Am. J. Trop. Med. Hyg., 95(5), 2016, pp. 1069–1076
doi:10.4269/ajtmh.16-0370

Malarial Infection and Curable Sexually Transmitted and Reproductive Tract Infections among Pregnant Women in a Rural District of Zambia

Enesia Banda Chaponda,[1,2]* R. Matthew Chico,[2] Jane Bruce,[2] Charles Michelo,[3] Bellington Vwalika,[4] Sungano Mharakurwa,[5,6] Mike Chaponda,[7] James Chipeta,[8] and Daniel Chandramohan[2]

[1]*Department of Biological Sciences, University of Zambia, Lusaka, Zambia;* [2]*Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom;* [3]*Department of Public Health, University of Zambia School of Medicine, Lusaka, Zambia;* [4]*Department of Obstetrics and Gynaecology, University of Zambia School of Medicine, Lusaka, Zambia;* [5]*Faculty of Health Sciences, Africa University, Mutare, Zimbabwe;* [6]*Department of Medical Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland;* [7]*Department of Clinical Sciences, Tropical Diseases Research Centre, Ndola, Zambia;* [8]*Department of Paediatrics and Child Health, University of Zambia School of Medicine, Lusaka, Zambia*

Abstract. Malarial infection and curable sexually transmitted and reproductive tract infections (STIs/RTIs) are important causes of adverse birth outcomes. Reducing the burden of these infections in pregnancy requires interventions that can be easily integrated into the antenatal care (ANC) package. However, efforts to integrate the control of malarial infection and curable STIs/RTIs in pregnancy have been hampered by a lack of evidence related to their coinfection. Thus, we investigated the prevalence of coinfection among pregnant women of rural Zambia. A prospective cohort study was conducted in Nchelenge District, Zambia, involving 1,086 first ANC attendees. We screened participants for peripheral malarial infection and curable STIs/RTIs (syphilis, *Chlamydia*, gonorrhea, trichomoniasis, and bacterial vaginosis), and collected relevant sociodemographic data at booking. Factors associated with malarial and STI/RTI coinfection were explored using univariate and multivariate regression models. Among participants with complete results (N = 1,071), 38.7% (95% confidence interval [CI] = 35.7–41.6) were coinfected with malaria parasites and at least one STI/RTI; 18.9% (95% CI = 16.5–21.2) were infected with malaria parasites only; 26.0% (95% CI = 23.5–28.8) were infected with at least one STI/RTI but no malaria parasites, and 16.4% (95% CI = 14.1–18.6) had no infection. Human immunodeficiency virus (HIV)-infected women had a higher risk of being coinfected than HIV-uninfected women (odds ratio [OR] = 3.59 [95% CI = 1.73–7.48], $P < 0.001$). The prevalence of malarial and STI/RTI coinfection was high in this population. An integrated approach to control malarial infection and STIs/RTIs is needed to reduce this dual burden in pregnancy.

INTRODUCTION

Maternal malarial infection and sexually transmitted and reproductive tract infections (STIs/RTIs) are important causes of adverse birth outcome in sub-Saharan Africa. Malarial infection in pregnancy is associated with intrauterine growth retardation,[1,2] preterm delivery,[2,3] stillbirth,[4] and low birth weight.[2,5] Syphilis,[4] *Chlamydia*,[6–10] gonorrhea,[6,9,11] trichomoniasis,[12,13] and bacterial vaginosis[14–16] have also been associated with adverse birth outcomes. To reduce the burden of malarial infection in areas of moderate (stable)-to- high transmission, the World Health Organization (WHO) recommends providing intermittent preventive treatment in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP) at all scheduled antenatal care (ANC) visits from the second trimester to delivery.[17] Despite a decade since the WHO first recommended IPTp-SP,[18] coverage remains disappointingly low in sub-Saharan Africa. The average coverage of at least two doses of IPTp-SP among pregnant women in 2013 was 24%, only slightly higher than a decade earlier when coverage was 14%.[19] The efficacy of IPTp-SP has been undermined by the emergence of SP-resistant parasites[20] and the effectiveness of three doses of IPTp-SP may be suboptimal in areas with very high SP resistance.[21] Thus, there is need to identify alternative drugs for IPTp in areas where parasites have lost SP sensitivity.

Antenatal syphilis screening is a standard policy throughout sub-Saharan Africa. A recent meta-analysis, however, estimated that only 39.5% of pregnant women throughout the region were screened during ANC visits.[22] Other curable STIs/RTIs are diagnosed during ANC consultations using syndrome-based algorithms recommended by the WHO.[23] However, the algorithms have poor sensitivity among pregnant women for detecting *Chlamydia*, gonorrhea, trichomoniasis, and bacterial vaginosis.[24] Therefore, a considerable burden of curable STIs/RTIs remains undetected and untreated in pregnancy.

A systematic review and meta-analysis of 171 studies showed that the prevalence of malarial infection and curable STIs/RTIs is unacceptably high among pregnant women attending ANC facilities in sub-Saharan Africa.[25] This analysis also highlighted the paucity of data on the frequency of malarial and curable STI/RTI coinfection (syphilis, *Chlamydia*, gonorrhea, trichomoniasis, and bacterial vaginosis).[25] In the context of increasing malaria parasite resistance to SP, particularly in east and southern Africa, and the limited diagnostic precision of the syndromic management among pregnant women, new strategies to control these infections in pregnancy are needed. One option under consideration is to provide combination therapy that is safe and effective against malarial infection and curable STIs/RTIs.[26] Quantifying the prevalence of malarial and STI/RTI coinfection is an important step toward tailoring integrated interventions. Thus, we conducted a prospective cohort study to estimate the prevalence of malarial infection, curable STIs/RTIs, and their coinfection in pregnant women and their effects on pregnancy outcomes. We provide evidence of the frequency of coinfection among pregnant women who present at ANC and the effect of HIV infection on the prevalence of malarial and STI/RTI coinfection. We also explore predictors of malarial and STI/RTI

*Address correspondence to Enesia Banda Chaponda, Department of Biological Sciences (Room 405), University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia. E-mail: enesia.chaponda@gmail.com

coinfection and assess whether there is an association between coinfection and adverse birth outcomes.

METHODS

The study setting and sample size calculations have been described elsewhere.[27] Briefly, the study site was the catchment area for two health centers, Nchelenge and Kashikishi, in Nchelenge District, which is located on the shores of Lake Mweru, northern Zambia, and has a population of 173,680.[28] We invited women to participate in the study from two health centers at ANC booking. Pregnant women (N = 1,086) were enrolled if they provided informed written consent, stated they had not been exposed to anti-malarial and/or antibiotic therapy within the previous 4 weeks, agreed to have a member of the study team record their HIV test results following routine HIV screening, and had a gestational age of < 32 weeks. Study participants were screened for syphilis using rapid plasma reagin (RPR) methods according to national norms. Women were notified of their test results if found to be RPR positive and referred for treatment. Screening for malarial infection and curable STIs/RTIs, apart from syphilis, is not standard and, therefore, retrospective batch analyses were conducted on relevant samples. Women who had fever or any other symptoms during the antenatal period were given care according to national norms. All women enrolled were followed until delivery.

Sample collection and laboratory methods. Health facility staff conducted routine HIV screening with finger-prick blood with Determine® HIV-1/2 (Abbott Diagnostic Division, Hoofddorp, The Netherlands) tests and confirmed positivity using Uni-Gold™ Recombigen® HIV-1/2 (Trinity Biotech USA Inc., New York, NY) assays. Trained field workers administered a questionnaire to participating women in a private room at the ANC facility to collect information on sociodemographics, malaria infection prevention interventions, HIV status, and obstetric history.

Study staff collected blood samples for *Plasmodium falciparum* malaria diagnosis by polymerase chain reaction (PCR). It is important to note that in Zambia, *P. falciparum* is responsible for approximately 95% of all malaria cases.[29] Details of the diagnostic method have been described elsewhere.[27] Malarial infection prevalence was defined as PCR-detected *P. falciparum* parasitemia because the technique is highly sensitive and consistent in the detection of parasites.[30,31] For the diagnosis of vaginal and cervical infections of interest, a trained midwife collected one vaginal swab and one cervicovaginal swab from each participant using nonlubricated vaginal specula. A dry cotton swab was placed in the vaginal cavity for about 10 seconds and then immediately rolled onto a slide for use in diagnosis of bacterial vaginosis. The midwife then collected a cervicovaginal sample using a Dacron® (Medical Wire & Equipment, Wiltshire, England, United Kingdom) swab for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by PCR. Each Dacron swab was placed in a cryovial (Narang Medical Limited, New Delhi, India) with a unique identification number that corresponded to each participant and the date of collection. Samples were then stored at −20°C for 6–12 hours. Within 24 hours of collection, we extracted the DNA from cervicovaginal swabs using the Quick-gDNA™ (Zymo Research Corp., Irvine, CA) Miniprep kit and stored the extracts at −20°C awaiting nucleic acid amplification.

Clinic staff collected venous blood (approximately 4 mL) from each participant for RPR analysis (Omega Diagnostics Limited, Alva, Scotland, United Kingdom). Field workers provided seropositive women with a note to return to the health center accompanied by their partner for free syphilis treatment with benzathine penicillin G (2.4 million units IM weekly × 3 weeks). We used *Treponema pallidum* hemagglutination assay (TPHA) (Chronolab Systems, Barcelona, Spain) to confirm syphilis infection on all RPR-seropositive samples. For quality control, we had 5% (55/1,077) of serum samples independently analyzed by the national STI reference laboratory at the University Teaching Hospital in Lusaka. Of the 117 RPR-positive samples, 30 (26%) were tested for TPHA quality control. Vaginal smear samples for the diagnosis of bacterial vaginosis were air dried and Gram stained using safranin as a counter stain. We classified results based on the Nugent criteria.[32] As with syphilis, we randomly selected 5% (55/1,085) of slides for independent reading at the microbiology laboratory of the University Teaching Hospital.

The extracted DNA samples were transported on dry ice to the Tropical Gastroenterology and Nutrition Group laboratory at the University Teaching Hospital, where we used in-house standard end-point PCR assays to detect the presence of *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* among cervicovaginal samples with PCR techniques previously described.[33–35]

We pretested all the PCR assays on known positive samples before they were applied to research samples, and used positive and negative controls at the extraction, amplification, and electrophoresis stages. A negative and a positive control were included in every batch of 46 samples. For quality control, we randomly selected 5% of the samples (55/1,084) and processed them using the Seeplex® STI Master Panel 1 V2.0 (Seegene Technologies Inc., Concord, CA). The Seeplex STI Master Panel 1 is a multiplex conventional PCR system for the detection of seven organisms including *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* from urine, vaginal swabs, and liquid-based cytology specimens. At delivery, infants were weighed and birth characteristics recorded. Stillbirth was defined as fetal death at ≥ 28 weeks gestation,[36] preterm as delivery at < 37 weeks, low birthweight as birthweight < 2,500 g, and intra-uterine growth retardation was defined as an infant with low birth weight born at ≥ 37 weeks gestation.[2,37] Gestational age after birth was recorded based on earlier assessment at enrolment by ultrasound.

The study protocol was approved by the University of Zambia Biomedical Research Ethics Committee (No. 004-02-13) and the London School of Hygiene and Tropical Medicine Observational/Interventions Research Ethics Committee (No. 6292).

Statistical analysis. Study data were double entered and verified in EpiData version 3.1 software,[38] then cleaned, processed, and analyzed using Stata Software version 13.[39] We generated necessary composite variables prior to all data analysis and created an index of household wealth using principal components analysis using data on source of income, level of education as well as fixed and durable assets.[40] We estimated the frequency distributions of baseline characteristics among all study women and then stratified results first by health center and then HIV status.

A four-level variable was constructed to define whether a woman was 1) coinfected, 2) had malarial infection but no STI/RTI, 3) had only STI/RTI and no malarial infection, 4) or had no infection. The relationship between coinfection and HIV status was examined. A χ^2 test was used to assess statistical significance of all associations.

Univariate logistic regression was used to identify potential predictors of malaria and STI/RTI coinfection, and each variable was assessed using a likelihood ratio test. All potential predictors of coinfection which were significant at the 10% level in the univariate analysis were input into a multivariable model and their adjusted effect on the risk of coinfection estimated. Potential predictors that were found to be independently associated with coinfection ($P < 0.05$) were entered in a final model. The associations between adverse birth outcome and malarial infection, STI/RTI and coinfection were also tested using logistic regression.

RESULTS

A total of 1,086 pregnant women were recruited between November 2013 and April 2014, a period which spans the high malaria transmission season. Study staff followed up participants until the last women delivered in November 2014. Figure 1 shows a flow chart of participation distribution. Less than 1% ($N = 9$) of first ANC attendees who met all other criteria for participation refused to take part in the study. The median age of participants was 25 (IQR [interquartile range] = 20–25) years, 61.7% were multigravidae and slightly over 80% of them were married. Sociodemographic details of study women are presented in Table 1. Table 2 summarizes the prevalence of malarial infection and STIs/RTIs among study participants.

The prevalence estimates of composite and individual STI/RTI and malarial coinfection by HIV status are shown in Table 3. The prevalence of HIV infection among participants was 13.2% (95% confidence interval [CI] = 11.3–15.3). Among women who tested positive for HIV, combined with those with known HIV-positive status, 42.7% were receiving antiretroviral therapy at the time of recruitment. The prevalence of malarial and any one STI/RTI coinfection was higher among HIV-infected (50.0%, 95% CI = 41.6–58.4%) than HIV-uninfected women (37.0%, 95% CI = 33.9–40.1%) with a 95% CI of the difference ranging between 3.0% and 20.3%. The prevalence of coinfection with malaria parasites and each individual STI/RTI was also higher among HIV-infected than HIV-uninfected women. However, these differences in the prevalence between HIV-infected and HIV-uninfected women were only statistically significant in the cases of malarial and syphilis or bacterial vaginosis coinfections. The STI/RTI that coexisted the most with malarial infection in this study population was bacterial vaginosis and gonorrhea occurred the least with malarial infection.

In univariate logistic models, the following factors were associated with malarial and STI/RTI coinfection at a 10% significance level ($P < 0.1$): 1) age group, 2) gravidity, 3) bed-net ownership, 4) bed-net usage, and 5) HIV status. After adjusting for all known potential risk factors in a multivariable model, only HIV infection was independently associated with malarial and STI/RTI coinfection. HIV-infected women were at a higher risk of being coinfected with malaria parasites and at least one curable STI/RTI compared with

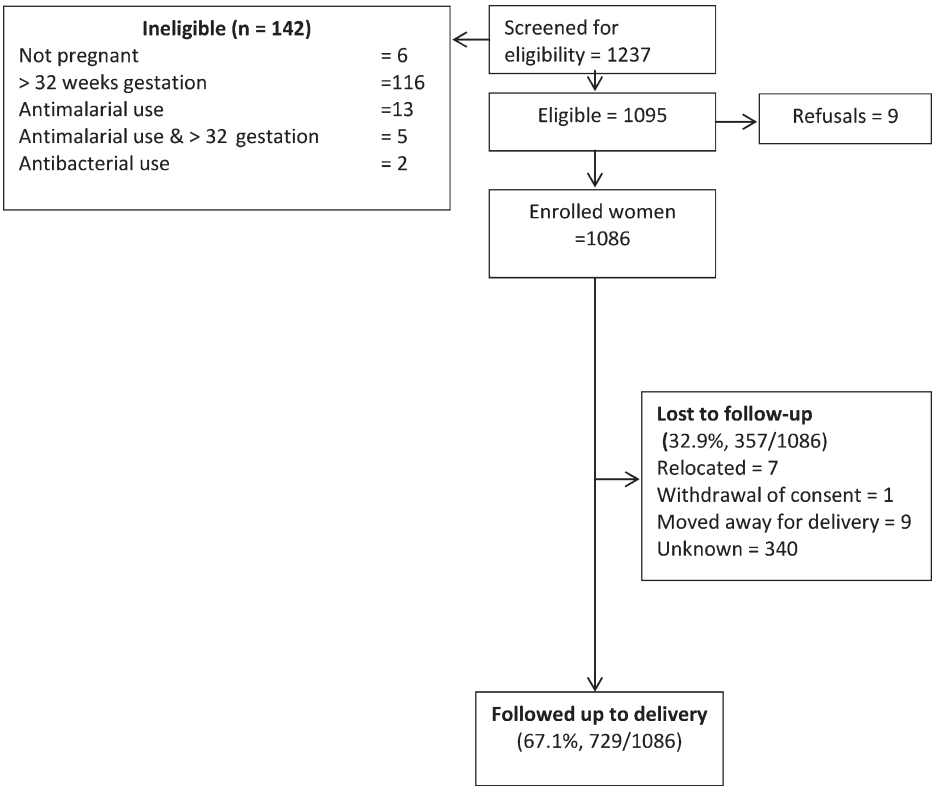

FIGURE 1. Participation flowchart.

TABLE 1
Characteristics of pregnant women at their first antenatal care visit

Characteristics	All pregnant women, *n* (%)
Total	1,084
Recruitment site	
Kashikishi	746 (68.8)
Nchelenge	338 (31.2)
Age	
Median (IQR)	25 (20–30)
Marital status	
Single	203 (18.7)
Married	873 (80.6)
Separated/divorced/widowed	8 (0.7)
Gravidity	
Primigravidae	261 (24.1)
Secundigravidae	165 (15.2)
Multigravidae	658 (60.7)
Years of schooling	
0–6 years	425 (39.2)
7 years and above	659 (60.8)
Bed-net ownership	
No	550 (50.7)
Yes	534 (49.3)
Net usage on previous night	
No	665 (61.5)
Yes	416 (38.5)
Missing*	3
IRS in past 1 year	
No	665 (61.5)
Yes	416 (38.5)
Missing*	51
Wealth index	
Lowest	217 (20.1)
Second	220 (20.3)
Middle	214 (19.8)
Fourth	215 (19.9)
Highest	216 (20.0)
Missing*	2

IQR = interquartile range; IRS = indoor residual spraying; NA = not applicable.
*Missing values are only presented as numbers and were not included in the calculation of percentages.

HIV-uninfected women (odds ratio [OR] = 3.59 [95% CI = 1.73–7.48], $P < 0.001$) (Table 4).

Results for quality control testing were highly concordant. There was a 96.4% (53/55) concordance in the Nugent score results obtained from the repeat reading of slides for detection of bacterial vaginosis. The two readings both conflicted between the normal and the intermediate class. Syphilis RPR results were 100% (55/55) concordant; TPHA testing was 100% (30/30) concordant. For *C. trachomatis*, agreement of test results between the in-house methods and the Seeplex STI Master Panel 1 kit was 96.4% (54/55). In the case *of N. gonorrhoeae* and *T. vaginalis*, there was 100% concordance between the in-house methods and the commercial kit.

Loss to follow-up in this study was much higher (33%) than expected (10%). However, there were no differences in the characteristics of women who were followed up to delivery and those who were lost to follow-up. Of the 729 participants followed to delivery, 98.4% ($N = 717$) had singleton deliveries and 1.6% ($N = 12$) had twin deliveries. Overall, the prevalence of adverse birth outcome among the 717 women with singleton deliveries was 35.0% ($N = 251$). Among 717 singleton deliveries, preterm delivery, low birth weight, intrauterine growth retardation, and stillbirth were observed in 22.1% ($N = 158$), 21.9% (157/716), 15.2% ($N = 85$), and 1.7%, ($N = 12$), respectively.

TABLE 2
Prevalence of malarial infection and sexually transmitted and reproductive tract infections among first ANC attendees of Nchelenge District, Zambia

Infection	Category	All ($N = 1,084$) *n* (%)	95% Confidence interval
Bacterial vaginosis	Normal	375 (34.7)	31.8–37.5
	Intermediate	184 (17.0)	14.9–19.4
	BV	524 (48.3)	45.2–51.2
	Missing*	1	
Trichomoniasis	Positive	269 (24.8)	22.3–27.5
	Missing*	1	
Chlamydia	Positive	56 (5.2)	3.9–6.7
	Missing*	1	
Gonorrhea	Positive	34 (3.1)	2.2–4.4
	Missing*	1	
Confirmed syphilis	Positive	76 (7.1)	5.6–8.7
	Missing*	7	
Malarial infection	Positive	621 (57.8)	54.8–60.7
	Missing*	10	
Composite STI	Positive	374 (34.5)	31.7–37.4
	Missing*	1	
STI/RTI	Positive	702 (64.8)	61.7–67.4
	Missing*	1	

STI/RTI = sexually transmitted and reproductive tract infection.
*Missing values are only presented as numbers and were not included in the calculation of percentage. Some values were missing due to missing samples.

In the univariate analysis, women who were monoinfected (malarial infection or at least one STI/RTI) and coinfected had 24% and 31% increased risk of experiencing an adverse birth outcome, respectively, compared with uninfected women, but this was not statistically significant. We found no association between malarial infection or STI/RTI and adverse birth outcome in the univariate analysis as summarized in Table 5.

DISCUSSION

The estimated prevalence of malarial and curable STI/RTI coinfection among all gravidae was considerable (38.7%; 95% CI = 35.7–41.6%). To our knowledge, there has only been one previous study that reported the prevalence of malarial and curable STI/RTI coinfection (syphilis only). That was in Tanzania where 48.3% of RPR-positive women also had placental malarial infection.[25] In contrast, we found the prevalence of malarial and syphilis coinfection to be 10.5% based on RPR testing and peripheral parasitemia diagnosed by PCR among pregnant women at ANC booking. Malarial and syphilis coinfection based on RPR and TPHA results in this study was 4.0%. The difference in the prevalence of malarial and syphilis (RPR-based seropositivity) coinfection between the two studies may partially be explained by the fact that the study in Tanzania was conducted in 1997–2000, when malaria endemicity was higher than 2013, and the prevalence of placental parasitemia can be higher than peripheral parasitemia.

Although malaria parasite transmission occurs year-round, the incidence of malaria rises during the rainy season, which is when this study was conducted. Thus, the observed prevalence of malarial and STI/RTI coinfection may have been higher than might be found during other times of the year. The prevalence of malarial and STI/RTI coinfection may have been influenced by the fact that some of the HIV-positive women (42.7%) were on antiretroviral therapy at recruitment. The observed prevalence of malarial and STI/RTI coinfection

TABLE 3
Prevalence of malarial and curable STI/RTI coinfection among pregnant women at first antenatal care visit

Coinfection	Category	All women (N = 1,084) n (%)	HIV uninfected (N = 941) n (%)	HIV infected (N = 143) n (%)	95% CI for difference	P value†
Malarial infection and all STIs/RTIs						
	Coinfection	414 (38.7)	345 (37.0)	69 (50.0)	3.0–20.3	0.003
	Malaria only	202 (18.9)	182 (19.5)	20 (14.5)	−1.7–10.8	0.154
	STI/RTI only	278 (26.0)	237 (25.4)	41 (29.7)	−3.9–11.8	0.271
	No infection	177 (16.4)	169 (18.1)	8 (5.8)	6.8–16.8	< 0.001
	Missing*	13	8	5		
Malarial infection and confirmed syphilis	Yes	43 (4.0)	31 (3.3)	12 (8.8)	1.3–10.9	0.002
	Missing*	12	6	5		
Malarial infection and *Chlamydia*	Yes	37 (3.4)	30 (3.2)	7 (5.1)	−1.1–6.7	0.263
	Missing*	11	6	5		
Malarial infection and gonorrhea	Yes	22 (2.1)	17 (1.8)	5 (3.6)	−0.6–6.2	0.162
	Missing*	11	6	5		
Malarial infection and trichomoniasis	Yes	164 (15.3)	142 (15.2)	22 (15.9)	−5.3–7.4	0.818
	Missing*	11	6	5		
Malarial infection and bacterial vaginosis	Yes	313 (29.2)	250 (26.8)	63 (45.7)	9.1–26.1	< 0.001
No	Missing*	14	9	5		

CI = confidence interval; HIV = human immunodeficiency virus; STI/RTI = sexually transmitted and reproductive tract infection.
*Missing values are only presented as numbers and were not included in the calculation of percentage. Some values were missing due to missing samples.
†P value for two-sample test of proportions.

could have been slightly higher than what was observed if fewer women were on antiretroviral treatment or lower if more women were undergoing treatment.

Since about 95% of pregnant women in Zambia have at least one ANC visit[41] and the refusal rate in this study was less than 1%, our results likely reflect the dual burden of malarial infection and curable STIs/RTIs among all pregnant women in this setting. Furthermore, inclusion of positive and negative controls in the molecular diagnosis procedures and the fact that results obtained from repeat reading and testing were virtually reproducible enhance the validity of the dual burden of malarial infection and STI/RTI among pregnant women in this population.

Quantifying the frequency of malarial and STI/RTI coinfection is important in the context of ongoing research for alternatives to SP for use in IPTp. Our study provides evidence in support of antimalarial and antibacterial drug combinations that may offer the added benefit of reducing the burden of curable STIs/RTIs in pregnancy, especially in resource-poor settings such as where STIs/RTIs are highly prevalent and routine screening may not be sustainable. Furthermore, up to 50% of stillbirths have been attributed to untreated maternal syphilis in some areas[42,43] and coverage of syphilis screening of pregnant women during ANC in sub-Saharan Africa is estimated to be quite low (39.5%).[22] In fact, malaria and syphilis are the two leading infectious causes of stillbirth in sub-Saharan Africa, implicated in 420,000 stillbirths, or three of every 10 stillbirths, every year.[4] Presumably fewer pregnant women with syphilis are given appropriate treatment; however, the proportion of women testing positive for syphilis who receive treatment could be increased by the use of point of care tests rather than laboratory-based RPR tests as demonstrated in several countries including Zambia.[44] An integrated solution for the management of malarial infection and STI/RTI in pregnancy is clearly needed for the benefit of pregnant women in areas with poor resources and overlapping prevalence of malarial infection and STIs/RTIs.

Bed-net use,[45,46] parity, and age[1] have been known to be associated with malarial infection. Parity,[47,48] the number of sexual partners,[49] and early sexual debut[47,49] have been previously associated with STIs/RTIs among pregnant women. No association was found between malarial and STI/RTI coinfection and factors such as parity, age, the number of sexual partners, early sexual debut, bed-net ownership, bed-net use, and wealth quintile in this study.

TABLE 4
Multivariate analyses of risk factors for malarial and curable STI/RTI coinfection among first ANC attendees (N = 1,071)

Potential risk factor	Category	n (%)	Adjusted OR	P value
Age group	≤ 20	295 (27.5)	1.00	0.149
	21–25	304 (28.3)	0.95 (0.52–1.72)	
	26–30	244 (22.7)	0.83 (0.43–1.61)	
	≥ 30	231 (21.5)	0.58 (0.30–1.14)	
Gravidity	Primigravidae	258 (24.1)	1.00	0.681
	Secundigravidae	165 (15.4)	0.96 (0.52–1.88)	
	Multigravidae	648 (60.5)	0.77 (0.41–1.46)	
Bed-net ownership	No	734 (68.5)	1.00	0.937
	Yes	337 (31.5)	0.83 (0.47–1.47)	
Bed-net use on previous night	No	658 (61.6)	1.00	0.386
	Yes	410 (38.4)	0.98 (0.56–1.74)	
	Missing	3		
HIV status	Negative	933 (87.1)	1.00	< 0.001
	Positive	138 (12.9)	3.93 (1.87–8.27)	

Coinfection = infection with malaria parasites and at least one STI/RTI; HIV = human immunodeficiency virus; NA = not applicable; OR = odds ratio; STI/RTI = sexually transmitted and reproductive tract infection. Only HIV infection was found to be independently associated with malaria and STI/RTI coinfection therefore we did not adjust for any other variable in the final model. Missing values are only presented as number and were not included in the calculation of percentages and analysis.

TABLE 5
Univariate analyses of the association of malarial infection, STIs/RTIs, and their coinfection with adverse birth outcomes ($N = 717$)

Potential risk factor	Category	*n* (%)	Unadjusted OR	*P* value*
Malarial and STI/RTI coinfection‡	No infection	117 (16.5)	1.00	0.507
	Single infection†	326 (46.1)	1.24 (0.79–1.96)	
	Coinfection‡	266 (37.5)	1.31 (0.82–2.09)	
	Missing§	8		
STI/RTI	Negative	259 (36.1)	1.00	0.663
	Positive	458 (63.9)	1.07 (0.78–1.48)	
Peripheral malarial infection	Negative	301 (42.4)	1.00	0.181
	Positive	409 (57.6)	1.24 (0.90–1.69)	
	Missing§	7		

OR = odds ratio; STI/RTI = sexually transmitted and reproductive tract infection. Diagnoses were conducted at first ANC attendance and women were followed up to delivery.
*Overall *P* value for univariate model.
†Infection with at least one STI/RTI or malaria.
‡Infection with at least one STI/RTI and malaria.
§Missing values are only presented as numbers and were not included in the calculation of percentages and in the analyses. Missing values were due to missing samples.

HIV-infected women were at a higher risk of malarial infection and STIs/RTIs. However, as previously reported,[27] the sample size was not large enough to conclude that the increased risk of malarial infection among HIV-infected women was statistically significant. Infection with HIV has been associated with both malarial infection[50,51] and curable STIs/RTIs.[52,53] It is, therefore, not a surprise finding that HIV was a risk factor for malarial and STI/RTI coinfection in our study.

The fact that HIV was strongly associated with malarial and STI/RTI coinfection among pregnant women on their first ANC visit highlights the importance of treatment and prevention of these infections in pregnant HIV-infected women. In areas where malaria parasites have lost sensitivity to SP, alternative therapies that combine a broad-spectrum antibiotic with an efficacious antimalarial should be investigated. One possible combination would involve azithromycin plus dihydroartemisinin–piperaquine. Azithromycin is efficacious against *T. pallidum*, *C. trachomatis* and *N. gonorrhoea* and may offer some protection against *T. vaginalis* and *Gardnerella vaginalis*, a bacterium that is commonly implicated in bacterial vaginosis.[54] Azithromycin is efficacious against *Plasmodium vivax,* but needs a potent antimalarial partner drug to clear *P. falciparum*.[55] Dihydroartemisinin–piperaquine has been shown to be an efficacious antimalarial and potential replacement for SP as IPTp.[56,57] Therefore, dihydroartemisinin–piperaquine plus azithromycin could be a good replacement for SP as IPTp.

Clinicians and staff involved in the provision of ANC services also need to be proactive in the management of these infections, especially in HIV-infected women. Moreover, the importance of community education on the ways that malarial infection, HIV, and STI/RTIs can be prevented cannot be overemphasized.

The lack of association between infections and adverse birth outcome could partially be attributed to the interventions in the ANC package including IPTp-SP, iron and folic acid supplementation, and syphilis treatment. Administration of IPTp-SP reduces the adverse effects of malarial infection in pregnancy including third trimester maternal anemia, placental parasitemia, and the incidence of low birth weight.[58–61] In this study, IPTp-SP coverage was high, with 99% of women who were followed to delivery receiving at least one dose of IPTp-SP during the pregnancy duration. Furthermore, studies have suggested that SP could offer additional protection against infections other than malarial infection resulting in the protective effect against adverse birth outcome, which becomes more apparent in areas where parasites have lost sensitivity.[26,56]

Diagnosis of infections was limited to the first ANC visit and women were classified as infected or uninfected based on results from the screening done at first ANC. This potentially may have resulted in the misclassification of individuals who were classified as uninfected but acquired an infection later in pregnancy and which may have resulted in the reduction in the strength of association between infection and adverse birth outcome. A second screening later in pregnancy is recommended in future studies.

The loss to follow-up in this study was much higher than expected mainly due to unforeseen interruptions in transportation during the follow-up to delivery period. Additional factors may be related to myths and traditional beliefs among residents of this district that influence birthing practices and cause women to prefer delivery at home in seclusion or away from their home villages.[62]

Given the high prevalence of malarial infection, STIs/RTIs and their coinfection in pregnancy alternatives to SP that address the dual burden of malarial infection and curable STIs/RTIs should be prioritized.

Received May 10, 2016. Accepted for publication August 9, 2016.

Published online September 26, 2016.

Acknowledgments: We thank the participants, the ANC staff at Nchelenge and Kashikishi health centers, and the Nchelenge District Health Management Team. We also thank Professor Paul Kelly and the Tropical Gastroenterology and Nutrition Group laboratory management for granting usage of their laboratory for the molecular work.

Financial support: This study was financially supported by the International Centers of Excellence for Malaria Research (ICEMR) in southern Africa, and the U.S. National Institutes of Health from NIH/NIAID grant U19AI089680 (Malaria Transmission and the Impact of Control Efforts in southern Africa) with John Hopkins University Bloomberg School of Public Health and the University of Zambia School Of Medicine (UNZA-SoM), and the Research Support Center at the UNZA-SoM through the southern African Consortium for Research Excellence (SACORE), which is part of the African Institutions Initiative grant of the Wellcome Trust (Company No. 2711000), a charity (No. 210183) registered in England, as well as the Commonwealth Scholarship Commission in the United Kingdom.

Authors' addresses: Enesia Banda Chaponda, Department of Biological Sciences, University of Zambia School of Natural Sciences, Lusaka, Zambia, E-mail: enesia.chaponda@gmail.com. R. Matthew Chico, Department of Disease Control, Faculty of Public Health and

Policy, London School of Hygiene and Tropical Medicine, E-mail: matthew.chico@lshtm.ac.uk. Jane Bruce, Department of Disease Control and Vector Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom, E-mail: jane.bruce@lshtm.ac.uk. Charles Michelo, Department of Public Health, University of Zambia School of Medicine, Lusaka, Zambia, E-mail: ccmichelo@yahoo.com. Bellington Vwalika, Department of Obstetrics and Gynaecology, University of Zambia School of Medicine, Lusaka, Zambia, E-mail: bvwalika@rzhrg-mail.org. Sungano Mharakurwa, Faculty of Health Sciences, Africa University, Mutare, Zimbabwe, and Department of Medical Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, E-mail: mharakurwas@africau.edu. Mike Chaponda, Department of Clinical Sciences, Tropical Diseases Research Centre, Ndola, Zambia, E-mail: mikechaponda@yahoo.com. James Chipeta, Department of Paediatrics and Child Health, University of Zambia School of Medicine, Lusaka, Zambia, E-mail: assistdeanresearch@smuth-mru.org.zm. Daniel Chandramohan, Department of Disease Control, London School of Hygiene and Tropical Medicine, London, United Kingdom, E-mail: daniel.chandramohan@lshtm.ac.uk.

REFERENCES

1. McGregor IA, 1984. Epidemiology, malaria and pregnancy. *Am J Trop Med Hyg 33:* 517–525.
2. Watson-Jones D, Weiss HA, Changalucha JM, Todd J, Gumodoka B, Bulmer J, Balira R, Ross D, Mugeye K, Hayes R, Mabey D, 2007. Adverse birth outcomes in United Republic of Tanzania–impact and prevention of maternal risk factors. *Bull World Health Organ 85:* 9–18.
3. McGregor IA, Wilson ME, Billewicz WZ, 1983. Malaria infection of the placenta in The Gambia, west Africa; its incidence and relationship to stillbirth, birthweight and placental weight. *Trans R Soc Trop Med Hyg 77:* 232–244.
4. Lawn JE, Blencowe H, Waiswa P, Amouzou A, Mathers C, Hogan D, Flenady V, Froen JF, Qureshi ZU, Calderwood C, Shiekh S, Jassir FB, You D, McClure EM, Mathai M, Cousens S, 2016. Stillbirths: rates, risk factors, and acceleration towards 2030. *Lancet 387:* 587–603.
5. Brabin BJ, 1983. An analysis of malaria in pregnancy in Africa. *Bull World Health Organ 61:* 1005–1016.
6. Johnson HL, Ghanem KG, Zenilman JM, Erbelding EJ, 2011. Sexually transmitted infections and adverse pregnancy outcomes among women attending inner city public sexually transmitted diseases clinics. *Sex Transm Dis 38:* 167–171.
7. Investigators of the Johns Hopkins Study of Cervicitis and Adverse Pregnancy Outcome, 1989. Association of *Chlamydia trachomatis* and *Mycoplasma hominis* with intrauterine growth retardation and preterm delivery. *Am J Epidemiol 129:* 1247–1257.
8. Blas MM, Canchihuaman FA, Alva IE, Hawes SE, 2007. Pregnancy outcomes in women infected with *Chlamydia trachomatis*: a population-based cohort study in Washington state. *Sex Transm Infect 83:* 314–318.
9. Liu B, Roberts CL, Clarke M, Jorm L, Hunt J, Ward J, 2013. *Chlamydia* and gonorrhoea infections and the risk of adverse obstetric outcomes: a retrospective cohort study. *Sex Transm Infect 89:* 672–678.
10. Rours GI, Duijts L, Moll HA, Arends LR, de Groot R, Jaddoe VW, Hofman A, Steegers EA, Mackenbach JP, Ott A, Willemse HF, van der Zwaan EA, Verkooijen RP, Verbrugh HA, 2011. *Chlamydia trachomatis* infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. *Eur J Epidemiol 26:* 493–502.
11. Elliott B, Brunham RC, Laga M, Piot P, Ndinya-Achola JO, Maitha G, Cheang M, Plummer FA, 1990. Maternal gonococcal infection as a preventable risk factor for low birth weight. *J Infect Dis 161:* 531–536.
12. Cotch MF, Pastorek JG 2nd, Nugent RP, Hillier SL, Gibbs RS, Martin DH, Eschenbach DA, Edelman R, Carey JC, Regan JA, Krohn MA, Klebanoff MA, Rao AV, Rhoads GG, 1997. *Trichomonas vaginalis* associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. *Sex Transm Dis 24:* 353–360.
13. Sutton MY, Sternberg M, Nsuami M, Behets F, Nelson AM, St Louis ME, 1999. Trichomoniasis in pregnant human immunodeficiency virus-infected and human immunodeficiency virus-uninfected congolese women: prevalence, risk factors, and association with low birth weight. *Am J Obstet Gynecol 181:* 656–662.
14. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG 2nd, Rao AV, McNellis D, Regan JA, Carey JC, Klebanoff MA; for the Vaginal Infections and Prematurity Study Group, 1995. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *N Engl J Med 333:* 1737–1742.
15. Riduan JM, Hillier SL, Utomo B, Wiknjosastro G, Linnan M, Kandun N, 1993. Bacterial vaginosis and prematurity in Indonesia: association in early and late pregnancy. *Am J Obstet Gynecol 169:* 175–178.
16. Minkoff H, Grunebaum AN, Schwarz RH, Feldman J, Cummings M, Crombleholme W, Clark L, Pringle G, McCormack WM, 1984. Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. *Am J Obstet Gynecol 150:* 965–972.
17. World Health Organization, 2012. *Intermittent Preventive Treatment of Malaria in Pregnancy Using Sulfadoxine-Pyrimethamine (IPTp-SP)*. Updated WHO Policy Recommendation (October 2012). Available at: http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf?ua=1.
18. World Health Organization, 2004. *A Strategic Framework for Malaria Prevention and Control During Pregnancy in the Africa Region*. Brazzaville, Congo: World Health Organization Regional Office for Africa.
19. van Eijk AM, Hill J, Larsen DA, Webster J, Steketee RW, Eisele TP, ter Kuile FO, 2013. Coverage of intermittent preventive treatment and insecticide-treated nets for the control of malaria during pregnancy in sub-Saharan Africa: a synthesis and meta-analysis of national survey data, 2009–11. *Lancet Infect Dis 13:* 1029–1042.
20. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, Kayentao K, Ouma P, Coulibaly SO, Kalilani L, Mace KE, Arinaitwe E, Mathanga DP, Doumbo O, Otieno K, Edgar D, Chaluluka E, Kamuliwo M, Ades V, Skarbinski J, Shi YP, Magnussen P, Meshnick S, Ter Kuile FO, 2015. 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 62:* 323–333.
21. Kayentao K, Garner P, van Eijk AM, Naidoo I, Roper C, Mulokozi A, MacArthur JR, Luntamo M, Ashorn P, Doumbo OK, ter Kuile FO, 2013. Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis. *JAMA 309:* 594–604.
22. Kuznik A, Habib AG, Manabe YC, Lamorde M, 2015. Estimating the public health burden associated with adverse pregnancy outcomes resulting from syphilis infection across 43 countries in sub-Saharan Africa. *Sex Transm Dis 42:* 369–375.
23. World Health Organization, 1991. Management of patients with sexually transmitted diseases. Report of a WHO Study Group. *World Health Organ Tech Rep Ser 810:* 1–103.
24. Pettifor A, Walsh J, Wilkins V, Raghunathan P, 2000. How effective is syndromic management of STDs? A review of current studies. *Sex Transm Dis 27:* 371–385.
25. Chico RM, Mayaud P, Ariti C, Mabey D, Ronsmans C, Chandramohan D, 2012. Prevalence of malaria and sexually transmitted and reproductive tract infections in pregnancy in sub-Saharan Africa. *JAMA 307:* 2079–2086.
26. Chico RM, Moss WJ, 2015. Prevention of malaria in pregnancy: a fork in the road? *Lancet 386:* 2454–2456.
27. Chaponda EB, Chandramohan D, Michelo C, Mharakurwa S, Chipeta J, Chico RM, 2015. High burden of malaria infection in pregnant women in a rural district of Zambia: a cross-sectional study. *Malar J 14:* 380.
28. Central Statistical Office (CSO), 2011. *2010 Census of Population and Housing*. Lusaka, Zambia: Central Statistical Office, 12.
29. Ministry of Health, 2008. *Health Management Information System (HMIS)*. Lusaka, Zambia: Ministry of Health.

30. Mercereau-Puijalon O, Fandeur T, Bonnefoy S, Jacquemot C, Sarthou JL, 1991. A study of the genomic diversity of *Plasmodium falciparum* in Senegal. 2. Typing by the use of the polymerase chain reaction. *Acta Trop 49:* 293–304.
31. Roper C, Elhassan IM, Hviid L, Giha H, Richardson W, Babiker H, Satti GM, Theander TG, Arnot DE, 1996. Detection of very low level *Plasmodium falciparum* infections using the nested polymerase chain reaction and a reassessment of the epidemiology of unstable malaria in Sudan. *Am J Trop Med Hyg 54:* 325–331.
32. Nugent RP, Krohn MA, Hillier SL, 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol 29:* 297–301.
33. Chaudhry U, Saluja D, 2002. Detection of Neisseria gonorrhoeae by PCR using orf1 gene as target. *Sex Transm Infect 78:* 72.
34. Kengne P, Veas F, Vidal N, Rey JL, Cuny G, 1994. Trichomonas vaginalis: repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. *Cell Mol Biol (Noisy-le-grand) 40:* 819–831.
35. Schachter J, 1997. DFA, EIA, PCR, LCR and other technologies: what tests should be used for diagnosis of *Chlamydia* infections? *Immunol Invest 26:* 157–161.
36. World Health Organization, 2016. *Maternal, Newborn, Child and Adolescent Health*. Available at: http://www.who.int/maternal_child_adolescent/epidemiology/stillbirth/en/. Accessed May 18, 2016.
37. Watson-Jones D, Gumodoka B, Weiss H, Changalucha J, Todd J, Mugeye K, Buve A, Kanga Z, Ndeki L, Rusizoka M, Ross D, Marealle J, Balira R, Mabey D, Hayes R, 2002. Syphilis in pregnancy in Tanzania. II. The effectiveness of antenatal syphilis screening and single-dose benzathine penicillin treatment for the prevention of adverse pregnancy outcomes. *J Infect Dis 186:* 948–957.
38. Lauritsen JM, Bruus M, 2003–2005. *EpiData (version 3.1). A Comprehensive Tool for Validated Entry and Documentation of Data*. Odense, Denmark: The EpiData Association.
39. StataCorp, 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP.
40. Braveman PA, Cubbin C, Egerter S, Chideya S, Marchi KS, Metzler M, Posner S, 2005. Socioeconomic status in health research: one size does not fit all. *JAMA 294:* 2879–2888.
41. Central Statistical Office (CSO) [Zambia], Ministry of Health (MOH) [Zambia], ICF International, 2014. *Zambia Demographic and Health Survey 2013–14*. Rockville, MD: Central Statistical Office, Ministry of Health, and ICF International.
42. McDermott J, Steketee R, Larsen S, Wirima J, 1993. Syphilis-associated perinatal and infant mortality in rural Malawi. *Bull World Health Organ 71:* 773–780.
43. Watson-Jones DCJ, Balthazar G, Weiss H, Rusizoka M, Ndeki L, Whitehouse A, Balira R, Todd J, Ngeleja D, Ross D, Buvé A, Hayes R, Mabey D, 2002. Syphilis in pregnancy in Tanzania. I. Impact of maternal syphilis on outcome of pregnancy. *J Infect Dis 186:* 940–947.
44. Mabey DC, Sollis KA, Kelly HA, Benzaken AS, Bitarakwate E, Changalucha J, Chen XS, Yin YP, Garcia PJ, Strasser S, Chintu N, Pang T, Terris-Prestholt F, Sweeney S, Peeling RW, 2012. Point-of-care tests to strengthen health systems and save newborn lives: the case of syphilis. *PLoS Med 9:* e1001233.
45. Rowland M, Bouma M, Ducornez D, Durrani N, Rozendaal J, Schapira A, Sondorp E, 1996. Pyrethroid-impregnated bed nets for personal protection against malaria for Afghan refugees. *Trans R Soc Trop Med Hyg 90:* 357–361.
46. Lindblade KA, Mwandama D, Mzilahowa T, Steinhardt L, Gimnig J, Shah M, Bauleni A, Wong J, Wiegand R, Howell P, Zoya J, Chiphwanya J, Mathanga DP, 2015. A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi. *Malar J 14:* 31.
47. Hokororo A, Kihunrwa A, Hoekstra P, Kalluvya SE, Changalucha JM, Fitzgerald DW, Downs JA, 2015. High prevalence of sexually transmitted infections in pregnant adolescent girls in Tanzania: a multi-community cross-sectional study. *Sex Transm Infect 91:* 473–478.
48. Walsh MS, Hope E, Isaia L, Righarts A, Niupulusu T, Temese SV, Iosefa-Siitia L, Auvaa L, Tapelu SA, Motu MF, Edwards C, Wernick M, Huston WM, Suaalii-Sauni T, Hill PC, 2015. Prevalence of *Chlamydia trachomatis* infection in Samoan women aged 18 to 29 and assessment of possible risk factors: a community-based study. *Trans R Soc Trop Med Hyg 109:* 245–251.
49. Wangnapi RA, Soso S, Unger HW, Sawera C, Ome M, Umbers AJ, Ndrewei N, Siba P, Li Wai Suen CS, Vallely A, Wapling J, Ryan C, Mueller I, Rogerson SJ, 2015. Prevalence and risk factors for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* infection in pregnant women in Papua New Guinea. *Sex Transm Infect 91:* 194–200.
50. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, Rogerson SJ, Steketee RW, 2004. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. *Am J Trop Med Hyg 71:* 41–54.
51. Steketee RW, Wirima JJ, Bloland PB, Chilima B, Mermin JH, Chitsulo L, Breman JG, 1996. Impairment of a pregnant woman's acquired ability to limit *Plasmodium falciparum* by infection with human immunodeficiency virus type-1. *Am J Trop Med Hyg 55:* 42–49.
52. Wasserheit JN, 1992. Epidemiological synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. *Sex Transm Dis 19:* 61–77.
53. Kapiga SH, Shao JF, Lwihula GK, Hunter DJ, 1994. Risk factors for HIV infection among women in Dar-es-Salaam, Tanzania. *J Acquir Immune Defic Syndr 7:* 301–309.
54. Chico RM, Hack BB, Newport MJ, Ngulube E, Chandramohan D, 2013. On the pathway to better birth outcomes? A systematic review of azithromycin and curable sexually transmitted infections. *Expert Rev Anti Infect Ther 11:* 1303–1332.
55. Chico RM, Pittrof R, Greenwood B, Chandramohan D, 2008. Azithromycin-chloroquine and the intermittent preventive treatment of malaria in pregnancy. *Malar J 7:* 255.
56. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A, Williamson J, ter Kuile FO, 2015. 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 386:* 2507–2519.
57. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P, Clark TD, Feeney ME, Charlebois ED, Rizzuto G, Muehlenbachs A, Havlir DV, Kamya MR, Dorsey G, 2016. Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy. *N Engl J Med 374:* 928–939.
58. Gutman J, Mwandama D, Wiegand RE, Abdallah J, Iriemenam NC, Shi YP, Mathanga DP, Skarbinski J, 2015. In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitaemia in pregnant women in Machinga District, Malawi. *Malar J 14:* 197.
59. Kayentao K, Kodio M, Newman RD, Maiga H, Doumtabe D, Ongoiba A, Coulibaly D, Keita AS, Maiga B, Mungai M, Parise ME, Doumbo O, 2005. Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali. *J Infect Dis 191:* 109–116.
60. Parise ME, Ayisi JG, Nahlen BL, Schultz LJ, Roberts JM, Misore A, Muga R, Oloo AJ, Steketee RW, 1998. Efficacy of sulfadoxine-pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection. *Am J Trop Med Hyg 59:* 813–822.
61. ter Kuile FO, van Eijk AM, Filler SJ, 2007. Effect of sulfadoxine-pyrimethamine resistance on the efficacy of intermittent preventive therapy for malaria control during pregnancy: a systematic review. *JAMA 297:* 2603–2616.
62. Mwewa D, Michelo C, 2010. Factors associated with home deliveries in a low income rural setting-observations from Nchelenge district, Zambia. *Med J Zambia 37:* 234–239.