Title

Application of Causal Inference Methods to Evaluate the Impact of Malaria Control Interventions on Pregnancy Outcomes

Author

Roh, Michelle

Publication Date

2020

Peer reviewed|Thesis/dissertation

Application of Causal Inference Methods to Evaluate the Impact of Malaria Control Interventions on Pregnancy Outcomes

by
Michelle Roh

DISSERTATION
Submitted in partial satisfaction of the requirements for degree of
DOCTOR OF PHILOSOPHY

in

Epidemiology and Translational Science

in the

GRADUATE DIVISION
of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Approved:

DocuSigned by:
Roly Gosling
772625E79391428...

Roly Gosling
Chair

DocuSigned by:
Grant Dorsey
[illegible]

Grant Dorsey

DocuSigned by:
Maria Glymour
[illegible]

Maria Glymour

DocuSigned by:
Shiboski, Stephen
013F9EE740B848B...

Shiboski, Stephen

Committee Members

Dedication

I am grateful to the following funding sources that made my research and dissertation possible: the Eunice Kennedy Shriver National Institute of Child Health and Human Development under a Ruth L. Kirschstein National Research Service Award (F31 HD096861); the National Institutes of Health, University of California, San Francisco-Gladstone Institute of Virology & Immunology Center for AIDS Research (P30-AI027763); and the Earle C. Anthony Social Sciences Fellowship.

First and foremost, I would like to thank the members of my Dissertation Committee, Roly Gosling, Grant Dorsey, Maria Glymour, and Stephen Shiboski. Your mentorship over the years have been invaluable for my growth as an epidemiologist. Dr. Gosling, I always tell everyone how incredibly lucky I am to have you as a mentor, academic advisor, and Committee Chair. Thank you for helping to build my confidence as a researcher and allowing me the freedom to explore new ideas. Your mentorship has taught me to not only conduct rigorous research, but to always advocate for the people and communities involved in our research. Dr. Dorsey, thank you for being a phenomenal mentor. Your dedication to our mentor-mentee relationship has greatly improved my skills as a researcher and has well prepared me for an independent career in academia. Your insightful and detailed comments on my abstracts, manuscripts, and grant applications has taught me how to be a better writer and how to convey my work to the broader public health community. Dr. Glymour, thank you for your mentorship as a Committee Member and PhD program director. I've learned so much from you through the PhD seminars and our one-on-one meetings. You have always challenged

me to be rigorous in my research and your enthusiasm for causal inference has impacted the way I approach research questions, which will definitely have a lasting impact on my future research. Dr. Shiboski, thank you for your amazing mentorship and guidance throughout the statistical aspects of my dissertation. Your expertise in conveying complex biostatistical methods into something more ‘palatable’ has been immensely helpful and has challenged me to do the same in my future work.

To my all my colleagues from the DEB PhD program, especially Luis Rodriguez, Stephen Asiimwe, Megha Mehrota, Vignesh Arasu, Yea-Hung Chen, Francois Rerolle, Amanda Irish, Adrienne Epstein, Rae Wainner, and Raj Kalapatapu. You have been an incredible group to learn with and from. Thank you for letting me bounce ideas off of you and peer-reviewing my work. I am so lucky to have been surrounded with such brilliant researchers and I value all of you as friends and colleagues.

To my colleagues and collaborators at the Malaria Elimination Initiative, Liverpool School of Tropical Medicine, London School of Hygiene and Tropical Medicine, and Infectious Disease Research Collaboration, especially Hugh Sturrock, Adam Bennett, Chris Cotter, Jimee Hwang, Michelle Hsiang, Ingrid Chen, Jennifer Smith, Feiko ter Kuile, Matthew Chico, and Arthur Mpimbaza. It has been such a pleasure to work and learn from all of you. Thank you for supporting this dissertation and providing ample opportunities to work on other cool projects.

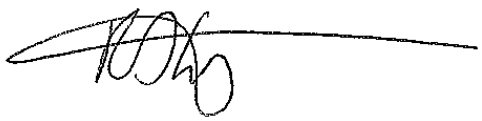
This dissertation was not possible without the love and support of my family and friends. To my loving mother and father, Sun and Won Roh, I could not have asked for better parents. Thank you for encouraging me to pursue my dreams and teaching me the value of hard work. To my sisters, Stephanie and Gina Roh, and brother-in-law, Paul Chung. Thank you for being my sounding board, feeding and housing me during the COVID-19 pandemic, and generally making life fun with our collective dogs: Sarang, Powder, and Bailey. Thank you especially to my friends Trena Mukherjee, Fausat Bello, Natalie Rivadeneira, Guat Vallejo-Howard, Max Murphy, Hector Sanchez, Sean Wu, Forrest Jones, and Josh Wei for lending your support at all hours of the day, the many hours proofreading, and making sure I balance work with some fun.

Lastly, I would like to thank the women and children whose data contributed to the findings of this dissertation and the dedicated study staff, researchers, and members of the Infectious Disease Research Collaboration in Uganda and Kenya Medical Research Institute, and the Centers for Disease Control and Prevention.

Acknowledgement of Previously Published Materials

A version of Chapter 1 in this dissertation was published in *The Lancet Global Health* on July 1, 2020. The Dissertation Committee Members supervised the research that forms the basis of this dissertation chapter. The published material is substantially the product of Michelle Roh's period of study at the University of California, San Francisco and was primary conducted and written by her. The work she completed for this published manuscript is comparable to a standard dissertation chapter.

Approved:



Roly Gosling, MD PhD, Dissertation Chair

Application of Causal Inference Methods to Evaluate the Impact of Malaria Control Interventions on Pregnancy Outcomes

Michelle Roh

ABSTRACT

Sub-Saharan Africa is faced with a challenging road ahead if it is to meet its United Nations Sustainable Development Goals of reducing rates of neonatal mortality to 12 per 1,000 live births by 2030. Currently, the region contributes to 90% of the global burden of newborn deaths and progress toward reducing these rates has been slow [1]. Although the causes of newborn death are multifactorial, in malaria-endemic areas, infection with the *Plasmodium falciparum* parasite during pregnancy is a leading cause of low birthweight (LBW) and stillbirth, which are both risk factors for neonatal mortality [2, 3]. Each year, malaria in pregnancy is estimated to cause nearly one million LBW deliveries, 220,000 stillbirths, and 110,000 neonatal deaths [3-6].

To prevent the adverse consequences of malaria in pregnancy, the World Health Organization (WHO) recommends all pregnant women living in areas of moderate and high malaria transmission receive an effective method of vector control, namely long-lasting insecticidal nets (LLINs), and malaria chemoprevention known as intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) [7]. Though LLINs and IPTp-SP have been shown to be highly effective in trial settings [8, 9], their current efficacy is threatened by the emergence of mosquito resistance to pyrethroids, the insecticide most commonly used in LLINs, and parasite resistance to the SP antimalarial in Eastern and Southern Africa. This has led researchers to evaluate

alternative strategies for malaria control, including new types of LLINs that can overcome pyrethroid resistance, more efficacious antimalarials, and non-pyrethroid-based vector control tools. However, little is known of how these alternative strategies will affect pregnant women and their birth outcomes.

The overall goal of this dissertation is to apply advanced causal inference methods to determine the effectiveness of these alternative strategies for preventing adverse pregnancy outcomes among women at-risk for malaria. The dissertation is organized into three chapters, each describing one of these alternative strategies. The first chapter pools data from three randomized controlled trials that compared IPTp efficacy of SP to dihydroartemisinin-piperaquine (DP), a highly efficacious antimalarial. Data from the pooled analysis found that while DP is a more efficacious antimalarial, it did not confer greater benefits on pregnancy outcomes compared to SP. Through applying causal mediation analyses, we found that this was due to the greater, 'non-malarial' and possible antimicrobial effects of SP which were counteracting DP's greater antimalarial effects. Findings from this study suggest future IPTp regimens should consider adding an antimicrobial to the IPTp-DP regimen to achieve a greater impact on improving birth outcomes. The second chapter compares the effectiveness of conventional, pyrethroid-based LLINs to a new type of LLIN additionally treated with piperonyl butoxide (PBO), a chemical shown to restore pyrethroid sensitivity. Using quasi-experimental analyses such as interrupted time series analyses and difference-in-differences models, the study found PBO LLINs conferred a 22% greater reduction in LBW and a 33% greater reduction in stillbirth compared to conventional (non-PBO) LLINs, supporting the latest

WHO recommendation for deployment of PBO LLINs. The third chapter evaluates the impact of indoor residual spraying (IRS), an existing but highly underutilized malaria vector control intervention, on preventing adverse birth outcomes. We used a novel, machine learning method to relax some of the strict assumptions of traditional quasi-experimental analyses to find that high-coverage IRS can reduce LBW incidence up to 17%, a finding consistent with conferring full protection against malaria via LLINs, IPTp, or both. Given the scale-up of LLINs and IPTp has been traditionally low across sub-Saharan Africa, our results suggest IRS can play a complementary role in preventing malaria-associated adverse pregnancy outcomes and thus, efforts should be made in expanding its use.

Together these findings provide important policy implications for identifying alternative and potentially more effective malaria interventions for pregnant women, particularly in light of growing pyrethroid and SP antimalarial resistance.

Table of Contents

CHAPTER 1: Intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy improves birthweight via non-malarial mechanisms: A mediation analysis 1

ABSTRACT 1

INTRODUCTION 3

METHODS 4

RESULTS 10

DISCUSSION 14

APPENDIX 26

CHAPTER 2: Impact of long-lasting insecticidal nets treated with and without PBO on preventing adverse birth outcomes 36

ABSTRACT 36

INTRODUCTION 38

METHODS 39

RESULTS 46

DISCUSSION 50

APPENDIX 64

CHAPTER 3: Temporal associations between indoor residual spraying of insecticide for malaria prevention and low birthweight risk: A quasi-experimental study from 11 districts in Uganda 66

ABSTRACT 66

INTRODUCTION 68

METHODS 69
RESULTS 76
DISCUSSION 79
APPENDIX 90
REFERENCES 97

List of Figures

Figure 1.1 Directed acyclic graph depicting relationship between IPTp and birth outcomes as mediated by malaria 18
Figure 1.2 Flowchart of participants 19
Figure 1.3 Crude differences in placental malaria between IPTp arms 21
Figure 1.4 Crude differences in mean birthweight between IPTp arms 22
Figure 1.5 Crude differences in LBW and PTD between IPTp arms 23
Figure 1.6 Antimalarial effect of IPTp on birthweight, LBW, and PTD 24
Figure 1.7 Non-malarial effect of IPTp on birthweight, LBW, and PTD 25
Appendix Figure 1.1 Sensitivity analyses of the crude effect of IPTp regimens on active placental malaria 34
Appendix Figure 1.2 Sensitivity analyses of the antimalarial and non-malarial effect where mediator is defined as active placental infections only 35
Figure 2.1 Map of study area 54
Figure 2.2 Flow chart of health facility selection 55
Figure 2.3 Estimates from interrupted time series models evaluating the effect of the LLIN campaign on LBW 58
Figure 2.4 Estimates from difference-in-differences models comparing the effect of PBO and conventional LLINs on LBW 59
Figure 2.5 Estimates from interrupted time series models evaluating the effect of the LLIN campaign on mean birthweight 60
Figure 2.6 Estimates from difference-in-differences models comparing the effect of PBO and conventional LLINs on mean birthweight 61

Figure 2.7 Estimates from interrupted time series models evaluating the effect of the LLIN campaign on stillbirth 62
Figure 2.8 Estimates from difference-in-differences models comparing the effect of PBO and conventional LLINs on stillbirth 63
Figure 3.1 Map of study area 84
Figure 3.2 Flow diagram of selected health facilities 85
Figure 3.3 Effect estimates from counterfactual estimators evaluating the impact of the Uganda IRS Project on birth outcomes 87
Figure 3.4 Results from HIV subgroup analyses..... 88
Figure 3.5 Results from gravidity subgroup analyses 89
Appendix Figure 3.1 Trends in LBW and stillbirth incidence of the treated group estimated by MC-NNM generated synthetic controls 96

List of Tables

Table 1.1 Baseline characteristics of the study population for each study by randomized treatment arm 20

Table 2.1 Study population characteristics across LLIN groups stratified by pre- and post-campaign periods 56

Table 2.2 Comparison of conventional and PBO LLIN groups based on the primary malaria efficacy endpoint in the LLINEUP trial 57

Table 3.1 Maternal characteristics and birth outcomes of IRS and non-IRS (control) groups stratified by pre- and post-campaign periods 86

List of Abbreviations

ATT	Average treatment effect in the treated
CI	confidence interval
DAG	directed acyclic graph
DiD	difference-in-differences
DP	dihydroartemisinin-piperaquine
GAM	generalized additive models
GAMM	generalized additive mixed models
HSD	health sub-district
IPTp	intermittent preventive treatment during pregnancy
IRR	incidence rate ratios
IRS	indoor residual spraying
ISTp	intermittent screening and treatment during pregnancy
ITN	insecticide-treated net
ITS	interrupted time series
LAMP	loop-mediated isothermal amplification
LBW	low birthweight
LLIN	long-lasting insecticidal net
MC-NNM	matrix completion method with nuclear norm minimization
MD	mean difference
MoH	Ministry of Health
NDE	natural direct effect
NIE	natural indirect effect

PBO	piperonyl butoxide
PCR	polymerase chain reaction
PTD	preterm delivery
RR	relative risk ratio
SD	standard deviation
SE	standard error
SP	sulfadoxine-pyrimethamine
WHO	World Health Organization

CHAPTER 1: Intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy improves birthweight via non-malarial mechanisms: A mediation analysis

Michelle E. Roh, Feiko O. ter Kuile, Francois Rerolle, M. Maria Glymour, Stephen Shiboski, Roly Gosling, Julie Gutman, Abel Kakuru, Meghna Desai, Richard Kajubi, Anne L'Ianziva, Moses R. Kamya, Grant Dorsey, R. Matthew Chico

ABSTRACT

Background. The World Health Organization recommends intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) to prevent the adverse consequences of malaria infection. Parasite resistance to SP has prompted researchers to evaluate dihydroartemisinin-piperaquine (DP) as an alternative. Three trials in East Africa showed DP was superior to SP in preventing malaria, but not at improving birthweight. Mediation analyses were conducted to determine whether these findings were due to the greater non-malarial (potentially antimicrobial) effects of SP.

Methods. We defined treatment as random assignment to SP or DP before pooling individual participant-level data from 1,617 HIV-negative pregnant women in Kenya (one trial, n=806) and Uganda (two trials, n=811). We quantified the relative effect of treatment on birthweight (primary outcome) attributed to preventing placental malaria infection (mediator). We estimated antimalarial (mediated) and non-malarial (non-mediated) effects of IPTp on birth outcomes using causal mediation analyses, accounting for confounders. Two-stage individual participant data meta-analyses were used to calculate pooled-effect sizes.

Results. Overall, birthweight was higher among newborns of women randomized to SP compared to DP (+69 grams [95% CI: 26, 112]), despite placental malaria infection being 36% lower in the DP group (RR=0·64 [95% CI: 0·39, 1·04]). Mediation analyses showed SP conferred greater non-malarial effects than DP (+87 grams [95% CI: 43, 131]), whereas DP conferred minimally larger antimalarial effects than SP (+8 grams [95% CI: -9, 26]), though more frequent dosing increased antimalarial effects (+31 grams [95% CI: 3, 60]).

Conclusion. IPTp with SP appears to have potent non-malarial effects on birthweight. Future research should evaluate monthly DP with SP (or another compound with non-malarial effects) to achieve greater protection against malarial and non-malarial causes of lower birthweight.

INTRODUCTION

In sub-Saharan Africa, malaria infection during pregnancy is a major cause of LBW. For pregnant women, *Plasmodium falciparum*-infected red blood cells sequester in the placenta, causing inflammatory cellular responses leading to increased risk of preterm delivery (PTD; <37 gestational weeks) and intrauterine growth restriction, which are both causes of low birthweight (LBW; <2,500 grams) [10-12]. To prevent adverse consequences of malaria infection, the World Health Organization recommends the provision of intermittent preventive treatment (IPTp) with SP to all pregnant women living in areas of moderate-to-high malaria transmission, administered at scheduled antenatal visits from the second trimester to delivery [13].

Parasite resistance to SP in eastern and southern Africa has led researchers to evaluate alternative drug regimens for IPTp. DP remains the most promising candidate given its long-acting prophylactic effect and highly efficacious antimalarial activity. To-date, three trials [14-16], in areas of high SP resistance, have shown DP is well-tolerated and more effective in preventing malaria infection than sulfadoxine-piperaquine. However, this effect did not translate into better birth outcomes [14-16]. A plausible explanation is that these studies lacked sufficient statistical power to detect differences in birth outcomes, as most of these studies were powered to detect differences in malaria outcomes, which were more prevalent. An alternative explanation is that SP, through sulfadoxine's broad-spectrum antimicrobial activity, improves birth outcomes via mechanisms independent of its antimalarial activity (i.e. via non-malarial mechanisms), and in these studies, non-malarial effects have offset the greater

antimalarial effects of DP on birth outcomes. Recent studies support this alternative hypothesis, suggesting IPTp with SP remains protective against LBW risk in areas of low malaria transmission [17] and/or high parasite resistance to SP [18-20].

The objectives of this study were to assess whether SP exhibits greater non-malarial benefits on birth outcomes than DP and whether DP exhibits greater antimalarial benefits on birth outcomes than SP. To evaluate this, we employed mediation analyses [21, 22] to estimate the non-malarial and antimalarial effects of these two treatments. Mediation analyses is an epidemiological method that uses statistical modelling to quantitatively examine the extent to which certain intermediate variables mediate the overall effect of a treatment on an outcome. Mediation analysis is conducted by pre-specifying a mediator and estimating the effect that a treatment has on an outcome either indirectly (via the mediator) or directly (via the non-mediated pathway). In this paper, the term 'indirect effect' is defined as the effect of IPTp on birthweight that is attributed to preventing placental malaria (i.e. antimalarial effect). The term 'direct effect' is defined as the effect of IPTp on birthweight that is not attributed to preventing placental malaria (i.e. non-malarial effect). Here, we present analyses of the relative overall, indirect, and direct effects of these two IPTp regimens on birth outcomes.

METHODS

Study Population

Individual participant-level data were collected from three trials conducted in Siaya County, Kenya (Kenya-STOPMiP) [14]; Tororo District, Uganda (Uganda-BC1) [15]; and

Busia District, Uganda (Uganda-BC3) [16]. In Siaya County, ~96% of parasites carry the quintuple antifolate mutation (*pfdhfr* 51I, 59R, and 108N and *pfdhps* 437G and 540E) and 5.8% have the sextuple mutation (*pfdhps A581G*) [18]. In Tororo, Uganda, ~78% of parasites carry the quintuple mutation, whereas none have the sextuple mutation [23]. No data were available on *pfdhf/pfdhps* mutations in Busia, though Tororo and Busia are adjacent districts.

Trial eligibility was restricted to HIV-negative pregnant women resident in the study region or health facility catchment area with no history of receiving IPTp during their current pregnancy.

In Kenya-STOPMiP, women between 16-32 gestational weeks were enrolled and randomized to receive: IPTp with DP, IPTp with SP, or intermittent screening and treatment (ISTp) with DP. Women assigned to IPTp arms received IPTp at enrollment and then at each subsequent antenatal visit at intervals of 4-6 weeks.

In the Ugandan studies, women between 12-20 gestational weeks were enrolled. In Uganda-BC1, women were randomized to either IPTp with SP every eight weeks, IPTp with DP every eight weeks, or IPTp with DP every four weeks. In Uganda-BC3, women were randomized to either IPTp with SP or IPTp with DP every four weeks. Women assigned to IPTp every eight weeks began IPTp at 20 gestational weeks whereas women assigned to IPTp every four weeks began IPTp at 16 or 20 gestational weeks, depending on their gestational age at enrollment.

For all studies, each dose of SP was three tablets of 500 mg sulfadoxine and 25 mg of pyrimethamine given as a single dose. In Kenya-STOPMiP, dosing of DP was based on bodyweight at enrollment (two, three, or four tablets of 40mg dihydroartemisinin and 320mg piperaquine a day for bodyweights of 24-35.9 kg, 36-74.9 kg; or 75+ kg, respectively) and given once a day for three days. In the Ugandan studies, each dose of DP was three tablets of 40 mg dihydroartemisinin and 320 mg piperaquine given once a day for three days. Single-dose SP and the first dose of DP were administered under direct observation at the clinic, while the second and third doses of DP were self-administered at home. The Ugandan trials were placebo-controlled such that all participants received a three-dose course. Participants in Kenya were visited at home two days after enrollment to verify drug adherence, and every fifth participant was visited at home on subsequent visits. For the Ugandan studies, standardized assessments were conducted to determine adherence.

Our mediation analysis included women who had singleton live births, received ≥1 IPTp dose(s), and a known status of either past or active placental malaria infection. This was determined by including women who either had a histopathological assessment of placental malaria using placenta tissue and/or women who tested positive for placental malaria by either microscopy, loop-mediated isothermal amplification, or polymerase chain reaction methods using placental blood. Women were excluded if they were: assigned to the Kenya-STOPMiP ISTp arm, assigned to the Uganda-BC1 IPTp with DP every four weeks arm, or had an unknown status of either past or active placental

malaria (i.e. missing placental histopathology results and negative for placental malaria by microscopy and molecular methods).

Ethical approvals

Ethical approvals were granted by the Kenya Medical Research Institute, Makerere University School of Biomedical Sciences, the Uganda National Council for Science and Technology, the US Centers for Disease Control and Prevention, and the University of California, San Francisco.

Measurement of Treatment, Mediator, and Confounders

We defined treatment as random assignment to IPTp with either SP or DP. The mediator in our analysis was defined as the presence of past or active placental malaria infection. A woman was determined to have a past or active placental malaria infection if she had pigment and/or parasites in her placenta determined by histopathology of the placental tissue [24] and/or if she tested positive for parasites by microscopy or molecular methods in her placental blood. Peripherally-detected malaria infection was considered as a potential mediator (**Appendix Text 1.1**), but we found that in our sample of women, parasitemia without the presence of placental malaria was not associated with lower birthweight, while women with placental malaria, regardless of whether they had peripherally-detected malaria, were more likely to have a lower birthweight baby.

Confounding variables were identified *a priori* based on causal assumptions represented in a directed acyclic graph (**Figure 1.1**). Due to treatment randomization, confounders were limited to those that affected mediator-outcome associations. These included gestational age at enrollment, maternal age, maternal parasitemia at enrollment, gravidity, education, and household wealth. Gravidity was dichotomized as primigravidae (first pregnancy) or multigravidae (one or more previous pregnancies). Household wealth was reported as tertiles and calculated using principal components analysis of common household items.

Outcomes

The primary outcome was continuous measure of birthweight at delivery measured in grams. Secondary outcomes included LBW (<2,500 grams) and PTD (<37 gestational weeks). Further details on outcome measurements are reported in the trials [14-16].

Statistical Analysis Plan

We used causal mediation analysis [25-27] to deconstruct the crude differences in birth outcomes between IPTp regimens (i.e. overall treatment effect) into (a) the differences in birth outcomes between IPTp regimens that is mediated by preventing placental malaria (i.e. indirect or 'antimalarial' effect) and (b) the differences in birth outcomes between IPTp regimens that is not mediated by preventing placental malaria infection (i.e. direct or 'non-malarial' effect) (**Figure 1.1**; **Appendix Text 1.2**).

We estimated crude differences in birth outcomes between IPTp regimens using linear or log-binomial regression models with random assignment as the sole predictor. For mediation analyses, we used the mediation R package [28] to estimate indirect and direct effects (**Appendix Text 1.2**). We ran separate models to specify the dependence of placental malaria and birth outcomes based on treatment and pre-specified confounders (as described in the assumed causal graph; **Figure 1.1**). Predicted values from these models were used in a Monte-Carlo framework to calculate indirect and direct effect estimates and corresponding 95% confidence intervals which we report as mean differences for birthweight and relative risks for LBW and PTD.

For all models, treatment-gravidity and treatment-mediator interaction terms were tested wherever possible and incorporated if the *p*-values ($p_{\text{interaction}}$) of these terms were <0·10. We modelled continuous predictors as three-knot restricted cubic splines if the *p*-value of the *F*-test for the joint-effect of the non-linear components was <0.05. Mediation effect estimates and 95% confidence intervals were generated for each study with a quasi-Bayesian approach using 1,000 simulations. Effect modification by gravidity of indirect and direct effect estimates were tested using the test.modmed() function [28] with corresponding *p*-values reported as $p_{\text{difference}}$. For the mediator and primary outcome (placental malaria and birthweight, respectively), we report effect estimates separately for each study and by gravidity, regardless of whether there was evidence of a statistical interaction. Analyses of secondary outcomes (LBW and PTD) were not reported separately by gravidity as they were relatively uncommon outcomes.

We generated pooled-effect estimates using two-stage individual participant data meta-analyses. Individual participant data were used to derive effect estimates for each study and combined using a DerSimonian-Laird random-effects model from the meta R package [29]. Between-study heterogeneity was measured using the I^2 statistic. Analyses were performed using Stata 14.0 (StataCorp, College Station, TX, USA) and R (version 3.5.0; R Project for Statistical Computing; http://www.r-project.org/).

RESULTS

Our primary analysis included 1,617 women: 806 from Kenya-STOPMiP, 178 from Uganda-BC1, and 633 from Uganda-BC3 (**Figure 1.2**). Of the 2,641 women enrolled across the three studies, 1,024 were excluded due to the following reasons: randomized to non-IPTp arm (*n*=516); enrolled in the Uganda-BC1 monthly DP arm and did not have a monthly SP arm as a study-specific comparison (*n*=100); withdrew from study before delivery (*n*=211); had a spontaneous abortion (*n*=21), stillbirth (*n*=22), or non-singleton pregnancy (*n*=50); did not have placental malaria assessed (*n*=103); or did not receive any study drugs (*n*=1). The proportion of women excluded from each category were similar between IPTp groups ($p>0.05$).

Baseline characteristics and the number of IPTp doses given were balanced between IPTp groups ($p>0.10$) (**Table 1.1**). In Kenya-STOPMiP, 100% of women participating in random home visits adhered to taking their second and third IPTp doses. Self-reported adherence to second and third IPTp doses was 99% in Uganda-BC1 and 98% in Uganda-BC3.

IPTp Effect on Placental Malaria (Mediator)

Overall, DP was associated with a 36% lower risk of placental malaria infection compared to SP, but this finding did not reach statistical significance ($RR_{DP:SP}$=0.64 [95% CI: 0.39, 1.04]). There was substantial heterogeneity between studies (I^2=92%, p<0.0001) and effects differed between primi- and multigravidae in the Ugandan studies (**Figure 1.3**). In Kenya-STOPMiP, DP was not associated with a substantially lower risk of placental malaria ($RR_{DP:SP}$=0·91 [95% CI: 0.75, 1.09]) and effects were similar between primi- and multigravidae ($p_{interaction\text{-}STOPMiP}$=0·68). In the Ugandan studies, DP was associated with significantly lower risk of placental malaria compared to SP ($RR_{DP:SP}$=0.63 [95% CI: 0.44, 0.91] in Uganda-BC1 and $RR_{DP:SP}$=0.45 [95% CI: 0.38, 0.55] in Uganda-BC3) and effects were larger in multigravidae compared to primigravidae (**Figure 1.3**; $p_{interaction\text{-}BC1}$=0.0095, $p_{interaction\text{-}BC3}$<0.0001).

Overall Effect of IPTp on Birth Outcomes

Though DP was associated with a lower risk of placental malaria compared to SP, infants born to mothers randomized to DP were of lower birthweight (mean $difference_{SP\text{-}DP}$=69 grams [95% CI: 26, 112]) (**Figure 1.4**). Effect estimates were similar between studies (I^2=0%; p=0·58). In Kenya-STOPMiP, the mean difference was 87 grams [95% CI: 24, 150]) and effects were similar between primi- and multigravidae ($p_{interaction\text{-}STOPMiP}$=0.82). In the Ugandan studies, birthweight was higher in the SP group compared to DP among multigravidae, but not among primigravidae, though these differences did not reach statistical significance ($p_{interaction\text{-}BC1}$=0.43, $p_{interaction\text{-}BC3}$=0.13).

Risks of LBW and PTD did not significantly differ between IPTp regimens, overall or for any of the three individual studies (**Figure 1.5**).

'Antimalarial' Effect of IPTp on Birth Outcomes

Overall, DP did not exhibit a significantly larger antimalarial effect on birthweight than SP (mean difference$_{DP\text{-}SP}$=8 grams [95% CI: -9, 26]), though effect estimates varied between studies (I^2=51%; p=0.13) (**Figure 1.6**). The effect size was larger and statistically significant in the Uganda-BC3 study where IPTp was given monthly (mean difference$_{DP\text{-}SP}$=31 grams [95% CI: 3, 60]). In the other studies, where the majority of women received ≤3 IPTp doses (**Table 1.1**), the mean difference was 2 grams with confidence intervals that included the null. There was no evidence that antimalarial effects differed between primi- and multigravidae in any of the three studies ($p_{difference}$>0.63).

Antimalarial effects on LBW showed similar patterns to birthweight. In pooled analyses, the antimalarial effects on LBW were similar between IPTp groups ($RR_{DP:SP}$=0.98 [95% CI: 0.88, 1.08]), though effect estimates were heterogeneous between studies (I^2=57%; p=0.10) (**Figure 1.6**). Compared to the Kenya-STOPMiP and Uganda-BC1 studies that showed null differences between IPTp regimens ($RR_{DP:SP}$=1.02 and $RR_{DP:SP}$=1.03, respectively), the Uganda-BC3 study (where IPTp was dosed monthly) showed DP conferred a greater and statistically significant antimalarial effect on LBW than SP ($RR_{DP:SP}$=0.88 [95% CI: 0.78, 0.99]). Antimalarial effects on PTD risk were similar

between IPTp regimens ($RR_{DP:SP}$=1.08 [95% CI: 0.95, 1.22]) overall and across the three studies (I^2=0%; p=0.93) (**Figure 1.6**).

'Non-Malarial' Effect of IPTp on Birth Outcomes

Overall, SP conferred a greater non-malarial effect on birthweight compared to DP (mean $difference_{SP-DP}$=87 grams [95% CI: 43, 131]) (**Figure 1.7**). Effects were similar across studies (I^2=0%; p=0.51) and there was no evidence that the non-malarial effects differed between primi- and multigravidae in the Kenya-STOPMiP and Uganda-BC1 study ($p_{difference}$>0.33). In the Uganda-BC3 study, SP conferred a greater statistically significant non-malarial effect than DP in multigravidae, but not in primigravidae (mean $difference_{SP-DP}$=133 grams [95% CI: 51, 216] versus -10 grams [95% CI: -143, 123], respectively; $p_{difference}$=0.094).

The non-malarial effect on LBW had a similar relationship to the continuous measure of birthweight (**Figure 1.7**). Overall, SP conferred a 22% (or 100*(1-1/1.28)) greater non-malarial effect on LBW risk compared to DP ($RR_{DP:SP}$=1.28 [95% CI: 0.85, 1.93]), though confidence intervals included the null. LBW effect estimates were similar between studies (I^2=0%; p=0.40).

Overall, SP was associated with a 13% (or 100*(1-1/1.15)) greater non-malarial effect on PTD risk compared to DP ($RR_{DP:SP}$=1.15 [95% CI: 0.50, 2.65]), though confidence intervals around all effect estimates included the null (**Figure 1.7**). Effects varied between studies (I^2=48%; p=0.15), particularly between Kenyan and Ugandan studies.

Sensitivity Analyses

Sensitivity analyses were performed to test the robustness of our effect estimates. First, we restricted the definition of our mediator to active placental infections only, as past infections include those that may have been present prior to study enrollment. We found that though DP was associated with a substantially lower risk of active infections compared to SP (**Appendix Figure 1.1**), mediation effect estimates on birthweight did not substantively differ from the primary analyses (**Appendix Figure 1.2**).

Second, we tested the robustness of our effect estimates to unmeasured mediator-outcome confounding (**Appendix Text 1.3**). We found that the strength of an unmeasured confounder would have to be implausibly large to explain away our observed 'non-malarial' effect, but not our antimalarial effect estimate.

DISCUSSION

By pooling data from three randomized controlled trials, we found evidence that IPTp influences birthweight through both antimalarial and non-malarial mechanisms. Crude analyses of data from these trials showed that, despite the substantially larger protective effect of DP on placental malaria, birthweight was higher for infants of women randomized to SP. Mediation analyses were performed to explain this seemingly paradoxical relationship. We found that via mechanisms mediated by malaria prevention, monthly IPTp with DP (as observed in the Uganda-BC3 study) was associated with a modest, but statistically significant increase in birthweight (31 grams) compared to SP. In contrast, there was little difference in the antimalarial effect on

birthweight between IPTp groups in studies with less frequent IPTp dosing (i.e. Kenya-STOPMiP and Uganda-BC1). In contrast, we found that via mechanisms not mediated by malaria, SP was associated with a significant increase in birthweight (87 grams) compared to DP, and this effect was similar across studies. Antimalarial effects on PTD risk did not follow birthweight or LBW trends, suggesting the mechanism by which IPTp affects birthweight may be through promoting intrauterine fetal growth, rather than timing of delivery. There was some evidence in Uganda that the non-malarial effects were greater in multigravidae, which may reflect a greater attributable fraction of non-malarial causes of LBW compared to primigravidae, for whom malaria may be a more predominant cause of LBW.

Though we do not know the exact non-malarial mechanisms by which SP is improving birthweight, it is likely the antibiotic properties of sulfadoxine are, at least in part, responsible for these observed effects [17, 30, 31]. Sulfadoxine belongs to a group of agents (sulfonamides) that have been previously used to treat *Trichomonas vaginalis* [32], *Gardnerella vaginalis* (a bacterium associated with bacterial vaginosis) [33], *Neisseria gonorrhoeae* [34], and *Chlamydia trachomatis* [35]. Recent studies show that these infections are prevalent among pregnant women in East Africa (range of 3.7%-50.8%) [36]. Although SP is unlikely to be curative of these infections, antenatal dosing has been shown to reduce adverse pregnancy outcomes among women who had these non-malarial infections at antenatal booking [37]. There are likely other mechanisms at play, which deserves future study, including those affected by sulfadoxine's broad-spectrum antibacterial activity [38]. For example, sulfadoxine may altering the maternal

intestinal or vaginal microbiome to stimulate fetal growth [30, 31] or modulate maternal immunity, similar to effects described for the related antifolate combination, trimethoprim-sulfamethoxazole [20]. Though the mechanisms of SP remain unclear, our findings show that IPTp with SP may be used to prevent the non-malarial causes of lower birthweight, which may be just as important as, if not more important than, preventing placental malaria infection.

In the Uganda-BC3 study, 73% of women received ≥6 IPTp doses, whereas in the Kenya-STOPMiP and Uganda-BC1 study, 73% and 100% of women received ≤3 IPTp doses. More frequent dosing may explain why DP's antimalarial effect was larger in the Uganda-BC3 study than the other studies. Our findings support the results the original Uganda-BC1 study [15] which found that monthly dihydroartemisinin was associated with lower malaria and adverse birth outcome risk than three-dose DP. Thus, in order to take advantage of the full antimalarial benefits of IPTp with DP, particularly in areas of high SP resistance and/or high malaria burden, doses should be given at monthly intervals and as early in the second trimester as possible.

This study had limitations. First, mediation effect estimates may have been subject to unmeasured confounding, though sensitivity analyses suggest our non-malarial effects were fairly robust. Second, it is also possible that placental malaria (mediator) was measured with error, which would have likely biased the antimalarial and non-malarial effect toward the null. However, a sensitivity analysis using a more specific definition of the mediator (active placental malaria infections) showed similar results. Third, our

meta-analyses effect estimates were derived from only three studies and should be interpreted with caution. Lastly, our study may have had low statistical power to detect true differences between gravidity subgroups and future studies are needed to confirm our findings.

In summary, mediation analyses enabled us to quantify the greater benefits of SP against the non-malarial causes of lower birthweight compared to DP, as well as the greater benefits of monthly DP against placental malaria as a cause of lower birthweight compared to SP. These findings have two important policy implications. First, this study suggests IPTp with SP may be beneficial in areas of low malaria transmission, where IPTp is not currently recommended, as long as the prevalence of these non-malarial causes are high. Second, the study suggests that in areas of high SP resistance and/or high malaria burden, rather than replacing SP with DP, a combination of these two regimens for monthly IPTp administration may be more efficacious in improving birthweight. Future IPTp trials will need to validate the efficacy and safety of this combination. Provided IPTp with SP and DP is safe and efficacious together, this regimen or other combinations that target both malarial and non-malarial causes of lower birthweight (e.g. DP plus azithromycin [IMPROVE: NCT03208179] [39] or DP plus metronidazole [The ASPIRE Trial: NCT04189744] [40]) may achieve a greater public health impact than giving either therapy alone.

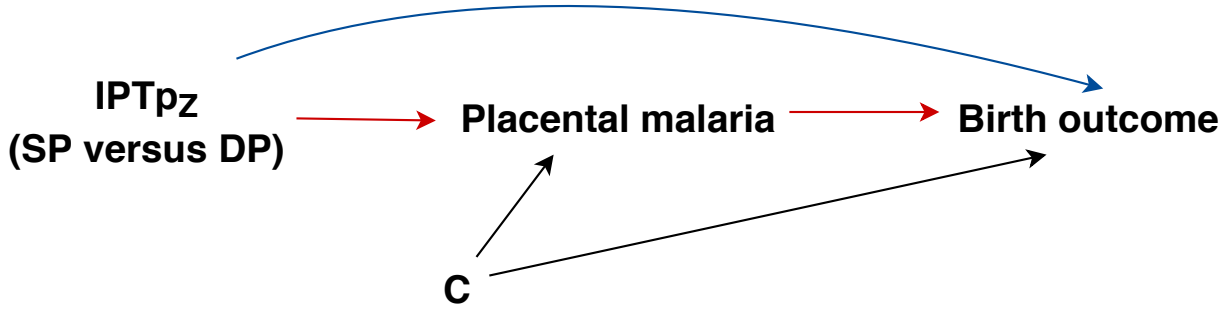


Figure 1.1 Directed acyclic graph depicting the relationship between random assignment to IPTp regimen ($IPTp_Z$) and birth outcomes as mediated by placental malaria infection during pregnancy. Subscript Z denotes randomization and C represents a vector of baseline mediator-outcome confounders (e.g. gestational age at enrollment, maternal age, presence of maternal parasitemia at enrolment, education, household wealth, and gravidity). The red path indicates the causal effect of IPTp on birthweight that is mediated by the prevention of placental malaria infection (i.e. antimalarial effect) and the blue path indicates the causal effect of IPTp on birthweight that is not mediated through placental malaria infection (i.e. non-malarial effect). Note: IPTp=intermittent preventive treatment; DP=dihydroartemisinin-piperaquine; SP=sulfadoxine-pyrimethamine.

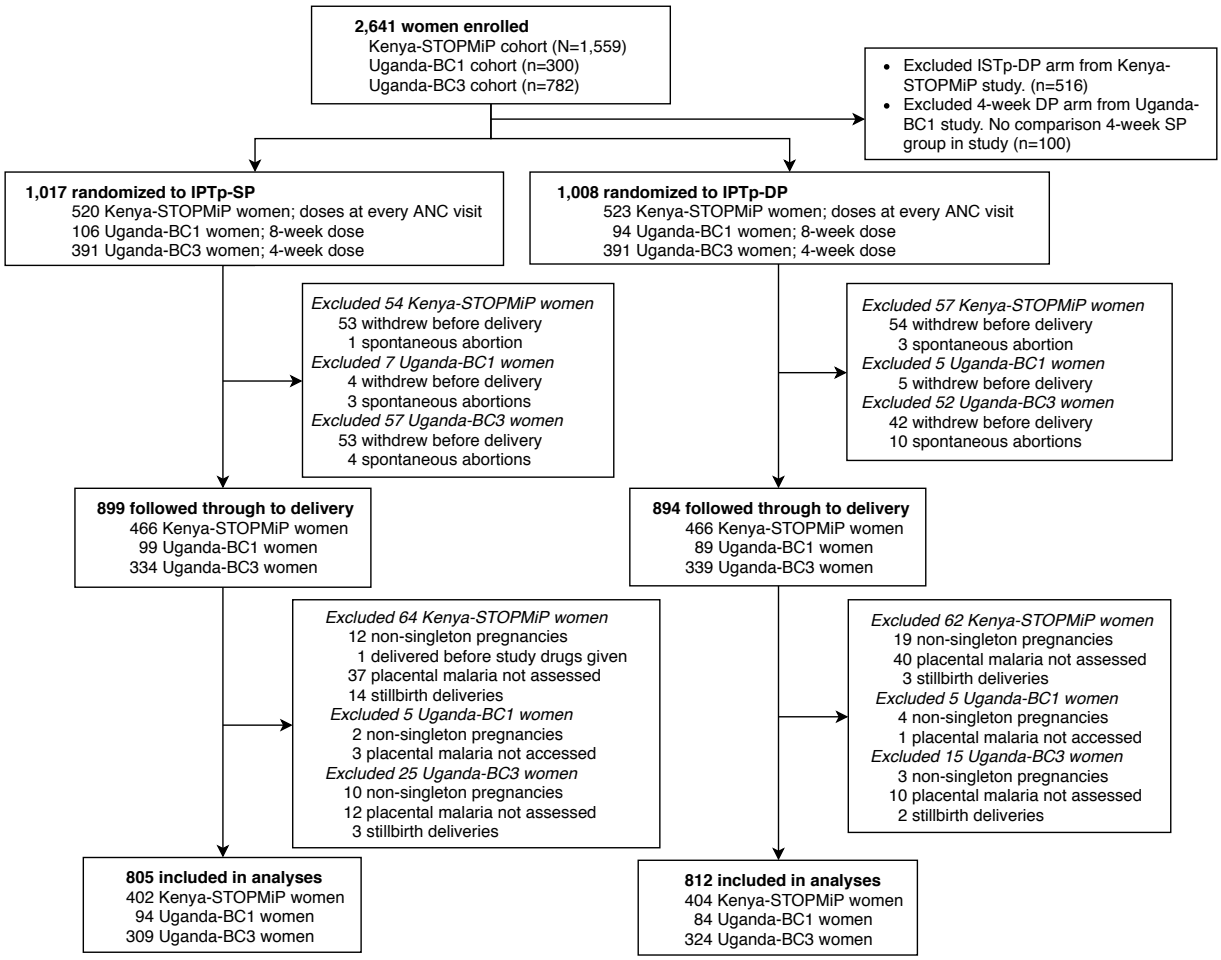


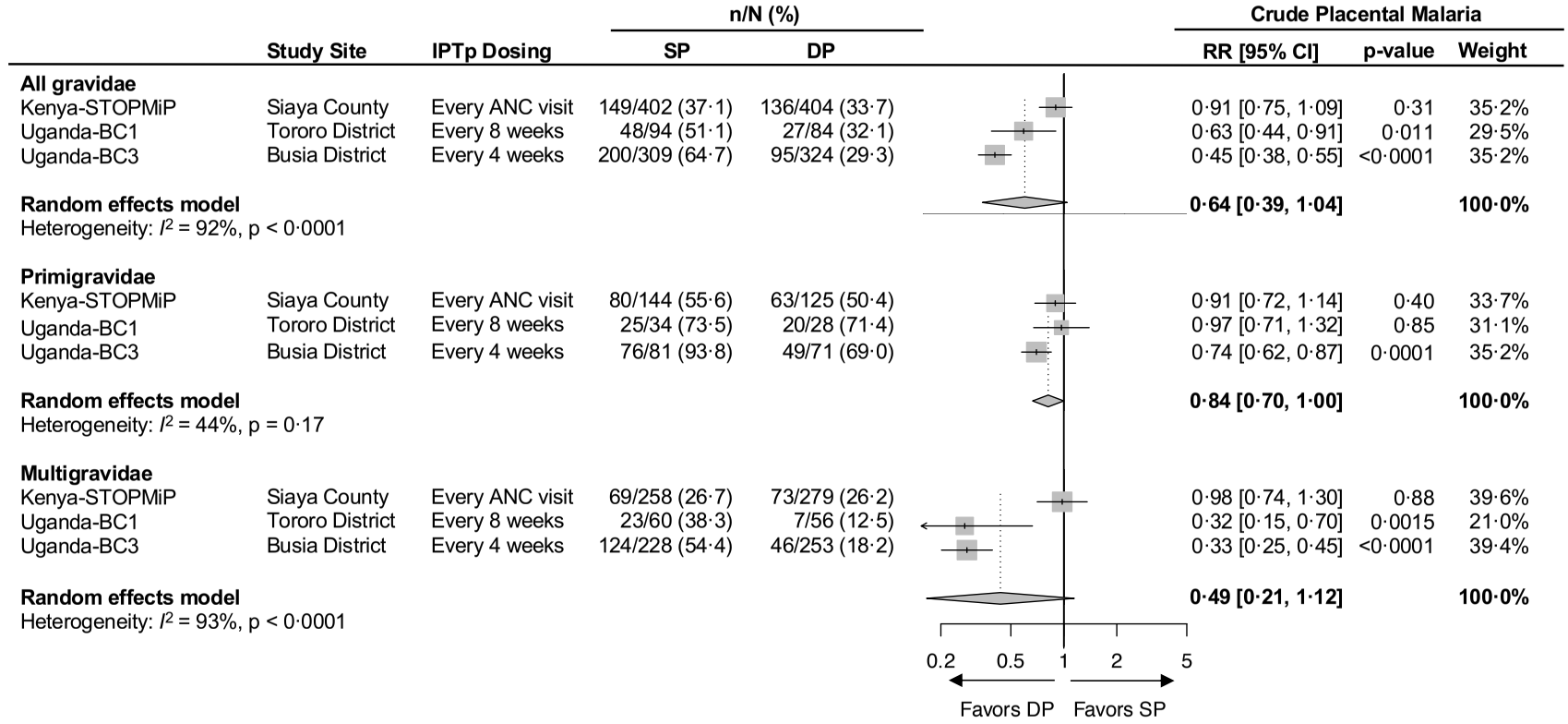
Figure 1.2 Flowchart diagram of participants from the Kenya-STOPMiP, Uganda-BC1, and Uganda-BC3 IPTp trials who were included in the primary analysis.

Table 1.1 Baseline characteristics of the study population for each trial by randomized treatment arm.

Characteristics	Kenya-STOPMiP		Uganda-BC1		Uganda-BC3	
	SP (n=402)	DP (n=404)	SP (n=94)	DP (n=84)	SP (n=309)	DP (n=324)
At enrollment						
Age at enrollment (years), mean (SD)	23·7 (5·9)	23·3 (5·4)	21·5 (3·7)	22·2 (4·4)	24·0 (6·0)	24·0 (5·7)
Weight (kg), mean (SD)	61·6 (9·0)	61·7 (9·3)	55·5 (6·9)	55·6 (6·9)	56·0 (7·8)	55·8 (7·8)
Gestational age (weeks), median (IQR)	22·7 (19·7, 26·3)	22·9 (19·9, 26·1)	14·9 (13·4, 16·9)	14·9 (14·0, 16·6)	15·7 (13·4, 17·9)	15·1 (13·4, 17·1)
Wealth index tertiles, n (%)						
Lowest	133 (33·2%)	124 (30·8%)	36 (38·3%)	28 (33·3%)	103 (33·3%)	112 (34·6%)
Middle	139 (34·8%)	133 (33·1%)	27 (28·7%)	32 (38·1%)	102 (33·0%)	110 (34·0%)
Highest	128 (32·0%)	145 (36·1%)	31 (33·0%)	24 (28·6%)	104 (33·7%)	102 (31·5%)
Level of education, n (%)						
None or primary level	241 (60·2%)	233 (58·2%)	75 (79·8%)	67 (79·8%)	238 (77·0%)	244 (75·3%)
Secondary and beyond	159 (39·8%)	167 (41·8%)	19 (20·2%)	17 (20·2%)	71 (23·0%)	80 (24·7%)
Gravidity, n (%)						
Primigravidae (1st pregnancy)	144 (35·8%)	125 (30·9%)	34 (36·2%)	28 (33·3%)	81 (26·2%)	71 (21·9%)
Multigravidae (2+ pregnancies)	258 (64·2%)	279 (69·1%)	60 (63·8%)	56 (66·7%)	228 (73·8%)	253 (78·1%)
Slept under a net during previous night, n (%)	288 (71·6%)	292 (72·3%)	81 (86·2%)	77 (91·7%)	104 (33·7%)	108 (33·3%)
Maternal parasitemia*, n (%)	126 (32·6%)	120 (31·2%)	52 (55·3%)	50 (59·5%)	254 (82·2%)	257 (79·3%)
Maternal hemoglobin (g/dL), mean (SD)	10·6 (1·5)	10·6 (1·5)	11·8 (1·5)	11·9 (1·1)	11·4 (1·4)	11·4 (1·2)
Following enrollment						
Number of IPTp doses received, n (%)						
1	20 (5·0%)	11 (2·7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
2	158 (39·3%)	179 (44·3%)	6 (6·4%)	5 (6·0%)	2 (0·6%)	0 (0%)
3	110 (27·4%)	114 (28·2%)	88 (93·7%)	79 (94·1%)	3 (1·0%)	3 (0·9%)
4	85 (21·1%)	68 (16·8%)	0 (0%)	0 (0%)	6 (1·9%)	8 (2·5%)
5	25 (6·2%)	29 (7·2%)	0 (0%)	0 (0%)	81 (25·9%)	79 (24·2%)
6	4 (1·0%)	3 (0·7%)	0 (0%)	0 (0%)	156 (49·8%)	152 (46·5%)
7	0 (0%)	0 (%)	0 (0%)	0 (0%)	65 (20·8%)	85 (26·0%)

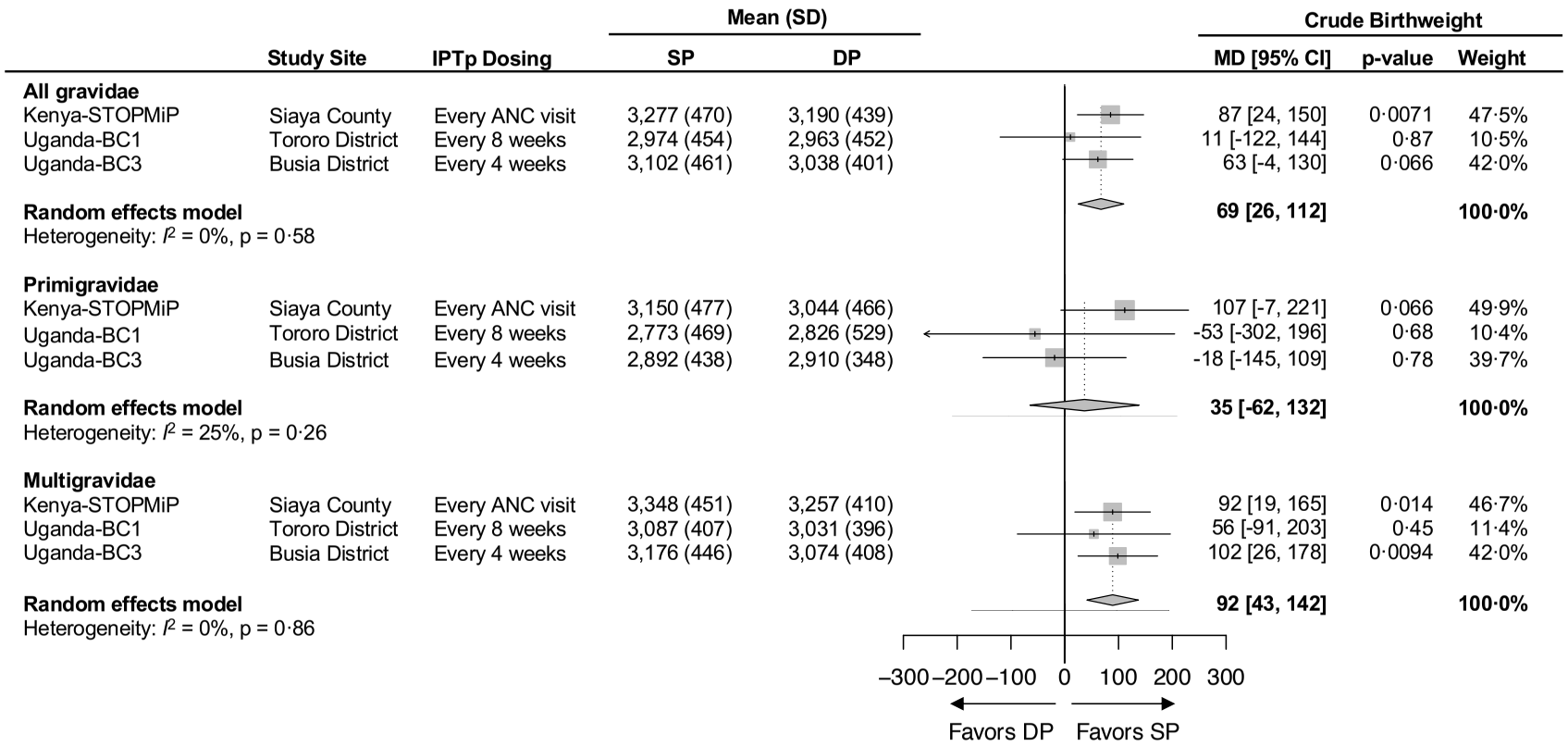
Note: DP=dihydroartemisinin-piperaquine; g/dL=grams per deciliter; IQR=interquartile range; kg=kilograms; SD=standard deviation; and SP=sulfadoxine-pyrimethamine.

*Malaria parasitemia assessed by loop-mediated isothermal amplification in Uganda-BC1 study and by quantitative polymerase chain reaction in the Kenya-STOPMiP and Uganda-BC3 studies.



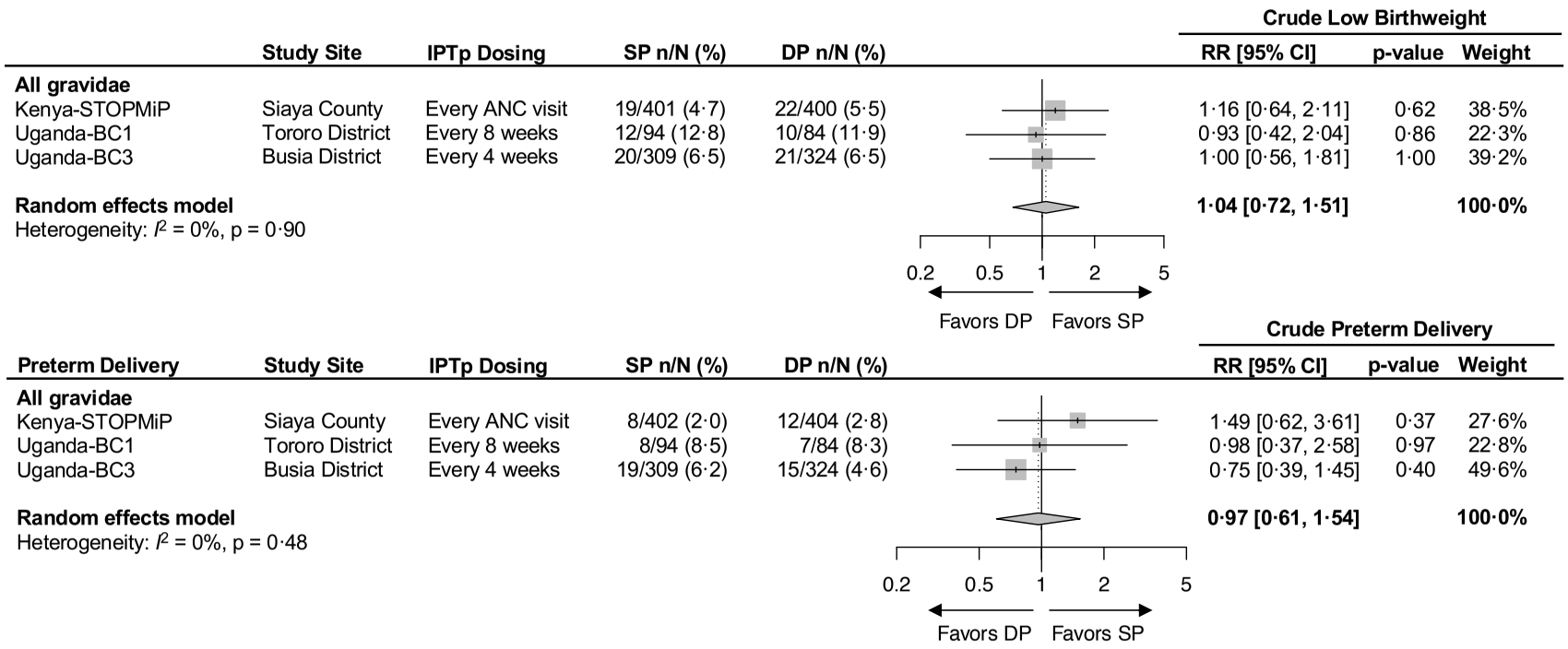
Note: ANC=antenatal care; CI=confidence interval; DP=dihydroartemisinin-piperaquine; IPTp=intermittent preventive treatment; PM=placental malaria; RR=relative risk ratio; and SP=sulfadoxine-pyrimethamine

Figure 1.3 Forest plot of crude differences in placental malaria risk between IPTp arms by study and gravidity subgroup.



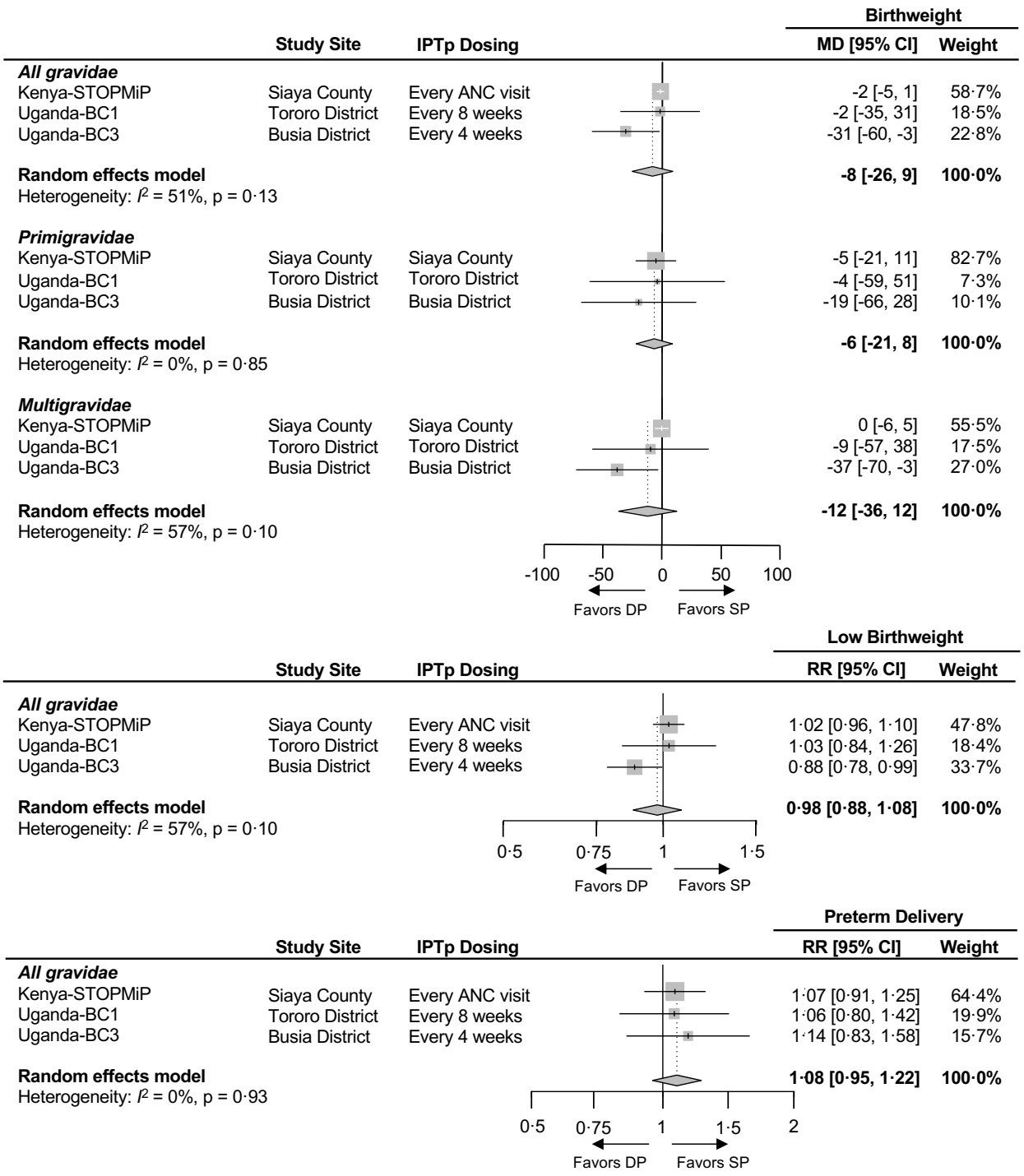
Note: ANC=antenatal care; CI=confidence interval; DP=dihydroartemisinin-piperaquine; IPTp=intermittent preventive treatment; MD=mean difference; SD=standard deviation; and SP=sulfadoxine-pyrimethamine

Figure 1.4 Forest plot of crude differences in mean birthweight between IPTp arms by study and gravidity subgroup.



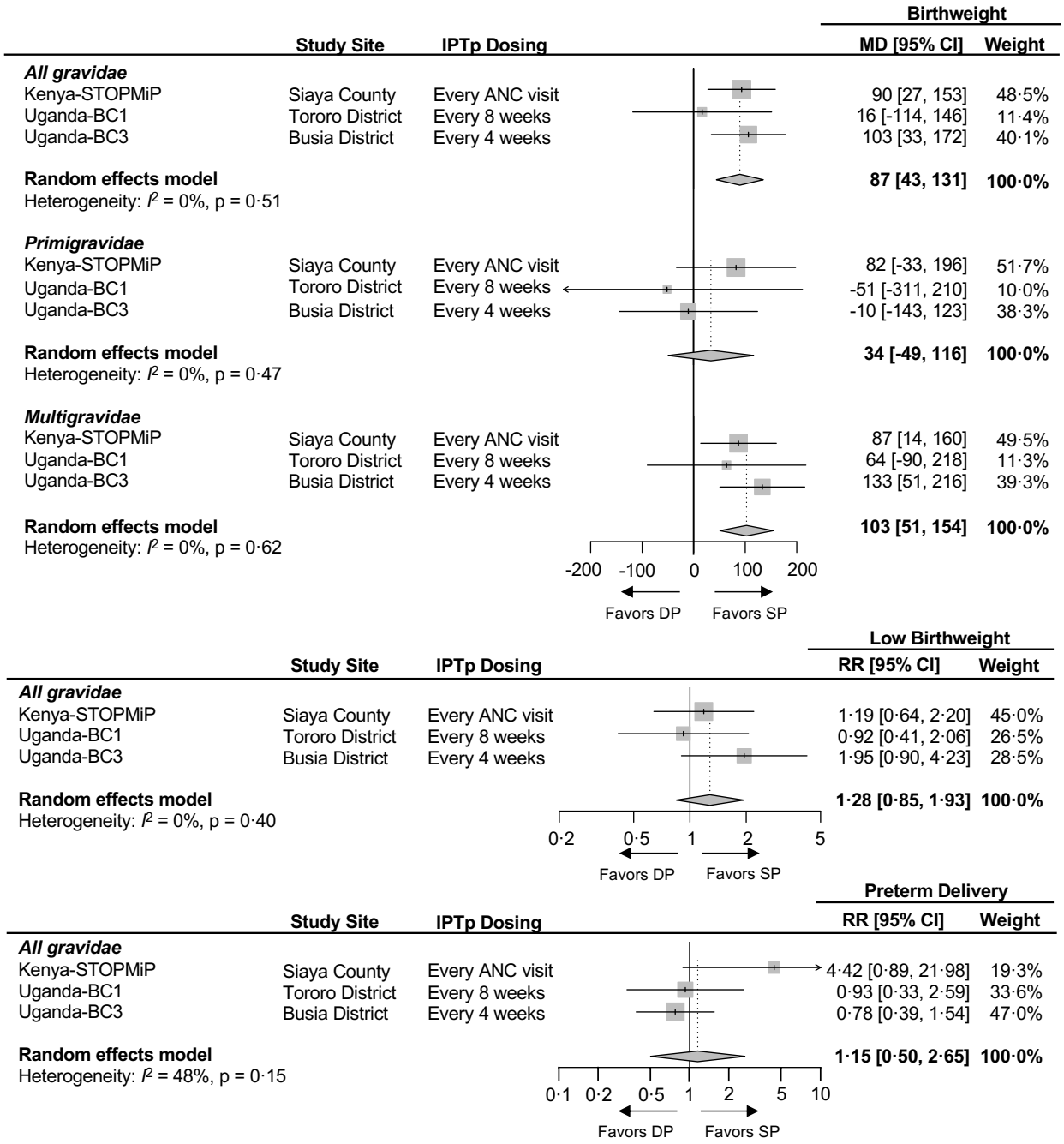
Note: ANC=antenatal care; CI=confidence interval; DP=dihydroartemisinin-piperaquine; IPTp=intermittent preventive treatment; RR=relative risk ratio; and SP=sulfadoxine-pyrimethamine

Figure 1.5 Forest plot of crude differences in low birthweight and preterm delivery between IPTp arms by study.



Note: ANC=antenatal care; CI=confidence interval; DP=dihydroartemisinin-piperaquine; IPTp=intermittent preventive treatment; MD=mean difference; RR=relative risk ratio; and SP=sulfadoxine-pyrimethamine.

Figure 1.6 Forest plot of the mediated/antimalarial effect of IPTp regimens on birthweight, low birthweight, and preterm delivery. Birthweight mediation effect estimates presented by gravidity subgroup.



Note: ANC=antenatal care; CI=confidence interval; DP=dihydroartemisinin-piperaquine; IPTp=intermittent preventive treatment; MD=mean difference; RR=relative risk ratio; and SP=sulfadoxine-pyrimethamine.

Figure 1.7 Forest plot of the non-mediated/non-malarial effect of IPTp regimens on birthweight, low birthweight, and preterm delivery. Birthweight mediation effect estimates presented by gravidity subgroup.

CHAPTER 1 APPENDIX

Appendix Text

Appendix Text 1.1 Association between mother's malaria infection status (mediator) and birthweight (outcome) to define the choice in the mediator.

Women were categorized into four groups that defined her malaria infection status based on the indication of whether she had peripherally detected malaria and/or placental malaria. Here, we define peripheral malaria as the detection of *P. falciparum* parasites in the maternal peripheral blood by PCR at any point between study drug initiation to delivery. Placental malaria was defined as the indication of past or active infections by placental histopathology and/or the presence of parasites in the placental blood detected by microscopy, PCR, and/or LAMP.

Mother's malaria infection status	N=1605	Mean Birthweight (SD)	Adjusted mean difference[1]	
			Beta (SE)	p-value
No peripheral or placental malaria	584 (36.4)	3213 (437)	Ref	--
Peripheral, but no placental malaria	369 (23.0)	3173 (435)	43.1 (33.7)	0.20
No peripheral, but placental malaria	208 (27.7)	3129 (490)	-23.2 (28.6)	0.54
Peripheral and placental malaria	444 (13.0)	3024 (461)	-57.4 (35.5)	0.11

Note: SD=standard deviation; SE=standard error

[1] All models adjusted for the same covariates used in primary analyses and included study-specific fixed effects.

From our adjusted analysis, we found that placental malaria (and not peripheral malaria) was associated with lower birthweights. Our findings support prior studies which suggests that the mechanism by which *P. falciparum* malaria increases the risk of low birthweight is via the sequestration of the *P. falciparum* parasite in the placenta [12, 24]. Thus, we chose to define our mediator as placental malaria.

Appendix Text 1.2 Details of causal mediation analysis methods.

For this study, we estimated the natural indirect (NIE) and direct (NDE) effects as described by Pearl et al [27, 41]. The NIEs and NDE evaluate the mediated and non-mediated effects of the treatment on the outcome when the mediator takes on the value it would have naturally taken under specified counterfactual treatment values [41] (e.g. the NIE and NDE would estimate the relative mediated and non-mediated effect of IPTp with sulfadoxine-pyrimethamine versus IPTp with dihydroartemisinin-piperaquine on birth outcomes, had the risk of placental parasitemia in this study population been what it would have naturally been had women received either sulfadoxine-pyrimethamine or dihydroartemisinin-piperaquine).

To estimate total, direct, and indirect effects, we used the mediation package [28] in R. The associated mediate() function uses a potential outcomes framework [22] to estimate counterfactual values of the outcome under different treatment and mediator scenarios. This requires the specification of two models.

The first model represents the mediator as a function of the treatment and specified predictors (which in our case, were the covariates identified as mediator-outcome confounders). Predictions from this mediator model were used to estimate counterfactual mediator values for each woman: $M_{t=1}$ and $M_{t=0}$, where M_t=the counterfactual mediator value had the individual received treatment value t).

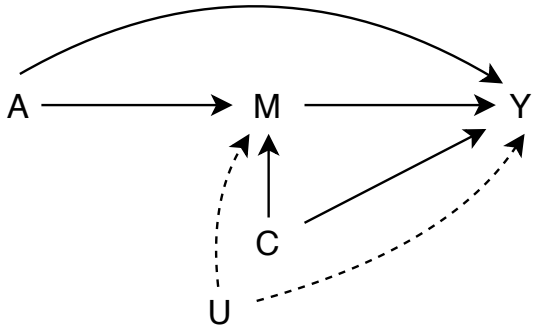
The second model fits the outcome as a function of the treatment, mediator, and mediator-outcome confounders. This fitted model was then used to predict four (possibly) counterfactual outcomes for each woman: $Y_{t=1,M(t=1)}$, $Y_{t=0,M(t=1)}$, $Y_{t=1,M(t=0)}$, and $Y_{t=0,M(t=0)}$; where $Y_{t,M(t)}$ is the outcome for specified counterfactual values of treatment (t=0,1) and mediator ($M_{t=0,1}$). Counterfactual mediator values predicted from the first model ($M_{t=1}$ and $M_{t=0}$) were used to predict counterfactual outcomes in the second model. These counterfactual values are then used to estimate the NDE = $\bar{Y}_{t=1,M(t=0)}$ - $\bar{Y}_{t=0,M(t=0)}$ and NIE= $\bar{Y}_{t=1,M(t=1)}$ - $\bar{Y}_{t=1,M(t=0)}$, where $\bar{Y}$ represents the outcome mean.

Mediation effects and 95% confidence intervals were estimated with a quasi-Bayesian Monte Carlo approach using 1,000 simulations. NIE and NDE risk ratios were calculated by modifying the functions to calculate NDE = $\bar{Y}_{t=1,M(t=0)}$ / $\bar{Y}_{t=0,M(t=0)}$ and NIE = $\bar{Y}_{t=1,M(t=1)}$ / $\bar{Y}_{t=1,M(t=0)}$. Effect modification of NIE and NDE were tested using the test.modmed() function, with p-values reported as $p_{\text{difference}}$.

Appendix Text 1.3 Sensitivity Analysis to Assess the Degree of Unmeasured Mediator-Outcome Confounding Needed to Explain Away Indirect (Mediated)/Direct (Non-mediated) Effect Estimates.

Identification of direct and indirect estimates relies heavily on the following assumptions on mediator-outcome confounding:

(A1) There must not be any unmeasured confounding between treatment and outcome.
(A2) There must not be any unmeasured confounding between treatment and mediator.
(A3) There must not be any unmeasured confounding between mediator and outcome.



Consider the above directed acyclic graph (DAG) representing the relationships between treatment, mediator, and outcome. In this example, A=treatment, M=mediator, Y=outcome, C=measured confounders, and U=unmeasured confounders. For randomized controlled trials, expect conditions A1-A2 to hold as a result of treatment randomization, whereas condition A3 may be violated in the presence of mediator-outcome confounders. Here, we present sensitivity analyses to assess the robustness

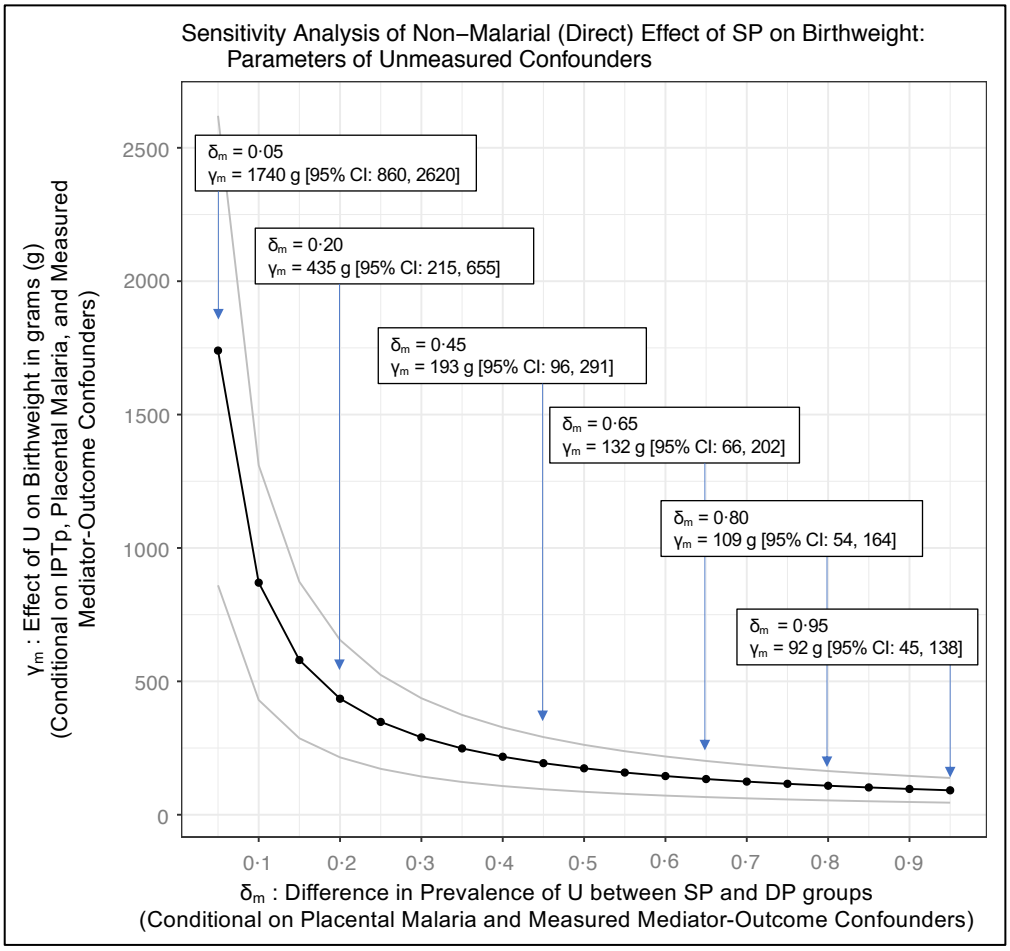
of our estimated direct and indirect effect estimates on birthweight to the presence of unmeasured mediator-outcome confounding.

Birthweight. Sensitivity analyses for the direct and indirect effects on birthweight were conducted using methods previously described by VanderWeele et al [26]. In brief, let B_{add} be a bias factor that denotes the difference between the observed effect estimate and the effect estimate we would have obtained had adjustment for the unmeasured confounder (U) been made. To estimate this bias factor (B_{add}), we used the formula, $B_{add}^{NDE} = \gamma_m \delta_m$ for the natural direct effect and $B_{add}^{NIE} = -B_{add}^{NDE} = -\gamma_m \delta_m$ for the natural indirect estimate, which estimates the effect of U on our outcome (Y), conditional on our treatment, mediator and measured outcome-confounders (γ_m) and the difference in the prevalence of U between treatment groups, conditional on mediator and measured mediator-confounders (δ_m). Sensitivity analyses assumed that the unmeasured confounder (U) was binary and that there was no interaction between treatment (A) and the unmeasured confounder (U).

Using the formulas above, we varied the sensitivity parameters (γ_m and δ_m) to quantify the extent of bias that an unmeasured confounder would be required to have to explain away completely the direct or indirect effect and to shift completely the confidence interval to include the null.

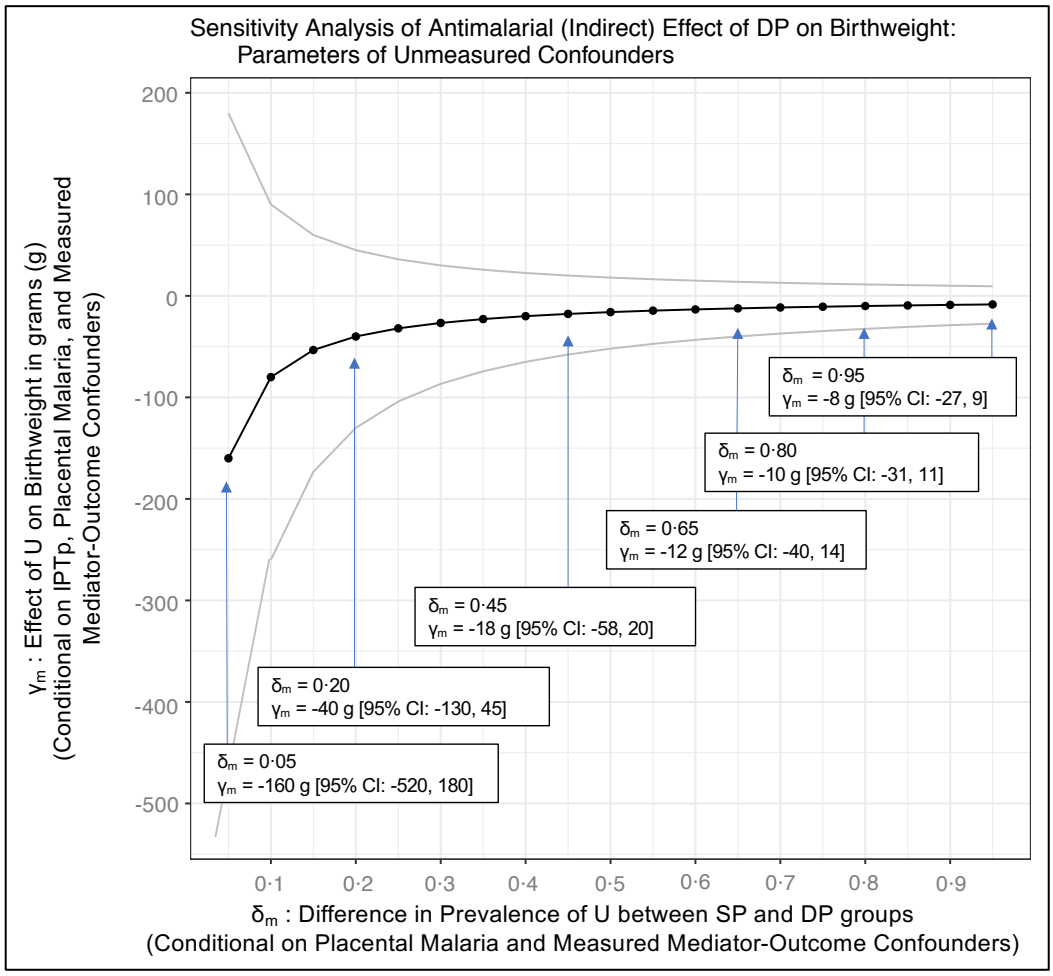
<u>Sensitivity parameters on the direct effect on birthweight.</u> Using the sensitivity analysis approach described above, we found that relatively large parameters of either γ_m or δ_m

would be needed to explain away the relative non-malarial effect of sulfadoxine-pyrimethamine compared to dihydroartemisinin-piperaquine (see Figure below). Small differences in the prevalence of the unmeasured confounder between sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine would require very strong, and likely implausible associations between the unmeasured confounder and birthweight (e.g. if the difference in the prevalence of the confounder between IPTp groups (δ_m) is 0.05, the effect of the confounder on birthweight (γ_m) would need to be 1740 grams to explain away the observed direct effect). Equally, large differences in the prevalence of the unmeasured confounder between IPTp groups would be needed to explain away the observed relative non-malarial benefit of SP vs DP (e.g. if δ_m=0.95, γ_m=92 grams). Thus, it seems unlikely that the magnitude of the non-malarial benefit of SP observed in our analyses is entirely due to unmeasured mediator-outcome confounding.



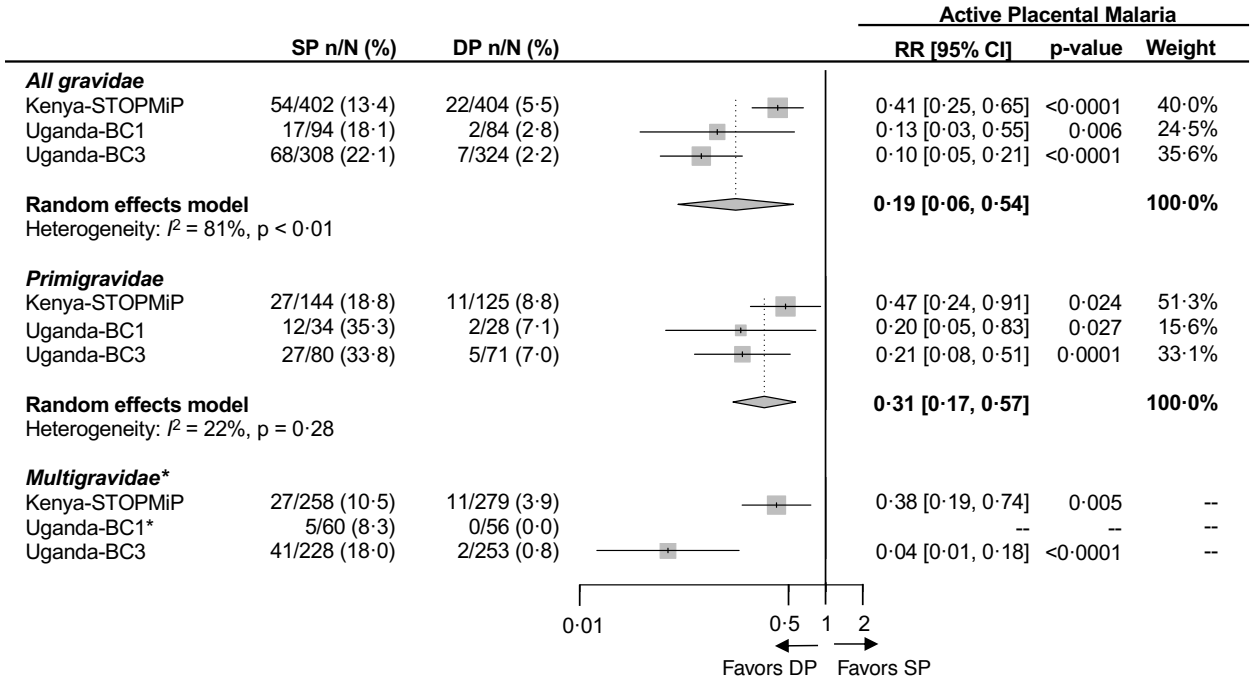
Abbreviations: DP=dihydroartemisinin-piperaquine; SP=sulfadoxine-pyrimethamine; U=unmeasured confounder
Note: Point estimates are shown in black lines, and grey lines denote the upper and lower bounds of the 95% CI.

Sensitivity parameters on the indirect effect on birthweight. Sensitivity analyses were conducted on the observed antimalarial effects between IPTp regimens (see Figure below). We note that observed antimalarial effect, of which the confidence interval included the null, may be sensitive to unmeasured confounding, as moderate effect sizes of unmeasured confounder on birthweight or moderate to high differences in the prevalence of the unmeasured confounder between sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine groups could explain away the observed antimalarial effects on birthweight.



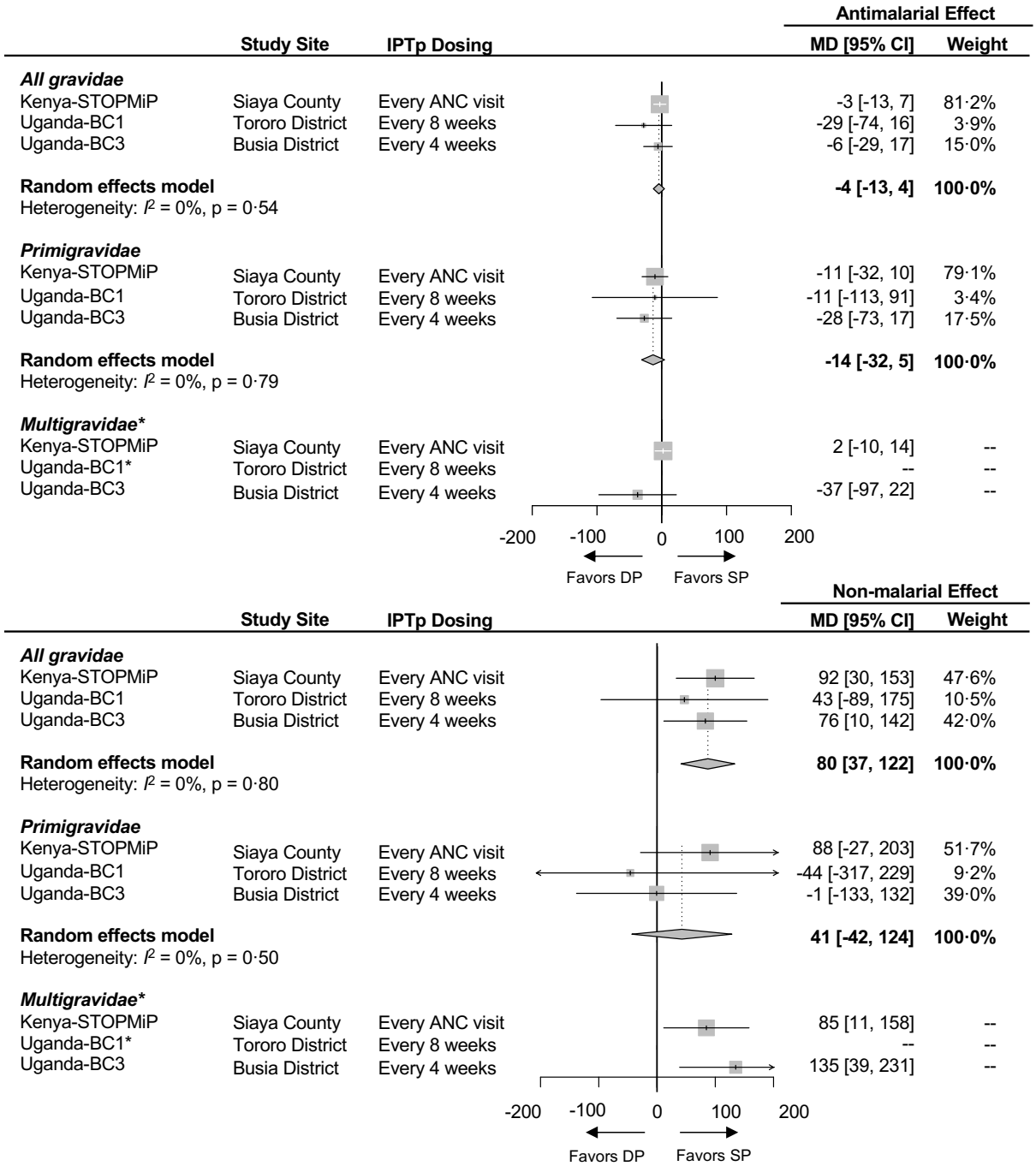
Abbreviations: DP=dihydroartemisinin-piperaquine; SP=sulfadoxine-pyrimethamine; U=unmeasured confounder

Note: Point estimates are shown in black lines, and grey lines denote the upper and lower bounds of the 95% CI.



Note: ANC=antenatal care; CI=confidence interval; DP=dihydroartemisinin-piperaquine; PM=active placental malaria; RR=relative risk ratio; and SP=sulfadoxine-pyrimethamine.
* Relative risk ratio not calculated for Uganda-BC1 multigravidae and multigravidae random effects model as zero events of active placental infection occurred among Uganda-BC1 multigravid women randomised to dihydroartemisinin-piperaquine.

Appendix Figure 1.1 Sensitivity analysis of the crude effect of IPTp regimens on active placental malaria.



Note: ANC=antenatal care; CI=confidence interval; DP=dihydroartemisinin-piperaquine; IPTp=intermittent preventive treatment; MD=mean difference; and SP=sulfadoxine-pyrimethamine.
* Mediation effect estimates were not calculated for Uganda-BC1 multigravidae or for multigravidae random effects model as zero events of the mediator occurred among Uganda-BC1 multigravid women randomised to dihydroartemisinin-piperaquine.

Appendix Figure 1.2 Sensitivity analysis of antimalarial and non-malarial effect estimates where the mediator is defined as active placental infections only.

CHAPTER 2: Impact of long-lasting insecticidal nets treated with and without PBO on preventing adverse birth outcomes

Michelle E. Roh, Brenda Oundo, Grant Dorsey, Stephen Shiboski, Roly Gosling, M. Maria Glymour, Sarah G. Staedke, Adam Bennett, Hugh Sturrock, Arthur Mpimbaza

ABSTRACT

Background. Widespread mosquito resistance to pyrethroid insecticides have called into question the efficacy of long-lasting insecticidal nets (LLINs) and its ability to prevent malaria-associated adverse birth outcomes among pregnant women. In 2017, the Ugandan Ministry of Health conducted a long-lasting insecticidal net (LLIN) campaign. A subset of health subdistricts (HSDs) included in the campaign received LLINs additionally treated with piperonyl butoxide (PBO), a chemical synergist known to partially restore pyrethroid sensitivity. This study aimed to quantify the overall impact of the LLIN campaign on improving pregnancy outcomes and to determine whether PBO LLINs conferred a greater protective effect than conventional (non-PBO) LLINs.

Methods. Birth registry data were retrospectively collected from health facilities across 12 HSDs, 29 months before and 9 months after the LLIN campaign (from 2015 to 2018). Of the 12 HSDs, six received conventional LLINs, five received PBO LLINs, and one received a mix of conventional and PBO LLINs. Interrupted time series (ITS) analyses were used to estimate slope changes in monthly low birthweight (LBW; defined as <2,500 grams) and stillbirth incidence before and 1-3, 4-6, and 7-9 months after the LLIN campaign. Generalized additive models were used to model the effect of the campaign, adjusting for HSD-level differences, seasonal variation, non-linear time

trends, and time-varying maternal characteristics. Difference-in-differences (DiD) analyses were used to assess whether PBO LLINs conferred a greater protective effect than non-PBO LLINs.

Results. Of the 39,085 deliveries included in the study, 4.6% were LBW (n=1,727) and 3.3% were stillbirths (n=1,279). Compared to pre-intervention trends, ITS analyses indicated distribution of any LLIN was associated with reduced LBW (IRR=0.68 [95% CI: 0.49, 0.95]) and stillbirth (IRR=0.56 [95% CI: 0.40, 0.79]) incidence in the 7-9 months following LLIN distribution. Benefits of the campaign were also observed at months 1-3 and 4-6 post-campaign, though effects were greatest for women who delivered 7-9 months after the campaign. Stratified and DiD analyses demonstrated PBO LLINs conferred greater protection compared to conventional LLINs. For example, in the 7-9 months following LLIN distribution, DiD analyses indicated PBO LLINs conferred a 22% greater reduction against LBW and a 33% greater reduction against stillbirth compared to conventional LLINs (IRR_{LBW}=0.78 [95% CI: 0.51, 1.18]; $IRR_{stillbirth}$=0.67 [95% CI: 0.46, 0.98]). Conventional LLINs appeared to confer some protection, though confidence intervals around these effect estimates all included the null.

Conclusion. Our study found a universal LLIN distribution campaign led to lower incidence of LBW and stillbirth, particularly among women distributed LLINs early in pregnancy. However, in this setting of intermediate-to-high pyrethroid resistance, PBO LLINs were more effective than conventional LLINs, further supporting the WHO recommendation for the deployment of PBO LLINs. Future research is needed on whether the switch to PBO LLINs will be more cost-effective.

INTRODUCTION

Malaria in pregnancy is a leading cause of stillbirth and low birthweight (LBW) in sub-Saharan Africa [6]. Despite advances in malaria control [1, 42], over 11 million pregnant women continue to be infected with malaria every year [43], resulting in nearly one million LBW and 220,000 stillbirth deliveries [3, 4, 6, 14, 43]. To prevent the adverse consequences of malaria infection in pregnancy, the World Health Organization (WHO) recommends all pregnant women living in moderate-to-high malaria transmission settings receive long-lasting insecticidal nets (LLINs) as early as possible during pregnancy [13].

In recent years, the spread of resistance of *Anopheles* mosquitoes to pyrethroids has resulted in major concerns over the efficacy of LLINs. As of 2018, pyrethroids have been the only commercially available insecticide recommended for use on LLINs. Although prior studies have shown pyrethroid-based LLINs to be protective [8, 44], recent evidence from Uganda [45] calls into question the current efficacy of these LLINs.

Pyrethroid resistance operates primarily through two main mechanisms: (1) knockdown resistance caused by single-point mutations in the voltage-gated sodium channel where pyrethroids bind and (2) metabolic resistance through mutations in cytochrome P450 (CYP450) genes which act to rapidly increase the clearance of pyrethroids [46-48]. In 2017, the WHO released a conditional recommendation [49] for the use of pyrethroid LLINs additionally treated with piperonyl butoxide (PBO), a chemical synergist known to

inhibit CYP450 enzyme activity [50]. To date, two cluster randomized controlled trials from Tanzania [51] and Uganda [52] demonstrated PBO LLINs were more effective than conventional (non-PBO) LLINs at reducing parasite prevalence. In the Uganda study, PBO LLINs conferred a 26% [95% CI: 13, 28] lower parasite prevalence in children 2-10 years of age compared to conventional LLINs. However, reductions from baseline were also observed in the conventional LLIN group [52], suggesting conventional LLINs may still be protective. Though this study provides clear evidence that both PBO and conventional LLINs are protective against childhood malaria prevalence, little is known about the outcomes among pregnant women who were also distributed LLINs during the campaign.

Using birth registry data collected from a subset of clusters from the Ugandan study, this study aimed to quantify the impact of the LLIN campaign on improving birth outcomes and provide novel insight into whether PBO LLINs conferred a greater protective effect than conventional LLINs.

METHODS

Study Setting

Between March 2017-2018, the Uganda National Malaria Control Program and research collaborators conducted the LLINEUP study, a large-scale cluster randomized controlled trial comparing PBO LLINs to conventional (non-PBO) LLINs for the prevention of malaria [52]. This study randomized 104 health subdistricts (HSDs) in the eastern (n=38) and western (n=66) regions of Uganda to receive PBO LLINs (PermaNet

3.0 or Olyset Plus) or conventional LLINs (PermaNet 2.0 or Olyset Net). Due to errors during study implementation, four HSDs received different LLINs from what they were randomized to and three clusters received a mixture of PBO and conventional LLINs (i.e. individuals within HSD received either PBO or conventional LLINs).

Based on data availability, the current study selected a subset of HSDs from the eastern study sites (12/38; 32%) to evaluate the impact of the LLIN campaign on adverse birth outcomes (**Figure 2.1**). Of these 12 HSDs, six received conventional LLINs (Amuria, Jinja Municipality, Kagoma, Ngora, Samia-Bugwe North, and Soroti Municipality), five received PBO LLINs (Bugweri, Busia Municipality, Kapelebyong, Kigulu North, and Soroti), and one HSD received a mix of PBO and conventional LLINs (Samia-Bugwe South). Timing of the LLIN campaign varied across HSDs, with five HSDs receiving LLINs in late March 2017 and seven HSDs receiving LLINs in mid-May 2017. Baseline entomological surveys among *Anopheles gambiae* (*s.l.*) and *An. funestus* (*s.l.*) mosquitoes demonstrated intermediate-to-high levels of pyrethroid resistance. In the eastern region of Uganda, the allele frequencies of *kdr* mutations, *Vgsc*-L1014S and *Vgsc*-L1014, was 1.00 and 0.06, respectively, while the allele frequency of the mutation for metabolic-based pyrethroid resistance, *Cyp4j5*-L43F, ranged from 0.60-0.80 [46].

Ethical Approvals

Approvals for the current study were granted by the Uganda National Council for Science and Technology, Makerere University College of Health Sciences, and the University of California, San Francisco.

Data Sources

Health Management Information System

To assess birth outcome trends before and after the LLIN campaign, we used health facility birth records collected 29 months before and 9 months after LLIN distribution (which ranged from January 2015 to February 2018). Non-referral health facilities from each HSD were selected if they were government-operated, included a maternity ward, located >5 kilometers from a neighboring HSD (to mitigate bias from exposure misclassification), and had a mean delivery rate of >200 births per year. Of the 34 health facilities that met these criteria, 31 (91%) were selected for further screening (three were not sampled due to budgetary limitations) (**Figure 2.2**). Due to concerns over data quality [53, 54], health facilities were screened by a research team member and excluded if for >33% of the study period: (1) complete months of data were missing or (2) important covariates (e.g. gravidity, HIV) or outcome (e.g. birthweight) values were not recorded. Of the 31 screened health facilities, 8 were excluded due to missing complete months of data (n=8) and 2 were excluded due to systematic missingness of outcome or covariate data (n=2). The final analytic sample included data from 21 health facilities (**Figure 2.2**).

From each health facility, individual-level birth registry data were retrospectively collected from Form 072: Integrated Maternity Registry of the Health Management Information System (HMIS), an integrated surveillance system used to collect relevant disease and health information from all public health facilities in Uganda [55]. This registry, recorded by trained nurses and midwives, included data on delivery outcomes

(e.g. date of delivery, birthweight [rounded to the nearest tenth of a kilogram], and stillbirth) and maternal characteristics (e.g. age, gravidity, and HIV status). Data on prior bed net use or intermittent preventive treatment (IPTp) was not recorded in this form.

LLINEUP Cross Sectional Surveys

Given only a subset of LLINEUP study HSDs were used for this analysis, we used cross sectional survey data collected from the original trial to re-analyze the primary malaria efficacy endpoint among the study HSDs included in the current study. This was done to assess whether inferences in malaria outcomes differed between the original trial of 104 HSDs and the 12 included in this study. In the LLINEUP study, parasite prevalence was collected using cross sectional surveys conducted 0, 6, and 12 months after LLIN distribution. For each survey, 50 households with at least one child 2-10 years of age and adult (aged ≥18 years) present were randomly sampled from each HSD. All children 2-10 years of age from each household were asked to provide a fingerprick blood sample to test for malaria parasites by microscopy. Additional details of the cross-sectional survey and laboratory procedures can be found in the LLINEUP trial [52].

Treatment Variable

For interrupted time series analyses, treatment was defined as the cumulative nine-month period after LLIN distribution. To assess for dose-dependent effects, treatment was further categorized into three-month intervals (to approximate pregnancy trimesters), so that the impact of the campaign could be assessed at months 1-3, 4-6, and 7-9 after LLIN distribution. For difference-in-difference analyses comparing PBO

versus conventional LLINs, treated and control units were defined as HSDs that received PBO LLINs and conventional LLINs, respectively.

Outcomes

For the current study, the primary birth outcome was LBW (defined as birthweight <2,500 grams) assessed among live births. Secondary outcomes included birthweight as a continuous measure (live births only) and stillbirth. Birth outcomes were assessed only among singleton deliveries.

Statistical Analysis Plan

Impact of LLIN Campaign

Interrupted time series (ITS) analyses [56-59] were used to quantify the impact of the LLIN campaign on reducing the incidence of adverse birth outcomes. An ITS study uses time series data observed during the pre-intervention period to establish an underlying trend and assumes that this trend would have remained unchanged had it not been 'interrupted' by the intervention of interest [57]. The counterfactual (i.e. the trend in birth outcomes had the LLIN campaign never occurred) is estimated by extrapolating the pre-intervention trend onto the post-intervention period. The impact of the intervention is estimated by taking the difference between the extrapolated counterfactual trend and the observed trend during the post-intervention period.

ITS analyses were conducted using segmented regression [56] to estimate the change in slope between pre- and post-campaign periods. Negative binomial generalized

additive models (GAM) were used to estimate monthly LBW and stillbirth incidence using data aggregated to the HSD. Models included the following predictors: time (in months) since the start of the study, time after the LLIN campaign (i.e. treated period), mean maternal age, proportion of primigravidae, proportion of HIV-positive women, calendar month (e.g. January, February, March, etc.) to account for seasonal trends [57], HSD-level fixed effects (to account for group-level variation), and an offset term for the log of the total number of deliveries recorded that month. Continuous time-varying covariates were modeled as smooth functions to accommodate possible non-linear relationships with the outcome. A first-order autoregressive AR(1) structure was applied to the residuals to account for serial autocorrelation. Separate models were used to determine the impact of distributing any LLINs, conventional LLINs, or PBO LLINs.

Effect estimates for birthweight (as a continuous measure) were modeled using individual-level data. Generalized additive mixed models (GAMM, with Gaussian errors and identity link function) were used to estimate changes in mean birthweight. Both GAM and GAMM models were fitted using the mgcv package in R. Quantile GAM regression (fitted using qreg2 package in Stata) was also used for further evaluation of campaign effects at varying birthweight percentiles (2.5^{th}, 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 80^{th}, 90^{th}, and 95^{th}). Standard errors specifying clustering at the health facility-level were used to compute 95% confidence intervals.

Individual-level data were also used to test for effect modification by gravidity and HIV. Effect estimates were reported separately if the p-value of the joint F-test of the

interaction terms was >0.1. All individual-level models adjusted for the same fixed effects as incidence models but included random intercepts for each health facility.

Comparison of PBO and conventional LLINs

To determine whether PBO LLINs conferred a greater protective effect than conventional LLINs, we used difference-in-differences (DiD) analyses. Unlike the ITS approach which uses the pre-intervention trend to estimate the counterfactual, DiD uses pre-post trends observed in a contemporaneous control group. Since both groups received LLINs, the counterfactual trend estimated by DiD represents what would have occurred in the PBO group had they received conventional LLINs. DiD analyses adjust for two types of confounding: group-varying, but time-invariant (i.e. baseline differences between PBO and conventional LLIN HSDs) and time-varying, but group-invariant (i.e. changes in IPTp scale-up over time).

DiD models included the same covariates as the primary analysis (e.g. HIV, gravidity, maternal age), however, treatment was defined as the duration since PBO LLIN distribution or 0 otherwise. HSDs that received a mix of PBO and conventional LLINs (i.e. Samia-Bugwe South) were excluded from DiD analyses. The validity of DiD estimates relies on the identifying assumption that PBO and conventional LLIN groups would have shared common trends had the PBO group received conventional LLINs. Though the validity of this assumption cannot be proven, we found little evidence that trends between PBO and conventional LLIN groups differed during the pre-intervention

period (p-values from time x PBO interaction terms: p_{LBW}=0.94, $p_{birthweight}$=0.34, and $p_{stillbirth}$=0.26).

All tests were two-sided (α=0.05) and p-values less than 0.05 were considered statistically significant. Analyses were conducted in R (version 3.5.3) and Stata (StataCorp LLC, version 14.0).

RESULTS

Descriptive Analysis

Over the 38 months of observation (January 2015 to February 2018), data on 39,085 singleton deliveries were available from five HSDs that received conventional LLIN (n=13,156), six HSDs that received PBO LLINs (n=18,353), and one HSD that received a mixture of conventional and PBO LLINs (n=7,576). Approximately 3.3% of deliveries were stillbirths (n=1,279) and of the total live births (n=37,806), 4.6% (n=1,727) were LBW.

Table 2.1 shows the characteristics and birth outcomes of the study population in each LLIN group during the pre- and post-campaign periods. Overall, mean maternal age and HIV prevalence were similar across LLIN groups, pre- and post-campaign periods. The proportion of primigravidae was generally higher during post-campaign months, but this finding was consistent between conventional and PBO LLIN groups. Samia-Bugwe South, the HSD that received a mixture of PBO and conventional LLINs, had a higher

proportion of primigravidae compared to both PBO and conventional LLIN groups, but this finding was similar between the pre- and post-campaign period.

Malaria (parasite prevalence in children 2-10 years of age)

Findings from the reanalysis of primary malaria outcome among the 12 HSDs included in this study were similar to those observed in the parent trial [52]. At baseline, parasite prevalence among children 2-10 years of age (the primary malaria endpoint of the LLINEUP study) was 36% across the 12 HSDs; 32% in the conventional LLIN group and 35% in the PBO LLIN group (**Table 2.2**). Compared to baseline, parasite prevalence was lower 6- and 12-months after the campaign in both conventional and PBO LLIN groups. After adjusting for baseline differences, distribution of PBO LLINs was associated with a greater reduction in parasite prevalence compared to the conventional LLIN group at months 6 and 12 after LLIN distribution, though a statistically significant difference was only observed at the 12-month survey (21% versus 13%; adjusted prevalence ratio=0.53 [95% CI: 0.37, 0.75]).

Low Birthweight

In the nine months following LLIN distribution, there was a 21% reduction in LBW incidence (incidence rate ratio (IRR)=0.79 [95% CI: 0.64, 0.99]) (**Figure 2.3**). Though reductions in LBW were seen at months 1-3 and 4-6 after the campaign, the effect size was largest among women who delivered 7-9 months after the campaign (IRR=0.68 [95% CI: 0.49, 0.95]). Individual-level models testing for effect heterogeneity showed little evidence that campaign effects on LBW differed between gravidity subgroups

(p=0.66) or HIV-positive women (p=0.16). Stratified ITS analyses indicated both PBO and conventional LLINs were associated with lower LBW incidence compared to their respective pre-intervention trends, though reductions in the PBO LLIN group appeared to be greater at each 3-month treatment interval. DiD analyses, which compared pre-post trends between LLIN groups, reflected similar findings, indicating for women delivering 7-9 after the campaign, distribution of PBO LLINs was associated with a 22% greater reduction in LBW incidence compared to conventional LLINs (IRR=0.78 [95% CI: 0.51, 1.18]) (**Figure 2.4**).

Birthweight

Overall, the campaign was associated with a modest increase in mean birthweight during the cumulative nine-month post-campaign period (mean difference (MD)=22 grams [95% CI: -8, 52]) (**Figure 2.5**). The effect was largest among women who delivered 7-9 months after the campaign (MD=87 grams [95% CI: 62, 111]). In the stratified ITS analysis, increases in mean birthweight were observed in both PBO and conventional LLIN groups for women delivering 7-9 months after the campaign. However, PBO LLINs did not appear to confer a greater benefit than conventional LLINs ($MD_{conventional}$=64 grams [95% CI: -20, 149] versus MD_{PBO}=46 grams [95% CI: 7, 84]), a finding which was also confirmed by DiD analyses (**Figure 2.6**). Exploratory quantile regression analyses were used to further examine the relationship between PBO and conventional LLINs at values other than the mean (i.e. at specified percentiles) (**Appendix 2.1**). Though confidence intervals around quantile regression coefficients

were too wide to provide any conclusive evidence, we found PBO LLINs appeared to benefit lower birthweight babies more than conventional LLINs (≤10th percentile).

Stillbirth

ITS analyses indicated distribution of any LLINs was associated with a 16% reduction in stillbirth incidence (IRR=0.84 [95% CI: 0.60, 1.16]) during the cumulative nine-month post-campaign period (**Figure 2.7**). Reductions in stillbirth were seen across all treated periods, though effects were largest among women who delivered 7-9 months after the campaign (IRR=0.56 [95% CI: 0.40, 0.79]). Individual-level models testing for effect heterogeneity indicated little evidence to suggest the effects of the campaign differed between gravidity subgroups (p=0.99) or HIV-positive women (p=0.15). Stratified ITS analyses showed that both PBO and conventional LLINs were generally associated with stillbirth reductions, except among women of the conventional LLIN group who delivered 7-9 months after the campaign (IRR=1.34 [95% CI: 0.58, 3.05]). However, uncertainty around this effect estimate was too large to provide any conclusive evidence as confidence intervals included both harmful and beneficial effects. DiD analyses indicated PBO LLINs conferred a 33% greater reduction in stillbirth incidence compared to conventional LLINs, especially among women who delivered 4-6 and 7-9 months after the campaign ($IRR_{4\text{-}6\ months}$=0.67 [95% CI: 0.45, 0.99]; $IRR_{7\text{-}9\ months}$=0.67 [95% CI: 0.46, 0.98]) (**Figure 2.8**).

DISCUSSION

In 2017, the Ugandan Ministry of Health conducted a nationwide LLIN campaign. In a subset of eastern sites, we found that the campaign was associated with a 21% reduction in LBW incidence and a 31% reduction in stillbirth incidence over a nine-month period. Effect sizes in our study were similar those reported by prior meta-analysis [8] of randomized controlled trials conducted between 1998-2003 which found insecticide-treated nets were associated with a 20% reduction in LBW risk and 32% reduction in stillbirth risk. In addition, we found the benefit of the campaign was greatest for women who delivered 7-9 months after the campaign, supporting the general consensus that the provision of LLINs early in pregnancy will lead to better birth outcomes. Comparison of PBO and conventional LLINs based on stratified ITS and DiD analyses demonstrated that while conventional LLINs appeared to confer some protection, PBO LLINs were more beneficial for reducing LBW and stillbirth incidence in this region of intermediate-to-high prevalence of metabolic-based pyrethroid resistance mutations. Unexpectedly, a modest increase in stillbirth incidence was observed 7-9 months after conventional LLIN distribution. Given the wide confidence intervals around this estimate (0.58-3.05) and inconsistency with effects observed in earlier periods and existing literature [8], its likely this result was attributable to chance.

Distribution of PBO and conventional LLINs were associated with increases in birthweight, particularly among women who delivered 7-9 months after the campaign. However, distribution of PBO LLINs were not associated with greater birthweight benefits compared to conventional LLINs. Our exploratory quantile regression analyses

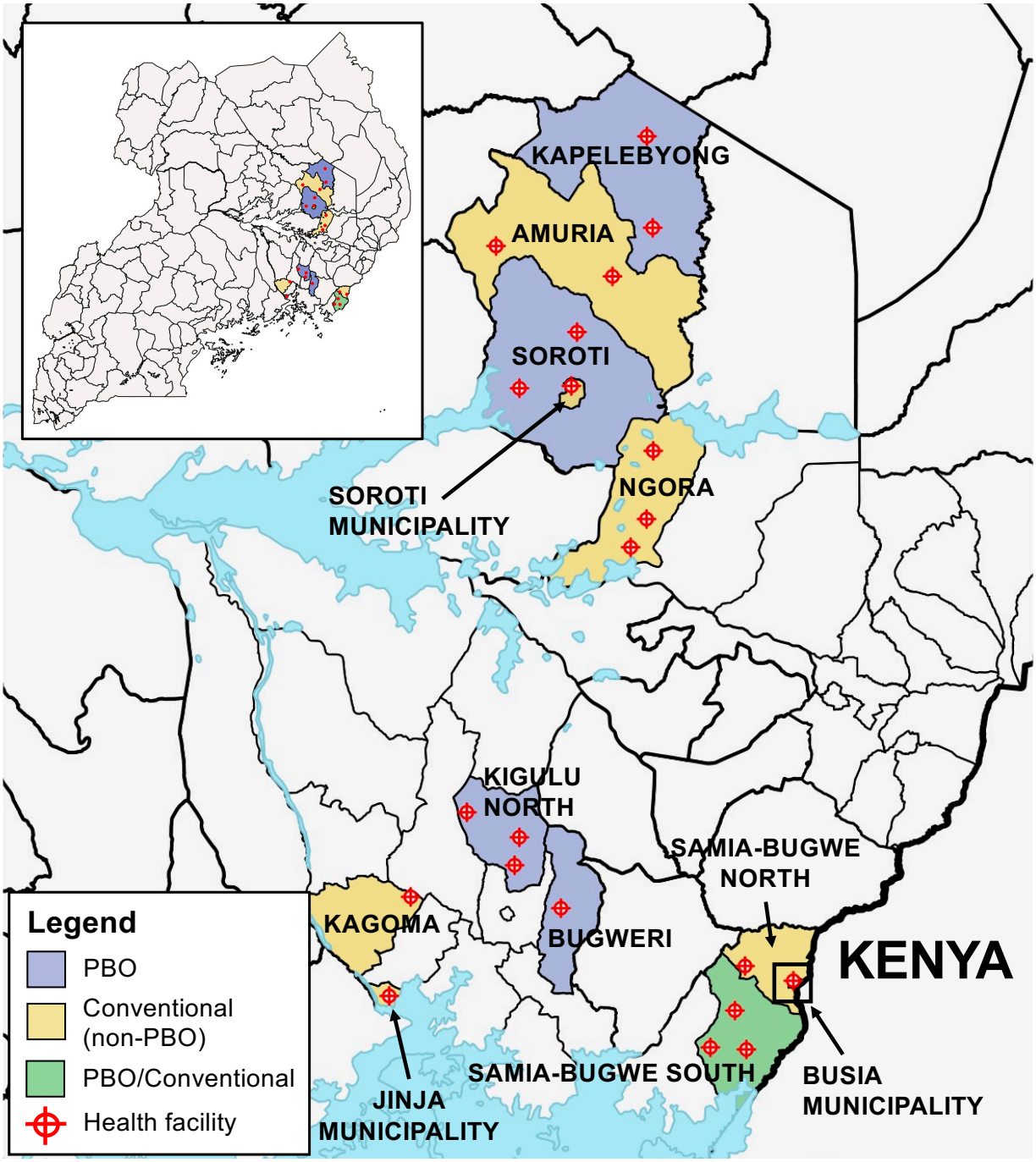
suggest PBO LLINs may confer greater protection than conventional LLINs for women delivering babies at lower birthweight percentiles. However, confidence intervals around quantile regression coefficients were too large to provide any conclusive evidence. Thus, more research with a larger sample size and more precise birthweight measurements will be needed to confirm our analyses.

The study had a few limitations. First, a number of health facilities were excluded from study HSDs. Though this process was done to ensure the collection of high-quality data, it may have limited the external validity of our findings. Furthermore, inferences of our study are limited health facility-based deliveries and may not be generalizable to home-based deliveries, which in Uganda, make up approximately 30% of all births [60]. Though it is unlikely that the effect of LLIN campaign would have differed solely based on place of delivery, it may be a proxy for some underlying factor (e.g. antenatal care attendance and receipt of other malaria interventions) that could have modified the effects of the LLIN campaign. Second, though the HMIS database enabled us to capture a comprehensive set of delivery information, variables in this registry may have been measured with error (e.g. gravidity, HIV, and birthweight). Measurement error of gravidity and HIV may have reduced our ability to adequately control for these time-varying covariates and thus, accurately assess for effect modification. In addition, rounded birthweight measurements likely increased random error, which may in part explain the wide confidence intervals around effect estimates [61]. Third, geographic information on the residence of women delivering at health facilities was not collected due to frequent missingness of these data. Thus, the HSD where the woman gave birth

may not accurately represent their HSD of residence. We attempted to mitigate this type of exposure misclassification by selecting health facilities at least five kilometers away from neighboring HSDs, however, this type of non-differential misclassification may have biased our effect estimates toward from the null. Fourth, due to the limited number of HSDs analyzed in this study, we did not test for differences between conventional and PBO LLIN brands (PermaNet and Olyset). Lastly, despite our effect estimates being highly consistent with prior work [8], we cannot rule out bias due to residual or unobserved confounding.

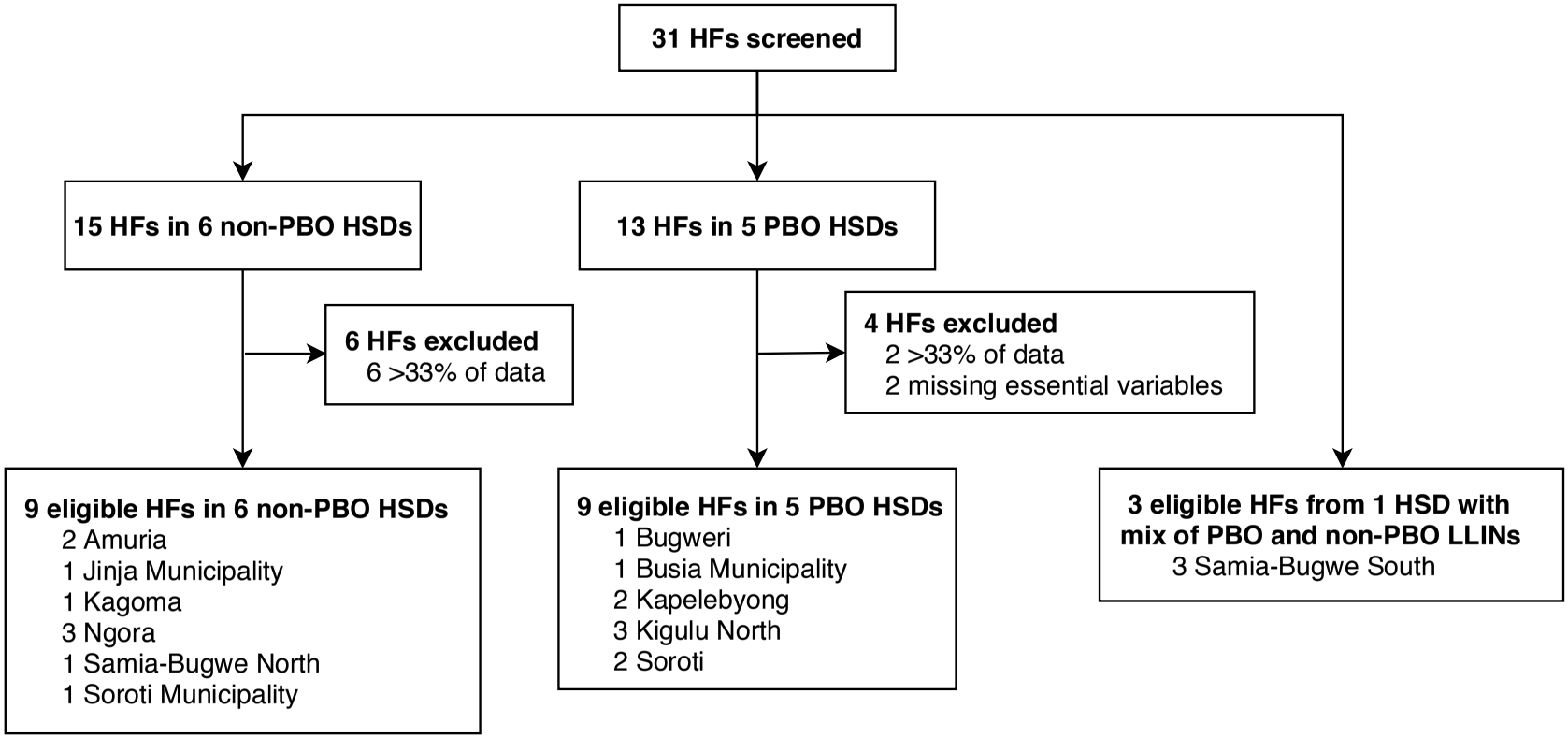
Though our study was limited by its non-randomized design and small number of clusters, this is the first study to show the beneficial effects of PBO LLINs on birth outcomes. Our findings are consistent with the parent study [52] and the latest WHO recommendation [49] which support the hypothesis that in areas of intermediate-to-high levels of metabolic-based pyrethroid resistance, PBO LLINs are likely confer greater protection than conventional LLINs. As national malaria control programs begin deploying PBO LLINs in recommended areas, countries should consider reporting the level and type of pyrethroid resistance in conjunction with impact outcomes to provide further evidence of where PBO LLINs may be more effective than conventional LLINs. Furthermore, these evaluations should not only consider reporting the impact of PBO LLINs on malaria outcomes, but reporting more downstream health outcomes, especially among high-risk groups (e.g. pregnant women and children). This will provide a greater evidence base on the public health impact of PBO LLINs which will help to finalize WHO's recommendation on PBO LLINs. Lastly, as PBO LLINs are likely to be

more expensive than conventional LLINs, additional studies are needed on the cost-benefit of switching to PBO LLINs.



Abbreviations: PBO=piperonyl butoxide

Figure 2.1 Map of the study health sub-districts (HSDs) (n=12) and health facilities (n=21). Purple shaded areas indicate HSDs that received PBO long-lasting insecticidal nets (LLINs); yellow shaded areas indicate HSDs that received conventional (non-PBO) LLINs; and the green shaded area indicates the HSD that received a mix of PBO and conventional LLINs. Red points indicate the geographic location of study health facilities where delivery information was collected.



Abbreviations: HF=health facility; HSD=health-subdistrict; LLIN=long-lasting insecticidal net; PBO=piperonyl butoxide

Figure 2.2 Flowchart of health facility selection.

Table 2.1 Study population characteristics across LLIN groups stratified by pre- and post-campaign periods. Summary statistics are presented as monthly averages/proportions across health subdistricts.

	Conventional LLINs		PBO LLINs		Conventional + PBO LLINs	
	Pre-period	**Post-period**	**Pre-period**	**Post-period**	**Pre-period**	**Post-period**
Total number of observations	9129	4027	13880	4473	5754	1822
Maternal age in years, mean (SD)	24.6 (1.2)	24.3 (0.8)	24.4 (0.8)	24.1 (0.6)]	23.5 (0.5)	23.5 (0.4)
% Primigravidae, mean (SD)	22.0 (10.3)	26.8 (9.3)	20.6 (7.6)	26.1 (7.6)	30.9 (4.3)	32.2 (2.4)
% HIV, mean (SD)	3.4 (2.6)	3.3 (3.1)	3.7 (2.4)	3.7 (1.8)	3.9 (1.5)	3.8 (1.6)
Birth Outcomes						
LBW infants per 100 births, mean (SD)	4.3 (3.8)	3.8 (3.3)	4.5 (4.1)	4.0 (3.0)	5.5 (2.3)	4.5 (2.0)
Stillbirths per 100 deliveries, mean (SD)	2.1 (8.7)	1.4 (2.4)	4.3 (4.6)	4.0 (4.4)	4.0 (2.3)	4.9 (2.1)
Birthweight in grams, mean (SD)	3119 (92)	3134 (83)	3194 (156)	3139 (106)	3070 (58)	3152 (84)

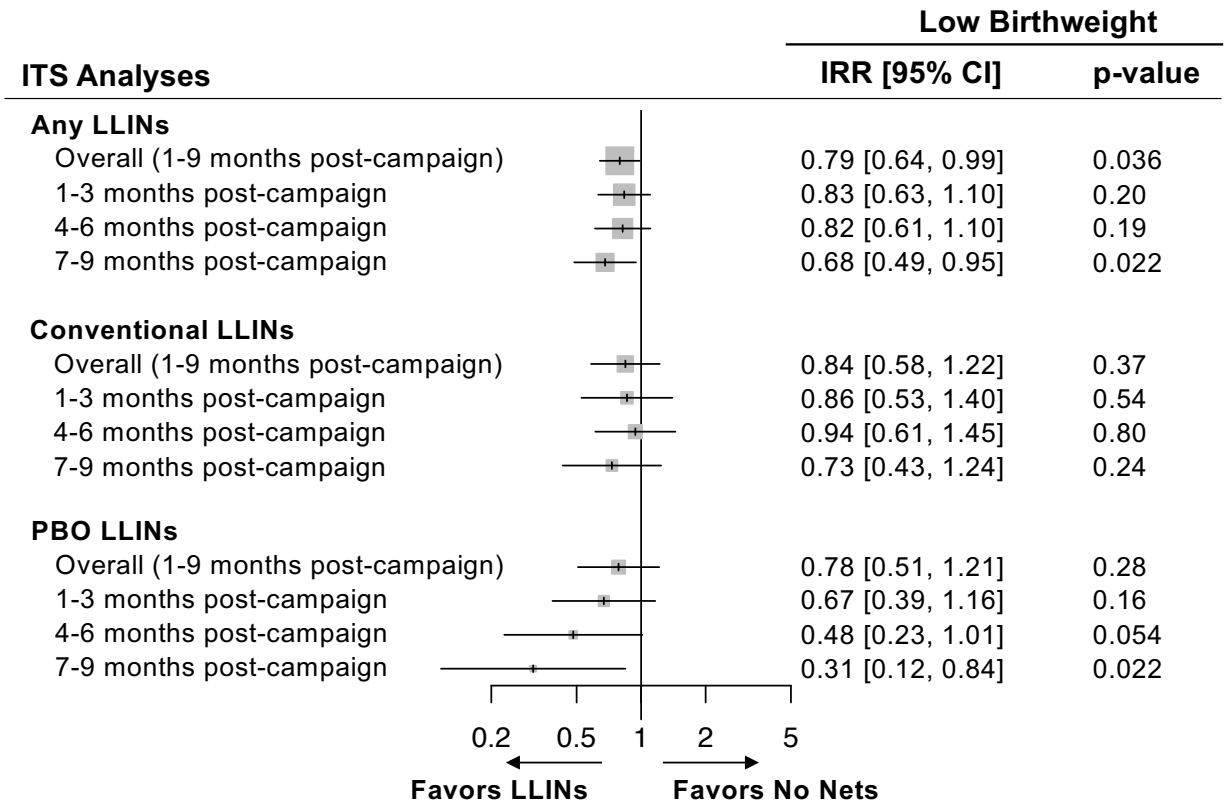
Abbreviation: LLIN=long-lasting insecticidal net; PBO=piperonyl butoxide; SD=standard deviation

Table 2.2 Comparison of conventional and PBO LLIN groups based on the primary malaria efficacy endpoint in the LLINEUP trial (e.g. prevalence of parasitemia in children 2-10 years of age). Analyses are presented by survey month and LLIN type.

Survey month	n/N (%)	PR* [95% CI]	p-value
Baseline			
Any LLIN	399/1098 (36%)	--	--
Conventional LLIN	167/530 (32%)	Ref	--
PBO LLIN	172/485 (35%)	1.13 [0.62, 2.05]	0.68
6 months			
Any LLIN	218/968 (23%)	--	--
Conventional LLIN	108/485 (22%)	Ref	--
PBO LLIN	83/399 (21%)	0.78 [0.53, 1.14]	0.20
12 months			
Any LLIN	178/984 (18%)	--	--
Conventional LLIN	98/476 (21%)	Ref	--
PBO LLIN	55/427 (13%)	0.53 [0.37, 0.75]	<0.001

Abbreviation: LLIN=long-lasting insecticidal net; PBO=piperonyl butoxide; PR=prevalence ratio

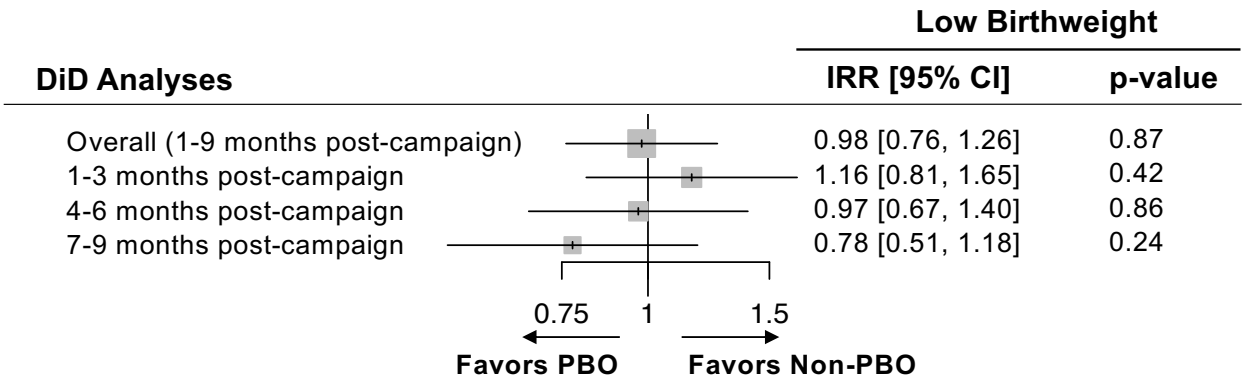
* Prevalence ratios were estimated using log-binomial regression models with generalized estimating equations to account for within-cluster correlation, and adjusted for baseline cluster-level parasite prevalence, as done in the parent trial [52].



Abbreviations: IRR=incidence rate ratio; ITS=interrupted time series analyses; LLIN=long-lasting insecticidal net; PBO=piperonyl butoxide

* Negative binomial generalized additive models were used to estimate incidence rate ratios (IRRs). Models adjusted for time (in months) since the start of the study period; maternal age; gravidity; HIV; calendar month; health-subdistrict fixed effects; and log of total number of deliveries per month included as an offset term.

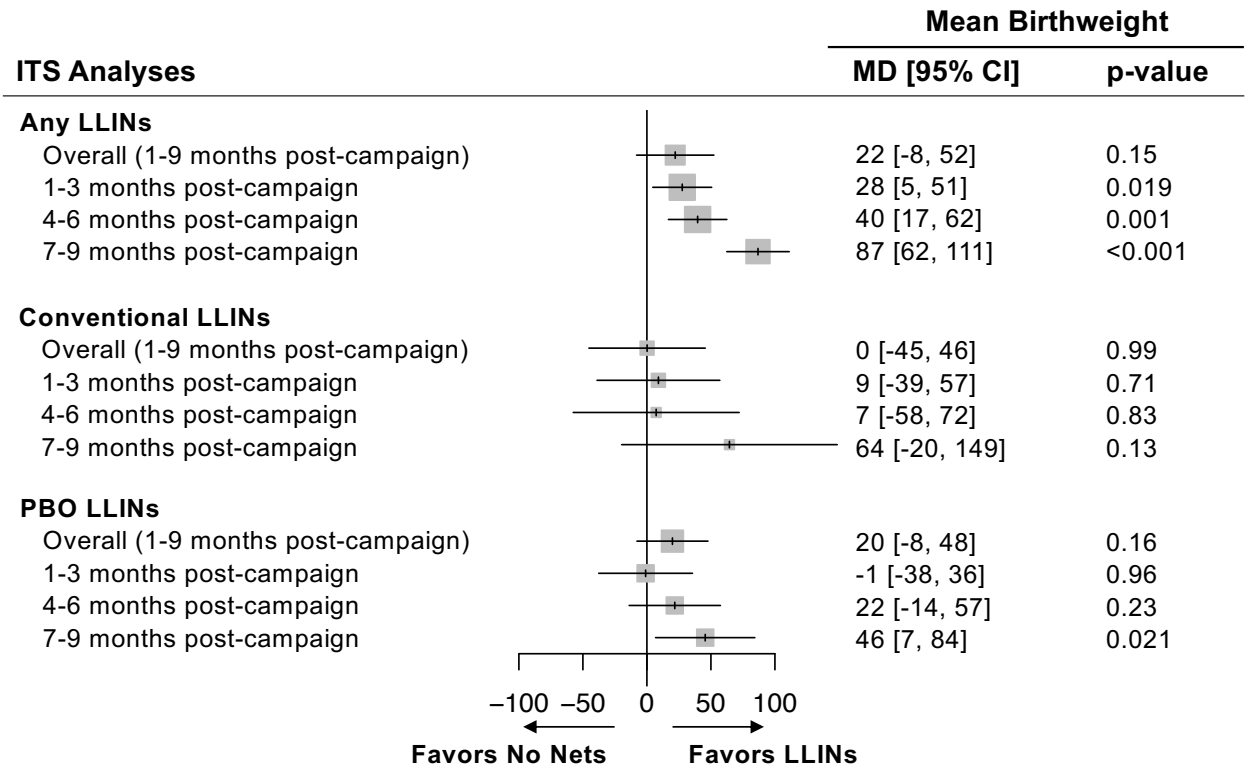
Figure 2.3 Estimates from interrupted time series models evaluating the effect of the LLIN campaign on low birthweight incidence. Estimates are also reported separately for each LLIN type and category of post-campaign exposure periods (i.e. 1-3, 4-6, and 7-9 months post-campaign).



Abbreviations: DiD=difference-in-differences; IRR=incidence rate ratio; PBO=piperonyl butoxide

* Negative binomial generalized additive models were used to estimate incidence rate ratios (IRRs). Models adjusted for time (in months) since the start of the study period; maternal age; gravidity; HIV; calendar month; health-subdistrict fixed effects; and log of total number of deliveries per month included as an offset term.

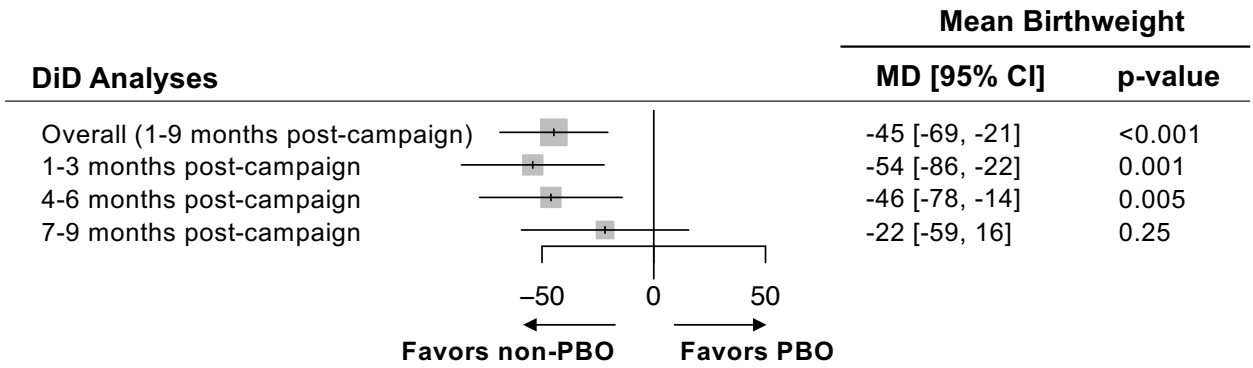
Figure 2.4 Estimates from difference-in-difference models comparing the effect of PBO and conventional LLINs on low birthweight incidence. Estimates are also reported separately for each post-campaign exposure periods (i.e. 1-3, 4-6, and 7-9 months post-campaign).



Abbreviations: ITS=interrupted time series;; LLIN=long-lasting insecticidal net; MD=mean difference; PBO=piperonyl butoxide

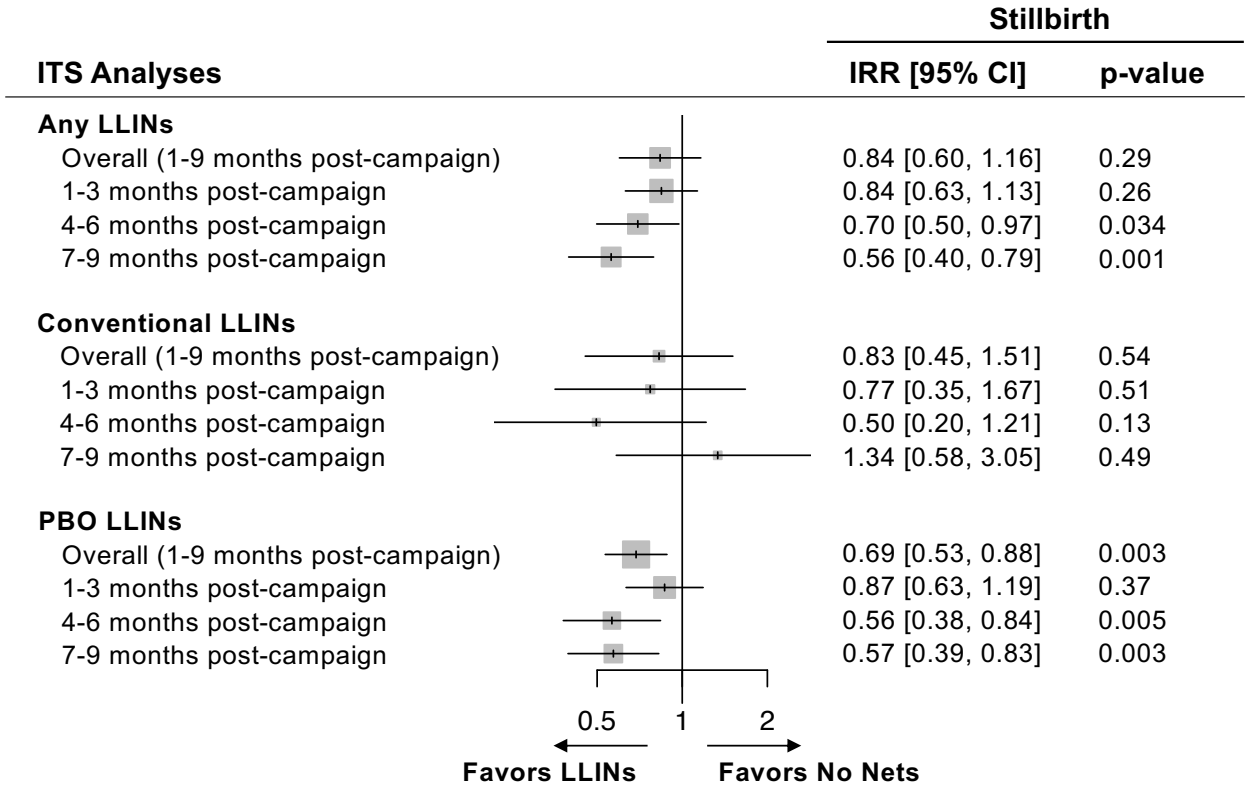
* Generalized additive mixed models were used to estimate differences in mean birthweight. Models adjusted for time (in months) since the start of the study period; maternal age; gravidity; HIV; calendar month; health-subdistrict fixed effects; and included random intercepts for each health facility.

Figure 2.5 Estimates from interrupted time series models evaluating the effect of the LLIN campaign on mean birthweight. Estimates are also reported separately for each LLIN type and category of post-campaign exposure periods i.e. 1-3, 4-6, and 7-9 months post-campaign).



Abbreviations: DiD=difference-in-differences; MD=mean difference; PBO=piperonyl butoxide
* Generalized additive mixed models were used to estimate differences in mean birthweight. Models adjusted for time (in months) since the start of the study period; maternal age; gravidity; HIV; calendar month; health-subdistrict fixed effects; and included random intercepts for each health facility.

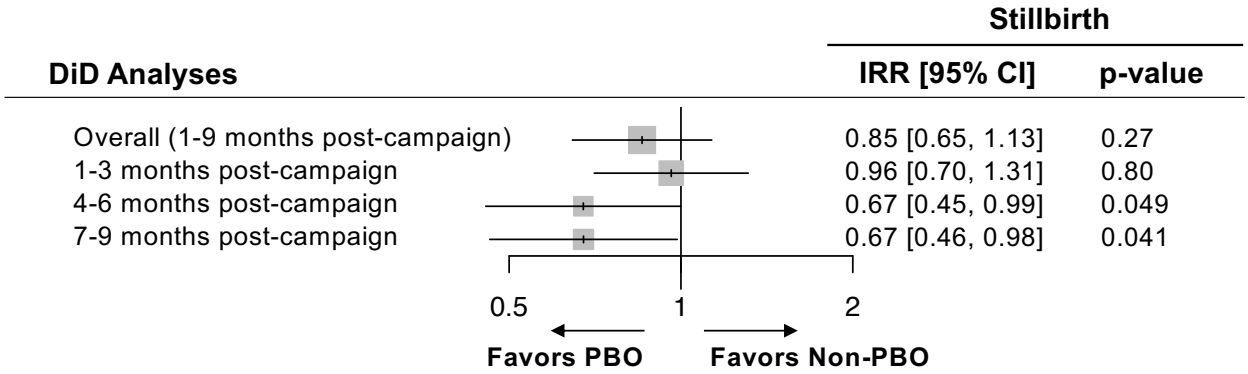
Figure 2.6 Estimates from difference-in-difference models comparing the effect of PBO and conventional LLINs on mean birthweight. Estimates are also reported separately for each post-campaign exposure periods (i.e. 1-3, 4-6, and 7-9 months post-campaign).



Abbreviations: IRR=incidence rate ratio; ITS=interrupted time series; LLIN=long-lasting insecticidal net; PBO=piperonyl butoxide

* Negative binomial generalized additive models were used to estimate incidence rate ratios (IRRs). Models adjusted for time (in months) since the start of the study period; maternal age; gravidity; HIV; calendar month; health-subdistrict fixed effects; and log of total number of deliveries per month included as an offset term.

Figure 2.7 Estimates from interrupted time series models evaluating the effect of the LLIN campaign on stillbirth incidence. Estimates are also reported separately for each LLIN type and category of post-campaign exposure periods i.e. 1-3, 4-6, and 7-9 months post-campaign).



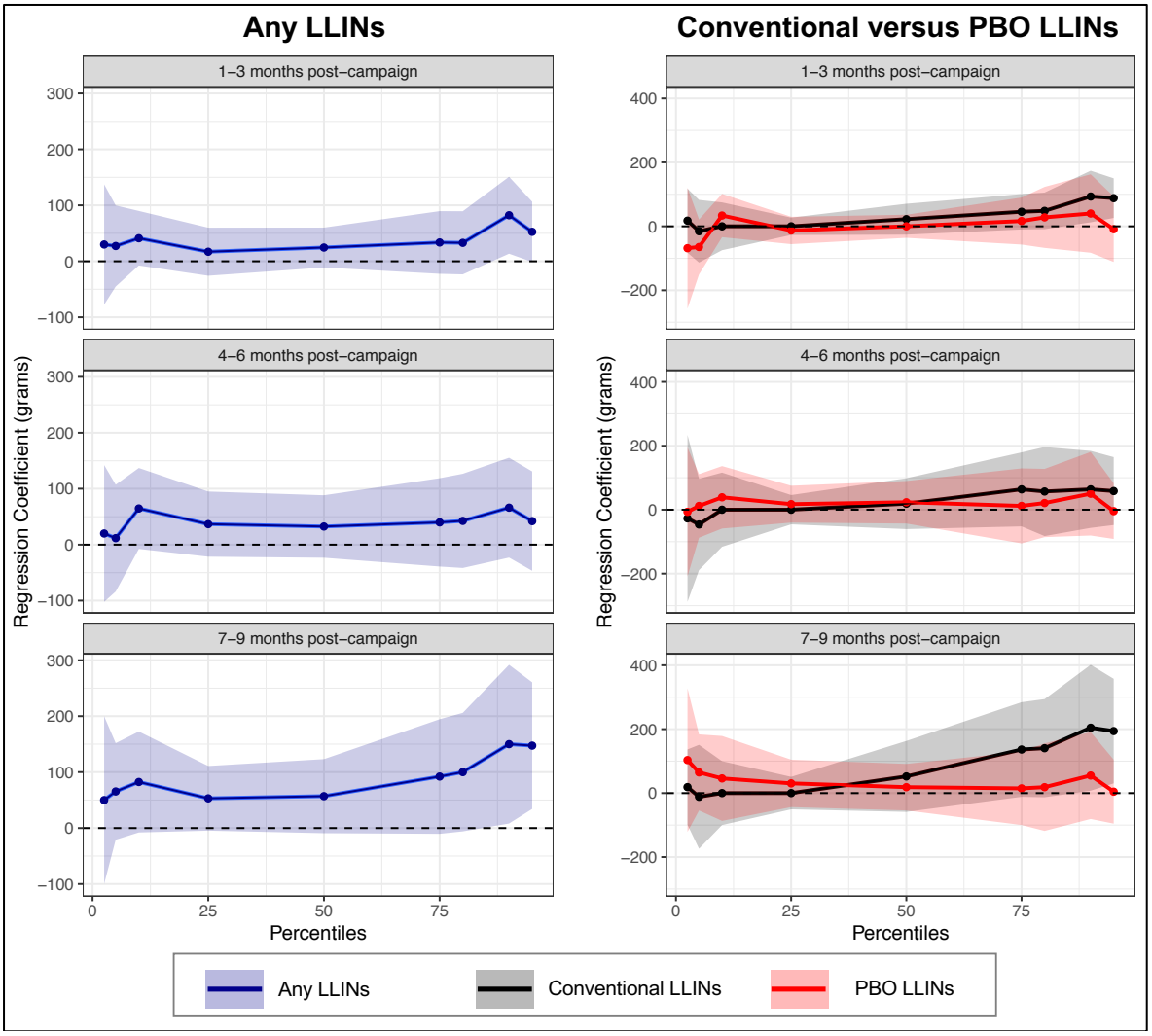
Abbreviations: DiD=difference-in-differences; IRR=incidence rate ratio; PBO=piperonyl butoxide
* Negative binomial generalized additive models were used to estimate incidence rate ratios (IRRs). Models adjusted for time (in months) since the start of the study period; maternal age; gravidity; HIV; calendar month; health-subdistrict fixed effects; and log of total number of deliveries per month included as an offset term.

Figure 2.8 Effect estimates from difference-in-difference models comparing the effect of PBO and conventional LLINs mean birthweight. Estimates are also reported separately for each post-campaign exposure periods (i.e. 1-3, 4-6, and 7-9 months post-campaign).

CHAPTER 2 APPENDIX

Appendix 2.1 Results from interrupted time series analyses estimating the effect of the LLIN campaign on varying birthweight percentiles by LLIN group and categories of post-campaign periods.

Quantile Regression Models. Effect estimates (and corresponding confidence intervals; shaded areas) were generated using quantile generalized additive regression models assessed at the 2.5th, 5th, 10th, 25th, 50th, 75th, 80th, 90th, and 95th percentile (see Figure below). Regression coefficients can be interpreted as the difference in birthweight (in grams) at the *q*th quantile. Models adjusted for time since the start of the study period, maternal age, gravidity, HIV, calendar month, and health sub-district fixed effect. Clustered standard errors were used to generate 95% confidence intervals, specifying clustering at the health facility-level.



Abbreviations: LLINs=long-lasting insecticidal nets; PBO=piperonyl butoxide

Findings. PBO LLINs appeared to confer greater benefits for lower birthweight babies (≤10th percentile), while conventional LLINs appeared to increase birthweight among higher birthweight babies (≥75th percentile). This finding was most apparent among women with early exposure to the campaign (i.e. women who delivered 7-9 months after the campaign). However, confidence intervals around effect estimates were too wide to provide any conclusive evidence.

CHAPTER 3: Temporal associations between indoor residual spraying of insecticide for malaria prevention and low birthweight risk: A quasi-experimental study from 11 districts in Uganda

Michelle E. Roh, Arthur Mpimbaza, Brenda Oundo, Amanda Irish, Max Murphy, Sean Wu, Justin White, Stephen Shiboski, M. Maria Glymour, Roly Gosling, Grant Dorsey, Hugh Sturrock

ABSTRACT

Background. Malaria in pregnancy is a significant risk factor for adverse birth outcomes in sub-Saharan Africa. To rapidly reduce malaria burden, countries have implemented population-level campaigns of indoor residual spraying with insecticide (IRS), a highly effective tool for malaria vector control. Though these campaigns have resulted in large in reductions of malaria burden, little is known of its impact on improving birth outcomes in the region. Between 2014-2015, the President's Malaria Initiative began a large-scale IRS campaign across several districts in Eastern Uganda. This aim of this study was to examine the effectiveness of this campaign on preventing adverse birth outcomes among women living in IRS-treated districts.

Methods. Health facility birth records (n=84,952) were obtained from routine surveillance data from five IRS and six non-IRS districts from January 2013-May 2017, around 27 months before and 24 months after IRS campaign. IRS effects on low birthweight (LBW), birthweight, and stillbirth were estimated using difference-in-differences (DiD) analyses and compared to a matrix completion method (MC-NNM), a

type of synthetic control method which relaxes assumptions inherent in DiD. Subgroup analyses were conducted to assess differences in effects among HIV-positive and primigravid women.

Results. DiD models indicated the IRS campaign was associated with a 26% reduction in LBW (IRR=0.74 [95% CI: 0.63, 0.86]) and a modest increase in mean birthweight (61 grams [95% CI: 47, 74]) over a two-year period. However, the campaign was not associated with changes in stillbirth incidence (IRR=0.98 [95% CI: 0.82, 1.16]). Similar estimates were observed using MC-NNM. Subgroup analyses indicated the effects of the campaign did not substantively differ by gravidity. The campaign appeared to reduce stillbirth risk in HIV-positive women, but not LBW.

Conclusion. In addition to its effects on malaria prevention, high-coverage IRS appears to have substantial downstream effects on LBW reduction. Our findings suggest the magnitude of this benefit are comparable to full malaria prevention via currently recommended interventions for pregnant women. This study demonstrates IRS is highly beneficial in reducing the burden of downstream effects of malaria, supporting the continued use of IRS in these districts and potential expansion to other regions where the overlapping burden of malaria and LBW is high.

INTRODUCTION

In sub-Saharan Africa, malaria in pregnancy is a major risk factor for adverse birth outcomes. In 2018, an estimated 11 million pregnant women were exposed to the *Plasmodium falciparum* parasite, resulting in nearly 220,000 stillbirths [3] and one million low birthweight (LBW) infants [43]. To prevent the adverse consequences of malaria infection, the World Health Organization (WHO) recommends all pregnant women living in moderate-to-high malaria transmission areas receive insecticide-treated bed nets (ITNs) and intermittent preventive treatment (IPTp), a malaria chemoprevention strategy where women are given intermittent doses of the antimalarial, sulfadoxine-pyrimethamine [13].

Despite efforts made by malaria-endemic countries to implement these interventions more widely, target levels of ITNs and IPTp coverage has been exceedingly low. In 2018, only 34% of pregnant women received the recommended three or more doses of IPTp and only 61% of pregnant women reported sleeping under a bed net [43]. Poor coverage of these interventions is further compounded by growing mosquito resistance to pyrethroids (the insecticide most commonly used in ITNs) [62, 63] and parasite resistance to sulfadoxine-pyrimethamine [20, 64]. Thus, there is an urgent need to identify additional tools for malaria prevention in pregnant women.

Indoor residual spraying of insecticide (IRS) is a key component of malaria control and elimination. The process involves applying insecticide to household surfaces that can potentially serve as a resting place for mosquitoes [43]. Studies have shown IRS to be

highly effective in reducing malaria morbidity [65, 66] and infant mortality [66], yet very few studies have evaluated its impact on reducing adverse birth outcomes.

In 2014, the US President's Malaria Initiative, the Ugandan Ministry of Health, and the UK Department for International Development launched the Uganda IRS Project, a large-scale IRS campaign conducted across 14 districts in Eastern Uganda. Shortly after its initiation, large reductions in malaria transmission were observed [67]. Small observational studies in one of these districts (Tororo) found that among women concurrently receiving ITNs and IPTp, IRS could offer up to an additional 60% reduction in adverse birth outcomes [68, 69]. However, these studies were conducted in one district with small sample sizes and may have been prone to residual confounding as both studies lacked a contemporaneous control group.

The present study aimed to more rigorously quantify the impact of the Uganda IRS Project on improving pregnancy outcomes. We overcome limitations of prior studies by evaluating a larger number of IRS-treated districts, including contemporaneous data from neighboring non-IRS districts, and using rigorous causal inference methods to generate more plausible counterfactual control groups.

METHODS

Study Setting

Between 2014 and 2015, the President's Malaria Initiative began a large-scale IRS campaign across 14 districts in Eastern Uganda. Five of these districts (Tororo,

Kaberamaido, Serere, Bugiri, and Namutumba) were selected for this study based on budget, feasibility, and geographical representativeness of the original 14 treated districts (**Figure 3.1**). In these five districts, timing of the campaign was staggered, whereby the first round of IRS was initiated in December 2014 (in Tororo and Kaberamaido), in April 2015 (in Serere), and in May 2015 (in Bugiri and Namutumba). Since its initiation, IRS has continued to be implemented in these districts and reported coverage has been high (>90% of households) [70, 71]. Data from six neighboring districts that were not part of the Uganda IRS Project (Amuria, Busia, Iganga, Jinja, Ngora, and Soroti) were used to generate appropriate control groups.

Ethical Approvals

Study approvals were granted by the Uganda National Council for Science and Technology, the Makerere University College of Health Sciences, and the University of California, San Francisco.

Study Design

To estimate the impact of the Uganda IRS Project, we used difference-in-differences (DiD) analyses [72] to compare the average pre-post changes in birth outcomes observed in the IRS-treated group to average pre-post changes observed in a control group (i.e. districts that did not receive IRS). By comparing the difference in slopes between these two groups, DiD can estimate unbiased effects even in the presence of group-varying, but time-invariant confounders (e.g. baseline differences in the level of malaria transmission intensity) and time-varying, but group-invariant confounders (e.g.

changes in the scale-up of other malaria interventions over time as long as they occurred across all units). Though a common method to evaluating population-level interventions [72], causal inference from DiD relies on the parallel trends assumption. The parallel trends assumption requires that the average slope in the control group provides a good approximation of the average counterfactual slope of the IRS-treated group had it not received IRS. Specifically, this requires the absence of unmeasured time- and group-varying confounding. Though this assumption cannot be formally proven (given counterfactual outcomes of the IRS group during the post-intervention period are not directly observed), it can be tested during the pre-intervention period. If the parallel trends assumption is violated during the pre-intervention period, this suggests DiD estimates may be biased.

To relax the parallel trends assumption, we also computed effect estimates using a novel, machine learning approach to estimating counterfactuals known as the matrix completion method with nuclear norm minimization (MC-NNM) [73]. This method is similar to DiD in that it uses outcome regression to predict missing counterfactuals, but unmeasured group- and time-varying confounders are controlled for using a more flexible modeling approach and is in many ways similar to the synthetic control method [74, 75]. The full methodological details can be found elsewhere [73, 76], but a brief description is provided in **Appendix Text 3.1**.

Data Source

To collect data on birth outcomes, birth registry data were obtained from 36 non-referral public health facilities: 15 from IRS districts and 21 from control districts (**Figure 3.2**). Due to budgetary limitations, not all health facilities were sampled from each district. To select study health facilities, we first generated a list of all known public health facilities in each district. From these, only Health Centers III and IV (non-referral health facilities that include a maternity ward) that averaged >200 births per year were selected. To mitigate exposure misclassification, facilities <5 kilometers away from a neighboring district were excluded. Upon determining a list of eligible health facilities, we randomly sampled three health facilities from each district. Health facilities were screened by a study coordinator who reviewed registries to determine the quality of the data. Health facilities deemed to have low quality data (e.g. missing complete months of data for >25 months during study period, systematically missing covariates or outcomes, and/or low delivery rates) were excluded and the next eligible health facility from the list was sampled until we reached three health facilities per district. If three health facilities could not be reach in each district, health facilities from neighboring districts were sampled. Of the 52 health facilities that were screened, 36 health facilities were included in the analysis (**Figure 3.2**). Of the 16 health facilities that were excluded, 12 were missing complete months of data for >25 months during study period and 3 were systematically missing key variables (e.g. birthweight and/or gravidity). Upon screening, one health facility had a delivery rate of ≤200 births per year and was therefore excluded.

From each health facility, individual-level birth registry data from all singleton deliveries which occurred between January 2013 to May 2017 were retrospectively collected from Form 072: Integrated Maternity Registry of the Health Management Information System (HMIS). The HMIS is an integrated surveillance system used by the Ministry of Health to collect relevant disease and health information from all public health facilities in Uganda [55]. The birth registry, managed by trained nurses and midwives, included data on delivery outcomes (e.g. date of delivery, birthweight [rounded to the nearest tenth of a kilogram], and stillbirth information) and maternal characteristics (e.g. age, gravidity, and HIV status).

Treatment Variable

Treatment was defined as a categorical variable corresponding to the period when IRS was implemented in IRS-treated districts, otherwise 0. Assuming IRS effects would be dose-dependent (i.e. longer exposure to IRS would be more beneficial), the effects of the IRS campaign were assessed in first- and second-year post-IRS initiation.

Outcomes

The primary outcome was incidence of LBW (defined as birthweight <2,500 grams) assessed among live, singleton births. Secondary outcomes included mean birthweight (assessed among live births only) and stillbirth incidence.

Statistical Analysis Plan

Difference-in-differences

DiD analyses estimating the impact of the IRS campaign on incidence outcomes were conducted using data aggregated by health facility and month. Negative binomial generalized additive models (GAM) were used to estimate monthly LBW and stillbirth incidence based on the following covariates: time (in months) since the start of the study; a categorical treatment variable indicating the post-IRS period for the treated group and 0 otherwise; mean maternal age; proportion of primigravidae; proportion of HIV-positive women; calendar month (e.g. January, February, March, etc.) to account for seasonal trends; health facility-level fixed effects; and an offset term for the log of the total number of deliveries recorded per health facility-month. Continuous time-varying covariates were modeled as smooth functions to accommodate their possible non-linear relationships with the outcome.

Effect estimates for mean birthweight were modeled using individual-level data. Generalized additive mixed models (GAMM, specifying a Gaussian distribution and identity link function) were used to estimate the impact of the IRS campaign on mean birthweight. GAMM models included the same covariates as incidence models but included random intercepts for each health facility. Both GAM and GAMM models were fitted using the mgcv package in R.

To formally test whether the parallel trends assumption was held during the pre-IRS period, we repeated outcome models using only pre-IRS data. An interaction term

between time and a binary indicator of whether the delivery occurred in an IRS district ($\beta_{month \times IRS}$) was used to test for differences in the average birth outcome trends between IRS and control districts. Models included the same covariates as the primary analyses but excluded the treatment variable indicating the post-IRS period for the treated group.

Matrix Completion with Nuclear Norm Minimization

To address possible violations in the parallel trends assumption of DiD, we estimated the effects of LBW and stillbirth incidence using MC-NNM. Analyses were conducted using the gsynth package in R. Average treatment effects in the treated group (ATT) were estimated by first dividing the observed outcome of the treated group by its estimated counterfactual outcome value at each month and averaging these effects across all post-IRS months. 95% confidence intervals were obtained using 1000 block bootstrapped percentiles to account for clustered observations at the health facility-level. Synthetic controls estimated by MC-NNM are provided in **Appendix Figure 3.1**.

Subgroup Analyses

In areas of high *Plasmodium falciparum* malaria transmission, HIV-positive and primigravidae women have less parity-specific immunity to malaria and are thus at a higher risk of malaria-associated adverse birth outcomes [77]. To investigate whether the IRS campaign differentially impacted birth outcomes among HIV-positive women and primigravidae, DiD models were conducted using individual-level data. Log-binomial GAMMs were used to estimate the effect of the campaign on LBW and stillbirth

risk. GAMMs specifying a Gaussian distribution and identity link function were used to estimate the impact of the campaign on mean birthweight. Models included the same covariates as the primary analyses but included an interaction term between the pre-specified subgroup and the treatment variable (i.e. a binary variable indicating the post-IRS period for the treated group and 0 otherwise). Stratified analyses were conducted for each subgroup regardless of whether p-values (reported as $p_{IRS \times HIV}$ and $p_{IRS \times Primigravidae}$) indicated presence of a statistical interaction.

All tests were two-sided (α=0.05). Analyses were conducted in Stata (StataCorp LLC, Version 14.0) and in R (version 3.5.3).

RESULTS

Descriptive Analysis

The final sample size included 84,952 singleton deliveries recorded between January 2013 and May 2017, approximately 27 months before and 24 months after the initiation of the IRS campaign. Approximately 3.9% of deliveries were stillbirths (n=3,331). Of the 81,621 live births, 3,921 (4.8%) were LBW.

The demographic characteristics and birth outcomes of the study population are presented in **Table 3.1**. Mean maternal age was similar across IRS and control groups and pre- and post-IRS periods. The mean proportion of primigravidae was lower during the pre-intervention period, but this finding was consistent across both IRS and control

groups. Mean prevalence of HIV was slightly higher in the control group compared to the IRS group, but this finding was consistent across pre- and post-IRS periods.

Impact of IRS on Birth Outcomes

Figure 3.3 presents DiD and MC-NNM estimates of the effect the Uganda IRS Project on birth outcomes. Both models demonstrated the Uganda IRS Project was associated with marked reductions in LBW incidence over a two-year period (IRR_{DiD}=0.74 [95% CI: 0.63, 0.86] and $IRR_{MC\text{-}NNM=}$0.83 [95% CI: 0.70, 1.00]). Reductions were seen in the first- and second-year post-IRS, though MC-NNM models indicated effect estimates were slightly larger in the second year ($IRR_{1st\ year}$=0.89 versus $IRR_{2nd\ year}$=0.76). The parallel trends assumption appeared to be violated during the pre-IRS period ($\beta_{month\ x\ IRS}$=-0.05; p<0.0001). Estimates from MC-NNM, which relaxes this assumption, were slightly more conservative than DiD.

Consistent with LBW estimates, DiD models demonstrated the Uganda IRS Project was associated with a 61 gram [95% CI: 47, 74] increase in mean birthweight over the two-year period (**Figure 3.3**). Benefits of the campaign on mean birthweight were seen in both the first- and second-year post-IRS, with effect estimates being slightly larger in the second year. Trends in mean birthweight did not appear to differ between IRS and control groups during the pre-IRS period ($\beta_{month\ x\ IRS}$=0.001; *p*=0.27)

DiD and MC-NNM models indicated the Uganda IRS Project was not associated with a substantive reduction in stillbirth incidence (IRR_{DiD}=0.98 [95% CI: 0.82, 1.16]) (**Figure**

3.3). This finding was consistent in the first- and second-year after IRS initiation. There was little evidence that the parallel trends assumption was violated during the pre-intervention period ($\beta_{month \times IRS}$=0.016; *p*=0.24). MC-NNM estimates were similar to DiD ($IRR_{MC\text{-}NNM=}$1.09 [95% CI: 0.83, 1.49]).

Subgroup Analyses

Subgroup analyses using individual-level data were conducted to investigate whether the effect of the IRS campaign on birth outcomes differed for HIV-positive women (**Figure 3.4**) and primigravidae (**Figure 3.5**). Of the 84,952 deliveries, 2,673 (3.1%) were among HIV-positive women. Overall, infants born to HIV-positive women had nearly a two-fold higher risk of LBW (RR=1.94 [95% CI: 1.66, 2.26]) and weighed around 124 grams [95% CI: 104, 144] less than infants born to HIV-negative women. However, infants born to HIV-positive women did not appear to have a greater risk of stillbirth than infants born to HIV-negative women (RR=1.01 [95% CI: 0.80, 1.27]). HIV subgroup analyses presented in **Figure 3.4** indicated the campaign was associated with reductions in LBW among HIV-negative women, but not HIV-positive women ($RR_{HIV\text{-}}$=0.73 versus RR_{HIV+}=1.43; $p_{IRS \times HIV}$=0.059). Similar patterns were observed with mean birthweight ($MD_{HIV\text{-}}$=63 grams versus MD_{HIV+}=-6 grams; $p_{IRS \times HIV}$=0.038). In contrast, the IRS campaign appeared to reduced stillbirth risk among HIV-positive women, but not HIV-negative women (RR_{HIV+}=0.86 versus RR_{HIV+}=1.15; $p_{IRS \times HIV}$=0.032), although confidence intervals around effect estimated in HIV-positive women included the null.

Of the total deliveries, 17,714 (21%) were among primigravidae. Overall, infants born to primigravidae had a higher risk of LBW (RR=1.80 [95% CI: 1.66, 1.96]) and weighed around 138 grams [95% CI: 128, 148] less than infants born to multigravidae, but did not appear to have a higher stillbirth risk (RR=0.92 [95% CI: 0.80, 1.05]). Despite the higher risk of adverse birth outcomes among primigravidae, there was little evidence to suggest the effects of the IRS campaign differed between gravidity subgroups on LBW risk ($p_{IRS \times Primigravidae}$=0.15), mean birthweight ($p_{IRS \times Primigravidae}$=0.14), and stillbirth risk ($p_{IRS \times Primigravidae}$=0.24). Stratified analyses presented in **Figure 3.5** showed the IRS campaign was associated with improvements in LBW risk and mean birthweight in both primi- and multi-gravidae, but neither group experienced a reduction in stillbirth risk.

DISCUSSION

In 2014, the Ugandan Ministry of Health began a large-scale IRS campaign in a highly malaria-endemic region of Eastern Uganda. After controlling for group- and time-varying confounding, our study found the campaign was associated with a 17% reduction in LBW incidence and a 61-gram increase in mean birthweight in the two years following IRS initiation. Results from our subgroup analyses indicated the IRS campaign was associated with statistically significant reductions in LBW among infants born to HIV-negative women and primi- and multi-gravidae, but not HIV-positive women. In contrast to birthweight estimates, the campaign was not associated with a reduction in stillbirth, except among HIV-positive women, though confidence intervals around this effect estimate included the null.

Overall, our findings are consistent with the current literature on the benefits of IRS [65, 78] and more broadly, the benefits of malaria prevention on pregnancy outcomes [44, 79]. Our results suggest the IRS campaign prevented a large proportion of malaria associated LBW, based on findings from a previous meta-analysis which found full malaria prevention with ITNs, IPTp, or both was associated with similar reductions in LBW risk (~17%). The magnitude of our effect sizes were generally smaller than those estimated by prior exploratory studies in Tororo [69, 80], one of the districts included in the current study. A possible explanation for this may be that residual confounding due to secular trends may have overestimated the campaign effects in prior simple, pre-post studies, whereas our DiD and MC-NNM analyses controlled for these confounders using data from a contemporaneous control group.

Contrary to prior studies [3], we did not find that the IRS campaign reduced overall stillbirth incidence. Several factors may have contributed to this finding. First, given IRS was initially conducted in areas of high malaria transmission, it is possible that women during the first two years of the campaign had acquired some partial antimalarial immunity against malaria, which in turn, may have confer protection against stillbirth. Results from a recent meta-analysis [3] support this hypothesis which found the effect of malaria on stillbirth risk was two-fold higher in areas of low malaria endemicity compared to areas of high malaria endemicity. Furthermore, this may explain why the campaign was associated with reduced stillbirth risk only among women with HIV, an infection which impairs antimalarial immunity [81]. A second plausible explanation is that the effect of malaria on stillbirth is dependent on the type of stillbirth delivery. A recent

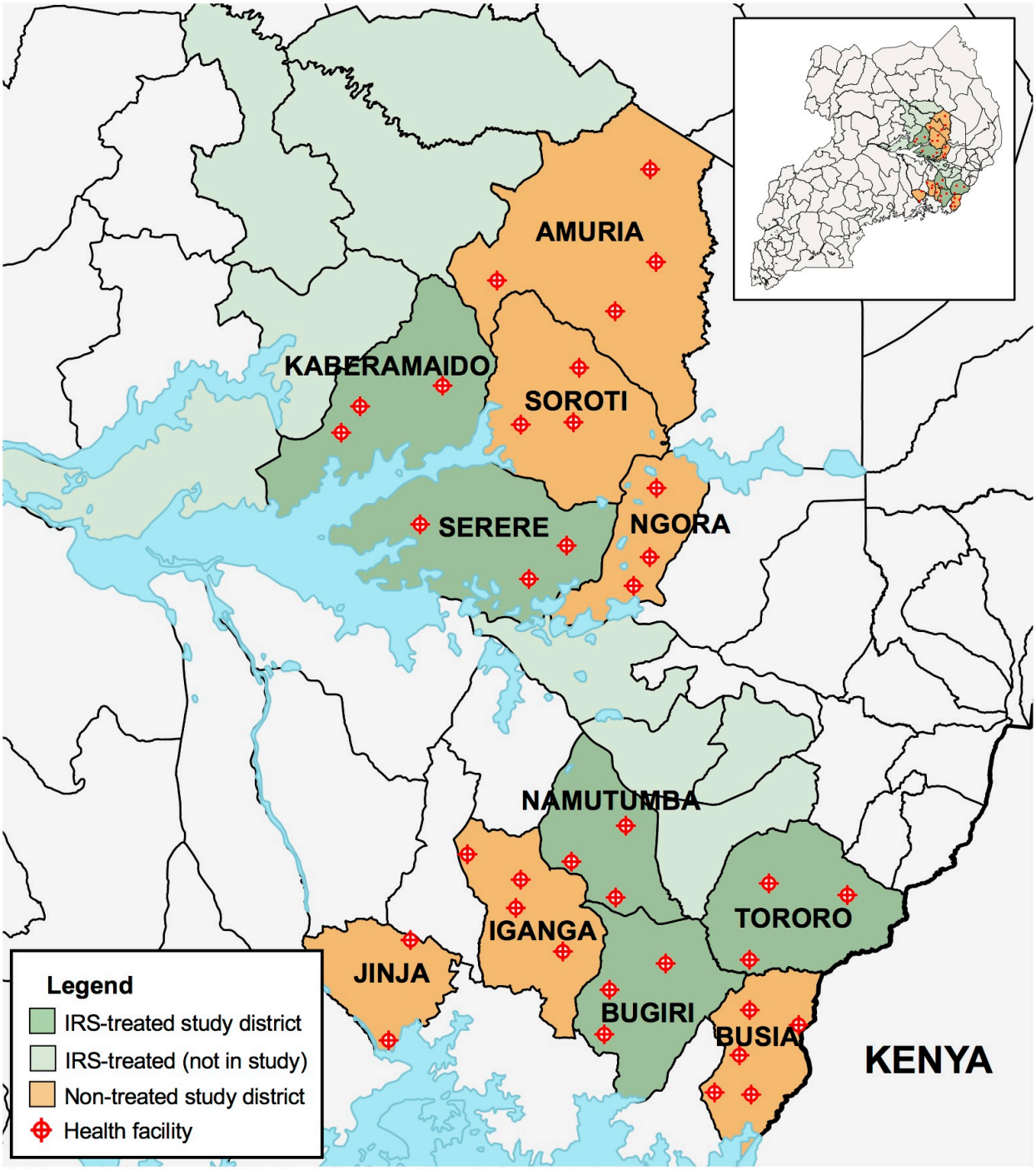
study from the Thai-Myanmar border [82] found that falciparum malaria was associated a higher risk of antepartum stillbirth (prelabor death *in utero*), but not intraparum stillbirth (death during labor). As the registry we used only captures delivery information (and not all antenatal care visits), it is possible many antepartum stillbirths may have been missed, resulting in attenuated effects.

Despite the established effects of IRS on malaria transmission, it is a vastly underutilized malaria vector control tool due to the high costs associated with its implementation. In 2018, only 5% of people at-risk for malaria in this region were protected by IRS (a decline from a peak of 10% in 2010) while 72% of people at-risk had access to an ITN [43]. This study supports existing evidence that IRS has major impacts on preventing the adverse consequences of infection, in particular, malaria associated LBW. In resource-limited settings, LBW is thought to contribute to 60-80% of all neonatal deaths [83, 84]. Among surviving infants, LBW increases the risk of a range of chronic diseases including respiratory and diarrheal disease [84], impaired growth and cognitive development [85-87], diabetes [88] and cardiovascular disease [89]. Thus, despite the high initial costs of its implementation, a highly effective tool like IRS could yield significant cost-savings [84]. Coincidentally, the President's Malaria Initiative has been conducting large-scale IRS campaigns in 13 other African countries [90]. Evaluation of these efforts should not only consider how these campaigns have impacted malaria outcomes, but how they have led to the improvement of other, more downstream health outcomes, which may help to justify its continued use in these areas and expansion to other malaria-endemic countries.

Despite efforts in applying rigorous causal inference methods to large datasets, the study had several limitations. First, we cannot rule out that our effect estimates may have been subject to unmeasured group and time-varying confounding, especially if there were secular trends that differed between groups during the pre- and post-campaign period. Second, our dataset only included health facility births from 5 of the 14 IRS districts, limiting our generalizability to the 9 other IRS districts as well as home births, which in Uganda make up approximately 30% of all deliveries [60]. Lastly, though the HMIS database enabled us to capture a comprehensive set of delivery information from a representative sample of facility-based births, variables collected in this registry may have been measured with error. We provide three examples of potential sources of measurement error and how this may have biased our results. First, in the registry, birthweight was recorded to the nearest tenth of a kilogram, which would have increased the variance around mean birthweight estimates, although LBW estimates would not have been affected [61]. Second, our exposure may have non-differentially misclassified women who delivered at health facilities located outside of their district of residence. Though we aimed to mitigate this bias by selecting health facilities at least 5 km away a neighboring district, it's likely that this type of non-differential misclassification error biased our effect estimates toward the null. Third, in 2014, around the time of the IRS campaign, the format of the HMIS 072 registry was changed to improve accurate reporting of gravidity versus parity. Inaccurate reporting of these variables during the pre-IRS period likely explains the observed change in the proportion of primigravidae between pre- and post-IRS periods (**Table 3.1**). Improvements in reporting may have resulted in non-differential misclassification error,

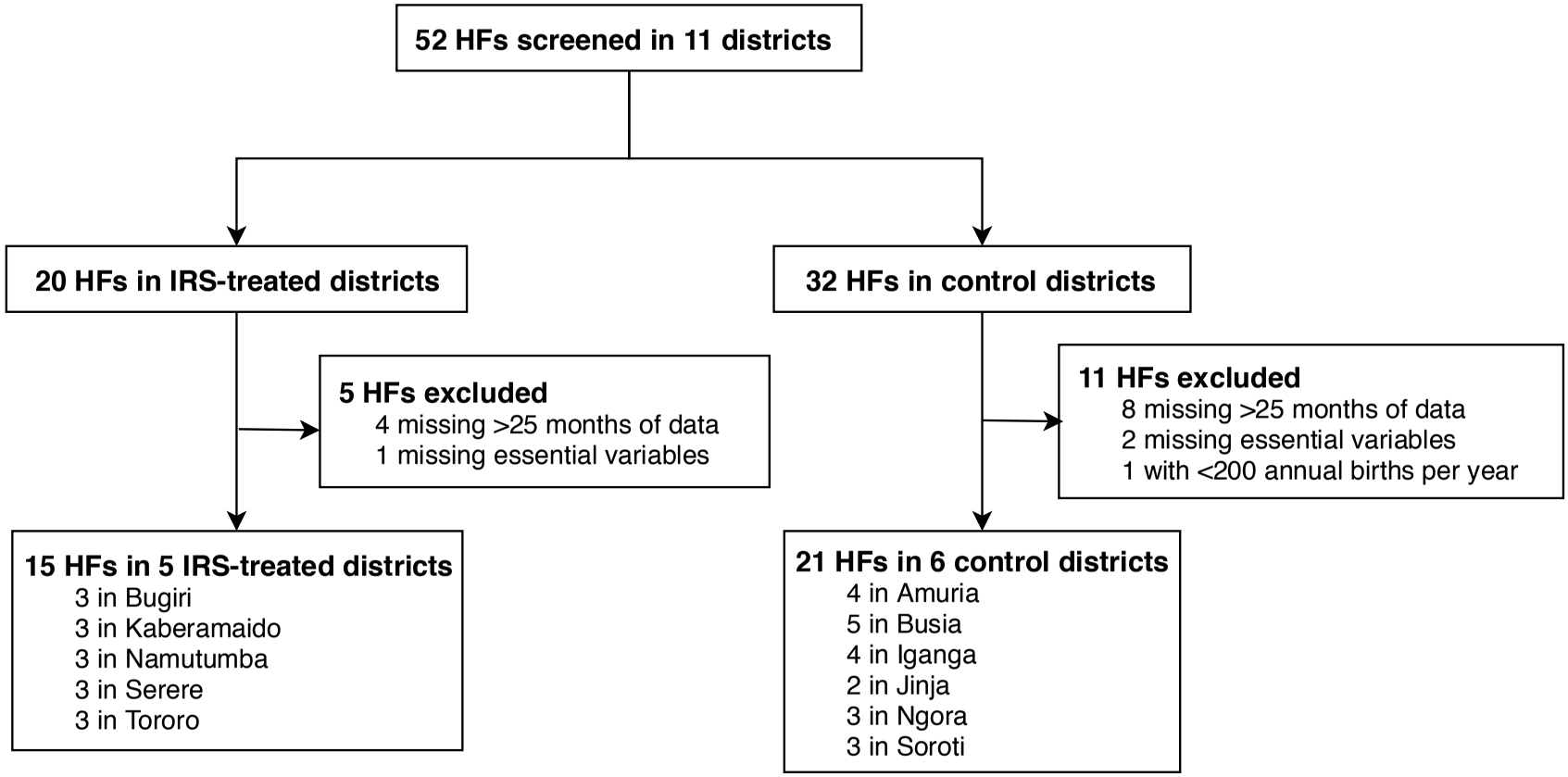
which likely underestimated true differences in treatment effects between primi- and multi-gravidae.

In summary, our study found that in an area of intense malaria transmission of Uganda, high-coverage IRS had substantial effects on LBW reduction. Clear benefits of IRS were observed among infants born to HIV-negative women, but not among HIV-positive women, further confirming the need for additional tools for LBW prevention in this particularly vulnerable group [91]. Overall, we found little evidence that IRS campaign reduced stillbirth incidence. More studies are needed to validate this null finding as it is unclear whether this was due to partial antimalarial immunity conferred prior to IRS or the type of stillbirth delivery information available in our dataset. Nevertheless, our study provides important and rigorous evidence highlighting the benefits of IRS on improving birthweight, warranting its further expansion and continued use in areas with a high overlapping burden of malaria and LBW.



Abbreviations: IRS=indoor residual spraying

Figure 3.1 Map of study districts and health facilities. Orange regions represent non-IRS treated districts. Green districts represent IRS-treated districts, where darker shaded districts indicate districts included in this study. Red points indicate locations of health facilities where data was collected.



Abbreviations: HF=health facility; IRS=indoor residual spraying

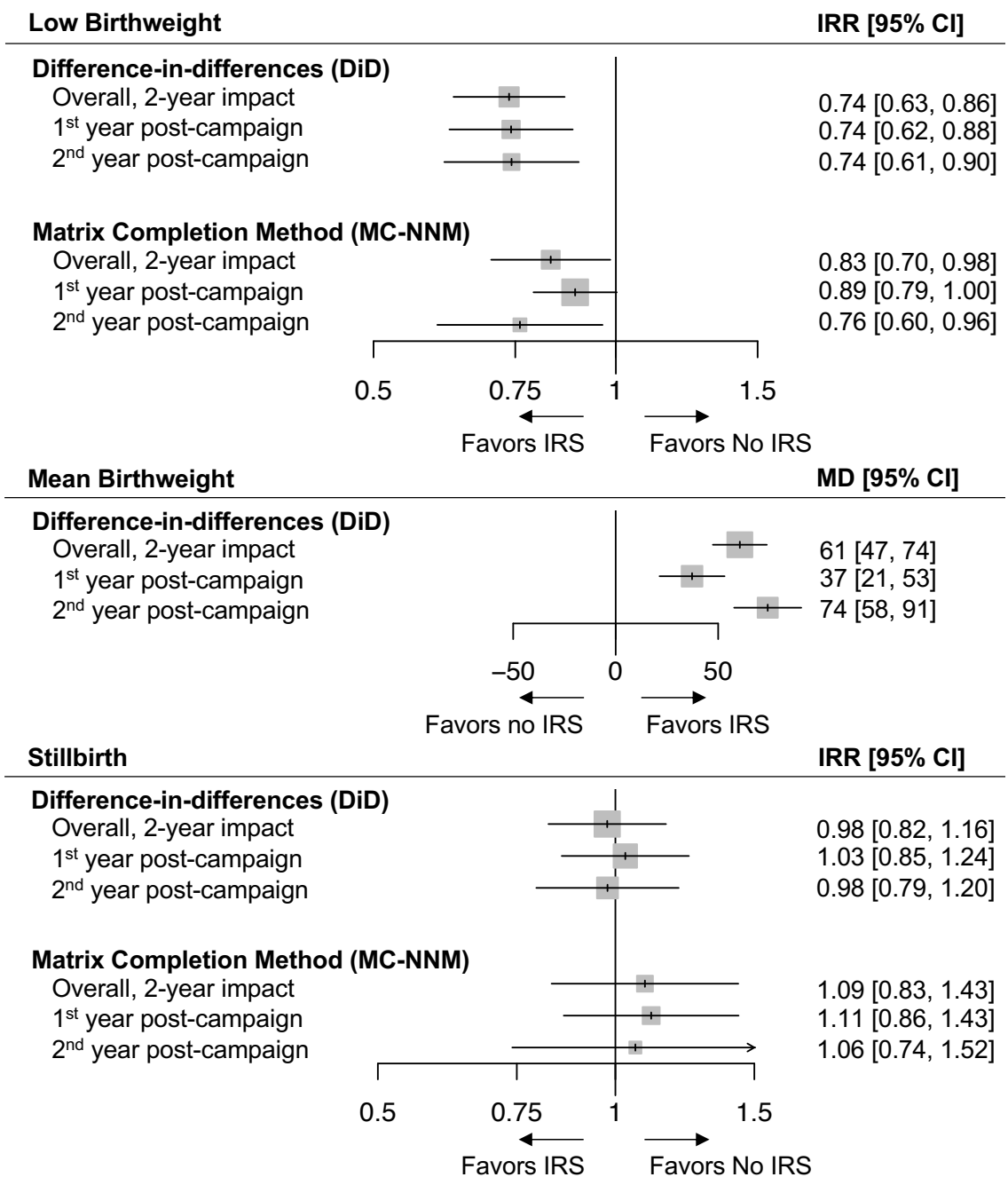
Figure 3.2 Flow diagram of the selection of health facilities.

Table 3.1 Maternal characteristics and birth outcomes of women who delivered between January 2013 and May 2017, stratified by IRS and non-IRS (control) groups and pre- and post-campaign periods. Summary statistics were first averaged across health facility-months and then averaged across the four groups.

	No IRS (Control)		IRS	
	Pre-IRS	**Post-IRS***	**Pre-IRS**	**Post-IRS**
Total number of observations	19,807	28,691	15,134	21,320
Maternal age in years, mean (SD)	24.5 (0.9)	24.3 (0.6)	24.6 (0.7)	24.5 (0.6)
% Primigravidae, mean (SD)	16.3 (9.0)	22.6 (6.7)	19.0 (7.3)	22.4 (4.8)
% HIV prevalence, mean (SD)	3.2 (2.2)	3.4 (1.9)	2.6 (2.4)	2.7 (1.8)
	Birth outcomes			
LBW infants per 100 births, mean (SD)	4.4 (4.5)	4.9 (3.5)	5.4 (5.0)	4.2 (3.2)
Birthweight in grams, mean (SD)	3132 (117)	3131 (100)	3103 (128)	3162 (102)
Stillbirths per 100 deliveries, mean (SD)	2.6 (2.5)	2.6 (2.4)	3.5 (2.9)	5.9 (4.0)

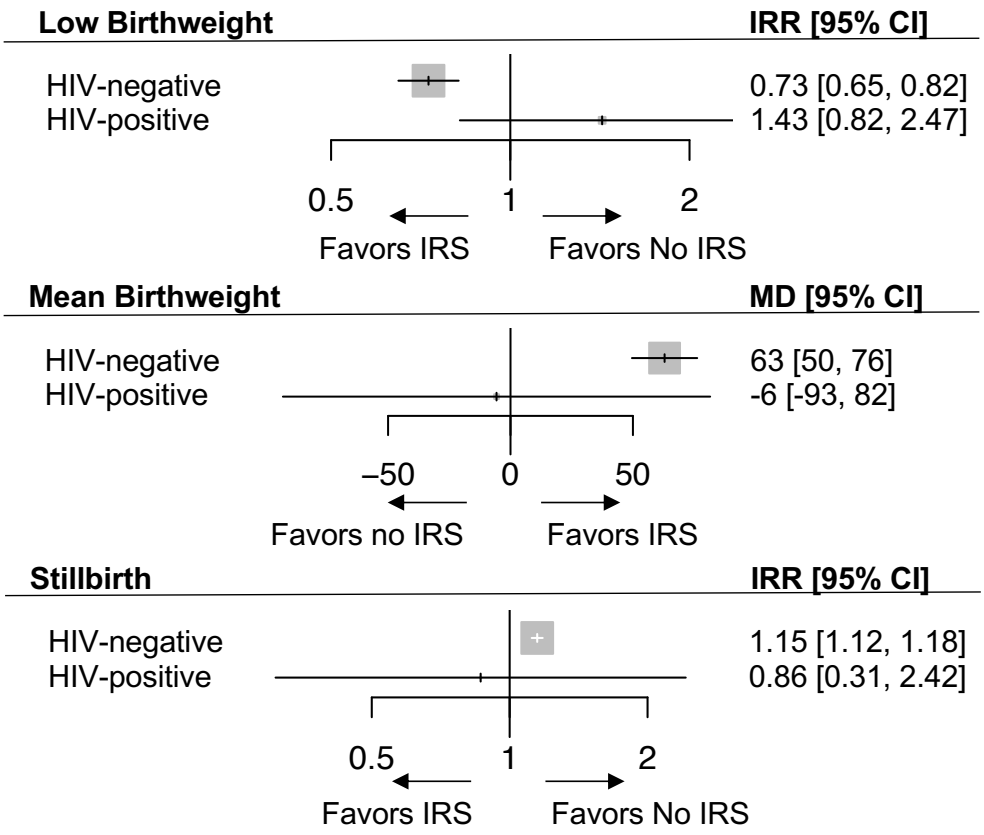
IRS=indoor residual spraying; LBW=low birthweight; SD=standard deviation

* Due to the staggered adoption of IRS, the post-IRS period for the control group in this table was defined as the midpoint between the earliest and latest date of first round of IRS.



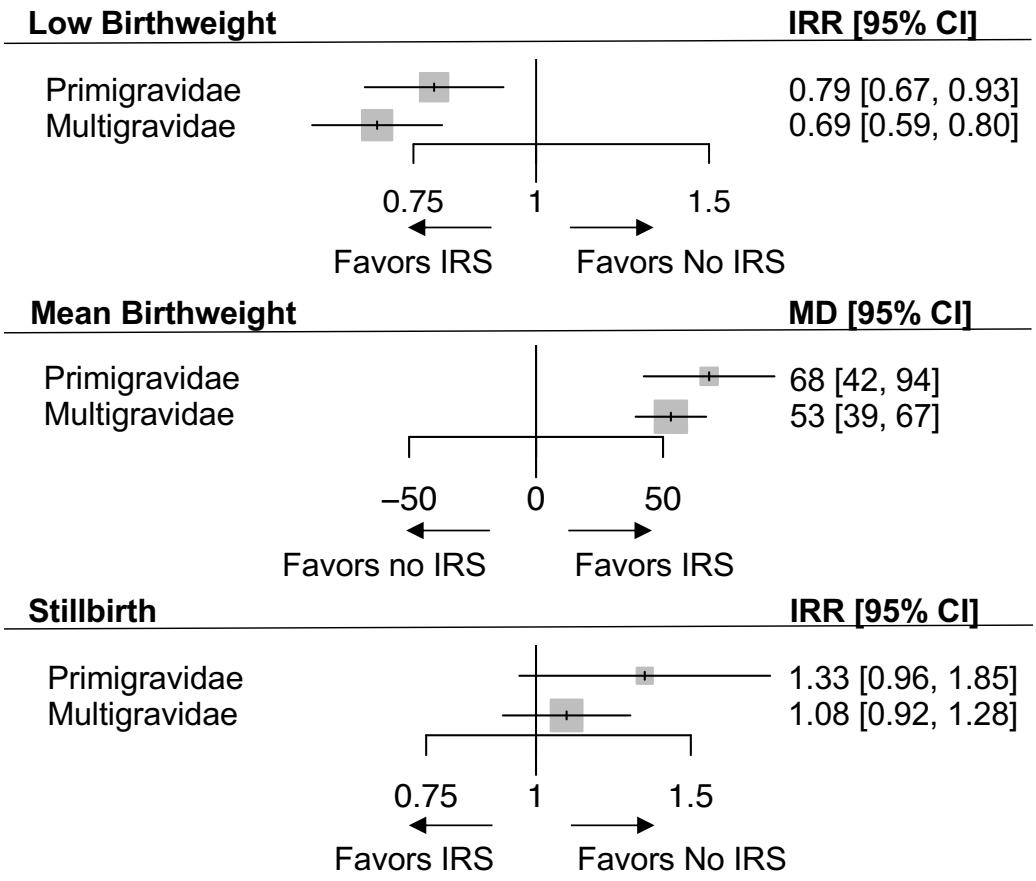
Abbreviations: IRR=incidence rate ratio; IRS=indoor residual spraying; MD=mean difference

Figure 3.3 Effect estimates from counterfactual estimators evaluating the impact of Uganda IRS Project on low birthweight incidence, mean birthweight, and stillbirth incidence. Effects were estimated from difference-in-difference models and the matrix completion method. Average treatment effects among the treated group are reported as incidence rate ratios for LBW and stillbirth incidence and mean difference for birthweight.



Abbreviations: IRR=incidence rate ratio; IRS=indoor residual spraying; MD=mean difference

Figure 3.4 Results from HIV subgroup analyses. Average treatment effects among the treated group were estimated using difference-in-differences models based on individual-level data. Results are provided based on the two-year impact of the Uganda IRS Project on low birthweight incidence, mean birthweight, and stillbirth incidence.



Abbreviations: IRR=incidence rate ratio; IRS=indoor residual spraying; MD=mean difference

Figure 3.5 Results from gravidity subgroup analyses. Average treatment effects among the treated group were estimated using difference-in-differences models based on individual-level data. Results are provided based on the two-year impact of the Uganda IRS Project on low birthweight incidence, mean birthweight, and stillbirth incidence.

CHAPTER 3 APPENDIX

Appendix Text

Appendix Text 3.1 Description of the Matrix Completion with Nuclear Norm Minimization (MC-NNM) Estimator.

Before discussing the methodology behind MC-NNM, we first need to provide an intuition of the data structure that MC-NNM uses to impute counterfactuals.

MC-NNM Data Structure. Suppose we observe Y outcomes from N districts over T months. We can feasibly contain these data in a matrix, where each row of the matrix represents the outcome values for a district over T months. Consider the below matrix Y, where Y_{it} represents the outcome for district *i* at month *t*. A second matrix, W, represents the actual treatment value for each Y_{it}, where W_{it}=1 if the treatment was implemented in district *i* at time *t* and 0 otherwise.

$$Y = \begin{bmatrix} Y_{11} & Y_{12} & Y_{13} & Y_{14} & \dots & Y_{1T} \\ Y_{21} & Y_{22} & Y_{23} & Y_{24} & \dots & Y_{2T} \\ Y_{31} & Y_{32} & Y_{33} & Y_{34} & \dots & Y_{3T} \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ Y_{N1} & Y_{N2} & Y_{N3} & Y_{N4} & \dots & Y_{N5} \end{bmatrix} \quad \text{and} \quad W = \begin{bmatrix} 0 & 0 & 1 & 1 & \dots & 1 \\ 0 & 0 & 0 & 1 & \dots & 1 \\ 0 & 0 & 0 & 0 & \dots & 0 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

Given that our goal is to estimate the counterfactual outcome value for each treated district had it not received treatment, we can consider the above matrix Y as representing only the observed values of two potential outcome matrices. Thus, we can partition the values of matrix Y into matrices Y(0) [which contain potential outcomes had

districts not received treatment] and Y(1) [which contain potential outcome values had districts received treatment].

$$Y(0) = \begin{bmatrix} Y_{11} & Y_{12} & ? & ? & \dots & ? \\ Y_{21} & Y_{22} & ? & ? & \dots & ? \\ Y_{31} & Y_{32} & Y_{33} & Y_{34} & \dots & Y_{3T} \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ Y_{N1} & Y_{N2} & Y_{N3} & Y_{N4} & \dots & Y_{N5} \end{bmatrix} \quad \text{and} \quad Y(1) = \begin{bmatrix} ? & ? & Y_{13} & Y_{14} & \dots & Y_{1T} \\ ? & ? & ? & Y_{24} & \dots & Y_{2T} \\ ? & ? & ? & ? & \dots & ? \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ ? & ? & ? & ? & \dots & ? \end{bmatrix}$$

Note that in the above Y(0) matrix, only the post-treatment outcome values of the treated group are missing, whereas in the Y(1) matrix, all but the observed values during the post-treatment period for the treated group are missing.

The goal of MC-NNM (or any counterfactual estimator) is then to best estimate the missing values of Y(0) (i.e. the outcomes of the treated districts had they never received treatment).

Comparison with Difference-in-Differences. DiD seeks to estimate the missing values of Y(0) using a fixed-effects regression model, where γ_i and δ_t represent group- and time-fixed effects.

$$Y_{it}(0) = \gamma_i + \delta_t + \varepsilon_{it} \quad \text{and} \quad E[\varepsilon|\gamma + \delta] = 0$$

By only including γ_i and δ_t, DiD assumes counterfactual values can be approximated group-varying, but time-invariant variability or time-varying, but group-invariant variability. If unobserved time- and group-varying heterogeneities are present, the DiD

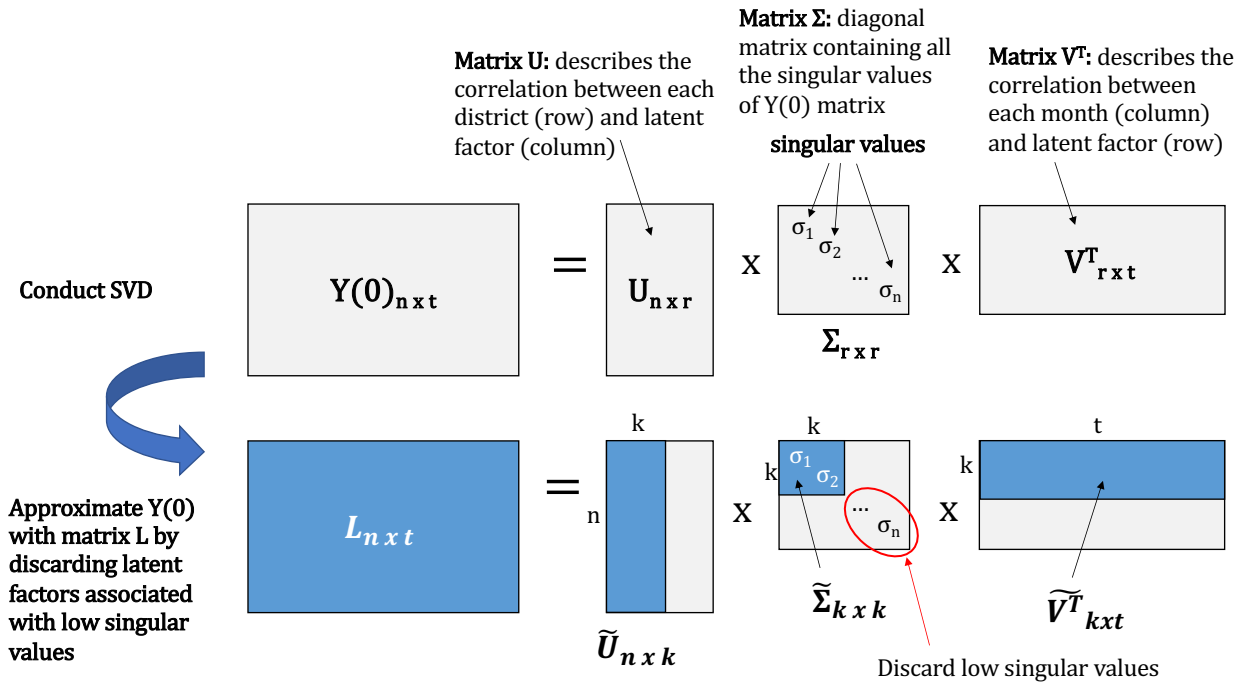
model will not accurately predict counterfactual estimates and thus the treatment effect may be biased.

MC-NNM Approach to Modeling Time- and Group-Varying Heterogeneity. MC-NNM seeks to generate a more reliable estimation of these counterfactual values by attempting to model time- and group-varying heterogeneities observed during the pre-intervention period into the estimation of counterfactual values.

If one can think of the columns of Y(0) as unique variables, then the approach is similar to factor analysis in that it seeks to represent the complex relationships among multiple observed, but highly correlated variables in terms of a smaller set of unobserved variables, called ‘latent factors’. For both approaches, each latent factor describes a set of observed variables that share a common variance. In MC-NNM, each latent factor corresponds to highly correlated time variables [columns of Y(0)] that vary in similar patterns across units. The purpose of these latent time factors is to identify time-varying patterns during the pre-intervention period that vary in the same way during the post-intervention period for control units. These factors are then used to impute missing counterfactuals for treated units during the post-intervention period. These latent factors could in theory represent seasonal effects, group- or time-level differences, and importantly, any group- and time-varying heterogeneities.

To identify these latent factors, MC-NNM uses singular value decomposition (SVD). Mathematically, SVD decomposes a matrix into a unique product of three matrices

[$Y(0)=U\Sigma V^T$], where matrix Σ is a diagonal matrix which contains the singular values of matrix Y(0). Each singular value represents the 'strength' of each latent factor (i.e. how much of the variance in the data each latent factor explains). The purpose of conducting SVD is to identify which latent factors explain the greatest proportion of the variance in the data (i.e. latent factors with the largest singular values) and discarding the ones associated with small singular values. Upon discarding latent factors associated with small singular values, a new matrix is constructed (call this matrix L) that approximates the original Y(0) matrix, but is not overly fitted to the data.



For MC-NNM, the optimal number of latent factors (defined as the rank of the matrix) is chosen based on a nuclear norm minimization method [73], an optimization procedure which seeks to minimize the mean squared prediction error with as few latent factors as possible by penalizing poorly determined singular values, much like how lasso penalizes poorly determined regression information. This is done in an effort to retain as

much information as possible while reducing the complexity of the model and to make the overall computation feasible. Details of the optimization procedure are described in the original paper [73].

Comparison with the Synthetic Control Method. By identifying patterns observed between districts during the pre-intervention period and applying these to estimating counterfactuals during the post-intervention period, MC-NNM is similar to the synthetic control method (SCM) [74]. The difference is that SCM constructs synthetic controls based on a weighted average of control units [74, 75]. In SCM, weights for control units are generated using a defined set of pre-intervention outcomes that can ensure a good fit between the synthetic control and its corresponding treated unit. Unlike SCM, where the analyst must choose which pre-intervention outcomes to construct weights upon, MC-NNM uses these latent factors to identify these patterns, which can be considered a more data-driven approach.

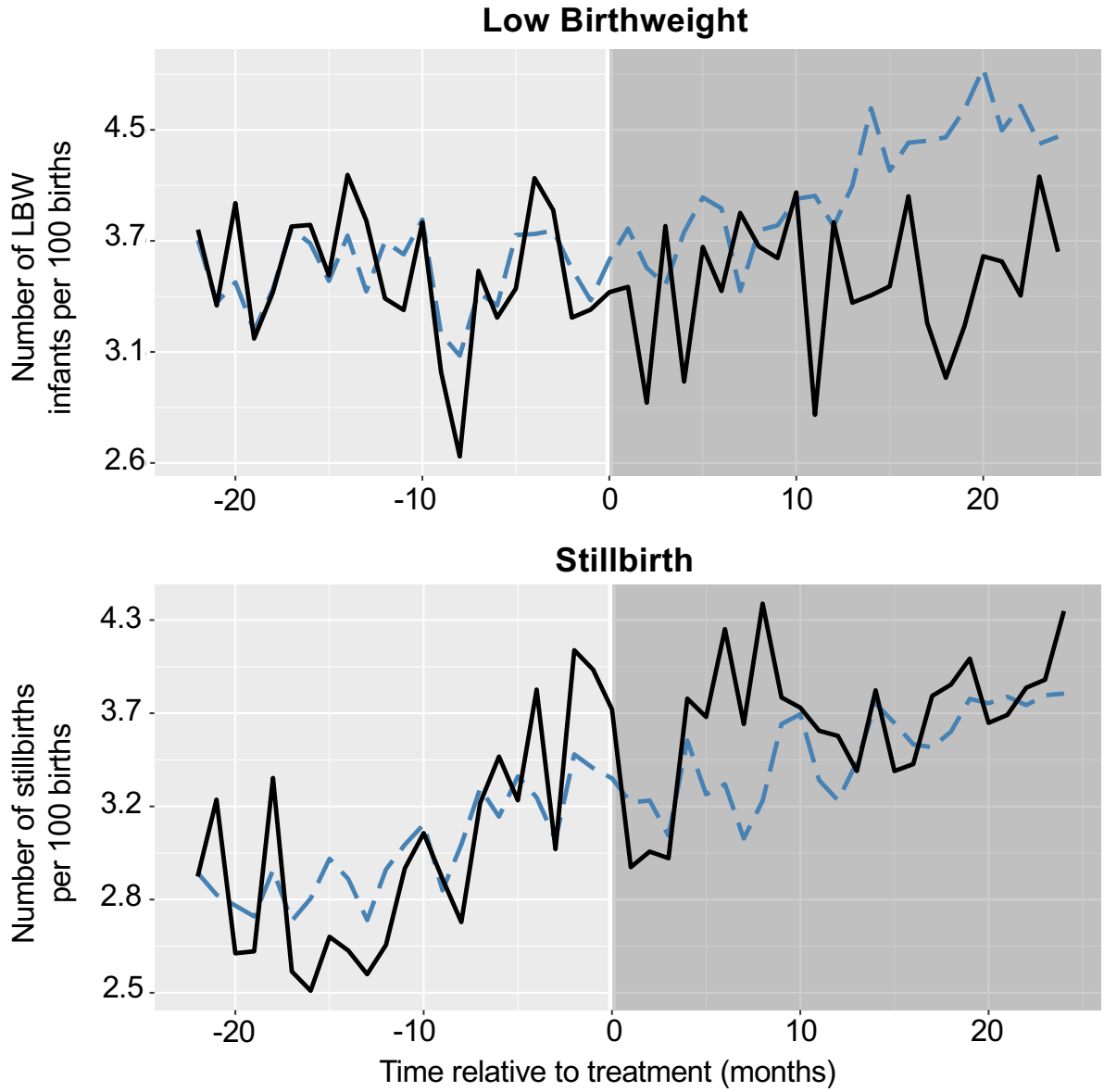
MC-NNM Outcome Model. Once matrix L is estimated, the outcome model for MC-NNM is as follows:

$$Y(0)_{it} = L_{it} + \gamma_i + \delta_t + \varepsilon_{it} \quad \text{and} \quad E[\varepsilon | L^* + \gamma + \delta] = 0$$

where L is a matrix approximated by SVD, and γ* and δ* are vectors of unit- and time-fixed effects, respectively. Note the above equation does not include any pre-specified covariates, however, they can be added as linear terms (similar to the way DiD incorporates observed covariates into the model).

Estimation of ATT. Once values of the $Y(0)_{it}$ are predicted, the average treatment effect of the treated (ATT) is estimated by subtracting Y(0) values from Y(1) for each treated district which is then averaged across all treated districts and time periods during the post-intervention period. 95% confidence intervals around ATT estimates are generated using block bootstrapped replications.

Appendix Figure 3.1 Trends in LBW and stillbirth incidence of the treated group estimated by MC-NNM generated synthetic controls. Black lines indicate the observed trend and blue lines indicate the estimated trend of the synthetic control.



REFERENCES

1. UNICEF: **Levels and Trends in Child Mortality: Report 2017.** (UNICEF, World Health Organization, World Bank Group, United Nations eds.). New York: UNICEF2017.
2. Taylor SM, ter Kuile FO: **Stillbirths: the hidden burden of malaria in pregnancy.** *Lancet Glob Health* 2017, **5:**e1052-e1053.
3. Moore KA, Simpson JA, Scoullar MJ, McGready R, Fowkes FJ: **Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis.** *Lancet Glob Health* 2017, **5(11):**1101-1112.
4. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, Hutchinson P, Steketee RW: **Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa.** *Lancet Infect Dis* 2012, **12:**942-949.
5. Desai M, Gutman J, L'lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A: **Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin–piperaquine versus intermittent preventive treatment with sulfadoxine–pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial.** *Lancet* 2016, **386:**2507-2519.
6. Global Partnership to Roll Back Malaria: *The contribution of malaria control to maternal and newborn health.* Geneva, Switzerland: World Health Organization; 2014.

7. World Health Organization: **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** *Geneva, Switzerland: WHO* 2013.

8. Gamble CL, Ekwaru JP, ter Kuile FO: **Insecticide-treated nets for preventing malaria in pregnancy.** *Cochrane Database Syst Rev* 2006.

9. Menéndez C, Bardají A, Sigauque B, Sanz S, Aponte JJ, Mabunda S, Alonso PL: **Malaria prevention with IPTp during pregnancy reduces neonatal mortality.** *PLoS One* 2010, **5:**e9438.

10. Guyatt HL, Snow RW: **Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa.** *Clin Microbiol Rev* 2004, **17:**760-769.

11. Guyatt HL, Snow RW: **The epidemiology and burden of Plasmodium falciparum-related anemia among pregnant women in sub-Saharan Africa.** *Am J Trop Med Hyg* 2001, **64:**36-44.

12. Sharma L, Shukla G: **Placental Malaria: A New Insight into the Pathophysiology.** *Front Med* 2017, **4:**117.

13. World Health Organization: **WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP).** (World Health Organization ed. Geneva, Switzerland 2013.

14. Desai M, Gutman J, L'lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A: **Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin–piperaquine versus intermittent preventive treatment with sulfadoxine–pyrimethamine for the**

control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. *The Lancet* 2016, **386:**2507-2519.

15. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P: **Dihydroartemisinin–piperaquine for the prevention of malaria in pregnancy.** *N Engl J Med* 2016, **374:**928-939.
16. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ategeka J, Nayabare P, Clark TD, et al: **Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: A randomized controlled trial.** *Lancet* 2019.
17. Stoner MC, Vwalika B, Smid M, Kumwenda A, Stringer E, Chi BH, Stringer JS: **Dosage of Sulfadoxine-Pyrimethamine and Risk of Low Birth Weight in a Cohort of Zambian Pregnant Women in a Low Malaria Prevalence Region.** *Am J Trop Med Hyg* 2017, **96:**170-177.
18. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, Kayentao K, Ouma P, Coulibaly SO, Kalilani L, Mace KE: **Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for malaria in pregnancy at clearing infections and preventing low birth weight.** *Clin Infect Dis* 2015, **62:**323-333.
19. Chico RM, Cano J, Ariti C, Collier TJ, Chandramohan D, Roper C, Greenwood B: **Influence of malaria transmission intensity and the 581G mutation on the**

efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis. *Trop Med Int Health* 2015, **20:**1621-1633.

20. van Eijk AM, Larsen DA, Kayentao K, Koshy G, Slaughter DE, Roper C, Okell LC, Desai M, Gutman J, Khairallah C: **Effect of Plasmodium falciparum sulfadoxine-pyrimethamine resistance on the effectiveness of intermittent preventive therapy for malaria in pregnancy in Africa: a systematic review and meta-analysis.** *Lancet Infect Dis* 2019.
21. VanderWeele TJ: **Mediation analysis: a practitioner's guide.** *Annual review of public health* 2016, **37:**17-32.
22. Imai K, Keele L, Yamamoto T: **Identification, inference and sensitivity analysis for causal mediation effects.** *Stat Sci* 2010**:**51-71.
23. Conrad MD, Mota D, Foster M, Tukwasibwe S, Legac J, Tumwebaze P, Whalen M, Kakuru A, Nayebare P, Wallender E: **Impact of Intermittent Preventive Treatment During Pregnancy on Plasmodium falciparum Drug Resistance–Mediating Polymorphisms in Uganda.** *J Infect Dis* 2017, **216:**1008-1017.
24. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW: **Malaria in pregnancy: pathogenesis and immunity.** *Lancet Infect Dis* 2007, **7:**105-117.
25. Pearl J: **Direct and indirect effects.** In *Proceedings of the seventeenth conference on uncertainty in artificial intelligence*Morgan Kaufmann Publishers Inc.; 2001: 411-420.
26. VanderWeele T: *Explanation in causal inference: methods for mediation and interaction.* Oxford University Press; 2015.

27. Robins JM, Greenland S: **Identifiability and exchangeability for direct and indirect effects.** *Epidemiology* 1992**:**143-155.

28. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K: **Mediation: R package for causal mediation analysis.** 2014.

29. Kontopantelis E, Reeves D: **metaan: Random-effects meta-analysis.** *Stata Journal* 2010, **10:**395-407.

30. Gutman J, Slutsker L: **Intermittent Preventive Treatment with Sulfadoxine–Pyrimethamine: More than Just an Antimalarial?** *Am J Trop Med* 2017, **96:**9-10.

31. Dingens AS, Fairfortune TS, Reed S, Mitchell C: **Bacterial vaginosis and adverse outcomes among full-term infants: a cohort study.** *BMC Pregnancy Childbirth* 2016, **16:**278.

32. Angelucci HM: **The Treatment of Trichomonas Vaginitis with a Sulfonamide Compound.** *Am J Obstet Gynecol* 1945, **50:**336-338.

33. Bhattacharyya M, Jones B: **Haemophilus vaginalis infection. Diagnosis and treatment.** *J Reprod Med* 1980, **24:**71-75.

34. Kampmeier R: **Introduction of sulfonamide therapy for gonorrhea.** *Sex Transm Infect* 1983, **10:**81-84.

35. Bowie W, Manzon L, Borrie-Hume C, Fawcett A, Jones H: **Efficacy of treatment regimens for lower urogenital Chlamydia trachomatis infection in women.** *Am J Obstet Gynecol* 1982, **142:**125-129.

36. Chico RM, Mayaud P, Ariti C, Mabey D, Ronsmans C, Chandramohan D: **Prevalence of malaria and sexually transmitted and reproductive tract**

infections in pregnancy in sub-Saharan Africa: a systematic review. *JAMA* 2012, **307:**2079-2086.

37. Chico RM, Chaponda EB, Ariti C, Chandramohan D: **Sulfadoxine-Pyrimethamine Exhibits Dose-Response Protection Against Adverse Birth Outcomes Related to Malaria and Sexually Transmitted and Reproductive Tract Infections.** *Clin Infect Dis* 2017, **64:**1043-1051.

38. Capan M, Mombo-Ngoma G, Makristathis A, Ramharter M: **Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin.** *Malar J* 2010, **9:**303.

39. **Improving PRegnancy Outcomes With Intermittent preVEntive Treatment in Africa (IMPROVE)** [https://clinicaltrials.gov/ct2/show/NCT03208179]

40. **The ASPIRE Trial - Aiming for Safe Pregnancies by Reducing Malaria and Infections of the Reproductive Tract** [https://clinicaltrials.gov/ct2/show/NCT04189744]

41. Pearl J: **The causal mediation formula—a guide to the assessment of pathways and mechanisms.** *Prev Sci* 2012, **13:**426-436.

42. Bhatt S, Weiss D, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle K, Moyes C, Henry A, Eckhoff P: **The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015.** *Nature* 2015, **526:**207-211.

43. World Health Organization: **World Malaria Report 2019.** (World Health Organization ed. Geneva, Switzerland2019.

44. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, Hutchinson P, Steketee RW: **Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa.** *Lancet Infect Dis* 2012, **12:**942-949.

45. Katureebe A, Zinszer K, Arinaitwe E, Rek J, Kakande E, Charland K, Kigozi R, Kilama M, Nankabirwa J, Yeka A: **Measures of malaria burden after long-lasting insecticidal net distribution and indoor residual spraying at three sites in Uganda: a prospective observational study.** *PLoS Med* 2016, **13:**e1002167.

46. Lynd A, Gonahasa S, Staedke SG, Oruni A, Maiteki-Sebuguzi C, Dorsey G, Opigo J, Yeka A, Katureebe A, Kyohere M: **LLIN Evaluation in Uganda Project (LLINEUP): a cross-sectional survey of species diversity and insecticide resistance in 48 districts of Uganda.** *Parasit Vectors* 2019, **12:**1-10.

47. Hemingway J, Ranson H: **Insecticide resistance in insect vectors of human disease.** *Annu Rev Entomol* 2000, **45:**371-391.

48. Sokhna C, Ndiath M, Rogier C: **The changes in mosquito vector behaviour and the emerging resistance to insecticides will challenge the decline of malaria.** *Clin Microbiol Infect* 2013, **19:**902-907.

49. World Health Organization: **Conditions for deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide: recommendations.** Geneva, Switzerland: World Health Organization; 2017.

50. Pennetier C, Bouraima A, Chandre F, Piameu M, Etang J, Rossignol M, Sidick I, Zogo B, Lacroix M-N, Yadav R: **Efficacy of Olyset® Plus, a new long-lasting**

insecticidal net incorporating permethrin and piperonil-butoxide against multi-resistant malaria vectors. *PLoS One* 2013, **8:**e75134.

51. Protopopoff N, Mosha JF, Lukole E, Charlwood JD, Wright A, Mwalimu CD, Manjurano A, Mosha FW, Kisinza W, Kleinschmidt I: **Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial.** *The Lancet* 2018, **391:**1577-1588.

52. Staedke SG, Gonahasa S, Dorsey G, Kamya MR, Maiteki-Sebuguzi C, Lynd A, Katureebe A, Kyohere M, Mutungi P, Kigozi SP: **Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign.** *The Lancet* 2020, **395:**1292-1303.

53. Kiberu VM, Matovu JK, Makumbi F, Kyozira C, Mukooyo E, Wanyenze RK: **Strengthening district-based health reporting through the district health management information software system: the Ugandan experience.** *BMC Med Inform Decis Mak* 2014, **14:**40.

54. Mpimbaza A, Miles M, Sserwanga A, Kigozi R, Wanzira H, Rubahika D, Nasr S, Kapella BK, Yoon SS, Chang M, et al: **Comparison of routine health management information system versus enhanced inpatient malaria surveillance for estimating the burden of malaria among children admitted to four hospitals in Uganda.** *Am J Trop Med Hyg* 2015, **92:**18-21.

55. **Uganda Ministry of Health Health Management Information System** [https://hmis2.health.go.ug/#/]

56. Wagner AK, Soumerai SB, Zhang F, Ross-Degnan D: **Segmented regression analysis of interrupted time series studies in medication use research.** *J Clin Pharm Ther* 2002, **27:**299-309.

57. Bernal JL, Cummins S, Gasparrini A: **Interrupted time series regression for the evaluation of public health interventions: a tutorial.** *Int J Epidemiol* 2017, **46:**348-355.

58. Penfold RB, Zhang F: **Use of interrupted time series analysis in evaluating health care quality improvements.** *Acad Pediatr* 2013, **13:**S38-S44.

59. Shadish C, Cook TD: *Experimental and Quasi-Experimental Designs for Generalized Causal Inference.* Belmont, CA: Wadsworth Cengage Learning; 2002.

60. Uganda Bureau of Statistics (UBOS), ICF: **Uganda Demographic Health Survey 2016.** (UBOS and ICF ed. Kampala, Uganda and Rockville, Maryland, USA2018.

61. Hutcheon JA, Chiolero A, Hanley JA: **Random measurement error and regression dilution bias.** *BMJ* 2010, **340:**c2289.

62. Ranson H, Lissenden N: **Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control.** *Trends Parasitol* 2016, **32:**187-196.

63. World Health Organization: **Global plan for insecticide resistance management in malaria vectors (GPIRM).** Geneva, Switzerland: World Health Organization 2012.

64. Okell LC, Griffin JT, Roper C: **Mapping sulphadoxine-pyrimethamine-resistant Plasmodium falciparum malaria in infected humans and in parasite populations in Africa.** *Sci Rep* 2017, **7:**1-15.

65. Pluess B, Tanser FC, Lengeler C, Sharp BL: **Indoor residual spraying for preventing malaria.** *Cochrane Database Syst Rev* 2010**:**CD006657.

66. Eisele TP, Larsen D, Steketee RW: **Protective efficacy of interventions for preventing malaria mortality in children in Plasmodium falciparum endemic areas.** *Int J Epidemiol* 2010, **39:**i88-i101.

67. Katureebe A, Zinszer K, Arinaitwe E, Rek J, Kakande E, Charland K, Kigozi R, Kilama M, Nankabirwa J, Yeka A, et al: **Measures of Malaria Burden after Long-Lasting Insecticidal Net Distribution and Indoor Residual Spraying at Three Sites in Uganda: A Prospective Observational Study.** *PLoS Med* 2016, **13:**e1002167.

68. Muhindo MK, Kakuru A, Natureeba P, Awori P, Olwoch P, Ategeka J, Nayebare P, Clark TD, Muehlenbachs A, Roh M, et al: **Reductions in malaria in pregnancy and adverse birth outcomes following indoor residual spraying of insecticide in Uganda.** *Malar J* 2016, **15:**437.

69. Roh ME, Shiboski S, Natureeba P, Kakuru A, Muhindo M, Ochieng T, Plenty A, Koss CA, Clark TD, Awori P: **Protective effect of indoor residual spraying of**

insecticide on preterm birth among pregnant women with HIV in Uganda: A secondary data analysis. *J Infect Dis* 2017.

70. US Agency for International Development, Centers for Disease Prevention and Control, US Department of Health and Human Services, Department US: **President's Malaria Initiative: Uganda Malaria Operational Plan FY 2017.** 2018.

71. US Agency for International Development, Centers for Disease Prevention and Control, US Department of Health and Human Services, Department US: **President's Malaria Initiative: Uganda Malaria Operational Plann FY 2018.** 2019.

72. Wing C, Simon K, Bello-Gomez RA: **Designing difference in difference studies: best practices for public health policy research.** *Annu Rev Public Health* 2018, **39**.

73. Athey S, Bayati M, Doudchenko N, Imbens G, Khosravi K: **Matrix Completion Methods for Causal Panel Data Models. arXiv e-prints, page.** *arXiv* 2017.

74. Abadie A, Diamond A, Hainmueller J: **Synthetic control methods for comparative case studies: Estimating the effect of California's tobacco control program.** *J Am Stat Assoc* 2010, **105:**493-505.

75. Bouttell J, Craig P, Lewsey J, Robinson M, Popham F: **Synthetic control methodology as a tool for evaluating population-level health interventions.** *J Epidemiol Community Health* 2018, **72:**673-678.

76. Liu L, Wang Y, Xu Y: **A practical guide to counterfactual estimators for causal inference with time-series cross-sectional data.** *Available at SSRN* 2020.

77. Fried M, Duffy PE: **Malaria during pregnancy.** *Cold Spring Harbor Perspect Med* 2017, **7:**a025551.

78. Steinhardt LC, Yeka A, Nasr S, Wiegand RE, Rubahika D, Sserwanga A, Wanzira H, Lavoy G, Kamya M, Dorsey G: **The effect of indoor residual spraying on malaria and anemia in a high-transmission area of northern Uganda.** *Am J Trop Med* 2013, **88:**855-861.

79. Cates JE, Westreich D, Unger HW, Bauserman M, Adair L, Cole SR, Meshnick S, Rogerson SJ, Malaria M, Initiative M: **Intermittent preventive therapy in pregnancy and incidence of low birth weight in malaria-endemic countries.** *Am J Public Health* 2018, **108:**399-406.

80. Muhindo MK, Kakuru A, Roh M, Natureeba P, Awori P, Olwoch P, Ategeka J, Nayebare P, Clark TD, Muehlenbachs A: **Reductions in malaria in pregnancy and adverse birth outcomes following indoor residual spraying of insecticide in Uganda.** *Malar J* 2016, **15:**437.

81. Desai M, Ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD: **Epidemiology and burden of malaria in pregnancy.** *Lancet Infect Dis* 2007, **7:**93-104.

82. Moore KA, Fowkes FJ, Wiladphaingern J, San Wai N, Paw MK, Pimanpanarak M, Carrara VI, Raksuansak J, Simpson JA, White NJ: **Mediation of the effect of**

malaria in pregnancy on stillbirth and neonatal death in an area of low transmission: observational data analysis. *BMC Med* 2017, **15:**98.

83. **Care of the preterm and low-birth-weight newborn** [https://www.who.int/maternal_child_adolescent/newborns/prematurity/en/]

84. Sicuri E, Bardají A, Sigauque B, Maixenchs M, Nhacolo A, Nhalungo D, Macete E, Alonso PL, Menéndez C: **Costs associated with low birth weight in a rural area of Southern Mozambique.** *PLoS One* 2011, **6**.

85. McCormick MC, Brooks-Gunn J, Workman-Daniels K, Turner J, Peckham GJ: **The Health and Developmental Status of Very Low—Birth-Weight Children at School Age.** *JAMA* 1992, **267:**2204-2208.

86. Kirk CM, Uwamungu JC, Wilson K, Hedt-Gauthier BL, Tapela N, Niyigena P, Rusangwa C, Nyishime M, Nahimana E, Nkikabahizi F: **Health, nutrition, and development of children born preterm and low birth weight in rural Rwanda: a cross-sectional study.** *BMC Pediatr* 2017, **17:**191.

87. Larroque B, Bertrais S, Czernichow P, Léger J: **School difficulties in 20-year-olds who were born small for gestational age at term in a regional cohort study.** *Pediatrics* 2001, **108:**111-115.

88. Mi D, Fang H, Zhao Y, Zhong L: **Birth weight and type 2 diabetes: A meta-analysis.** *Exp Ther Med* 2017, **14:**5313-5320.

89. Risnes KR, Vatten LJ, Baker JL, Jameson K, Sovio U, Kajantie E, Osler M, Morley R, Jokela M, Painter RC: **Birthweight and mortality in adulthood: a systematic review and meta-analysis.** *Int J Epidemiol* 2011, **40:**647-661.

90. The PMI VectorLink Project: **Annual Report: October 1, 2018 - September 30, 2019.** (The PMI VectorLink Project, Abt Associates I eds.). Rockville, Maryland, USA.

91. Ter Kuile FO, Taylor SM: **Gilding the Lily? Enhancing Antenatal Malaria Prevention in HIV-Infected Women.** *J Infect Dis* 2017, **216:**4-6.

Publishing Agreement

It is the policy of the University to encourage open access and broad distribution of all theses, dissertations, and manuscripts. The Graduate Division will facilitate the distribution of UCSF theses, dissertations, and manuscripts to the UCSF Library for open access and distribution. UCSF will make such theses, dissertations, and manuscripts accessible to the public and will take reasonable steps to preserve these works in perpetuity.

I hereby grant the non-exclusive, perpetual right to The Regents of the University of California to reproduce, publicly display, distribute, preserve, and publish copies of my thesis, dissertation, or manuscript in any form or media, now existing or later derived, including access online for teaching, research, and public service purposes.

DocuSigned by:
Michelle Roh
C0092959DE83434...
Author Signature

12/6/2020
Date