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

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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 Nehelelenghe 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 infected 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,2 preterm delivery,3 stillbirth,4 and low birth weight.5 Syphilis,6 Chlamydia,7–9 gonorrhea,8,9,11 trichomoniasis,12,13 and bacterial vaginosis14–16 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 parasites20 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
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. 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.

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 sites. For the diagnosis of vaginal and cervical infections is highly sensitive and consistent in the detection of *P. falciparum* parasitemia because the technique is highly sensitive and consistent in the detection of parasites. At 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. 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.

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, 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. 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, then cleaned, processed, and analyzed using Stata Software version 13. 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. 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, or 4) had no infection. The relationship between coinfection and HIV status was examined. A $\chi^2$ 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 infections. 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

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**FIGURE 1. Participation flowchart.**
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 coinfecion 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 coinfecion (syphilis only). That was in Tanzania where 48.3% of RPR-positive women also had placental malarial infection. In contrast, we found the prevalence of malarial and syphils coinfection to be 10.5% based on RPR testing and peripheral parasitemia diagnosed by PCR among pregnant women at ANC booking. Malarial and syphils coinfection based on RPR and TPHA results in this study was 4.0%. The difference in the prevalence of malarial and syphils coinfection (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 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 coinfecion (syphilis only). That was in Tanzania where 48.3% of RPR-positive women also had placental malarial infection. In contrast, we found the prevalence of malarial and syphils coinfection to be 10.5% based on RPR testing and peripheral parasitemia diagnosed by PCR among pregnant women at ANC booking. Malarial and syphils coinfection based on RPR and TPHA results in this study was 4.0%. The difference in the prevalence of malarial and syphils coinfection (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.

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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 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 malaria and STI/RTI coinfection among pregnant women in Zambia. 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, parity, and age have been known to be associated with malarial infection. Parity, the number of sexual partners, and early sexual debut 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

<table>
<thead>
<tr>
<th>Potential risk factor</th>
<th>Category</th>
<th>% (%)</th>
<th>Adjusted OR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>≤ 20</td>
<td>295 (27.5)</td>
<td>1.00</td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>21–25</td>
<td>304 (28.3)</td>
<td>0.95 (0.52–1.72)</td>
<td>0.681</td>
</tr>
<tr>
<td></td>
<td>26–30</td>
<td>244 (22.7)</td>
<td>0.83 (0.43–1.61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 30</td>
<td>231 (21.5)</td>
<td>0.58 (0.30–1.14)</td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td>Primigravidae</td>
<td>258 (24.1)</td>
<td>1.00</td>
<td>0.937</td>
</tr>
<tr>
<td></td>
<td>Secundigravidae</td>
<td>165 (15.4)</td>
<td>0.96 (0.52–1.88)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multigravidae</td>
<td>648 (60.5)</td>
<td>0.77 (0.41–1.46)</td>
<td></td>
</tr>
<tr>
<td>Bed-net ownership</td>
<td>No</td>
<td>754 (68.5)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>337 (31.5)</td>
<td>0.83 (0.47–1.47)</td>
<td>0.836</td>
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<tr>
<td>Bed-net use on previous night</td>
<td>No</td>
<td>658 (61.6)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>410 (38.4)</td>
<td>0.98 (0.56–1.74)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV status</td>
<td>Negative</td>
<td>933 (87.1)</td>
<td>1.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>138 (12.9)</td>
<td>3.93 (1.87–8.27)</td>
<td></td>
</tr>
</tbody>
</table>

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 percentages and analysis.
HIV-infected women were at a higher risk of malarial infection and STIs/RTIs. However, as previously reported, 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 and curable STIs/RTIs. 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.

Azithromycin is efficacious against *Plasmodium vivax*, but needs a potent antimalarial partner drug to clear *P. falciparum*. Dihydroartemisinin–piperaquine has been shown to be an efficacious antimalarial and potential replacement for SP as IPTp. 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. 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.

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.

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.

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### 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) | 0.42
|                                           | Coinfection‡ | 266 (37.5) | 1.31 (0.82–2.09) | 0.22
|                                           | Missing§ | 8 | | |
| STI/RTI                                   | Negative | 259 (36.1) | 1.00 | 0.663
|                                           | Positive | 458 (63.9) | 1.07 (0.78–1.48) | 0.64
| Peripheral malarial infection             | Negative | 301 (42.4) | 1.00 | 0.181
|                                           | Positive | 409 (57.6) | 1.24 (0.90–1.69) | 0.21
|                                           | 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.

§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.
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