of protection, time at risk was assumed to continue until this date. To assess association of piperaquine concentration with protection, the logrank test for trend, stratified by month, was used. Analysis of covariance was used to estimate the difference between groups in the mean of each biochemical and haematological parameter, with the day 0 value included as a covariate, pooled across months.

Serious adverse events: 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 (one in each group) occurred at home. In the SPAQ group, a boy aged 2yrs died 26 days after the August round, from dehydration, and a boy aged 3 yrs died 19 days after the September round, with gastro-enteritis. In the DHAPQ group, a boy aged 20

months died 24 days after the August round of SMC, with respiratory distress; a girl aged 5 months died 14 days after the September round of SMC, with gastroenteritis; a girl aged 1 year died 34 days after the October round with gastroenteritis; and a girl aged 21 months died 8 days after the October round, with pneumonia. None of these deaths was considered related to SMC. In the untreated cohort, a girl aged 3 yrs died in November, with malnutrition.

Additional references:

Lindegardh, N., A. Annerberg, et al. (2008). "Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of piperaquine in plasma stable isotope labeled internal standard does not always compensate for matrix effects." J Chromatogr B Analyt Technol Biomed Life Sci **862**(1-2): 227-36.

Plowe, C. V. and T. E. Wellems (1995). "Molecular approaches to the spreading problem of drug resistant malaria." Adv Exp Med Biol **390**: 197-209.

Table S1: Haematological and biochemical variables measured before and after SMC:

	DHAPQ			SPAQ			Adjusted difference	
Variable	Day 0	Day 7	N[#]	Day 0	Day 7	N[#]	between groups on day 7 SPAQ-DHPQ (95%CI)*	P-value
Haemoglobin g/dL	10.9	10.4	37	10.6	11.3	47	1.03 (0.51,1.55)	0.000
PCV %	33.1	32.5	37	32.4	34.4	47	2.31 (0.86,3.77)	0.002
Platelets10^3/ mm^3	234	319	37	276	304	47	-28 (-84,28)	0.316
White blood cells10^3/ mm^3	8151	8714	37	7665	9035	48	599 (-767,1963)	0.386
Red blood cells10^6/mm^3	4.284	4.242	37	4.082	4.370	48	230 (48,412)	0.014
Neutrophils, cells/ mm^3	3083	3474	32	2994	3505	41	53 (-925,1031)	0.914
Eosinophils, cells/ mm^3	233	173	32	194	223	41	54 (-42,151)	0.266
Basophils, cells/ mm^3	3.00	0.00	32	1.24	3.61	41	3.6 (-2.2,9.3)	0.219
Lymphocytes, cells/ mm^3	4752	4830	32	4283	4974	41	393 (-360,1147)	0.302
Monocytes, cells/ mm^3	129	118	32	118	129	41	15 (-38,69)	0.575
Creatinine, µmol/litre	34.2	34.3	17	42.6	50.2	26	13 (-2.1,29)	0.089
Tgoa, IU/litre	54.4	53.1	17	56.2	59.5	25	6.4 (-10,23)	0.449
Tgpa, IU/litre	29.8	35.9	17	45.5	43.4	25	5.4 (-12,23)	0.534
Vgm, Fl	77.6	77.1	37	78.6	79.0	47	1.3 (-0.81,3.3)	0.229
Tcmh pg/GR	25.6	24.8	37	26.2	26.0	47	0.70 (-0.06,1.5)	0.069
Ccmh g/dL	32.8	32.1	37	32.8	33.0	47	0.95 (0.03,1.9)	0.044

[#]No. of children sampled on both days

*difference between group means on day 7, adjusted for the day 0 value

Table S2: Incidence of mild adverse events in children who received DHAPQ or SPAQ

	DHAPQ			SPAQ			Risk difference (95%CI)		
	Aug	Sep	Oct	Aug	Sep	Oct	Aug	Sep	Oct
	N=707	N=726	N=735	N=685	N=713	N=709			
At least one symptom	20%	6.1%	4.1%	18%	5.0%	2.1%	2.80% (1.20%,12.10%)	-1.00% (-3.40%,1.40%)	-2.00% (-3.70%,-0.20%)
Cough	11%	2.8%	1.2%	12%	2.2%	0.80%	1.80% (1.80%,8.50%)	-0.50% (-2.10%,1.10%)	-0.40% (-1.40%,0.70%)
Diarrhoea	7.9%	1.8%	1.8%	8.5%	1.7%	0.60%	1.00% (0.70%,3.40%)	-0.10% (-1.50%,1.20%)	-1.20% (-2.30%,-0.10%)
Vomitting	4.2%	1.0%	0.70%	3.4%	0.60%	0.60%	1.70% (0.80%,2.30%)	-0.40% (-1.30%,0.50%)	-0.10% (-0.90%,0.70%)
Fever	2.8%	1.7%	0.80%	2.3%	1.5%	0.60%	1.00% (0.30%,1.80%)	-0.10% (-1.40%,1.20%)	-0.30% (-1.10%,0.60%)
Headache	1.3%	1.0%	0.30%	1.8%	0.30%	0.10%	0.30% (0.10%,1.60%)	-0.70% (-1.50%,0.10%)	-0.10% (-0.60%,0.30%)
Abdominal pain	1.7%	0.30%	0.10%	1.6%	0.00%	0.00%	0.70% (0.10%,1.50%)	-0.30% (-0.70%,0.10%)	-0.10% (-0.40%,0.10%)
Loss of appetite	1.0%	0.70%	0.10%	1.5%	0.10%	0.10%	0.60% (0.40%,0.90%)	-0.50% (-1.20%,0.10%)	0.00% (-0.40%,0.40%)
Pruritis	1.1%	0.60%	0.40%	0.9%	0.30%	0.00%	0.40% (0.10%,0.60%)	-0.30% (-0.90%,0.40%)	-0.40% (-0.90%,0.10%)
Drowsiness	0.30%	0.40%	0.10%	0.6%	0.00%	0.00%	0.30% (0.70%,0.40%)	-0.40% (-0.90%,0.10%)	-0.10% (-0.40%,0.10%)
Skin reaction	0.40%	0.30%	0.70%	0.4%	0.10%	0.10%	0.00% (0.10%,0.30%)	-0.10% (-0.60%,0.30%)	-0.50% (-1.20%,0.10%)
Jaundice	0.10%	0.00%	0.10%	0.3%	0.10%	0.00%	0.40% (0.10%,0.10%)	0.10% (-0.10%,0.40%)	-0.10% (-0.40%,0.10%)
Dizziness	0.30%	0.40%	0.10%	0.1%	0.00%	0.00%	0.30% (0.10%,0.10%)	-0.40% (-0.90%,0.10%)	-0.10% (-0.40%,0.10%)
Nausea	0.30%	0.30%	0.10%	0.1%	0.00%	0.00%	0.00% (0.00%,0.00%)	-0.30% (-0.70%,0.10%)	-0.10% (-0.40%,0.10%)

Table S3: Prevalence of anaemia at the end of the transmission season (ITT)

	SPAQ	DHAPQ	Control
No.	713	719	243
Mean Hb g/dL (SD)	9.4 (3.3)	9.5 (3.3)	9.8 (1.9)
% Hb<8	16%	15%	14%
% Hb<11	65%	63%	72%

Table S4: Prevalence of anaemia in relation to dose administered

SPAQ					
Sulfadoxine dose mg/kg*	No.	Mean Hb g/dL	Age-adjusted difference#	% <8g/dL	Age-adjusted prevalence ratio#
<23.8	215	9.7	-	0.14	1
23.8-26.8~	220	9.7	0.20 (-0.47,0.86)	0.13	1.0 (0.62,1.6)
>=26.8	211	9.2	0.52 (-0.15,1.2)	0.19	1.3 (0.84,2.0)

DHAPQ					
Piperaquine dose mg/kg*	No.	Mean Hb g/dL	Age-adjusted difference#	% <8g/dL	Age-adjusted prevalence ratio#
<48	220	8.9	-	0.22	1
48-55~	212	10.3	1.1 (0.45,1.7)	0.06	0.33 (0.18,0.60)
>=55	227	9.3	0.42 (-0.18,1.0)	0.19	0.84 (0.59,1.2)

*based on dose at round 1
based on 627 observations