Impact of *Plasmodium falciparum* Sulphadoxine-Pyrimethamine Resistance on the Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy in Africa: A Systematic Review and Meta-Analysis

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Abstract

Background

Plasmodium falciparum resistance to sulphadoxine-pyrimethamine (SP) threatens the efficacy of intermittent preventive treatment (IPTp) for malaria in pregnancy in Africa. We conducted a meta-analysis to assess the impact of SP resistance on IPTp-SP effectiveness.

Methods

We searched databases (1990 to March-01-2018) for clinical studies (aggregated data) or surveys (individual-participant data) containing information on low birthweight (LBW, primary outcome) and malaria by IPTp-SP dose, and for studies reporting SP-resistance molecular markers. We performed random-effects meta-analysis (clinical studies) or multivariate log-binomial regression (surveys) to obtain summarized dose-response data (Relative-Risk-Reduction:RRR) and multivariate meta-regression to explore modifying effects of SP-resistance (*dhps* substitutions A437G, K540E, A581G).

Findings

Of 1097 records, 57 studies were included in the aggregated-data meta-analysis (59,457 births). The RRR for LBW declined with increasing prevalence of *Pfdhps*-K540E (P-trend=0.0060) but not with *Pfdhps*-A437G (P-trend=0.35). The RRR in areas of high (*Pfdhps*-K540E >90%, n=11), moderate (Central/West Africa:*Pfdhps*-A437G≥90% or East/southern Africa:*Pfdhps*-K540E 30-90%, n=16) and low SP-resistances (n=30) were 7% (95% CI 0-13), 21% (14-29) and 27% (21-33) respectively (P-trend=0.0054, I^2=69.5%). In the individual-participant analysis of 13 surveys (42,394 births), IPTp-SP was associated with reduced LBW in areas with *Pfdhps*-K540E>90% & *Pfdhps*-A581G<10% (RRR=10%, 7-12), but not those with *Pfdhps*-A581G>=10% (pooled *Pfdhps*-A581G prevalence:37%, range 29-46) (RRR=0.5%, -16-14, n=3).

Interpretation

The effectiveness of IPTp-SP is reduced in areas with high SP-resistance, but IPTp-SP remains associated with reduced LBW in areas where *Pfdhps*-K540E prevalence exceeds 90%. IPTp-SP is not effective in areas with ≥37% prevalence of the highly-resistant sextuple *Pfdhps*-A581G-containing genotype.

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Research in context

Evidence before this study

We searched four databases (the Malaria in Pregnancy Library, PubMed, Web of Science, and Scopus) for studies in sub-Saharan Africa of the ecological relationship between molecular markers of SP resistance and the effectiveness of IPTp with SP on LBW, preterm birth, maternal malaria infection and maternal anaemia published in the English language up to March 01, 2018. The following search terms were used: "Malaria AND pregnan* AND (intermittent OR IPT) AND Review". Four studies, including one prospective multi-country study in eight sites, two meta-analyses and one modelling study were identified. The prospective study which included a 42-day therapeutic efficacy study, showed a strong ecological correlation between the prevalence of molecular markers for SP resistance and the ability of SP to clear existing infections or the duration of post-treatment prophylaxis in women receiving IPTp-SP. There was no clear trend between resistance and the effectiveness of SP to reduce LBW, maternal anaemia or *Plasmodium* infections, but none of the studies were conducted in areas where the highly resistant sextuple mutant parasites were common. One of the two meta-analyses compared, among other regimens, the effect of IPTp-SP against no intervention or placebo; no protective effect of IPTp-SP against LBW was detected in areas with >50% *Pfdhps*-K540E mutation prevalence, but this analysis was based on three studies, only one of which was in areas where the sextuple mutant parasites were common. By contrast, the other meta-analysis included nine studies and found no evidence for reduced effectiveness in areas with high SP resistance; the small number of studies in these highly resistant areas (n=3) was a limiting factor. The modelling study estimated the number of pregnancies occurring in the different SP resistance strata in Africa based on the *Pfdhps*-K540E and A581G mutations. It also modelled the relationship between the effectiveness of IPTp-SP on LBW at differing levels of resistance using an existing model of parity-dependent immunity to placental malaria combined with data from the multi-country observational study that assessed the ability of SP to clear existing infections among women receiving IPTp-SP. It did not study the relationship between the impact of SP resistance and the effectiveness of SP directly but did suggest that, even accounting for SP resistance, extending IPTp-SP to all women attending ANC would have a sizeable and cost-effective impact on maternal and infant health. This inference was valid in almost all malaria-endemic settings in sub-Saharan Africa with low to high resistance; the single exception was the highly resistant areas where parasites with the sextuple mutant genotypes are common.

Added value of this study

This is the most comprehensive study of the impact of SP resistance on the effectiveness of IPTp involving 57 studies, 13 surveys, and more than 100,000 births. The results of the aggregated data meta-analysis indicate substantial heterogeneity in effect size between studies, which may explain both the contradictory findings between the two previous smaller reviews and the ongoing controversy about the continued use of IPTp with SP in areas of high resistance We report for the first time a clear trend towards reduced effectiveness of IPTp-SP on LBW and *P. falciparum* infection with increasing prevalence of molecular SP resistance markers. A beneficial association of SP protection against LBW remains evident even in areas of high resistance where parasites with the quintuple *Pf*dhfr/*Pfdhps* mutant haplotype are essentially fixed. However, three observational cohort studies published elsewhere suggest that these beneficial effects are no longer apparent in individual women infected with the highly-resistant sextuple mutant parasites additionally carrying the quintuple mutant haplotype and the *Pfdhps*-A581G mutation.

Implications of the available evidence

The overall available evidence to date suggests that the effect of IPTp-SP in reducing malaria infection, anaemia, and LBW declines as resistance increases, but overall the effect of SP on decreasing LBW is surprisingly resilient. Use of IPTp-SP remains associated with lower risks of LBW even in areas where SP fails to clear a third of asymptomatic infections in women receiving SP for IPTp. These findings support the World Health Organization (WHO) recommendation to continue using SP for IPTp in these high resistance areas. The important exception is in areas with ≥37% prevalence of sextuple mutant parasites. In these areas, alternative preventive strategies are required now. The substantial levels of heterogeneity between studies, even in areas with similar levels of resistance, suggest that single observational studies of the relationship between SP doses and LBW may not be informative as a policy decision tool. Our improved understanding of the ecological relationship between molecular markers of SP resistance and the effectiveness of IPTp-SP suggests a molecular decision tool using just two or three markers in the *Pfdhps* gene could now be considered to guide IPTp-SP policy.

Introduction

Without pregnancy-specific protection, an estimated 45% of 32 million pregnancies in malaria-endemic sub-Saharan Africa are exposed to *Plasmodium falciparum* malaria,[1] leading to 900,000 malaria-associated low birthweight (LBW) deliveries annually[2] and associated consequences to infant health.[3] In these areas, the World Health Organization (WHO) recommends intermittent preventive treatment in pregnancy (IPTp) with antimalarials. IPTp with sulphadoxine-pyrimethamine (SP), the only antimalarial currently recommended for this strategy, is associated with marked reductions in maternal anaemia, LBW, and neonatal mortality.[4]

However, IPTp-SP effectiveness is threatened by SP resistance, particularly in East/southern Africa. In *P. falciparum* (*Pf*), SP resistance results from a series of single nucleotide polymorphisms in the parasites dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthase (*Pfdhps*) genes. Sextuple mutant parasites, defined as those that harbour the *Pfdhps*-A581G mutation in addition to the five most common substitutions (*Pfdhfr* substitutions N51I, C59R and S108N and *Pfdhps* substitutions A437G and K540E), are associated with enhanced levels of resistance *in-vitro*, SP treatment failure in patients with acute malaria,[5-7] and failure to inhibit parasite growth or prevent malaria-associated foetal growth restriction in pregnant women.[8-11] At the ecological level, a high prevalence of *Pfdhps*-K540E negates the efficacy of SP as IPT in infants and children,[12,13] undermines SP's ability to clear existing *P. falciparum* infections in asymptomatic pregnant women,[14,15] and shortens the post-treatment prophylactic period following IPTp.[14]

Despite these effects, there are currently no guidelines by which to use molecular prevalence data to inform the use of SP for IPTp.[16] The ecological relationship between molecular measures of SP resistance and the impact of IPTp-SP on clinically-relevant birth outcomes such as LBW is not clear; previous attempts to define these relationships reached conflicting conclusions,[17,18] possibly reflecting substantial between-study heterogeneity in the impact of SP on LBW.[14,18] To address this further, we used all available data derived from observational studies, clinical trials, and national surveys in sub-Saharan Africa to conduct a meta-analysis of the ecological relationship between molecular markers of SP resistance and the impact of IPTp-SP on LBW. We hypothesized that a higher prevalence of SP resistance, as expressed by the prevalence of molecular markers of sulphadoxine resistance, would be associated with an attenuation of the IPTp-SP associated reduction in LBW.

Methods

Search strategy and selection criteria

This analysis was completed following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (Appendix, page 41). The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO:CRD42016035540).

Studies of IPTp effectiveness

Two main sources of data on IPTp effectiveness were used: Aggregated data from observational studies and clinical trials, henceforth referred to as 'clinical studies', and individual-participant data from nationally representative surveys ('surveys'). The clinical studies were identified by two independent reviewers (AMvE and GK) by searching trial registries and electronic databases from 1990 to March 01, 2018 without language restrictions, and by scanning reference lists of articles and consultation with experts in the field (Appendix, page 2). Surveys were identified by searching all publicly available national-level survey datasets from malaria endemic countries in Africa[19-21]

conducted after the year 2000 (when WHO introduced the IPTp-SP policy) as described in detail elsewhere (DL).[4] See Appendix for sources and eligibility criteria (page 2-3).

Data on SP resistance and malaria transmission

Data on molecular markers of SP resistance were obtained from the clinical study reports or from the reports' authors. If these were not available, data were obtained from existing population prevalence maps of the *Pfdhps* mutations using the molecular surveyor tool of the World Wide Antimalarial Resistance Network (WWARN) and existing prediction surfaces of SP resistance mutation prevalence based on these data.[16,22-24] Malaria transmission intensity data were obtained from the Malaria Atlas Project.[25]

Data extraction and quality assessment

IPTp effectiveness data

From clinical studies, data extraction was conducted independently by two investigators (AMvE and GK or DECS). Authors of primary studies were contacted for missing information or if reported data did not fit the required format. The following information was extracted: 1st author, publication year, year of study start and end, study design, study and randomisation procedures (trials only), inclusion criteria (e.g. any restrictions by gravidity), insecticide treated net (ITN) use, numerator/denominator per outcome per SP dose, and details of control group's intervention (trials only). If available, SP resistance data were extracted. Study quality was assessed using an adaptation of the Newcastle-Ottawa Scale (AMvE and GK or DECS) (Appendix, page 3).[26]

From surveys, the following data were extracted: Reported number of SP doses received; composite of LBW (<2.5 kg) if measured birthweight was available or perceived small birth size (very small or small) if it was not available (the correlation between perceived and measured LBW has been described elsewhere[4]), and measured birthweight as a continuous variable.[4] Other data extracted included number of antenatal visits, tetanus vaccination, iron supplementation and ITN ownership, household socioeconomic status, mother's education, mother's age, parity, birth spacing, newborn sex, season of birth, and whether it was a single or multiple birth.

Prediction surfaces of SP resistance mutation prevalence

Prevalence data of *Pfdhps*-A437G, K540E and A581G mutations were extracted from the clinical studies in pregnant women, the literature, and existing molecular surveyor databases.[16,22-24] These mutations in *Pfdhps* were chosen over the major resistance mutations in *dhfr* because the *Pfdhps* mutations have a more geographically heterogeneous distribution, reflecting their more recent emergence in Africa.[27] The *Pfdhps*-K540E prevalence served as a proxy for the prevalence of quintuple *Pfdhps*/*Pf*dhfr mutant genotype.[7] In areas where the prevalence of this quintuple mutant was >50%, the prevalence of *Pfdhps*-A581G mutation served as a proxy for the sextuple mutant. In West Africa, *Pfdhps*-A581G may occur independent of the *Pfdhps*-K540E mutation. These mutations were not considered sextuple mutants if the *Pfdhps*-K540E prevalence was <=50%.[16] Presence was defined as a prevalence of ≥1%. Raster files from existing point data maps from areas that had previously been defined as 'super-resistant' (>10% prevalence of sextuple mutant) were obtained from the authors.[16] Two such areas were identified: in north-eastern Tanzania, and in the area crossing the borders of Southwest Uganda and East Rwanda, eastern Democratic Republic of Congo, and north-western Tanzania.[16]

Matching IPTp effectiveness, resistance and malaria transmission intensity data

The prevalence of each point mutation and the *Pf*PR_{2-10} (the *Plasmodium falciparum* parasite rate in 2-10 year olds)[25] was matched to each study by time (the same year for *Pf*PR_{2-10} and +/- 2 years for

point mutations) and location using latitude and longitude (within 300 km).[28] Location was defined as the site of the main research facility in the observational studies and trials, or the midpoint between study locations. The following order of preference was used to match resistance with clinical data: a) resistance data provided in the clinical study reports or by the authors for that location and time of study (data from individuals with a recent history of SP intake were excluded); b) estimates from continuous surface maps from WWARN's geospatial models for *Pfdhps*-A437G and *Pfdhps*-K540E;[24] and c) for *Pfdhps*-A581G, or for studies after 2012, data were used from existing population prevalence maps of *Pfdhps* (Appendix, page 9).[16,22,23] For national surveys, the mean prevalence of *Pf*PR_{2-10} and *Pfdhps* mutations was calculated for the administrative-2 boundary of the given survey using Malaria Atlas Project data[25] and WWARN's geospatial models.[24] All matching for surveys was performed using the Raster-Package of R (v3.3.2).[29]

Statistical Analysis

The primary outcome was LBW. Secondary outcomes included anaemia, malaria, preterm delivery, birthweight, haemoglobin level, and gestational age. Analyses of clinical studies was conducted in Stata v14. A two-stage random-effects meta-analysis was performed using generalized least square (GLST) regression for trend estimation of summarized dose-response data.[30,31] The effect sizes were expressed as Relative Risk Reduction (RRR, 100 x [1-RR]) for trend, Appendix, page 4). These were then combined across studies using random effect meta-analysis with heterogeneity quantified using the I^2 statistic. Potential modifying effects of SP resistance were examined using multivariate linear meta-regression adjusting for malaria transmission, study quality, average number SP doses and proportion of paucigravidae in a study.[32] The proportion of women using ITNs was not associated with resistance level and not included as co-variate. Subgroup analyses by gravidity (paucigravidae vs multigravidae) were conducted. For the assessment of the effect of IPTp-SP on continuous outcomes, only the 0 vs 2+ dose groups were compared. Further sensitivity analysis was conducted by excluding low quality studies and exploring the presence and impact of potential small-study effects due to publication and other bias[33] (Appendix, page 4).

The survey analysis was conducted in R and restricted to the higher resistance areas with >80% *Pfdhps*-K540E prevalence. Only the most recent live birth in the past <2 years was considered. To mitigate potential confounding of the effect of SP dose on birthweight, exact matching was employed (Appendix, page 4).[4] The modifying effect of SP resistance was first assessed for each survey using random effects log-binomial regression models for LBW and linear regression for birthweight with the matched birth strata included as a random intercept using the lme4 package in R.[34] IPTp exposures were considered as continuous variables similar to the aggregated meta-analysis. The effect measures were then further evaluated by resistance strata (quintiles) and compared using meta-regression.

Defining resistance categories

To stratify resistance into low, moderate, and high levels, different combinations of threshold levels (at 5% step increases) for the *Pfdhps* resistance mutations were explored in the aggregated-data meta-analysis. Because of distinct parasite populations and distributions of mutations in each region,[35] threshold analysis was conducted separately for Central/West and for East/southern Africa. Results were then combined to obtain a single categorical variable that represented the optimal thresholds based on the R-squared for each region.

Role of the funding source

Except for CDC and WWARN, the funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. CDC and WWARN staff participated in the conduct of the study. AMvE and DL had full access to all the data in the study. AMvE and FtK had final responsibility for the decision to submit for publication.

Results

Aggregated meta-analysis clinical studies

Included studies

Sixty-six of 1097 articles assessed for eligibility were included, comprising 58 observational studies and 8 trials (Figure 1; Appendix, page 6). Of these, 50 source studies from 17 countries (Appendix, page 30) were included in the analysis of LBW, involving 57 data points (henceforth referred to as studies) and 59,457 births. In Central/West Africa (n=31 studies), the median (range) of *Pfdhps*-A437G and *Pfdhps*-K540E prevalences were 57.9% (15.2-100) and 0.1% (0.0-18.9), compared to 85.4% (13.3-100) and 85.8% (0-100) in East/southern Africa (n=26) (Appendix, page 27). In East/southern Africa, *Pfdhps*-K540E prevalence closely mirrored that of *Pfdhps*-A437G, yet in Central/West Africa, high prevalence of *Pfdhps*-A437G occurred despite a paucity of *Pfdhps*-K540E (Appendix, page 31). As a proxy for the sextuple mutant, the *Pfdhps*-A581G mutation mainly occurred in areas with >80% *Pfdhps*-K540E in East/southern Africa (Appendix, page 27). Among SP recipients, the median (inter-quartile range) number of SP doses received (study-level) was 1.7 (1.3-2.4, Appendix, page 13). The number of SP doses was not correlated with prevalence of *Pfdhps*-A437G (r=-0.0295, p=0.83) or *Pfdhps*-K540E (r=0.1594, p=0.24).

IPTp-effectiveness on LBW

Overall, per incremental dose of SP, IPTp-SP was associated with a RRR for LBW of 21% (95% CI 17-25, N=57 studies) (Figure 2) (paucigravidae: RRR=22% [17-27], N=34; multigravidae RRR=18% [11-24], N=31, Appendix, page 17). There was substantial heterogeneity between studies (I^2=69.5%, Figure 2). Meta-regression showed a linear trend towards decreasing effectiveness with increasing prevalence of *Pfdhps*-K540E (Table 1, Figure 3, $P_{unadjusted}$=0.0060, $P_{adjusted}$=0.0031). No differences were seen by gravidity (Appendix, page 16). In contrast, no significant trend was observed for *Pfdhps*-A437G (Table 1, Figure 3). Of the different threshold options to stratify resistance into low, medium, and high, the most predictive combination was: low-resistance: *Pfdhps*-A437G <90% in central/west Africa or *Pfdhps*-K540E <30% in east and southern Africa (RRR=27%, 95% CI 21-33); moderate: *Pfdhps*-A437G ≥90 (Central/West) or *Pfdhps*-K540E ≥30% & <90% in East/Southern Africa (RRR=21%, 14-29); high: *Pfdhps*-K540E ≥90% in east and southern (RRR=7%, 0-13) (Figure 1; P-value for linear trend=0.0043, Table 1). This definition explained 22.8% (R^2) of the between study variance in univariate models and 55.2% in multi-variate models (Table 1). Very similar results were obtained if 20% or 40% was used for the lower *Pfdhps*-K540E threshold or 80% for the lower *Pfdhps*-A437G, or the presence (≥1%) of *Pfdhps*-A581G instead of *Pfdhps*-K540E ≥90 for the upper threshold (Appendix, page 18).

Similar conclusions could be drawn after excluding low-quality studies (Table 1). There was evidence for significant small-study effects (P<0.0001) , but this was observed in all three resistance strata (Appendix, page 32) and restricting the analysis to the larger 50% of studies did not change the observed trend towards decreasing efficacy with increasing resistance (Table 1). By region, we observed significant trends for LBW only in east/southern Africa (Appendix, page 16). Only five

studies were conducted in areas with >10% prevalence of sextuple mutant parasites (pooled *Pfdhps*-A581G prevalence 32%, 95% CI 17-48). There was substantial heterogeneity in their effect sizes (Appendix, page 33, I^2=68.8%) with an RRR of 35% (14-51) in three studies with small sample sizes in the reference group (average *Pfdhps*-A581G prevalence=22.9%) compared to RRR=-2% (95% CI -15-9) in the two remaining larger studies, both conducted in areas with the highest *Pfdhps*-A581G prevalence (average=45.3%) (P=0.05 for subgroup difference).

IPTp-effectiveness for other outcomes

A similar linear trend towards decreasing effectiveness with increasing prevalence of *Pfdhps*-K540E was observed for maternal moderate-to-severe anaemia and malaria infection (peripheral or placental or any malaria) at delivery. The RRR at delivery in low, moderate and higher resistance areas were 41 (28-51), 20% (1-35), 13% (3-22) for moderate-severe anaemia (P_{trend}=0.0049), and 20% (13-26), 18% (10-26), and 3% (-3-9) for malaria infection (P_{trend}=0.0164) (Appendix pages 21-26).

Individual participant data meta-analysis surveys

The analysis of the surveys focused on areas with >80% *Pfdhps*-K540E prevalence to further determine the impact of the sextuple mutant parasite in areas previously defined as 'super'-resistant (>10% *Pfdhps*-A581G prevalence).[16] Of 138 publicly available surveys, 39 met the inclusion criteria; 13 included areas with >80% *Pfdhps*-K540E or super resistance, all in east and southern Africa (2008-2015), comprising 42,394 singleton live births after exact matching (Figure 1). IPTp-SP in these areas was associated with a RRR of 11% (95% CI 8-13) of LBW; even in areas with >90% *Pfdhps*-K540E and *Pfdhps*-A581G<10%, IPTp-SP was associated with significantly lower risk of LBW (RRR=10%, 7-12) (Figure 4). However, in areas with >10% *Pfdhps*-A581G prevalence, IPTp-SP did not protect against LBW: RRR=0.5% (-16 to 14); this is illustrated by the outlier in the bubble plot of RRR for LBW which represents the pooled data from three surveys in two 'super' resistance areas (Figure 4). In these two study areas, the pooled prevalence of the *Pfdhps*-A581G mutation across all contemporary molecular studies was 37% (29-46) (Appendix, page 34).

Discussion

In our meta-analysis of aggregated data of 57 clinical studies, the increasing prevalence of two molecular markers of sulphadoxine resistance were associated with a clear reduction in the effectiveness of IPTp-SP to reduce LBW and malaria infection at delivery. In our parallel analysis from nationally-representative surveys containing individual participant data, IPTp-SP was still associated with a significant but modest (10%) protective effect on LBW in areas where the *Pfdhps*-K540E mutation is >=90% and the sextuple mutant is <10%.[16] However, these surveys also demonstrated that in areas where parasites harbouring the sextuple mutant are common (pooled estimate 37%), no protective effect of IPTp-SP could be seen on LBW. These findings are consistent with our understanding of the incremental increase in resistance to SP with successive mutations in the *dhfr* and *dhps* genes and with the previous studies showing that the efficacy of SP is compromised in individual women infected with these sextuple mutants.[8-11] These highly resistant areas are currently restricted to few foci in East Africa,[16] but their spread would have important implications for the continued use of SP for IPTp.

Compared to other SP resistance markers, fewer data are available on the distribution of the *Pfdhps*-A581G mutation. Therefore, the aggregated-data meta-analysis was limited in its ability to define and validate different thresholds for the *Pfdhps*-A581G mutation. The number of studies in areas in East/Southern Africa with >10% *Pfdhps*-A581G was small (N=5) and none were conducted in areas

with *Pfdhps*-A581G prevalence between 13 and 43%. Within these studies, there was also substantial between-study heterogeneity in the treatment effect on LBW: The three smaller studies with only four to eight LBW events in the reference groups[8,11,36] showed a pooled effect size of 35% (95% CI 14-51), while the studies with larger reference groups reported an effect size of -2% (-15 to 9) (Appendix, page 33).[40,41] The results of these latter two studies, which were conducted in areas with >45% *Pfdhps*-A581G, are consistent with the lack of effect on LBW in the survey analysis, which was based on much larger sample sizes and conducted in areas with an average of 37% *Pfdhps*-A581G prevalence.

Irrespective of SP resistance, we also observed large between-study heterogeneity in the treatment effect on LBW in the 57 clinical studies. This can in part be explained by the multi-causal nature of LBW and the varying population-attributable fractions of malaria towards LBW, which depends on transmission intensity and uptake of interventions such as ITNs. ITN use was not an effect modifier or confounder, but malaria transmission intensity was correlated with resistance (lower transmission levels were associated with higher resistance levels) and thus a potential confounder, which is why it was important to adjust for malaria transmission in models. Nevertheless, the inclusion of the four co-variates, including malaria transmission, study quality, average number of SP doses and proportion of paucigravidae, had minimal impact on the effect estimate of the impact of SP resistance on the effect of IPTp-SP on LBW (i.e. the slope of the meta-regression lines), suggesting that overall there was minimal confounding by these variables.

Although the effectiveness of IPTp on LBW decreased with increasing resistance, IPTp-SP remained associated with a 7% to 10% lower risk of LBW even in areas where the resistant quintuple haplotype is fixed. This small but resilient effect contrasts with the lack of impact (RRR=3%) on malaria infection in high resistance areas in the aggregated-data meta-analysis (Appendix, page19) and with the previously observed unfavourable parasitological response in asymptomatic pregnant women receiving IPTp-SP in these areas, where up to 50% of paucigravidae fail to clear their parasites by day 42.[14] This may suggest that suppression, rather than radical clearance of parasites, is required to mitigate the adverse effects on placental function and growth, as observed in multigravidae, who have acquired protective anti-malarial immunity over successive pregnancies. Alternatively, SP may have beneficial effects on birthweight that are independent of its antimalarial properties and therefore unaffected by parasite resistance (e.g. antimicrobial effects,[37-39] or through immune modulating effects similar to those described for cotrimoxazole[40]).

The differences in parasite populations identified in the scatter plot of the prevalence of *Pfdhps*-A437G and K540E mutations (Appendix, page 31) reflects the distinct geographical origins of two or three parasite populations in East and West Africa.[35] In East/southern Africa, the combination of the resistance alleles at *dhps* codons 540 and 581 could be considered to track SP resistance. In Central/West Africa, where the *Pfdhps*-K540E mutation is absent or rare, tracking *Pfdhps*-A437G may be informative. However, other mutations have started to emerge in West Africa, such as *Pfdhps*-I431V, which has been reported on a haplotype bearing mutant alleles at codons 581 and 613 but a wildtype allele at codon 540.[41,42] The clinical implications of such new haplotypes require further study.

These analyses have important limitations. The potential biases associated with observational data where the number of SP doses is not determined by the study have been discussed in detail elsewhere.[4] Although the use of exact matching and multivariate models will have reduced the potential for bias in the surveys, residual confounding cannot be excluded. Second, national surveys, are subject to measurement error and information bias from respondent recall and self-report.[44]

Similar limitations apply to the aggregated-data analysis, which could only adjust for study-level co-variates. For some studies, time-matched local resistance data were not available and were obtained from other sources, which are less precise. Some studies were considered of poor quality with a trend towards greater effectiveness with decreasing study quality, but sensitivity analysis showed that these were equally distributed across the resistance spectrum and did not impact on the conclusions. Similarly, there was evidence of a small-study effect, but this was also observed in all three resistance strata and restricting the analysis to the largest 50% of studies (which are least likely to be affected by publication bias) did not alter the conclusions. In addition, the meta-analysis suffered from design and reporting variation and small numbers in the extreme dose groups (0 dose and 3+ doses). This was partly mitigated by using a dose-response analysis that placed less emphasis on the extreme dose groups.

This is the most comprehensive study of the impact of SP resistance on the effectiveness of IPTp involving 57 clinical studies, 12 nationally-representative surveys, and more than 100,000 births. The data show that, despite the substantial heterogeneity between studies regarding the effectiveness of IPTp-SP on LBW, increasing prevalence of molecular markers of sulphadoxine resistance is correlated with a decrease in effectiveness of SP to reduce LBW and malaria infections. This suggests that molecular monitoring of SP resistance could be considered as a policy tool to guide the use of IPTp-SP. It is reassuring that a protective association with LBW can be detected even in high resistance areas where the quintuple mutant parasites are almost fixed. However, IPTp-SP is not likely to reduce malaria and malaria-associated LBW in areas where the sextuple mutant parasites with the *Pfdhps*-A581G mutation exceed 37% (the pooled estimate in the high resistance areas). For these areas, the search for alternative strategies or drugs to replace IPTp-SP is a pressing research priority for the control of malaria in pregnancy.

Declarations

Contributors

FtK conceived the concept of the study. AMvE, DL and FtK wrote the protocol. AMvE, DL, GK and DECS did the literature search, acquired the aggregated data, screened records and extracted data. AMVE, GK and DECS assessed the quality of included studies. CK and FtK acquired and combined the individual participant data from different observational studies. AMvE, DL and FtK did the statistical analysis. KK, MD, JG, SR, SM, ST, CR and LCO provided individual level participant clinical or molecular data. CR, LCO, and CHS set-up and maintained the interactive maps of the distribution of molecular resistance markers used in the analysis. AMvE, DL and FtK wrote the first draft of this manuscript. All authors provided critical conceptual input, interpreted the data analysis, and critically revised and approved the final version of the manuscript.

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grant from Bill & Melinda Gates Foundation to the Liverpool School of Tropical Medicine (Grant number 46099).

Role of the sponsor

The funding institutions had no role in the protocol development, the design and conduct of the review; data collection, analysis, interpretation, and preparation, review, or approval of the manuscript.

Registration

This study was registered in the International Prospective Register of Systematic Reviews (PROSPERO; 42016035540)

Conflicts of interest

We declare that we have no conflicts of interest.

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Supplemental information (see Appendix)

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Tables

TABLE 1: THE EFFECT OF SP RESISTANCE ON THE EFFECTIVENESS OF IPTP ON LBW IN SUB-SAHARAN AFRICA, 1997-2013, AGGREGATED DATA

		Meta-regression									
		Univariate					Multivariate*				
	N	Coefficient (95% CI)	P	Tau^2	I^2 %	R^2 %	Coefficient (95% CI)	p	Tau^2	I^2 %	R^2 %
***Pfdhps*-A437G *(continuous variable)*†**											
All studies	57	1.001 (0.999, 1.004)	0.35	0.02596	69.9	1.8	1.001 (0.999, 1.004)	0.25	0.01645	57.7	37.8
Excluding low quality studies‡	50	1.002 (0.999, 1.004)	0.13	0.02079	67.3	7.5	1.002 (1.000, 1.004)	0.08	0.01323	54.1	41.1
Restricted to largest 50% of studies§	29	1.003 (1.000, 1.005)	0.06	0.01615	73.9	13.3	1.002 (1.000, 1.005)	0.09	0.01137	62.1	39.0
***Pfdhps*-K540E *(continuous variable)*†**											
All studies	57	1.002 (1.001, 1.003)	0.0060	0.02142	66.2	19.0	1.002 (1.001, 1.003)	0.0031	0.01222	53.7	53.8
Excluding low quality studies‡	50	1.002 (1.000, 1.003)	0.0090	0.01732	61.9	22.9	1.002 (1.000, 1.003)	0.0160	0.01133	51.1	49.6
Restricted to largest 50% of studies§	29	1.002 (1.000, 1.003)	0.0223	0.01469	70.5	21.1	1.002 (1.000, 1.003)	0.0132	0.00909	58.7	51.2
***Resistance strata (low, moderate, high)*¶**											
All studies	57	1.10 (1.03, 1.18)	0.0054	0.02040	65.4	22.8	1.10 (1.03, 1.17)	0.0043	0.01184	52.9	55.2
Excluding low quality studies‡	50	1.10 (1.03, 1.18)	0.0075	0.01687	61.6	24.9	1.09 (1.02, 1.16)	0.0095	0.01067	49.7	52.5
Restricted to largest 50% of studies§	29	1.10 (1.02, 1.18)	0.0122	0.01386	69.7	25.6	1.10 (1.03, 1.17)	0.0067	0.00802	55.9	56.9

Abbreviations: N=number of studies. CI=confidence interval. *Pf=Plasmodium falciparum.* dhps=dihydropteroate synthase.

* Multivariate meta-regression: adjusted for malaria transmission intensity, average number of SP doses, study quality, and proportion of paucigravidae in study

† In meta-regression the SP-resistance variable is introduced as a linear continuous variable reflecting 1% step increases in prevalence of the resistance marker

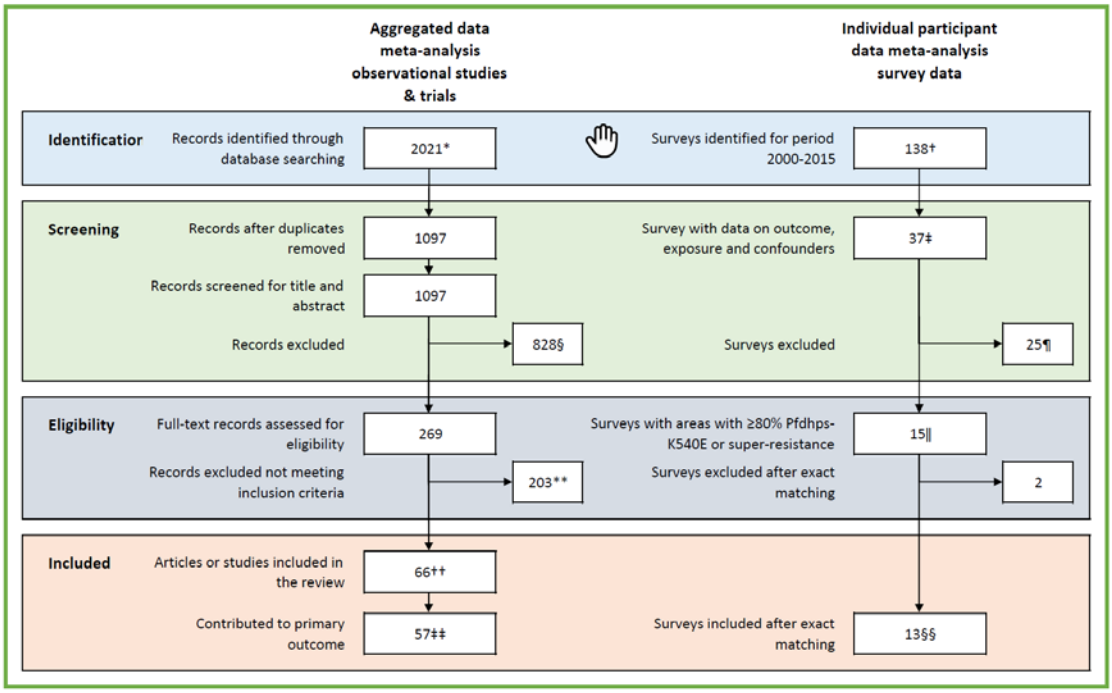
‡ Excludes studies with less than 3 out of 6 stars for quality.

§ To determine the impact of potential bias due to small-study effect, analysis is restricted to the largest 50% of studies, based on their standard error of the log relative risk for LBW.

¶ Definition sulphadoxine-pyrimethamine resistance using molecular markers: Low resistance: *Pfdhps*-A437G <90% in Central and West Africa or *Pfdhps*-K540E <30% in East and southern Africa; moderate: *Pfdhps*-A437G ≥90% in Central and West Africa or *Pfdhps*-K540E ≥30% and *Pfdhps*-K540E <90% in East and southern Africa; high: *Pfdhps*-K540E ≥90% in East and southern Africa

Figures

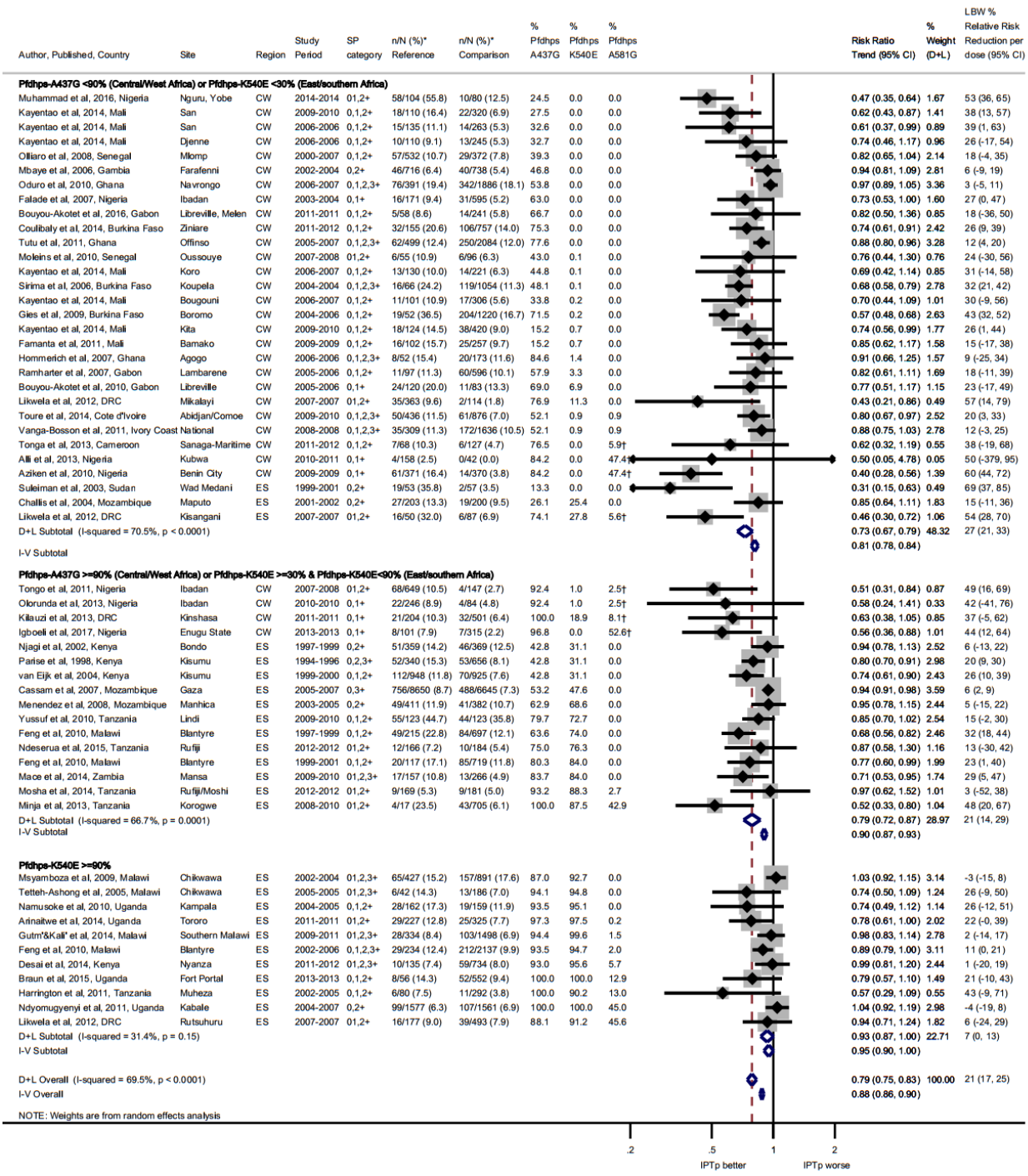
Figure 1: Prisma Flow diagram



Search terms aggregated data meta-analysis: "Malaria AND pregnan* AND intermittent AND (prevent* OR prophyla* OR chemoprevent* OR chemoprophyla* OR IPT*) AND (sulfadoxine OR sulphadoxine OR pyrimethamine OR SP)".

* 561 Malaria in Pregnancy Library, 440 PubMed, 518 Web of Science, 502 Scopus: Total 2021
† 63 Demographic and Health Surveys (DHS), 13 Malaria Indicator Surveys (MIS), 54 Multiple Indicator Cluster Surveys (MICS) (UNICEF) and 8 AIDS indicator surveys. ‡ 39 surveys with individual level data available and information on outcomes, exposures and potential confounders (276,383 single live births: 46% measured birthweight available, 54% perceived birth weight; mean birth weight 3,217 grams (SD 699), small birth size 14.1% of 276,383, LBW 9.4% of 128,347). § 828 records excluded: 238 studies on IPTp-SP coverage, or qualitative studies; 34 not in pregnant women; 14 on cost-effectiveness; 365 related to policy implementation or reviews; 10 outside Africa; 143 on treatment efficacy, drug resistance, pharmacokinetics or modelling; 24 in HIV-infected women. ¶ 25 excluded; 18 in areas with <80% prevalence of *Pfdhps*-K540E, and the remaining from Central and West Africa. ‖ 15 surveys comprising 49,481 births, 98.2% of which were singleton live births. ** 203 full-text records excluded: 36 outcome during pregnancy, but not at delivery; 36 no information on outcomes in relation to SP dose; 49 almost all participants received (no zero dose control); 12 studies with insufficient detail on the number of SP doses received; 68 overlapped with other studies that were included; 1 small sample size with inclusion of twin deliveries, 1 multiple co-interventions in the SP group. †† 66 studies comprising of 74 mutually exclusive data points. ‡‡ 57 data points contributing to the primary outcome of low birthweight; 4 from trials comparing IPTp-SP against placebo or passive case detection, 3 from the IPTp-SP arm of other trials, and 43 observational cross-sectional studies with exposure data recorded at the time of delivery. §§ 13 surveys included in the primary analysis comprising of 42,394 singleton live births; 19,429 with measured birthweight not available and 22,965 with measured birthweight available.
Abbreviations: IPTp=Intermittent preventive treatment. SP=sulphadoxine-pyrimethamine.

FIGURE 2 META-ANALYSIS OF THE RELATIVE RISK OF LOW BIRTHWEIGHT ASSOCIATED WITH EACH INCREMENTAL DOSE OF IPTP-SP IN ALL GRAVIDAE BY RESISTANCE STRATA IN 57 CLINICAL STUDIES IN SUB-SAHARAN AFRICA



Dhps=Dihydropteroate synthase. LBW=Low birthweight <2,500 grams. n/N=Total number of women with LBW/Total number of women contributing to group. CI=confidence interval. D+L=Dersimonian-Laird method for random effects models. I-V=inverse variance method for fixed effects models. CW=Central and West Africa. ES=East and southern Africa.

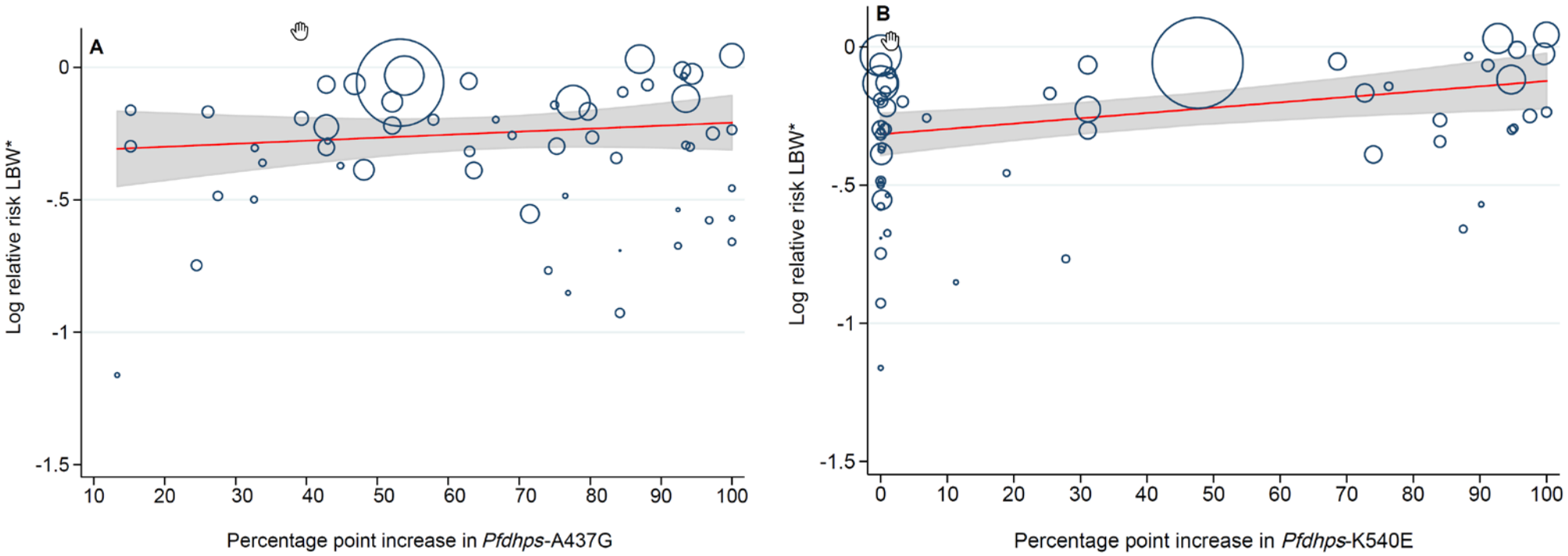
* Reference refers to the lowest SP dose category and reflects 0 or 0 or 1 dose as indicated in the 'SP category' column. Comparison column is included for illustration only and refers to all the other exposure groups pooled; for example, if the SP categories were 0, 1, 2+, then the reference column reflects the data in the 0-dose group, and

the comparison column reflects the data in the 1 dose and 2+ dose groups pooled. For full sample size per dose-group, and average dose, see Appendix, page 13.

† The high prevalence of *Pfdhps*A581G in these studies was not accompanied by a high prevalence in *Pfdhps*K540E, so this information was not interpreted as an indication of the presence of sextuple mutant parasites.

Notes: P-values following the I^2 statistics represent the Chi-square test for heterogeneity. Weights are from random effects analysis. Data-marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies; the first diamond represents random effects meta-analysis, and the second diamond fixed effect meta-analysis. The molecular markers refer to the estimated prevalence of markers in the study area matched as described in text and Appendix, page 9.

FIGURE 3: META-REGRESSION BUBBLE PLOT OF 57 CLINICAL STUDIES SHOWING THE RELATIONSHIP BETWEEN THE RELATIVE RISK (RR)* FOR LOW BIRTHWEIGHT OF IPTP AND THE PREVALENCE OF *PFDHPS*-A437G OR *PFDHPS*-K540E



LBW=low birthweight. *Pfdhps=P. falciparum* dihydropteroate synthase.

3A: Plot for *Pfdhps-A437G*: Slope (exponentiated): 1.001, 95% CI 0.999-1.004, adjusted R^2: 1.8%, P-value for linear trend: P=0.35

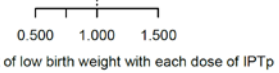
3B: Plot for *Pfdhps*-K540E: Slope (exponentiated): 1.002, 95% CI 1.001-1.003, adjusted R^2: 19.0%. P-value for linear trend: P=0.0060

*Log relative risk LBW=the log of the relative risk estimates for LBW across each of SP dose categories obtained using generalized least square (GLST) regression for trend estimation of summarized dose-response data. The size of the bubbles for individual studies is proportional to the random effects study weights. The greyed area represents the 95% confidence interval of the regression line. The positive slope of the meta-regression line for *Pfdhps*-K540E indicates that with increasing prevalence of *Pfdhps*-K540E, the effect of IPTp-SP on low birthweight decreases.

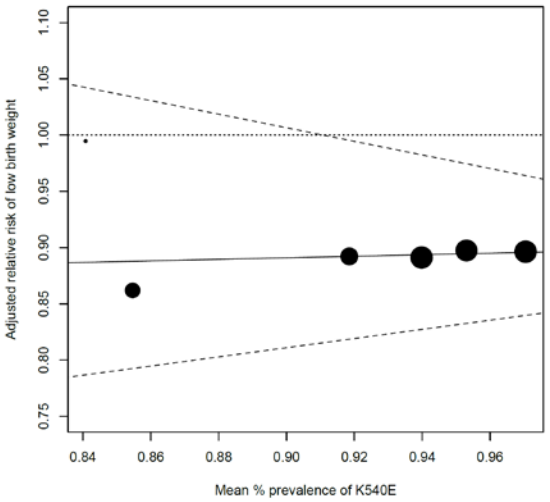
FIGURE 4: RELATIVE RISK LOW BIRTHWEIGHT ASSOCIATED WITH EACH DOSE OF IPTP-SP BY RESISTANCE STRATA (13 SURVEYS) WITH >80% PREVALENCE *PFDHPS*-K540E OR >10% *PFDHPS*A581G IN EAST & SOUTHERN AFRICA

Forest Plot

Quintiles % K540E prevalence (mean [range])	n surveys *	0 IPTp LBW / N (%)	1 IPTp LBW / N (%)	2 IPTp LBW / N (%)	3+ IPTp LBW / N (%)	Adjusted RR [95% CI]
1st (85.5 [80.2 - 89.7])	10	694 / 3958 (17.5)	217/2000 (10.9)	152 / 1520 (10.0)	63/701 (9.0)	0.862 [0.802, 0.926]
2nd (91.9 [89.7 - 93.1])	11	766 / 3923 (19.5)	202/1872 (10.8)	160 / 1522 (10.5)	81/711 (11.4)	0.892 [0.836, 0.952]
3rd (94.0 [93.1 - 94.6])	9	446 / 2438 (18.3)	347/2445 (14.2)	239 / 2096 (11.4)	144/1058 (13.6)	0.891 [0.844, 0.941]
4th (95.3 [94.6 - 95.8])	7	519 / 2552 (20.3)	322/2452 (13.1)	226 / 2076 (10.9)	155/1070 (14.5)	0.897 [0.849, 0.949]
5th (97.0 [95.8 - 98.5])	6	589 / 2850 (20.7)	248/1891 (13.1)	217 / 1834 (11.8)	157/1099 (14.3)	0.896 [0.849, 0.947]
Super (83.7 [70.7 - 96.3])	3	86 / 891 (9.7)	73/815 (9.0)	39 / 503 (7.8)	11/117 (9.4)	0.995 [0.856, 1.156]
RE Model						0.893 [0.870, 0.917]



Linear meta-regression Bubble Plot



Pfdhps=*P. falciparum* dihydropteroate synthase.

Super, 'super resistant' areas defined as prevalence of *Pfdhps*A581G >10% (south-western Uganda, northern Tanzania and eastern DRC).[16] The effect size of IPTp-SP in these super resistance areas is illustrated by the small dot in the bubble plot corresponding to a relative risk of 0.995 at a mean prevalence of 83.7% of *Pfdhps*-K540E.

Quintiles are calculated for areas with a prevalence >80% *Pfdhps*-K540E after excluding the surveys in the super resistant areas

Bubble Plot: The solid line represents the regression line, which shows no evidence for a linear trend (p = 0.8398). The corresponding dashed lines represent the 95% confidence interval.

Overall, IPTp-SP was associated with an RRR of 11% (95% CI 8-13) of LBW. The RRR in areas with >90% Pfdhps-K540E and Pfdhps-A581G<10% was 10% (95% CI 7-12).

*n: Indicates number of surveys that contributed to this quintile or group