Enesia Banda Chaponda

The epidemiology of malaria, curable sexually transmitted and reproductive tract infections and their coinfection among pregnant women in a catchment area in Nchelenge District, Zambia

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Declaration by Candidate

I Enesia Banda Chaponda confirm that the work presented in this thesis is my own. Where information has been derived from other sources I confirm that this has been indicated in the thesis.

Signed:---------- Date: ----------

Full Name: Enesia Banda Chaponda

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Scope of work conducted by the PhD candidate

This thesis is the work of the principle investigator, Enesia Banda Chaponda. This investigator designed the study with the help of the main and the co-supervisors. All the standard operation procedure, the questionnaire and all study forms were developed by this investigator. The translation of the questionnaire and consent forms from English to Bemba and back translation was done by personnel from the Zambia National Broadcasting Network with input from the principle investigator who ensured that what was communicated in the local language, Bemba, was what was intended. This investigator applied for ethical clearance from the London school of Hygiene and Tropical Medicine Observational/Interventions Research Ethics committee and the University of Zambia Biomedical Research Ethics Committee.

After obtaining ethical clearance this investigator also sought and obtained written permission to conduct the study from the Zambia Ministry of Health. Upon being granted permission by the ministry of health this investigator wrote letters (and personally delivered them) to the district health office, the office of the district commissioner, the administrators of St. Paul's Mission Hospital (only hospital in Nchelenge District) and administrators of the two health centres (recruitment sites for the study) to inform them about the study before commencement. This investigator paid a courtesy call on the chief in the area and organised and held pre-study meetings with community leaders (village headmen). With the help of members of the Tropical Disease Research centre study team members who were conducting research in the area, this investigator recruited her study team members, which included 4 full-time team members and those who were already working in the health system. Those who were already in the system were recruited to help out with the project activities during routine duty. These included midwives, general nurses, traditional birth attendants/counsellors, counsellors who were involved in the routine running of the ANC clinic in the two the health centres, a radiographer and laboratory technicians.

This investigator also trained team members on the general aspects of the project and specifics of their role in the project. This investigator managed the day to day running of the project, coordinated study team members and conducted weekly

feedback meetings with team members. This investigator was also involved in some of study activities including screening eligibility of participants, obtaining written consent and administration of questionnaires while coordinating the rest of the activities during the enrolment and sample collection process.

All the molecular work was conducted by this investigator. Molecular work included deoxy-ribonucleic acid extraction from dried blood samples and cervico-vaginal swabs for diagnosis of malaria and three sexually transmitted infections and diagnoses of malaria, Chlamydia, gonorrhoea and trichomoniasis infection by standard polymerase chain reaction. Over 75% of slides for diagnosis of bacterial vaginosis were stained by the principle investigator each week during the recruitment period. At the beginning of the project, slide staining for diagnosis of bacterial vaginosis was carried out by a technician who later left for school. Reading of slides for diagnosis of bacterial vaginosis was done by this investigator at the University of Zambia at intervals. Data was mainly entered by a data clerk; this investigator coordinated data entry, occasionally entered data and conducted regular data entry checks. Data cleaning, development of the analysis plan, data processing, data analysis and writing up of the thesis were all done by this investigator.

Activities coordinated by the candidate but conducted by hired individuals

Some study activities were conducted by individuals who were part of the team or those hired for a specific function. These included preparation of blood slides for diagnosis of malaria and slides reading; processing of placental tissue, slide preparation and reading of slides for diagnosis of placental malaria; rapid plasma reagin (RPR) and Treponema pallidum haemagglutination (TPHA) assay testing and gestational age measurement by ultra-sound. All of these functions were conducted by individuals with training and experience in performing these procedures.

Abstract

Introduction

Malaria and curable sexually transmitted and reproductive tract infections (STIs/RTIs) are important causes of adverse birth outcomes (ABO) and are both prevalent in most parts of sub-Saharan Africa. From a public health perspective, control of these infections requires interventions that are part of an integrated antenatal care package. The extent to which there may be coinfection increases the importance of such an integrated approach to reduce ABO.

A systematic review and meta-analysis published in 2012 showed that the prevalence of malaria and curable STIs/RTIs among antenatal attendees in sub-Saharan Africa is considerable. However, the prevalence of malaria and curable STI/RTI coinfection has not been reported in any epidemiological setting.

The primary objective of this thesis is to address this knowledge gap by estimating the prevalence of malaria, curable STIs/RTIs and their coinfection and to highlight the importance of an integrated approach to control malaria and STIs/RTIs in pregnancy. Secondary objectives of the study were to:

(1) determine risk factors for malaria, curable STIs/RTIs and their coinfection;

(2) estimate the prevalence of ABO and identify risk factors for ABO;

(3) measure the *in vivo* efficacy and the prophylactic effect of sulphadoxine-pyrimethamine (SP) in pregnant women, and

(4) characterise the molecular markers associated with parasite resistance to SP among pregnant women.

Methods

A prospective cohort study of 1,086 antenatal attendees was conducted in Nchelenge District, Zambia. Consenting women visiting two health centres for their first antenatal care (ANC) visit were screened for malaria and curable STIs/RTIs (Chlamydia, gonorrhoea, trichomoniasis, bacterial vaginosis [BV] and syphilis). Socio-demographic data and maternal characteristics were also collected at enrolment. Sulphadoxine-pyrimethamine was administrated as intermittent preventive treatment to eligible women and they were followed up at day 28 for a second

malaria screening to determine the therapeutic and prophylactic failure of SP. At delivery participants were screened for placental malaria and data on birth outcomes were recorded. Univariate and multivariate analyses were conducted to determine the association between the potential risk factors for infection and ABO.

Results

Of the 1086 women recruited 729 were successfully followed to delivery**.** The prevalence of malaria infection measured by microscopy was 31.8% (95% CI, 29.1-34.6) and by PCR was 57.8% (95% CI, 54.9-60.8). The risk of malaria infection was higher among pregnant women recruited from Nchelenge health centre compared to those attending the Kashikishi health centre (adjusted odds ratio [aOR] = 1.81; 95% CI, 1.38-2.37, *P* < 0.001), and HIV-infected women across health centres had a greater risk of malaria infection compared to HIV-uninfected women (aOR = 1.46; 95%, 1.00-2.13, *P* = 0.045).

Infection with at least one STI/RTI was observed in 64.8% (95% CI, 61-67.4) of the participating women. With the exclusion of BV the prevalence of infection with at least one curable STI was 34.5% (95% CI, 31.7-37.4). Infection with at least one STI was associated with BV. In comparison to uninfected women, women infected with BV were at a higher risk of being infected with at least one STI (aOR 1.44; 95% CI, 1.08-1.92, *P* = 0.012). HIV-infected women had a higher risk of infection with BV than HIV-uninfected women (aOR 1.87; 95% CI, 1.24-2.83, *P* = 0.003) and women infected with at least one STI had a higher risk of BV (aOR 1.40; 95% CI (1.07 -1.84, *P* = 0.01).

Among participants with complete results (n=1071), 38.7% (95% 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. The risk of malaria and curable STI/RTI coinfection was higher among HIV infected women than HIV-uninfected women (OR; 3.59 [95% CI, 1.73-7.48], *P* < 0.001).

The prevalence of composite ABO was 35.1%. Women shorter than 1.5m were at a higher risk of experiencing at least one ABO (aOR 1.55; 95% CI, 1.10-2.18, *P* = 0.02). The risk of having ABO among para II was less than half of the risk observed in

primiparous women (aOR 0.41; 95% CI, 0.27-0.61, *P* < 0.001) and much lower among multiparous women (aOR 0.32; 95% CI, 0.22-0.48, *P* < 0.001). Having taken two or more doses of SP during pregnancy was protective against ABO (aOR 0.47; 95% CI, 0.31-0.72, *P* = 0.001). None of the infections (malaria, curable STIs/RTIs and their coinfection) diagnosed at first ANC were associated with ABO.

The prevalence of highly resistant quintuple mutant was 68.8% among first ANC attendees. Despite the moderate prevalence of the quintuple mutant among pregnant women, SP cleared parasitaemia in 86% of the asymptomatic malaria cases among HIV-negative women

Conclusion

The prevalence of malaria, STI/RTI and their coinfection at first ANC in this study population was considerable. However, no association was found between ABO and infection with malaria or STI/RTI or their coinfection. This lack of association is partially a result of interventions within the ANC package including treatment of some STI/RTI, intermittent preventive treatment in pregnancy with SP and iron and folic acid supplementation. Sulphadoxine-pyrimethamine retains partial efficacy against *P. falciparum* malaria in this area with moderate prevalence of the quintuple mutant. While continuing the policy of offering intermittent preventive treatment with SP during pregnancy, an alternative preventive therapy that is effective against both malaria and STIs/RTIs needs to be considered.

Abbreviations

ABO	Adverse birth outcome
ANC	Antenatal clinic
BV	Bacterial vaginosis
CI	Confidence interval
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HPTN	HIV Prevention Trials Network
ID	Identification (number)
ITN	Insecticide treated net
IUGR	Intra-uterine growth retardation
IPTp-SP	Intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine
LBW	Low birth weight
MSP	Merozoite surface protein
OR	Odds ratio
PD	Preterm delivery
PCR	Polymerase chain reaction
RPR	Rapid plasma reagin
RR	Risk ratio
RTI	Reproductive tract infection
SP	Sulphadoxine-pyrimethamine
STI	Sexually transmitted infection
STI/RTI	Sexually transmitted and reproductive tract infection
TPHA	*Triponema pallidum* Haemagglutination assay
ZDHS	Zambia Demographic and Health Survey
ZEPRS	Zambia Electronic Perinatal Record System

Acknowledgement

I would like to thank my supervisor Professor Daniel Chandramohan for the challenges and sound advice throughout the programme. Thank you for being there whenever I needed direction. I thank the co-supervisors, Matthew Chico, Professor Charles Michelo and Dr. Bellington Vwalika. You made my journey interesting and much more bearable. I would like to thank Professor Philippe Mayaud for being part of my advisory panel. I would like to express my gratitude to Jane Bruce for offering guidance with the data analysis. Many thanks to the International Centres of Excellence for Malaria Research (ICEMR) in Southern Africa, and the US National Institutes of Health for the financial support 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). I would also like to thank my ICEMR mentor Dr. Sungano Mharakurwa for laboratory technical support. I am grateful for the financial support I received from the Research Support Centre 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 UK. Many thanks go to the administrators of the National Malaria Control Centre for the provision of some reagents and consumables.

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Many thanks to my dear friend, Kangwa Chintu, for her prayers and constant encouragement. Many thanks to Anita Ngulube for taking excellent care of my children, Mphatso Ngulube and Israel Munashe Ngulube, in my absence. To my husband, Joseph, I would like say thank you for taking care of the family during my time away from home and for being a pillar of strength, you are simply the best. And last but not least, I will always be thankful to God Almighty, besides whom there is no other, for His provision, for connecting me to many wonderful people and seeing me through the challenges that I have faced in my life and education.

Summary of content

This thesis aims to address a knowledge gap by measuring malaria and STIs/RTIs and their coinfection. Many studies have reported the prevalence of malaria and that of STI/RTI in pregnant women but the prevalence of their coinfection has not been estimated in an epidemiological setting. Secondary objectives were to: identify risk factors for malaria, curable STIs/RTIs and their coinfection; estimate the prevalence of ABO and identify risk factors; characterise the mutations that are associated with parasite resistance to SP and assess the *in vivo* efficacy, prophylactic and therapeutic failure of SP among pregnant women.

The thesis is organised as follows:

- Chapter 1 gives the background to the study by highlighting the burden of malaria, curable STIs/RTIs in pregnant women and their effect on birth outcome and the antenatal care package for their management. The background also highlights the gap in knowledge on malaria and curable STI/RTI coinfection and points out the weaknesses of the intermittent preventive treatment in pregnancy with SP (IPTp-SP) strategy for the alleviation of the consequences of malaria infection in pregnancy in the face of emerging parasite resistance to SP. The background also points out the inaccuracy of the syndromic approach for the management of STIs/RTIs. The chapter also gives the rationale for the study and the objectives.
- Chapter 2 describes the methods employed including the study design, a description of the study area, identification and recruitment of participant; and tools used in the collection of data and samples, detection of infections, data processing and analyses.

- Chapter 3 describes the characteristics of study participants according to recruitment sites and the prevalence of malaria, curable STIs/RTIs and their coinfection among participants.
- Chapter 4 describes the risk factors for malaria, curable STIs/RTIs and their coinfection in the study population.
- Chapter 5 describes results on the estimates of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) mutations associated with *P. falciparum* resistance to SP and gives estimates of the *in vivo* efficacy and the therapeutic and prophylactic failure of IPTp-SP. The idea behind this chapter is to give details of interest that is give an estimation of the level of SP resistance and the efficacy of SP in the study population.
- Chapter 6 gives estimates of individual and composite adverse birth outcome and describes risk factors for ABO.
- Chapter 7 discusses all the results and gives the limitations of the study, conclusions, policy recommendations and reflexivity.

Note

1. The data on malaria prevalence and risk factors for infection have been published in the *Malaria Journal (2015)* 14:380 DOI 10.1186/s12936-015-0866-1

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.

2. The data on malaria and STI/RTI coinfection has been published in the American Journal of Tropical Medicine and Hygiene.

Chaponda EB, Chico RM, Bruce J, Michelo C, Vwalika B, Mharakurwa S, et al. Malarial Infection and Curable Sexually Transmitted and Reproductive Tract Infections Among Pregnant Women in a Rural District of Zambia. Am J Trop Med Hyg. 2016;95(5):1069-76.

1 Introduction

1.1 Background

Maternal malaria and curable STIs/RTIs remain important causes of adverse birth outcomes (ABO). One of the interventions recommended by the World Health Organisation (WHO) for reducing the burden of malaria in pregnancy is intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP). However, the effectiveness of IPTp-SP has been undermined by the emergence of SP resistant parasites [1, 2]. Results from a systematic review and meta-analysis of trials showed that administration of three doses of IPTp-SP appear to be beneficial even in areas with high SP resistance. However, this does not imply that it may be so in areas with very high SP resistance [3]. Nevertheless, there is urgent need to identify alternative preventive therapy for SP.

Testing women for curable STIs/RTIs during ANC visits and providing appropriate care has been a public health challenge for decades in resource-poor settings. The WHO has developed syndrome-based algorithms for the detection of curable STIs/RTIs to assist countries with limited resources [4]. However, syndrome-based algorithm for vaginal discharge has poor sensitivity in detecting cervical infections [5]. Therefore, a considerable burden of STIs/RTIs in pregnancy remains undetected and untreated.

A systematic review and meta-analysis of studies reporting malaria or curable STIs/RTIs in pregnant women attending antenatal clinics in sub-Saharan Africa published in 2012 showed that the prevalence of malaria and curable STIs/RTIs during pregnancy is considerable [6]. The review also highlighted the paucity of data on coinfection of malaria and curable STIs/RTIs during pregnancy. In view of the challenges in the current approach, one of the possible ways forward is to find an initiative that integrates the prevention and control of malaria and STI/RTI in pregnancy. However, initiative for an integrated solution is hampered by a lack of information about the burden of their coinfection. This thesis aims to address this gap in knowledge and to highlight the importance of an integrated approach to control malaria and STIs/RTIs in pregnancy.

Malaria and curable STIs/RTIs are both prevalent in most parts of sub-Saharan Africa. The overlapping prevalence of malaria and curable STIs/RTIs has important public health implications especially in pregnant women.

Despite the fact that malaria and curable STIs/RTIs are important risk factors for ABO, no study identified in the systematic review by Chico *et al.* had reported the prevalence of malaria and STIs/RTIs coinfection, with the exception of one study which reported that 48.3% of women who tested positive to rapid plasma reagin (RPR) had placental malaria [6].

The primary objective of the current study was to estimate the prevalence of malaria, STIs/RTIs, and their coinfection among pregnant women in an area with overlapping prevalence of malaria and STIs/RTIs. Secondary objectives were to:

1. determine risk factors for malaria, curable STIs/RTIs and their coinfection;
2. estimate the prevalence of ABO and identify risk factors for ABO;
3. measure the *in vivo* efficacy and the prophylactic effect of sulphadoxine-pyrimethamine (SP) in pregnant women, and
4. characterise the molecular markers associated with parasite resistance to SP among pregnant women.

1.2 Risks associated with malaria and curable STIs/RTIs in pregnancy

1.2.1 Effects of curable STIs/RTIs on pregnancy outcomes

Curable STIs/RTIs are a major public health problem, especially in developing countries [7, 8]. In 2004 Mullick and colleagues reviewed the prevalence of STIs/RTIs and the relationship between these infections and pregnancy outcomes [7]. The paper reviewed evidence from both developed and developing countries. Although there were few studies from developing countries at the time of the review, the authors concluded that developing countries have a high prevalence of STIs/RTIs which have a significant impact on birth outcomes.

1.2.1.1 Syphilis

Mullick *et al.* identified two studies that looked at the impact of syphilis in pregnancy at a population level in Malawi and Tanzania [9, 10]. In Malawi women with active

syphilis were 11 times more likely to experience a stillbirth (adjusted odds ratio [aOR] 10.89; 95% CI, 6.61-17.93) and 26% of stillbirths were attributed to active syphilis [9]. In Tanzania, 17% of all adverse pregnancy outcomes were attributed to syphilis. Syphilis was associated with increased risk of stillbirth (adjusted risk ratio [aRR] 18.1; 95% CI, 5.5-59.60), low birth weight (LBW) in live born infants (aRR 3.3; 95% CI, 2.0-5.4), preterm delivery (PD) (aRR 6.1; 95% CI, 2.5-15.3) and intra-uterine growth retardation (IUGR) (aRR 2.1; 95% CI, 1.0-4.2) [10].

1.2.1.2 *Chlamydia trachomatis*

Two prospective studies from the United States (US) included in the same review showed that *Chlamydia trachomatis* has significant impact on pregnancy outcomes. In the first study researchers found that *C. trachomatis* infection was associated with both IUGR (aOR 2.4; 95% CI, 1.3-4.2) and PD (aOR 1.6; 95% CI, 1.0-4.2) [11]. Authors of the second study found that infection with *C. trachomatis* was associated with LBW (aOR 2.7; 95% CI, 1.3 to 5.7), premature rupture of membranes (PROM) (OR 2.4; 95% CI 1.7 to 5.4) and < 34 weeks preterm labour (aOR 4.0; 95% CI, 1.7-9.2) [12]. However, a third study (also done in the US) showed no such association between infection with *C. trachomatis* and ABO [13]. This lack of association could be explained by the fact that some of the infected women received prenatal antibiotic treatment. It is highly unlikely that Chlamydial-infected women in the first study mentioned above received treatment as this is not mentioned or referred to at all [11]. In the second study mentioned above that associated infection with *C. trachomatis* with LBW, PROM and preterm labour, women received treatment for gonococcal infections but Chlamydial infections were not treated until after the onset of labour or rupture of membranes [12].

In two studies from Kenya, infection with C. *trachomatis* was associated with postpartum endometritis [14] and 31% of 181 cases of neonatal conjunctivitis were found to have been caused by *C. trachomatis* [15].

1.2.1.3 *Neisseria gonorrhoea*

Three studies on the impact of *N. gonorrhoea* infection on pregnancy outcome were noted in the review by Mullick *et al*. 2005. One was based on case reports in the United Kingdom and documented sequelae of untreated gonorrhoea in pregnancy

which included PD, PROM, LBW, postpartum endometritis and gonococcal ophthalmia neonatorum [16]. A case control study of women at delivery in Kenya found an association between *N. gonorrhoeae* infection and LBW (aOR 2.9; 95% CI 1.2 - 7.2) and it was concluded that *N. gonorrhoeae* was responsible for 14% of LBW cases in the study population [17]. In South Africa a prospective study of 167 women looked at gonorrhoea diagnosed at first ANC visit and pregnancy outcome [18]. It was found that women with gonorrhoea delivered significantly smaller babies (mean weight 2252g versus 2970, $P < 0.005$).

1.2.1.4 *Trichomonas vaginalis*

Among the four studies of *Trichomonas vaginalis* in pregnancy included in this review, only one study was done in Africa [19]. The studies associated infection with *T. vaginalis* in pregnancy with PD and LBW [19-22]. In the Democratic Republic of Congo, trichomoniasis infection in pregnancy was associated with LBW in infants born to infected mothers (aOR 2.4; 95% CI, 1.2- 4.5) [19]. In the three studies in the US, one found that infection with *T. vaginalis* was associated with LBW among adolescents [22]; in the second study, women infected with *T. vaginalis* were significantly more likely to have PROM ($P < 0.03$) [21]. Trichomoniasis was associated with LBW (aOR 1.3; 95% CI, 1.1 to 1.5), PD (aOR 1.3; 95% CI, 1.1 -1.4) and PD of a LBW infant (aOR 1.4; 95% CI, 1.1 to 1.6) in the third study [20].

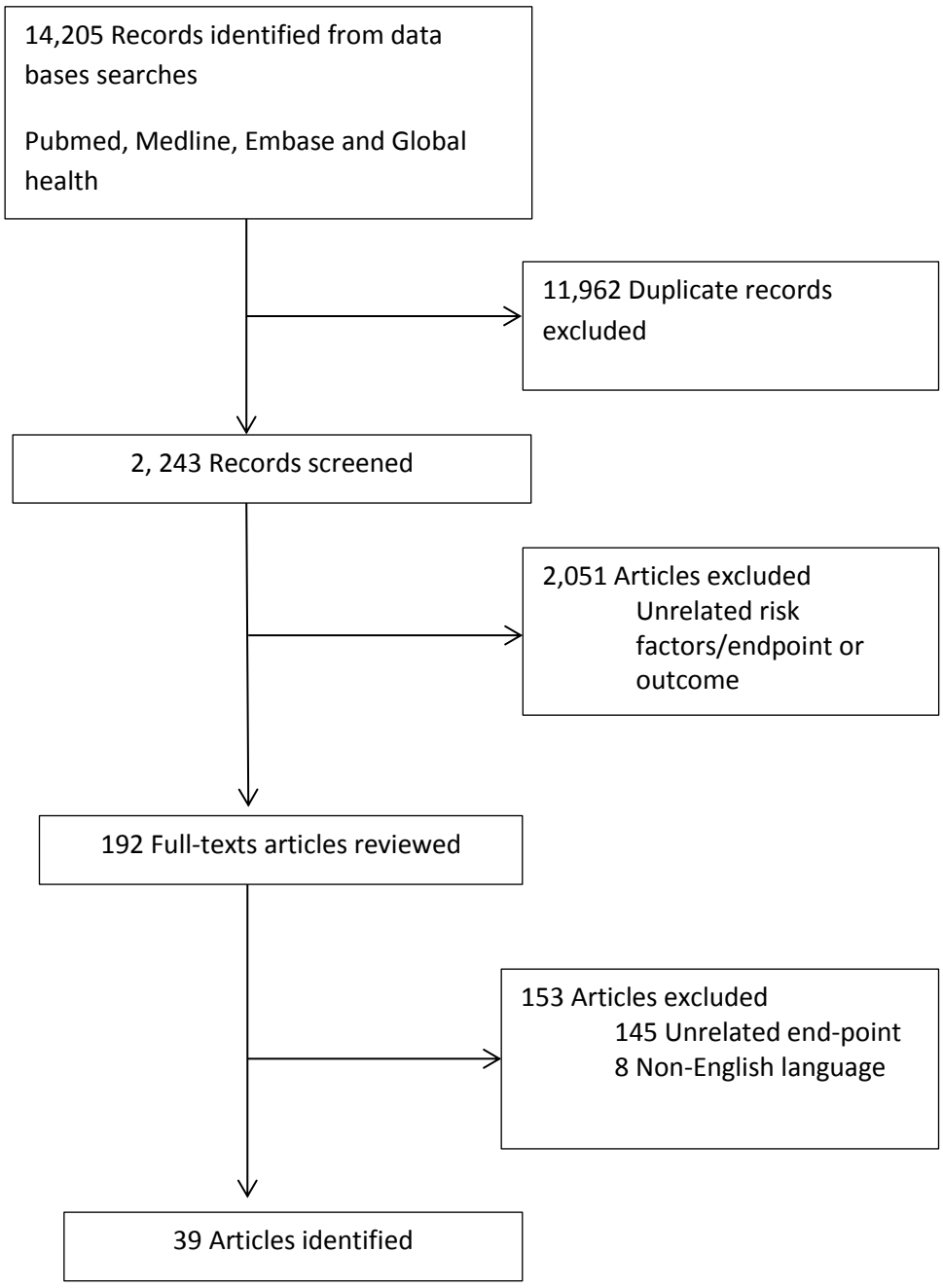
1.2.1.5 Bacterial vaginosis

Studies on the effect of bacterial vaginosis (BV) on birth outcomes identified in the review by Mullick *et al.* included a prospective, controlled treatment trial in the US. Bacterial vaginosis was associated with increased risk of both PD (aRR 1.9; 95% CI, 1.2-3.0) and PROM (aRR 3.5; 95% CI, 1.4-8.9) in women in the observation phase of the study [23]. A cohort study in the US found an association between BV and PD of LBW infants (aOR 1.4; 95 % CI, 1.1- 1.8) [24] and a prospective study in Indonesia associated BV diagnosed early, between 16 and 20 weeks gestation, with PD (OR 2.0; 95% CI, 1.0-3.9) but not with BV diagnosed at 28 to 32 weeks gestation (aOR 1.5, 95% CI, 0.7-3.0) [25].

1.2.2 Additional evidence for association of STIs/RTIs with ABO

To explore additional evidence for the effects of STIs/RTIs on pregnancy outcome, a literature search was undertaken. The last search was conducted in February 2016. The Pubmed, Medline, Embase and Global Health databases were searched using combinations of the following key words: "Pregnancy", "antenatal/prenatal", "sexually transmitted disease", "syphilis/*Treponema pallidum"*, "Chlamydia/*Chlamydia trachomatis"*, "Gonorrhoea/*Neisseria gonorrhoeae*/gonorrhoea", "trichomoniasis/*Trichomonas vaginalis"*, "bacterial vaginosis" and "birth/pregnancy outcome". The words "Malaria/*Plasmodium falciparum*" were also used to identify and include studies reporting the effect of malaria on pregnancy outcome in the search. The Medline, Embase and Global Health databases were searched via Ovid®. Appendix 1.1a shows search terms that were used for studies reporting infection with malaria or STI/RTI in pregnancy and birth outcomes. There was no restriction of the time when the studies were conducted. Titles and abstracts were screened for relevance and full texts were screened for those that were relevant to the subject. Articles with full texts published in non-English language were excluded. Studies that reported investigating the association between malaria or STI/RTI and ABO were identified. Figure 1.1 shows a flow chart of the literature search and identification of articles.

Figure 1. 1: Identification and screening of studies



Overall, 32 studies associating STIs/RTIs with ABO (including those that were identified in the review by Mullick *et al*.) and seven studies on malaria and ABO were identified. Below are brief details of 16 of the 32 studies on STIs/RTIs and ABO that were published after the review by Mullick *et al*., 2005.

1.2.2.1 Syphilis

In a multiple centre record based retrospective study of 368,151 births in Latin America and the Caribbean, syphilis was associated with stillbirth (aOR 1.88; 95% CI, 1.25-2.83) [26].

A review and meta-analysis of studies reporting estimates of ABO among untreated 1,715 women with syphilis and 22,515 women without syphilis, pooled estimates showed that among untreated pregnant women with syphilis, fetal loss and stillbirth were 21% more frequent, neonatal deaths were 9.3% more frequent and PD or LBW were 5.8% more frequent than among women without syphilis [27].

1.2.2.2 *Chlamydia trachomatis*

In a study of 343 pregnant women in South Africa, 36 (10.5%) delivered before 37 weeks gestation and *C. trachomatis* was found in 8 (22.2%) of women who had PD in contrast to 32 (10.4%) of women who had full-term deliveries (*P* = 0.037) [28]. Results from an unmatched case-control study conducted in Uganda showed no association between *C. trachomatis* infection and PROM (aOR; 2.05, 95% CI, 0.37-11.49) [29]. In a study of 3,913 pregnant women in the Netherlands *C. trachomatis* infection was associated with PD occurring before 32 weeks (aOR 4.35; 95% CI, 1.3 - 15.2) and 35 weeks gestation (aOR: 2.66; 95% CI, 1.1- 6.5), but not before 37 weeks (aOR 1.17; 95%CI, 0.6-2.4) and not with LBW (*P* = 0.25) [30]. In a case control study in Switzerland, *C. trachomatis* was associated with PD (aOR; 7.93, 95% CI, 1.34-46.76) [31]. A record-based retrospective cohort study conducted in Australia involving 354,217 participants, showed that women with Chlamydia had increased risk of PD (aOR 1.17, 95% CI, 1.01-1.37) and stillbirth (aOR 1.40, 95% CI, 1.00 -1.96) but no association was found between infection with Chlamydia and small for gestational age (aOR 0.99, 95% CI, 0.89-1.09) [32]. In a retrospective study in the US, electronic medical records from a sexually transmitted disease clinic were matched with state birth records for 730 women [33] Chlamydia was associated with LBW (aOR 2.07;

95% CI: 1.01 - 4.24). A population based retrospective cohort study in the US found that Chlamydial-infected women were at increased risk of PD (aRR 1.4; 95% CI: 1.08 - 1.99) and PROM (aRR 1.5; 95% CI 1.03 -2.17) compared with uninfected women [34]. No increased risk of LBW was observed in this study (aRR 1.12; 0.61-1.68). Another study done in the US found no association between *C. trachomatis* infection and PD (aOR 0.7; 95% CI 0.4–1.4) [35]. This could be attributed to the fact that 66% of the positive diagnoses were done early and treatment was administered before 20weeks gestation.

1.2.2.3 *Neisseria gonorrhoeae*

In a record-based retrospective cohort study in Australia involving 354,217 participants, no association was found between infection with gonorrhoea and stillbirth (aOR 2.35, 95% CI, 0.58-9.56) or gonorrhoea and small for gestational age (aOR 0.98, 95% CI, 0.58-1.68) [32]. Women infected with gonorrhoea had a higher risk of experiencing PD (OR 2.50, 95% CI, 1.39-4.50) [32]. Similarly, gonorrhoea was associated with PD (aOR 2.01; 95% CI 1.02 - 3.97), particularly when diagnosed in the first trimester (aOR 2.95; 95% CI: 1.30 - 6.70) in a study of 730 women in the US [33].

1.2.2.4 *Trichomonas vaginalis*

No addition studies on the effect of *T. vaginalis* on ABO were identified in the literature search. The only studies that were identified were included in the review by Mullick *et al*., 2005 and are already described above.

1.2.2.4 Bacterial vaginosis

In a prospective study of 1,536 Tanzanian women designed to determine risk factors for poor birth outcomes, no association was found between treated STIs/RTIs and ABO except untreated BV which was associated with PD (aOR; 2.9 95% CI, 1.3 - 6.6) [36]. In a prospective cohort study of 3,262 women in Denmark, BV was associated with LBW (aOR 1.95; 95% CI, 1.3-2.9), PD of a LBW infant (aOR 2.5; 95% CI, 1.6-3.9) and clinical chorioamnionitis (aOR 2.4; (95% CI, 1.4-4.1) [37]. In the United Kingdom, women with BV were not at a higher risk of experiencing PD (aOR 0.9, 95% CI, 0.4-2.2) but had increased risk of experiencing spontaneous abortion (aOR 4.0, 95% CI, 1.3-12.1) [38].

In a meta-analysis aimed at evaluating BV as a risk factor for PD, BV was associated with PD (aOR 2.2; 95% CI, 1.5-3.1) and spontaneous abortion (aOR 9.9; 95% CI, 2.0-49.3) [39]. The meta-analysis was updated after about four years and BV was still associated with PD (aOR 2.2; 95% CI, 1.6-3.9) and spontaneous abortion (aOR 6.3; 95% CI, 3.7-10.9) [40]. In Switzerland a study of 1,197 pregnant women found that BV was associated with preterm delivery (aOR 2.19; 95% CI, 1.2-4.0) [41].

1.2.3 Effects of malaria on pregnancy outcomes

Pregnant women in malaria endemic areas are a high risk group for *Plasmodium falciparum* infection and related consequences [42]. Pregnancy results in a transient depression of cell-mediated immunity which improves the likelihood of foetal allograft retention but also interferes with immune responses to various infectious diseases [43]. Red blood cells that have been infected by *P. falciparum* sequester in vascular spaces in the placenta mediated by adhesion to chondroitin sulphate A [44] leading to placental inflammation [45] and complications associated with malaria in pregnancy [46]. Consequences of malaria in pregnancy include maternal anaemia, IUGR [36, 47], PD [36, 48], stillbirth [49, 50] and LBW [36, 42]. Low birth weight is associated with a marked increase in neonatal death [51-54].

Studies that investigated the effect of malaria on pregnancy outcome identified in the literature search mentioned above were all conducted in Africa. In Tanzania a study of the effectiveness of syphilis screening and treatment conducted from 1997 to 2000, women with malaria had an increased risk of experiencing PD (aOR 3.2; 95% CI, 1.9-5.2), LBW (aOR 5.4; 95% CI, 3.1-9.5) and IUGR (aOR 2.8; 95% CI, 1.2-6.7) [36]. In another study designed to identify risk factors for stillbirth conducted in November and December of 2006 in Ghana, women with malaria had increased risk of stillbirth (aOR 1.9; 95% CI, 1.2-9.3) [50]. In a hospital based study also aimed at identifying risk factors for stillbirth, conducted between November 2007 to February 2008 in Sudan, infection with malaria was associated with increased risk of stillbirth (aOR 3.0; 95% CI, 1.0-8.9) [49].

Among 1,766 women in Malawi, placental blood *P. falciparum* infection was independently associated with LBW (aOR: 1.71) [55]. Secondary analysis of data for

2,149 women included in a community based randomised placebo controlled trial for the prevention of preterm birth. Results showed that persistent malaria (despite malaria prophylaxis) increased the risk of late preterm birth (aOR 1.99, 95% CI, 1.05-3.79) [56]. In a case-control study in Sudan conducted in 2010, a combination of histologically determined and sub-microscopic infections was significantly associated with LBW (aOR 2.45; 95% CI, 1.2-4.9) [57]. No association was found between malaria and LBW (aOR 1.0; 95% CI, 0.4-2.5), PD (aOR 1.7; 95% CI, 0.7-3.9), stillbirth (aOR 1.0; 95% CI, 0.3-3.0) and any ABO (aOR 1.3; 95% CI, 0.66-2.38) in a study in Sudan [58] . This lack of association was attributed to high IPTp-SP uptake in the study population.

Table 1-1 shows a summary of studies associating curable STIs/RTIs and malaria with ABO.

Table1- 1: Summary of studies associating curable STIs/RTIs and malaria with adverse pregnancy outcomes

Organism/ infection	LBW	IUGR	PD	Stillbirth	PROM	Spontaneous abortion	Country	year	Author, publication Year
Plasmodium falciparum	**5.4(3.1-9.5)**	**2.83 (1.2-6.7)**	**3.2 (1.9-5.2)**	NA	NR	NR	Tanzania	1997-2000	Watson-Jones *et al*., 2007[36]
	NR	NR	NR	**3.0 (1.0-8.9)**	NR	NR	Sudan	2007-2008	Bader *et al*., 2010 [49]
	NR	NR	NR	**1.9 (1.2-9.3)**	NR	NR	Ghana	2006	Yatichi *et al*., 2010 [50]
	1.71, P < 0.05	NR	NR	NR	NR	NR	Malawi	1987-1990	Steketee *et al*., 1996 [55]
	2.45 (1.2-4.9)	NR	NR	NR	NR	NR	Sudan	2010	Muhammed et al., 2013 [57]
	NR	NR	**2.0 (1.1-3.8)**	NR	NR	NR	Malawi	2004-2005	Van der Brook *et al*., 2014 [56]
	1.0 (0.4-2.5)	NR	1.7 (0.7-3.9)	1.0 (0.3-3.0)	NR	NR	Ghana	2011	Asundep *et al*., 2014 [58]
Treponema pallidum	NR	NR	NR	NR	NR	42% of cases	Zambia	1982	Ratnam *et al*., NR [59]
	NR	NR	1.4 (0.5-4.1)	NR	NR	NR	Kenya	1985	Elliot *et al*., 1990 [17]
	NR	NR	33%, 5 of 15 cases	NR	NR	NR	South Africa	1988	Donders *et al*., 1993 [18]
	NR	NR	NR	**11.0 (6.6-17.9)**	NR	NR	Malawi	1987-1990	McDermott *et al*., 1993 [9]
	4.01, *P* < 0.001	NR	NR	**3.3[a] , *P* = 0.028**	NR	NR	Kenya	1991	Temmerman *et al*., 1995 [60]
	3.3[a] (2.0-5.4)	**2.1[a] (1.0-4.2)**	**6.1[a] (2.5-15.3)**	**18.1[a] (5.5-59.6)**	NR	NR	Tanzania	1998-2000	Watson-Jones *et al*., 2002 [61]
	NR	NR	NR	**1.9 (1.3-2.8)**	NR	NR	Multiple	2009-2012	Arnesen *et al*., 2015[26]
Neisseria gonorrhoeae	**2.9(1.2-7.2)**	NR	NR	NR	NR	NR	Kenya	1985	Elliot *et al.* , 1990 [17]
	P = 0.005 [‡]	NR	56%, 5 of 9 cases	NR	NR	NR	South Africa	1988	Donders *et al.*, 1993 [18]
	0.8 (0.3-2.3)	NR	2.0 (1.0-4.0)	NR	NR	NR	USA	1996-2002	Johnson *et al*., 2011 [33]
	NR	NR	2.5 (1.4-4.5)	NR	NR	NR	Australia	1999-2008	Liu *et al*., 2013 [32]

Table 1-1: Summary of studies associating curable STIs/RTIs and malaria with adverse pregnancy outcomes continued

Organism/ infection	LBW	IUGR	PD	Stillbirth	PROM	Spontaneous abortion	Country	year	Author, publication Year
Chlamydia trachomatis	**2.7(1.3-5.7)**	NR	NR	NR	**2.4 (1.7-5.4)**	NR	USA	1983	Gravett *et al*., 1986 [12]
	NR	NR	**19.4% versus 8.0%, *P* = 0.03**	NR	**19.4% versus 8.0%, *P* = 0.03**	NR	USA	NR	Sweet *et al*., 1987 [62]
	NR	**2.4 (1.3-4.2)**	NR	NR	NR	NR	USA	1983-1985	IJH, 1989 [11]
	NR	NR	NR	NR	NR	1.8 (0.3-10.7)	USA	1995-1996	Sozio *et al*., 1998 [63]
	15.5 versus 13.2 *P* > 0.05	7.3 versus 5.8% *P* > 0.05	NR	20 versus 21% *P* > 0.05	NR	NR	Hungary	1994-1995	Kovacs *et al*., 1998[64]
	NR	NR	**22.2% 8 of 36 cases versus 10.4% 32 of 307 cases *P* = 0.037**	NR	NR	NR	South Africa	2002-2003	Odendaal *et al*., 2006 [28]
	1.1 (0.7-1.70)[a]	NR	**1.5 (1.1-2.0)** [a]	NR	**1.5 (1.0-2.2)** [a]	NR	USA	2003	Blas *et al*., 2007 [34]
	0.19 (0.1-1.5) 0.3 (0.1-1.2)	0.97 (0.1-7.9) **3.65 (1.1-12.0)**	0.59 (0.2-2.0) 1.15 (0.5-2.6)	NR NR	NR NR	NR NR	Tanzania	1997-2000	Watson-Jones *et al*., 2007 [36]
	NR	NR	0.7 (0.4-1.4)	NR	NR	NR	USA	2005-2008	Silveira *et al*., 2009 [35]
	2.07 (1.01-4.24)	NR	1.0 (0.6-2.0)	NR	NR	NR	USA	1996-2002	Johnson *et al*., 2011 [33]
	NA	NR	**4.4 (1.3-15.2)**[b] **2.7 (1.6-6.5)**[c]	NR	NR	NR	Netherlands	2003-2005	Rours *et al*., 2011 [30]
	NR	NR	**7.9 (1.3-46.9)**	NR	NR	NR	Switzerland	2006-2009	Baud *et al*., 2014 [31]
	NR	NR	NR	NR	2.1 (0.4-11.5)	NR	Uganda	2013	Nakubulwa *et al*., 2015 [29]

Table 1-1: Summary of studies associating curable STIs/RTIs and malaria with adverse pregnancy outcomes continued

Organism/ infection	LBW	IUGR	PD	Stillbirth	PROM	Spontaneous abortion	Country	year	Author, publication Year
Trichomonas vaginalis	NR	NR	NR	NR	P < 0.03	NR	USA	NR	Minkoff *et al*., 1984 [21]
	1.3(1.1-1.5)	NR	**1.3 (1.1-1.4)**	NR	NR	NR	USA	1984-1990	Cotch *et al*., 1997 [20]
	2.1(1.0-4.2.)	NR	NR	NR	NR	NR	Congo DR	1989-1990	Sutton *et al*., 1999 [19]
	1.11 (0.6-1.9) NR	0.61 (0.2-1.5) NR	1.27 (0.8-2.0) 2.38 (0.5-12.1)	**2.32 (1.0-5.7)** 5.57 (0.5-66.1)	NR NR	NR NR	NR NR	1997-2000	Watson-Jones *et al*., 2007 [36] [¥]
	1.5 (0.9-2.6)	NR	1.4 (0.7-2.8)	NR	NR	NR	USA	1996-2002	Johnson *et al*., 2011[33]
Bacterial vaginosis	NA	NR	NR	NR	**2.0 (1.1-3.7)**	NR	USA	1983	Gravett *et al*., 1986 [12]
	NR	NR	**2.0 (1.0-3.9)**[e]	NR	NR	NR	Indonesia		Riduan *et al*., 1993 [25]
	NR	NR	**1.9 (1.2-3.0)** [a] 1.5 (0.7-3.0)[f]	NR	**3.5 (1.4-8.9)** [a]	NR	USA	1991-1992	Mcgregor *et al*., 1995 [23]
	NR	NR	1.4 (0.9-2.05) Week 24, **1.8 (1.2-3.0)** Weeks 28	NR	NR	NR	Multiple	Multiple	Meis *et al*, 1995 [65]
	1.5 (1.2-1.7)	NR	**1.4 (1.1-1.8)**	NR	1.1 (0.8-1.6)	NR	USA	1984-1989	Hillier *et al*., 1995 [24]
	NR	NR	0.9 (0.4-2.2)	NR	NR	**4.0 (1.3-12.1**	UK	1998-2000	Oakeshott *et al*., 2004 [38]
	NR	NR	2.16 (0.9-3.6) [a]	NR	NR	NR	Sweden	1990-1991	Jacobson *et al*., 2002 [66]
	NR	NR	**2.2 (1.5-3.1)**	NR	NR	**9.9 (2.0-49.3)**	Multiple	Multiple	Leitich *et al*., 2003 [39]
	2.0 (1.3-2.9)	NR	**2.5 (1.6-3.9)***	NR	NR	NR	Denmark	1998-2002	Svare *et al*., 2006 [37]
	NR	NR	**2.2 (1.6-3.0)**	NR	NR	**6.3 (3.7-10.9)**	Multiple	Multiple	Leitich *et al*., 2007 [40]
	NR	NR	**2.19 (1.2-4.0)**	NR	NR	NR	Switzerland	NR	Daskalakis *et al*. , 2006 [41]
	1.08 (0.7-1.9) 2.02 (0.7-5.9)	1.09 (0.6-2.2) NR	0.91 (0.6-1.4) **2.95 (1.3-6.6)**	1.79 (0.8-4.2) NR	NR NR	NR NR	Tanzania	1997-2000	Watson-Jones *et al*., 2007 [36] [¥]

Table 1-1: Summary of studies associating curable STIs/RTIs and malaria with adverse pregnancy outcomes continued

IJH: Investigators of John Hopkins;
IUGR: Intrauterine growth retardation
LBW: Low birth weight
NR: Not reported
PROM: Premature rupture of membranes
PD: Preterm delivery
[a] Adjusted risk ratio, the other figures are adjusted odds ratios. Bracketed figures are 95% confidence Intervals for odds ratios.
[b] Preterm delivery before 32 weeks
[c] Preterm delivery before 35 week
[d] preterm delivery before 37 weeks
[e] Bacterial vaginosis diagnosed at 16 to 20 weeks
[f] Bacterial vaginosis diagnosed at 28 to 32 weeks
[g] Among treated women
*preterm delivery of low birthweight infant
[¥] first row represents untreated women and second row represents treated women
[†] 22.2% versus 10.4% prevalence of Chlamydia among women with preterm delivery and full term delivery
[‡] Mean birth weight of preterm babies versus full-term, 2252g and 2970g, *P* = 0.005
Bold type: statistically significant association

1.2.4 Effect of treatment of curable STIs/RTIs in pregnancy

The benefits of STIs/RTIs treatment on pregnancy outcome have been demonstrated in randomised trials. Three studies on the effects of treatment of STIs/RTIs were identified in the review by Mullick and colleagues [67-69]. A randomised trial in the US showed that treatment with erythromycin between 26 and 30 weeks gestation resulted in reduced frequency of PROM (6%) compared to placebo recipients (16%), P < 0.01 [68]. In a double-blind placebo controlled clinical trial, a single dose of ceftriaxone given to pregnant women in Kenya between 28 and 32 weeks led to a significant increase in mean birthweight [3.21kg versus 3.06kg, (P < 0.01)] [69]. A randomised trial of presumptive sexually transmitted therapy in pregnancy in Uganda, showed that treatment with a single dose of metronidazole combined with azithromycin and cefixime reduced rates of neonatal death (rate ratio 0.83; 95% CI, 0.71-0.97), LBW (rate ratio 0.68; 95% CI, 0.53-0.86), and PD (rate ratio 0.77; 95% CI, 0.56-1.05) [67].

Studies focusing on specific infections have shown benefits of treatment of STIs/RTIs in pregnancy. A cohort study in the US found an increased risk in the incidence of PROM in untreated women with positive Chlamydia cultures compared to those with positive cultures who received treatment or those with negative cultures [70]. Another study in the US showed a decreased risk of PROM in successfully treated Chlamydia positive patients compared to patients who were treated but had either recurrent or persistent Chlamydia infection at the end of pregnancy (aOR 0.31; 95% CI) [71]. In the third study in India the mean duration of gestation for PD was significantly higher among Chlamydial-infected women who received treatment in comparison to untreated women [35.5 versus 33.1 weeks (P < 0.05)] [72].

Treatment of syphilis has also been shown to be beneficial, particularly if done in the first trimester compared to the third [61]. A study in Tanzania demonstrated that there was no increased risk of adverse pregnancy outcomes for women treated for high titre active syphilis (OR 0.76; 95% CI, 0.4 -1.4) or low titre active syphilis (OR 0.95; 95% CI, 0.6 -1.5) compared with sero-negative women. Women were recruited, tested for syphilis and treated on the same day (mean recruitment age, 25.8 weeks) [61]. However, whilst treatment of women who were RPR (rapid plasma reagin) positive at antenatal clinics in Kenya significantly improved pregnancy outcome, it

did not eliminate the risk altogether [60]. Indeed, in this study, infected women who did not receive treatment had a marked higher risk of ABO (OR 4.1; 95% CI, 2.4-7.2) compared to uninfected women; but infected women who received treatment still experienced a 2.5-fold higher risk of ABO than uninfected women. Women who were treated but were still syphilis positive at delivery had significantly more ABO than those without syphilis (14.7 versus 6.2; $P < 0.05$) but less than untreated women (26.2% versus 14.7%; $P < 0.05$).

Treatment of BV with clindamycin in the treatment phase of a controlled drug trial in the US was associated with reduced PD (RR 0.5; 95% CI, 0.3-0.8) and PROM (RR 0.5; 95% CI, 0.2-1.4) [23]. Findings in another study in the US showed that women with clinically diagnosed BV, and were treated using metronidazole and erythromycin, had a lower incidence of PD (31% with treatment versus 49% with placebo, $P = 0.006$) [73].

Another study in the US designed to evaluate the effect of early BV screening (at < 22 weeks gestation) and treatment followed by re-screening and re-treatment if necessary, found that treatment of BV (with oral metronidazole or intravaginal metronidazole or clindamycin) was significantly associated with a reduction in the risk of PD (OR 0.5; 95% CI, 0.3-0.8) [74]. Similar results were found in another study in the United Kingdom where screening and treatment with intravaginal clindamycin were done early (13-20 weeks gestation)[75]. Treated women experienced a significant reduction in PD compared with the placebo group [4% versus 10%, ($P < 0.03$)] [76].

In a review of studies on screening, treatment, or ABO data in women with asymptomatic BV, no benefit was found in the treatment of asymptomatic BV [77].

Treatment of *T. vaginalis* infection in pregnancy has been shown to have no effect on gestational age and birthweight. A study in South Africa found that gestational age and birth weight of infants born to women who were treated with benzoyl-metronidazole and in those who did not receive treatment were similar [78]. Treatment was effective in clearing infection. A review aimed at determining whether antibiotic treatment for BV or *T. vaginalis* during pregnancy decreases the risk of PD and associated ABO concluded that there is no evidence to support the use of antibiotic treatment for these infections for the purpose of reducing the risk of PD

and associated ABO [75]. These conclusions were based on two studies. One US study evaluating treatment of asymptomatic trichomoniasis (selectively enrolled asymptomatic women) with metronidazole suggested that the drug failed to prevent PD [79]. In this study women treated with metronidazole were significantly more likely to deliver a preterm infant than untreated women (RR 1.8; 95% CI 1.2-2.7). The second study was a sub-analysis of a randomised trial in Uganda which showed that women who were treated for trichomoniasis with metronidazole were 2.5 times more likely to deliver a LBW infant (RR 2.49; 95% CI 1.12-5.50) [80]. Although the original study was not designed to assess the effect of *T. vaginalis* treatment, the authors inferred that this might have been due to metronidazole exposure. However, what was observed in this study may be attributable to some other factor rather than what was inferred by the authors since the risk of PD was not increased in other clinical trials of metronidazole treatment of bacterial vaginosis during pregnancy [73, 81-83]. Furthermore a sub-analysis of a study of pregnant women in four sub-Saharan African sites found that treatment of trichomoniasis with metronidazole did not influence the risk of PD or LBW, women randomised to the antibiotic arm were not more likely to deliver a preterm infant (20.9% versus 19.8%, $P = 0.84$) or to deliver an infant with a lower mean birth weight (2992 versus 2930 $P = 0.27$) [84]. The treatment effectively resolved *T. vaginalis* infection.

1.3 Prevalence of malaria and STIs/RTIs

1.3.1 General overview of malaria prevalence in pregnancy

Several reviews have reported the prevalence of malaria in pregnancy. Brabin reviewed 14 studies conducted before 1980 and found peripheral and placental parasitaemia ranging from 2-76% and 2-74%, respectively [42]. This review included two studies from Panama and 12 conducted in Africa. The wide range of prevalence of malaria in pregnancy is partly due to the difference in the level of malaria transmission in the study sites. Generally, the prevalence of malaria in pregnancy was higher in rural than urban areas. The median prevalence was at 27%. Another review of studies done between 1985 and 2000 in eight countries found maternal malaria infection, defined as placental or peripheral, in all gravidae ranged between

6 and 65% [54]. The median prevalence of maternal parasitaemia was 27%. A similar estimate of 26% was obtained for placental parasitaemia (range 5-52%) from another review of studies conducted between 1980 and 2001 [85]. Desai *et al.,* reviewed 13 studies conducted between 1986 and 2004 of *P. falciparum* infection in pregnancy in areas of low-transmission settings in Africa and found a median prevalence of peripheral and placental parasitaemia at 13.7% and 6.7%, respectively [86]. The point prevalence estimates in the review by Desai *et al.,* were generally lower since the review concentrated on low transmission areas.

The meta-analysis by Chico *et al*. reviewed studies conducted among antenatal attendees in sub-Sahara Africa [6] and reported pooled prevalence estimates for malaria and STIs/RTIs. The estimates were reported separately for West and Central Africa and for East and Southern Africa. This review included studies of women receiving antenatal care conducted between 1990 and 2011 and excluded studies that had selective enrolment, i.e. high risk groups such as HIV-seropositive women. These estimates were calculated using a standard method for correcting errors of magnitude based on the known specificity and sensitivity of individual diagnostic methods [87].

Pooled prevalence estimates for Eastern and Southern Africa from this review were as follows: placental malaria 25.8% (95% CI, 19.7-31.9) and peripheral malaria 32.0% (95% CI, 25.9-38.0). Prevalence estimates that were obtained for West and Central Africa were higher than those obtained for East and Southern; placental and peripheral parasitaemia was at 39.9% (95% CI, 34.2,-45.7) and 38.2% (95% CI, 32.3-44.1) respectively [6].

At the patient level, placental parasitaemia is almost always higher than measures of peripheral parasitaemia [88-90]. When this has not been the case in systematic reviews or meta-analyses, this is because not all studies conduct placental histology; therefore, fewer data points are often pooled for estimates of placental infection. If these happen to be studies where the prevalence of parasitaemia is lower, then the overall frequency will be lower than pooled measures of peripheral infection.

1.3.2 General overview of STI/RTI prevalence

The review (mentioned in earlier text) by Mullick *et al*. 2005 of studies published between 1987 and 2004 also examined the prevalence and the impact of STIs/RTIs on pregnancy outcomes in developing countries. Results from this review by Mullick *et al*. showed that up to 40% of pregnant women in Africa had trichomoniasis and bacterial vaginosis, 2.5–17% had serological evidence of syphilis, 3–6% while the prevalence of gonorrhoea and Chlamydia ranged from 2–7% and 3–29%, respectively. Studies conducted between 1990 and 2011 reporting point prevalence estimates for malaria and STI/RTIs among pregnant women were identified in a more recent review and meta-analysis by Chico *et al*., 2012. Pooled prevalence estimates for West and Central Africa from this meta-analysis are as follows: syphilis, 3.5% (95% CI, 1.8%-5.2%), gonorrhoea*e*, 2.7% (95% CI, 1.7%-3.7%), Chlamydia 6.1% (95% CI, 4.0%-8.3%), trichomoniasis 17.8% (95% CI, 12.4%-3.1%), BV, 37.6% (95% CI, 18.0%-57.2%). For East and Southern Africa, the estimates were as follows: syphilis 4.5% (95% CI, 3.9-5.1), gonorrhoea 3.7% (95% CI, 2.8- 4.6), Chlamydia 6.9% (95% CI, 5.1- 8.6), trichomoniasis 29.1 (95% CI, 20.9-37.2) and BV 50.8% (95% CI, 43.3- 58.4).

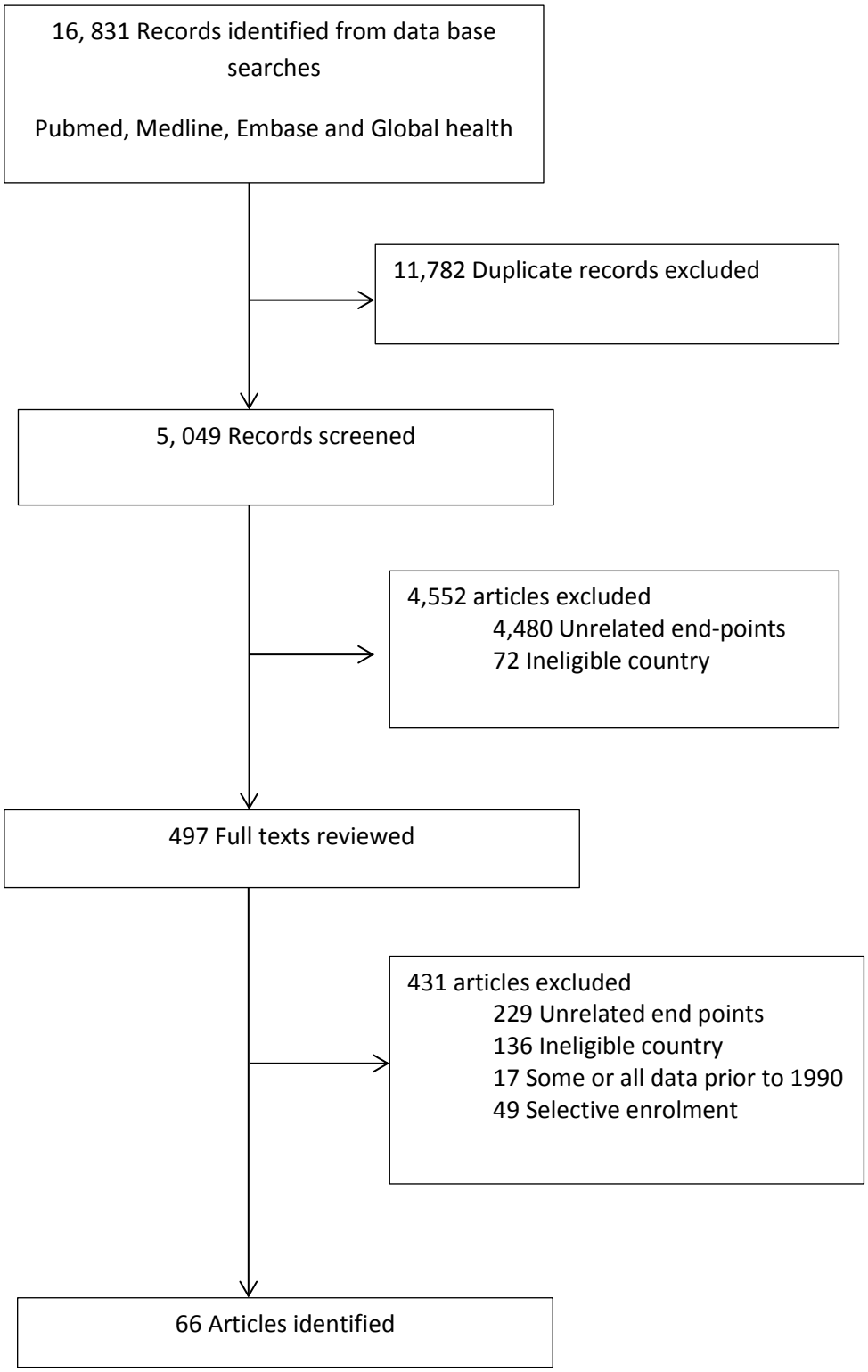
1.4 Prevalence of malaria and STIs/RTIs in pregnancy in Zambia and neighbouring countries

The review by Chico *et al.* covered a broader region in sub-Saharan Africa. In this study a review was conducted for a smaller region of sub-Saharan Africa, Zambia and neighbouring countries. This was done to focus on areas surrounding the study site in which a substantial number of prevalence studies have been conducted. To explore studies reporting the prevalence of malaria and STIs/RTIs in pregnancy conducted between 1990 and 2015 in Zambia and neighbouring countries, a literature search was conducted. Four data bases, Pubmed, Medline, Embase and Global Health were searched for studies reporting *P. falciparum*, *T. pallidum*, *N. gonorrhoea*, *Chlamydia trachomatis*, *T. vaginalis* and BV using medical subject headings and free texts. The Medline, Embase and Global Health data bases were searched simultaneously via Ovid® using combinations of the following key words; “Pregnancy”, “antenatal/prenatal”, “sexually transmitted disease”,

"syphilis/*Treponema pallidum"*, "Chlamydia/*Chlamydia trachomatis"*, "Gonorrhoea/*Neisseria gonorrhoeae*/gonorrhoea/gonorrhea", "trichomoniasis/*Trichomonas vaginalis"*, "bacterial vaginosis", "malaria", "*Plasmodium falciparum*" "test", "screen" and "Africa". Search terms that were used are shown in Appendix 1.1b. The last search was conducted in April 2016.

If an article reported two or more estimates e.g., if more than one diagnostic method was used, the highest/higher estimate was picked. Studies of women seeking treatment at STI facilities, non-pregnant women and those reporting estimates in high-risk groups such as commercial sex workers and in HIV-positive pregnant women were excluded. Multi-year studies reporting prevalence estimates that included data collected before 1990 were also excluded. Studies conducted in countries that are not Zambia's immediate geographic neighbours were also excluded, hence only studies conducted in Angola, Botswana, Democratic Republic of Congo (Congo DR), Malawi, Mozambique, Namibia, Tanzania and Zimbabwe were included. All the articles obtained from all the search terms were originally retained and duplicates were then removed and other studies were excluded based on the criteria stated above. Appendix 1.2a to 1.2g show point prevalence estimates for malaria and STIs/RTIs from studies conducted in Zambia and neighbouring countries and diagnostic methods used in each study. Figure 1.2 shows the identification and screening of studies in the review.

Figure 1. 2: Flow chart



1.4.1 Prevalence of malaria in pregnancy in Zambia and neighbouring countries

Studies in Zambia and neighbouring countries conducted between 1990 and 2015 have reported the prevalence of placental and peripheral malaria in all gravidae ranging from 9.8-52.0% and 4.7-51.9%, respectively (Appendix 1.2a and 1.2b). The wide range of prevalence of malaria in pregnancy is partly due to the difference in the intensity of malaria transmission in the various study sites. The median prevalence of placental malaria (from 12 studies) and peripheral parasitaemia (from 22 studies) was 20.3% and 20.5%, respectively.

1.4.1.1 Diagnostic methods for malaria and published sensitivity and specificity

Diagnostic methods for malaria and published sensitivity and specificity are presented within Appendix 1.3. The gold standard for malaria diagnosis, Giemsa stain microscopy, has sensitivity measures ranging from 50-90% and specificity of approximately 95% [91-93]. The sensitivity and specificity of Gram stain microscopy are greatly influenced by the skills and workload of microscopists [94] and therefore the sensitivity and specificity of the method are expected to be on the higher end when the method is carried out by trained individuals with reasonable workload. One advantage of standard microscopy is that the method requires a relatively short time for diagnosis when used in areas of high transmission and parasites are present in high concentrations (approximately 1,000 parasites per microlitre [µL] of blood). However, if parasite densities are very low, examination of each slide is labour-intensive [94]. The use of PCR for the diagnosis of malaria is highly sensitive in the detection of parasites. The method has been reported to have 91% sensitivity and specificity [95] and has the ability to measure infections where parasite counts are as low as five per µL of blood [96, 97], although the sensitivity of PCR has been shown to decrease markedly at parasite densities < 500/µL [98]. In a study in Taiwan a high proportion (58.2%) of microscope-positive samples were negative by PCR and 98.0% of these infections had fewer than 250 parasites/µl of blood [98]. In another study PCR failed to detect parasite DNA in two microscopy positive samples [99]. Other studies have reported that PCR may occasionally yield false negative results [98, 100, 101] sometime as a result of inappropriate DNA isolation [102, 103]. Among the 22 studies reporting prevalence of peripheral malaria among pregnant women in

Zambia and neighbouring countries Giemsa stain microscopy was used in 14, PCR in seven and RDT in one study (Appendix 1.2f)

Since Giemsa stain microscopy was used in the majority of these studies, it would be expected that there was some level of underestimation of the actual malaria prevalence in the region. However, the estimation can be considered reliable as they are expected to lie within acceptable ranges of the actual prevalence provided diagnoses were conducted under appropriate conditions.

The use of placental blood microscopy in placental malaria diagnosis has 63% sensitivity and 98-99% specificity [104]. Placental histology is reported to have 91% sensitivity and 98-99% specificity [104]. Placental blood microscopy and tissue histology (score based on more precise method, i.e. histology), placental histology, impression, smears with microscopy (score based on most precise method, i.e. histology) and placental impression and blood smears with microscopy (score based on more precise method, i.e. impression) have all been reported to have 91% sensitivity and approximately 100% specificity [6, 105].

In the case of studies that used more than one method for the detection of placental malaria the estimate based on the method with higher sensitivity and specificity was recorded. The majority of studies (eight out of the 14) that contributed to the range of the prevalence estimates were based on histology (five) and impression (three) both of which are reported to have the same sensitivity and specificity measures. Placental blood microscopy was used in two of the studies and PCR was use in the remaining four studies. Based on the published sensitivity and specificities of the methods one would expect the estimates to be slightly lower than the situation obtaining on the ground. However, these estimates can be considered reliable and the wide range between the highest and lowest recorded prevalence estimates are mainly due to differences in malaria prevalence in the different areas in which these studies were conducted.

1.4.1.2 The malaria situation in Zambia

In Zambia, malaria is endemic in all 10 provinces and *P. falciparum* is responsible for approximately 95% of all cases [106]. There are an estimated 4.3 million clinically

diagnosed cases of malaria and 6,150 deaths annually [106]. Transmission of malaria is year-round but peaks in the rainy season (November-April). An estimated 716,192 pregnancies in Zambia were at risk of malaria in 2010 based on projections from the 2000 census [107].

Since 2006, the National Malaria Control Programme has conducted a Malaria Indicator Survey (MIS) every two years to measure the prevalence of malaria by microscopy in children under five years of age in selected sites. The prevalence of malaria parasitaemia decreased in Zambia from 2006 to 2010 in some regions, while little change was observed in others [108]. Of concern is that parasitaemia declined between 2006 and 2008 in Eastern, Northern, and Luapula Provinces, but was higher in the 2010 survey with parasite rates in young children in Luapula Province exceeding 50% in 2010. Although a substantial decline of 66% in patient cases and deaths occurred in 2000-2008 following the introduction of multiple interventions, a malaria upsurge occurred in 2009-2010 when vector control interventions were disrupted following delays in the disbursement of funds [109]. There has been a 48.3% decline in the national overall malaria prevalence in children under five years of age between 2006 and 2012 (22.1% versus 14.9%) [109].

1.4.1.3 Malaria treatment policy in Zambia

Zambia revised its national malaria drug treatment policy between 2000 and 2005 to adopt artemisinin combination therapy (ACT) as the first-line treatment for uncomplicated malaria [110]. Quinine is the first-line treatment for uncomplicated malaria in pregnancy in the first trimester, whereas artemether-lumefantrine (AL) is for use in second and third trimesters. For complicated malaria, parenteral quinine is recommended in all trimesters; SP is used by policy for IPTp and also serves as a drug of choice for people who cannot tolerate AL and during periods of AL stock-outs in health facilities [111]. The Zambian malaria policy states that IPTp-SP doses are to be administered during pregnancy at scheduled ANC visits, spaced one month apart after 16 weeks of gestation.

1.4.1.4 Prevalence of *P. falciparum* resistance markers in Zambia

Monitoring SP resistance markers is important in areas where IPTp-SP is policy as these markers have been associated with compromised efficacy of SP for IPTp [1-3].

A few studies conducted in Zambia have estimated the prevalence of SP resistance markers in the general population and in pregnant women. In 2006, the frequency of dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR), mutations associated with parasite resistance to SP were found to range from 71-92% for the triple mutant DHFR and 39-71% for the double mutant DHPS in the general population in six districts in Zambia [112]. In another study conducted in 2006 in the southern part of Zambia, the prevalence of the DHFR triple mutant was found at 41.3% and the DHPS double mutant was at 16% in a random sample of 95 selected from 188 malaria positive samples [113]. The frequency of the quintuple mutant was at 6.5%. Higher prevalence estimates of the quintuple mutant have been found in pregnant women in Northern Zambia. In a study conducted between January 2010 and May 2011 in Mansa the prevalence of the quintuple mutant among 84 malaria positive samples was found in 63.1% (n = 53) and 2.3% (n = 2) of these had an additional 581-G mutation [114]. This was the first published record of the sextuple mutant in Zambia. Another study also conducted in February to April 2013 in the Northern part of Zambia, in Nchelenge District the quintuple mutant in a sample of 72 malaria positive pregnant women was found in 17% (n = 12) of the samples. The sextuple mutant was found in 3% (n = 2) of the samples [115].

1.4.2 Prevalence of curable STIs/RTIs in pregnancy in Zambia and neighbouring countries

Studies of pregnant women in Zambia and neighbouring countries conducted between 1990 and 2015 and identified in the literature search have reported prevalence of individual STIs/RTIs with wide ranges between the lowest and highest reported figures. From these studies it has been reported that 0-14.6% of pregnant women have syphilis; 0.5-18.6% have gonorrhoea and 1.7-17.5% have Chlamydia; while the prevalence of trichomoniasis and BV range between 2.4-32.2% and 4.3-37.6% respectively. The median prevalence of individual STIs/RTIs are as follows: syphilis 5.6%, gonorrhoea 2.8%, Chlamydia 6.6%, trichomoniasis 17.4% and BV 24.9%. Appendices 1.2c to 1.2g show point prevalence estimates of STIs/RTIs reported in studies conducted in Zambia and neighbouring countries and includes diagnostic methods used to estimate prevalence.

1.4.2.1 Diagnostic methods for STI/RTIs and published sensitivity and specificity

Diagnostic methods for STIs/RTIs employed in the various studies in Zambia and neighbouring countries and published sensitivity and specificity measures are shown within Appendix 1.3. Methods used for detection of syphilis are rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) test and confirmation is conducted using *Treponema pallidum* haemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA), determine Syphilis -*Treponema pallidum*, microhaemagglutination assay-*Treponema pallidum* (MHA-TP) and immunoglobulin G (ELISA) tests. When RPR is used for syphilis diagnosis and a confirmatory test is conducted both sensitivity and specificity are approximately 100% [6]. When used alone the sensitivity measure is 86-100% for RPR and 78-100% for VDRL and the specificity is 93-98% and 98% for RPR and VDRL respectively [116].

Thirty four prevalence estimates (Appendix 1.2c) of syphilis prevalence were reported from 33 studies, one study reported two separate point prevalence estimates, one for first ANC attendees and another for delivering women. Of these 34 estimates four were based on RPR only and one on VDRL test only. The rest of the estimates were based on either RPR or VDRL and a confirmatory test (TPHA or ELISA or Determine Syphilis TP or MHA-TP) in each case. Estimates obtained from RPR or VDRL only would be expected to be higher than those where a confirmatory test is used. Since the majority of studies used the combination of diagnostic tools known to have the highest sensitivity and specificity, one would expect that the reported estimates of syphilis prevalence among ANC attendees in Zambia and surrounding countries are reliable. Therefore, it can be concluded that the reported prevalence, whether low or high, is mainly due to the actual prevalence in the study setting rather than in differences in diagnostic methods.

The prevalence of Chlamydia among pregnant women in Zambia and surrounding countries has been reported to range between 1.7 and 17.5% (Appendix 1.2d). Cervical swab culture for diagnosis of Chlamydia has been reported to have sensitivity ranging between 74 and 94% while specificity is between 98 and 99%

[117]. Enzyme immunosorbent assay has been widely used for detection of Chlamydia and is reported to have sensitivity ranging from 71-87% and specificity ranging from 97-99% [117]. Urine based PCR has 78-89% sensitivity and 99-100% specificity and cervical swab based PCR has 99% sensitivity and 99-100% specificity [117]. The use of Deoxyribonucleic acid identification assay is reported to have 100% sensitivity and specificity [6]. In the 11 studies reporting the prevalence of Chlamydia among pregnant women in this region, a variety of methods were used to obtain these estimates in these studies as follows; culture was used in one, EIA in four, PCR in two, LCR in two, DNA-ID in one and ELISA was used in one. Based on the range of sensitivity measures for the methods that were used to measure prevalence, it is expected that there was some level of underestimation of the prevalence. However, some of the studies used DNA-ID, PCR and LCR which have higher sensitivity and specificity.

It is reported that 0.5-18.6% of pregnant women in Zambia and surrounding countries have gonorrhoea (Appendix 1.2e). Detection of gonorrhoea using gram stain is reported to have 50-70% sensitivity and 95-100% specificity [118]. Sensitivity and specificity of culture and gram stain (based on mean of culture) have been reported to be approximately 80% and 100%, respectively [6]. Ligase chain reaction with cervical swab has been reported to have the highest sensitivity and specificity, 95-100% and 98-100% respectively, while for PCR with cervical swab sensitivity and specificity have been estimated to be at 89-97% and 94-100%, respectively [118].

Of the 13 studies conducted in Zambia and surrounding countries, 14 point prevalence figures were reported for gonorrhoea among ANC attendees. In these studies culture was used in eight (with one study reporting two culture based estimates), culture and gram stain were used in two, PCR in two and LCR in one. Since the use of culture alone and culture combined with gram stain have lower sensitivity and specificity, and were used in the majority of studies, the individual prevalence estimates would be expected to be slightly lower than the actual prevalence but within acceptable ranges.

The prevalence of trichomoniasis is reported to range between 2.4 and 32.2%. Diagnosis of trichomoniasis has widely been done using wet mount microscopy which has sensitivity ranging from low to moderately high (38-82%) and high specificity (100%) [117]. All of the 16 estimates of trichomoniasis prevalence from 15 studies conducted in Zambia and neighboring countries were obtained using wet mount microscopy, except one estimate from a study conducted in Zambia in which PCR was used. Based on published sensitivity and specificity measures of the wet mount microscopy, it is expected that the actual prevalence of trichomoniasis in these settings were generally underestimated.

Bacterial vaginosis is the most prevalent of the STIs/RTIs with reports of prevalence ranging between and 4.3 and 37.6% among pregnant women of Zambia and surrounding countries. Over the years diagnosis of BV has been done using clue cell count, Amsel criteria [119] and the Nugent score [120]. The sensitivity and specificity of the Amsel criteria are reported to be 51% and 98% [121]. The Nugent method has higher sensitivity (86-89%) than the Amsel criteria but its specificity is reported to be between 94-96% [122-124].

Of the 12 point prevalence estimates from 11 studies reporting BV prevalence in Zambia and neighbouring countries, six were based on the Amsel criteria; four were based on the Nugent criteria and two were based on the clue cell count method. Based on published sensitivity and specificity, the reported prevalence estimates would generally be expected to be lower than the actual prevalence in the study population.

1.4.3 General assessment of prevalence literature

Sensitivity and specificity measures vary among different diagnostic tests. However, a diagnostic test conducted at different times under standard laboratory conditions would be expected to have similar sensitivity and specificity measures. It was noted that similar diagnosed methods have been used over time in the study period (1990-2015) within which prevalence studies reviewed in thesis were conducted. No particular method predominated a certain period in time (e.g. the first 10 or 15 years) among the various diagnostic methods used in the detection of the different

infections. In this context time has no influence on the prevalence estimates in the studies that were reviewed. It was also noted that the prevalence figures from studies conducted in the period between 1990 and 2015 do not show any downward or upward trend with time across the various infections from different sites in Zambia and surrounding countries.

In general, methods with low sensitivity would underestimate and those with low specificity would overestimate prevalence. Overall the methods used in the diagnosis of malaria and STIs/RTIs have been widely applied and, therefore, variation in estimates are likely due to true differences in the prevalence of the infections in the different study sites.

The median prevalence estimates obtained from studies in Zambia and neighbouring countries were lower across all the infections, in comparison to pooled prevalence estimates obtained from the meta-analysis by Chico *et al*. [6] This may be explained by the fact that Chico *et al*. corrected point estimates to increase the precision of each measure before generating pooled prevalence estimates. The median prevalence for each infection obtained from studies in Zambia and neighbouring countries either fell within (Chlamydia, gonorrhoea and placental malaria) or outside (syphilis, trichomoniasis, BV and peripheral malaria) the 95% CI of the pooled prevalence estimates obtained from the meta-analysis by Chico *et al*., 2012. This could further be explained by notable differences in number of studies included in the meta-analysis by Chico *et al*., and in the review conducted in this thesis i.e., syphilis (seven), trichomoniasis (four) and BV (six) and peripheral malaria (12). In the case of placental malaria the difference in the number of studies between the two was minimal (two) and the number of studies was the same in the case of gonorrhoea and Chlamydia. Another possible explanation is that the prevalence of some of the infections is simply higher in some regions/study sites than others.

1.5 Risk factors for STI/RTI and malaria

1.5.1 Risk factors for STIs/RTIs

Studies conducted in Zambia and neighbouring countries reporting prevalence of malaria and STIs/RTIs among pregnant women identified in this thesis were further reviewed for predictors of infections.

It was noted that not all the studies reported risk factors for infection. Some of the studies identified risk factors for individual infections while in others predictors of infection were identified for different combinations of infections e.g. Chlamydia and gonorrhoea [125-127]; HIV, HSV and syphilis [128]; BV, trichomoniasis and candidiasis [128]); syphilis, trichomoniasis and gonorrhoea [129]; HSV-type 2, trichomoniasis, Chlamydia, gonorrhoea, syphilis and HIV [130]. Risk factors for STIs/RTIs and malaria identified in studies conducted in Zambia and neighbouring countries are summarised and presented in Appendix 1.4.

Among these studies syphilis has been associated with the following factors: HIV infection [131], history of stillbirth [132, 133], self-reported previous spontaneous abortion [133], history of past STI [131], genital ulcers [131, 134], vaginal discharge [131], genital warts [131], married status or living with a partner [131, 135], infection with trichomoniasis [134], higher parity and gravidity [132], older age [136], lower education [136, 137], having more than one lifetime partner [128, 133], previous pregnancies [138] and having a partner who takes alcohol [128].

Infection with gonorrhoea was associated with having more than one lifetime partner [139]. Infection with gonorrhoea and/or Chlamydia was associated with following factors in one study; vaginal discharge, having had a new sexual partner in the last three months, being separated from one's partner for more than 3 months, presence of a purulent discharge, lower education, current use of a condom [125]. Other predictors of Chlamydial/gonococcal infection are older age group (above 25 versus ≤ 25 [126] and ≥ 30 compared to 20-29 group [127]), single status, being in a polygamous marriage, having more than one sexual partner over the previous year and having delivered more than five years earlier [126]. Trichomoniasis has been associated with polygamy [129], lower education level of sexual partner, abnormal discharge, genital ulcer and genital warts [140]. Infection with trichomoniasis and/or BV and/or candidiasis has been associated with early sexual debut (< 20 years) [128].

In a study in which risk factors for composite STI (HSV-type 2, trichomoniasis, Chlamydia, gonorrhoea, syphilis and HIV) were identified, the presence of any STI was associated with being in a long-term relationship as opposed to a short term one, increasing age difference between the girl and her partner and history of prior pregnancy [130].

Geographical location has been shown to be an important risk factor for STIs/RTIs when one area is compared to another, e.g. semi-urban and rural versus urban [138] or one study site versus another [136, 140].

1.5.2 Risk factors for Malaria infection

In the studies conducted in Zambia and neighbouring countries identified in this thesis, the following factors were associated with an increased risk of peripheral malaria; younger age [89, 115, 141], wet season [141] primigravidity/primiparity [142, 143], wealth quintile (all categories of wealth below the highest) [144] and malaria infection earlier in pregnancy [89, 145]. Bed net use was found to be protective against peripheral malaria infection [1].

A higher risk of placental parasitaemia has been associated with younger age [89], primiparity [141, 143, 146] and malaria infection earlier in pregnancy [89]. Iron deficiency [146], bed net use [1] and receiving IPTp-SP during pregnancy [1, 147] have been associated with a reduced risk of placental malaria infection.

1.5.3 Conceptual frameworks for infection

In order to identify both distal and proximal risk and protective factors associated with malaria and STI/RTI, two conceptual frameworks were constructed based on evidence from literature. Under these frameworks (Figures 1.3 and 1.4) it is assumed that demographic and socio-economic factors directly or indirectly (via maternal/partner characteristics) influence the risk of infection.

Figure 1. 3: Conceptual framework for STIs/RTIs

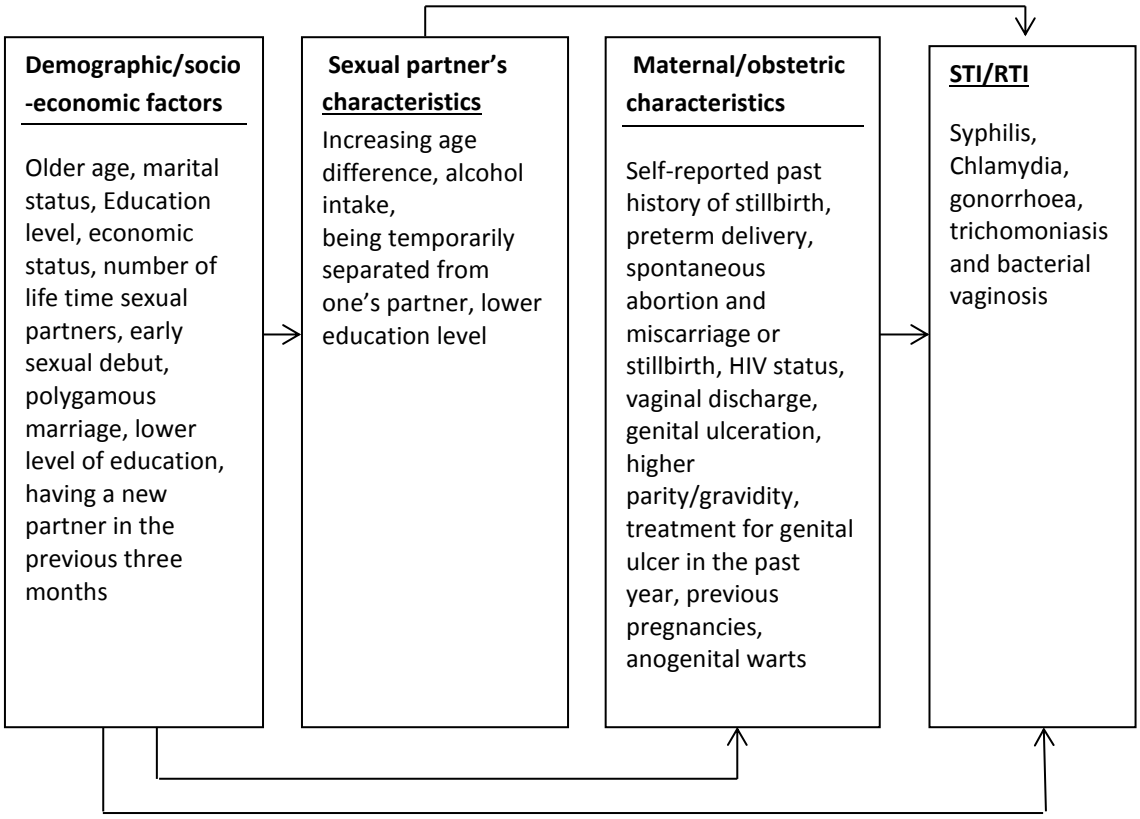
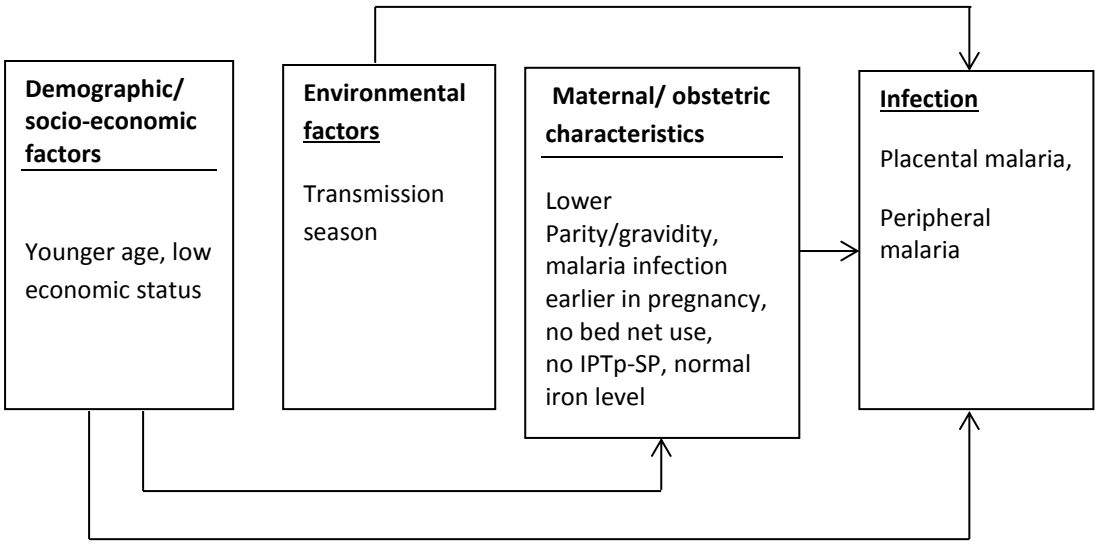
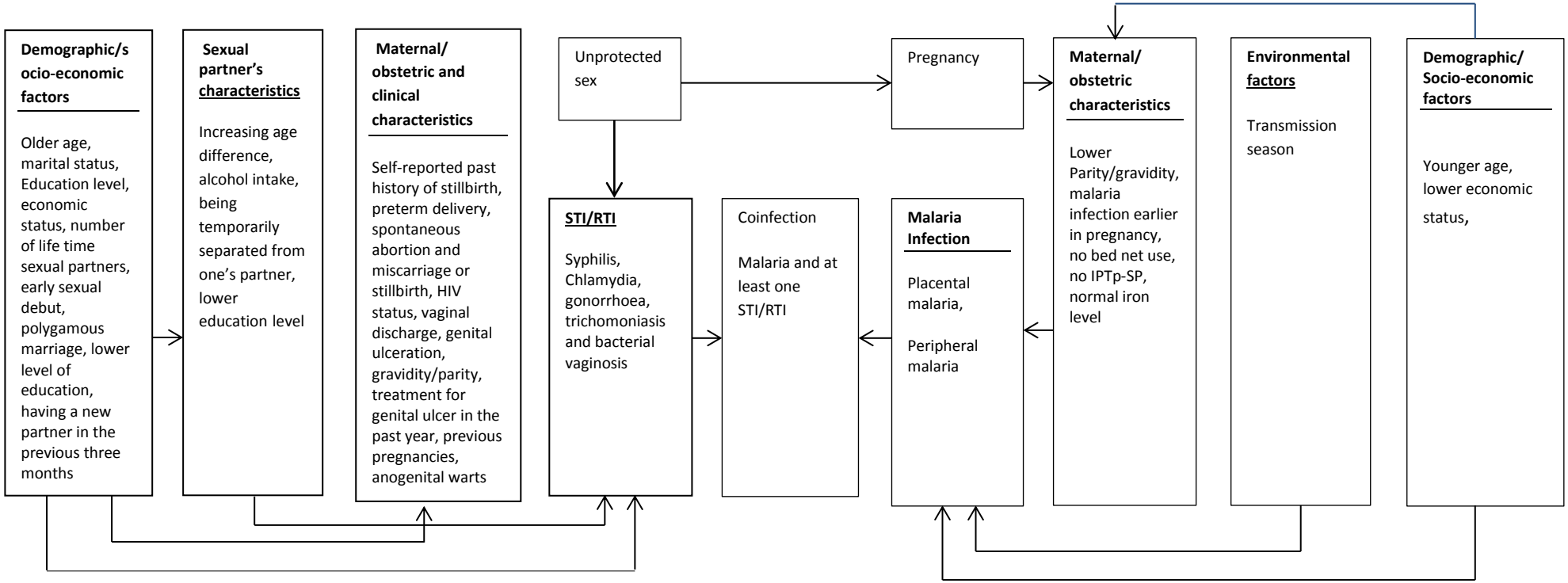


Figure 1. 4: Conceptual framework for malaria infection



As earlier mentioned the prevalence of malaria and STI/RTI has not been reported in any epidemiological setting therefore risk factors for coinfection are not known. It is plausible to assume that distal and proximal risk factors for malaria infection and STIs/RTIs play a role in their coinfection. Figure 1.5 shows a conceptual framework for malaria and STI/RTI coinfection based on risk factors for malaria and STI/RTI identified in literature. Unprotected sex may result in pregnancy and STIs. Pregnancy brings about reduced immunity [43] which makes pregnant women in a malaria endemic area susceptible to malaria infection.

Figure 1. 5: Conceptual frame work for malaria and STI/RTI coinfection



1.6 Prevalence of malaria and STIs/RTIs coinfection

It is clear from literature that there is paucity of information on the prevalence malaria and STIs/RTIs coinfection in pregnancy. In their review, Chico *et al.*, noted that only one study from Tanzania reported the prevalence of malaria among women who tested positive for syphilis [6]. In this retrospective cohort study, it was found that among 73 women with high-titre (≥ 1:8) active syphilis, 34% concomitantly had maternal malaria, 18% had past chronic placental malaria and 48% had histological evidence of current malaria and chronic changes indicative of malaria infection earlier in pregnancy [10]. However, the occurrence of other curable STIs/RTIs was not reported in this paper.

1.7 Current antenatal Care package

1.7.1 Antenatal care package for malaria in pregnancy

Intermittent preventive treatment in pregnancy with SP is recommended by the World Health Organization for reducing the risks associated with malaria in pregnancy [148]. Randomised controlled trials have shown IPTp-SP decreases the risk of maternal anaemia, placental parasitaemia, and LBW [149-152]. In a review of nine trials conducted in Africa IPTp-SP reduced the risk of placental malaria (RR 0.48; 95% CI, 0.35-0.68), LBW (RR 0.71; 95% CI, 0.55-0.92), and anaemia (RR 0.90; 95% CI, 0.81-0.99) [153]. At least one dose of IPTp-SP has been shown to protect against maternal parasitaemia (OR 0.20, 95% CI, 0.12-0.34, $P < 0.001$) in an observational study in Ghana [154] and to protect against LBW in Benin [155].

Administration of three doses instead of two doses of SP has been shown to reduce the prevalence of placental malaria, LBW, and preterm delivery by 50% in a high malaria transmission area of Mali [156]. In comparison to a 2-dose regimen, three doses of IPTp-SP are superior in reducing the effect of malaria in pregnancy and appear to be effective even in areas with high levels of SP resistance [3]. However, the protective effect of IPTp-SP has been compromised [157] and a decline in IPTp-SP efficacy has been reported in Africa [158]. In an area in Tanzania where 14 days parasitological SP treatment failure rate was as

high as 38.8% (95% CI, 26.8-50.8) [159], IPTp-SP was significantly associated with placental parasitaemia (*P* = 0.03), did not have an effect on LBW [OR 0.71 (95% CI 0.33-1.54) *P* = 0.39] and was significantly associated with foetal anaemia [1.91 (95% CI 1.14-3.19) *P* = 0.01] [2]. However, in Malawi the use of IPTp-SP was not associated with higher parasite densities, greater placental inflammation, or worsened delivery outcomes despite the increasing prevalence of SP-resistant *P. falciparum* haplotypes [160]. A recent systematic review and meta-analysis reports no evidence of paucigravidae being protected against delivering a LBW baby in areas where the parasite population of Arg-581 is greater than 10.1%. [161]. Therefore, an evidence-based policy revision is urgently needed.

1.7.2 Antenatal care package for curable STIs/RTIs in pregnancy

Screening and treatment of syphilis is recommended as a routine part of antenatal care [162, 163]. Detection of STIs/RTIs is most accurately done using technologically advanced and expensive diagnostic techniques. These techniques require trained staff, laboratory equipment, specific storage and transportation conditions [5]. In order to assist countries with limited resources, the WHO has developed syndrome-based algorithms for the diagnosis and management of *gonorrhoea*, *Chlamydia*, trichomoniasis and BV in symptomatic individuals. Unfortunately in women high proportions of gonococcal (80%) and Chlamydial (70-75%) infections are asymptomatic [164]. Approximately 50% of women infected with *T. vaginalis* are asymptomatic but even higher proportions (60-70%) of asymptomatic *T. vaginalis* infection and BV have been reported [165, 166]. The implication is that the use of syndromic management would lead to many asymptomatic cases going unnoticed, and thus, a large proportion of STIs/RTIs in pregnancy will remain undetected and untreated.

1.7.3 Antenatal care in Zambia

` In Zambia the ANC package includes the following; weight measurement, height measurement, blood pressure measurement, urine sample collection for analysis, blood sample collection for analysis, voluntary counselling and testing, provision of iron supplementation , IPTp-SP, discussion of birth preparedness plan, provision of treatment for intestinal parasites and tetanus toxoid vaccination [167]. Blood testing for screening of maternal syphilis, HIV and anaemia are conducted as part of ANC [168]. Although syndromic management is not documented as part of the ANC package, the syndromic approach for STI management is documented in the National HIV/AIDS/STI/TB Policy [169]. Therefore in practise the syndromic approach is used for diagnosis of other STIs whenever women attending ANC come with complaints of signs and symptoms that are indicative of STIs.

In a study where two national datasets with detailed antenatal care provider and user information i.e. the 2005 Zambia Health Facility Census and the 2007 Zambia Demographic and Health Survey (ZDHS), were analyzed to describe the level of ANC service provision at 1,299 antenatal facilities in 2005 and the quality of ANC received by 4,148 mothers between 2002 and 2007 [170]. In the study mentioned above, criteria were developed to describe quality of ANC and different levels of provision of ANC. This included information on ANC use, type of ANC provider, place of ANC provision, number of ANC visits, timing of first ANC visit and on the interventions received. Having received “good quality ANC” was defined as having attended at least the recommended four ANC visits with a skilled provider and received at least eight antenatal interventions, while the definition of “moderate quality ANC” required four visits with a skilled provider and five to seven antenatal interventions [170]. Only 45 antenatal facilities (3%) fulfilled their developed criteria for optimum ANC service, while 47% of facilities provided adequate service, and the remaining 50% offered inadequate service. Although 94% of mothers reported at least one ANC visit with a skilled health worker and 60% attended at least four visits, only 29% of mothers received good

quality ANC, and only 8% of mothers received good quality ANC and attended in the first trimester [170]. Results from this study showed that "effective ANC delivery", as estimated by their indicators, was way below ANC coverage.

Percent distribution of women aged 15-49 who had a live birth in the five years preceding the 2013-14 ZDHS by number of antenatal care (ANC) visits for the most recent live birth were slightly different between women in the urban and rural areas of Zambia. In the urban areas 0.9%, 1.6%, 40.7%, 55.9% and 1% of women aged 15-49 attended none, one, two to three and four or more ANC visits respectively. In the rural areas distribution was as follows: 1.7%, 2.0%, 40.2%, 55.2% attended none, one, two to three and four or more ANC visits respectively. The median gestational age at first ANC was 4.8 months and 4.7 months among urban and rural women [167] respectively.

The proportions of ANC visits among Zambian women seem to suggest that a good number of women do not receive adequate ANC interventions indicating lost opportunity for delivering interventions.

1.8 Birthing practices and prevalence of adverse pregnancy outcome in Zambia

1.8.1 Practices and beliefs about pregnancy and childbirth in Zambia

In Zambia western religious practices are widespread and Christianity co-exist with deep spirituality of local tribes [171]. In general there is belief in the Christian God and the spirit realm of ancestors, which has allowed for the acceptance of the existence of witchcraft [171]. Beliefs in witchcraft are often applied to pregnancy, labour and birthing practices.

Zambian women often deliver in seclusion or in the company of a few selected women at home [172]. Several factors contribute to the preference of a secretive home delivery. In general pregnancy due dates and the start of labour are kept secret for fear of witchcraft harming the child and women deliver with a few people present to avoid supernatural harm to the child [173].

Some of the beliefs contribute to the proportions of women who deliver at home as opposed to making use of health professionals and facilities. A population-based cross sectional study survey by Mwewa and Michelo conducted among women (n= 499) of Nchelenge District in 2008 to identify factors associated with home deliveries showed that the prevalence of home deliveries was 43%, 95% CI (38.62, 47.48) [174]. Among the factors cited for home deliveries were long distances to the health facilities coupled with lack of transport, abrupt unexpected labour, circumstances beyond control (such as embarrassment due to not having the required items that delivering mothers are expected to present with at health facilities) and myths and traditional beliefs [174].

The study also identified some practices that are a result of myths and traditional beliefs that prevent women from delivering at health facilities. Among the common traditional beliefs pointed out in the study by Mwewa and Michelo is what is locally called “incila”. According to information from a focus group discussion most women decided to deliver at home in order to be attended by their grandmothers and be treated for “incila”, which according to the participants, was a situation where the partner or husband of the pregnant woman or the pregnant woman herself engaged in sexual affairs with other people during the woman's pregnancy, as a result of which the woman could have difficulties at delivery [174].

It was stated by a focus group discussion participant aged 56 from Kabuta village in the study by Mwewa and Michelo that 'The process of preventing death by “incila” requires the woman to divulge confidential information to the women assisting her delivery so that she delivers well in addition to taking the medication and so some women even go further away from their own villages to other villages for confidential reasons because some traditional birth attendants (TBAs) do not keep secrets. If there were too many men that the woman may have had extramarital affairs with while pregnant and they could not all be counted, then she is required to put maize meal into a bowl as a gift, for everything to end there and this cannot be done at the clinics'.

A 59 years old male focus group discussion participants from Kambwali village stated that 'The other reason why women prefer to deliver at home is to have their babies protected from “icifutato”, which is a situation where the baby may die if the father of the child recommences sex with a different woman other than his spouse who has not even recovered and healed after delivery of the child. So the women will prefer to deliver at home so that the baby after being born is bathed in water that is medicated to prevent death by “icifutato”. There are other traditional beliefs identified in this population from the study mentioned above (including the use of sorcery out of envy and malice to bring doom upon the couple) that prevent women from delivering at health centres as a way of ensuring the “wellbeing” of the mother and the child by carrying out certain traditional practices which cannot be carried out at health facilities.

In another study conducted in Kalomo, Southern Zambia by Sialubanje *et al*., a negative attitude towards nurses; low risk perception regarding their personal susceptibility to pregnancy and labour complications; dependence on husbands for financial support and decision making; social cultural norms (unwillingness to be delivered by a young nurse or a male staff of the health facility) were cited among reasons why women delivered at home [175]. Furthermore trust in the TBAs and being familiar with TBAs were given as reasons that encouraged women to deliver at home [175].

In contrast to low risk perception regarding pregnancy and labour complications, over 80% of young focus group discussion respondents, a few older women (mainly those who had given birth at a health facility), and all the midwives explained that most young women delivered at the health facility because they had no experience giving birth and that they were afraid of labour complications if they gave birth at home [175]. Although the study by Mwewa and Michelo did not explore reasons that influenced women to deliver at a Health facility as in the case of the study by Sialubanje *et al*., it is likely that women who deliver at

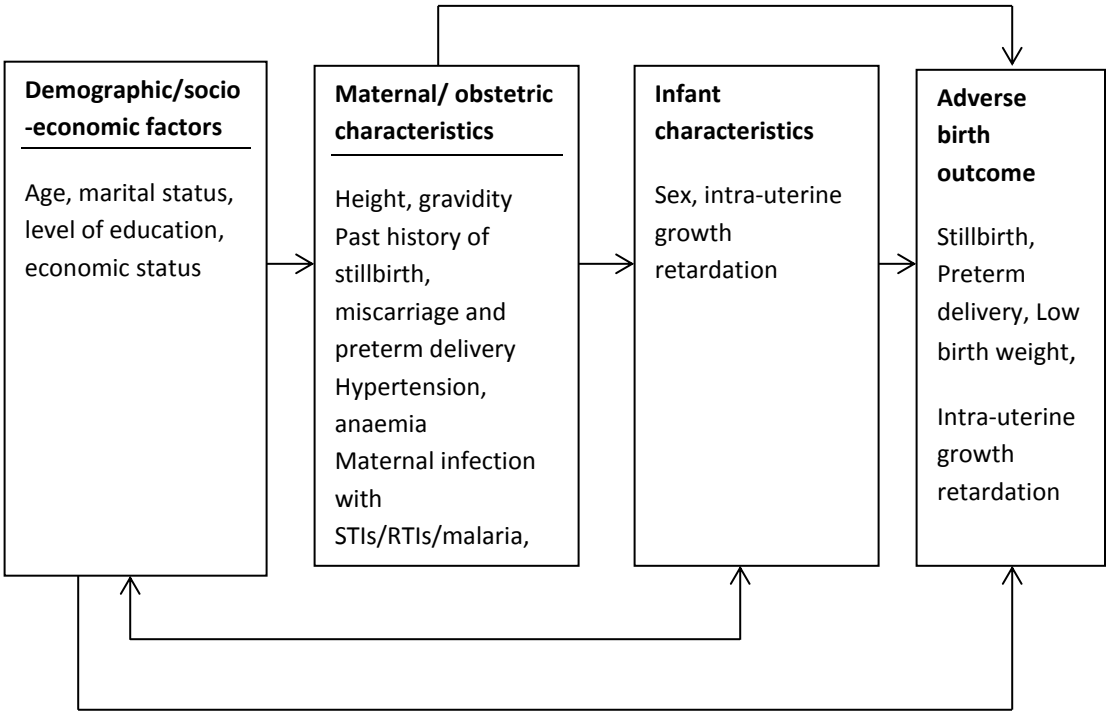
health facilities in the northern part of Zambia are encouraged to do so for similar reasons.

In 2010 the Zambian Ministry of Health announced that it had no intention of training TBAs because certain situations which expectant mothers faced required the attention of qualified medical personnel [176]. The ministry charged that TBAs contributed to high levels of maternal mortality rate in the country hence the need for expectant mothers to deliver from health centres. The Ministry of Health carried out massive sensitisation campaigns on the dangers of delivering from home through safe motherhood action groups [176]. In the study by Sialubanje *et al.*, all Focus group discussions and in-depth interviews were aware of the new policy but some TBAs in the focus discussion groups argued that women still delivered at home and some TBAs still conducted deliveries [175].

1.8.2 Prevalence of adverse birth outcomes in Zambia

The 2013-14 ZDHS reported the prevalence of LBW among deliveries at 10% in Zambia based on figures from Lusaka, Copperbelt and Southern Provinces [167]. A similar estimate was found in the Zambia Electronic Perinatal Record System (ZEPRS) study conducted across 25 facilities in the Lusaka public health sector from June 2007 to January, 2010. Records of 115,552 pregnant women showed that the proportion of LBW and PD was 10.8% and 34.0% respectively, and the incidence of stillbirth was at 24 per 1000 deliveries [177].

Figure 1. 6: Conceptual framework for adverse birth outcomes



Adopted from Olusanya and Ofovwe, 2010 [178]

Adverse birth outcomes that are generally described in literature in relation to curable STIs/RTIs and malaria and are of interest in this study are IUGR, PD, stillbirth and LBW.

In order to identify both distal and proximal risk factors associated with adverse birth outcomes an approach similar to the conceptual framework described by Olusanya and Ofovwe, 2009 [178] (Figure 1.1) was adopted. Under this framework it is assumed that demographic and social economic status directly or indirectly (via maternal and infant characteristics) influence birth outcomes. Maternal obstetric characteristics have direct impact on infant status. The variables of interest have been guided by evidence from published literature. Maternal socio-demographics included age, marital status, education, economic status, number of sexual partners and age at sexual debut. Maternal factors included height, past history of stillbirth, miscarriage and PD, hypertension,

anaemia, infection with curable STIs/RTIs, malaria and HIV. Infant factors included IUGR and sex. The sex of the baby is an independent determinant of ABO.

1.9 Rationale and objectives

1.9.1 Rationale

The individual prevalence of malaria and curable STIs/RTIs among antenatal care attendees in sub-Saharan Africa is considerable with the combined prevalence of curable STIs/RTIs being equal to, if not greater than malaria [6]. However, the prevalence of curable STIs/RTIs and malaria coinfection in pregnancy is entirely unknown. Despite the interest in the associations between malaria and birth outcomes, and also between curable STIs/RTIs and birth outcomes, no study has assessed the occurrence of malaria and curable STIs/RTIs coinfection and identified its risk factors.

Currently, the recommended antenatal care package includes IPTp with SP for the control of malaria, screening and treatment of syphilis, and syndromic management for other curable STIs/RTIs. However, in the context of emerging malaria parasite resistance to SP and the limited accuracy of the syndromic management, new strategies to control these infections in pregnancy are needed. One of the strategies proposed is preventive therapy that is safe and effective against malaria and curable STIs/RTIs. Azithromycin–based combination therapy offers these properties and is a possible candidate replacement for SP [179]. Thus characterising the prevalence of malaria, curable STIs/RTIs and their coinfection in pregnant women will not only fill this gap, but will also provide evidence in support of an integrated solution for prevention and control of these infections in pregnancy.

Despite the individual public health importance of malaria and curable STIs/RTIs, their impact on pregnancy outcomes when they occur together has not been assessed.

Measuring molecular markers of SP resistance is proposed to monitor the efficacy of IPTp-SP. Although the frequency of SP resistance markers at which IPTp would cease to be effective is unknown, monitoring trends of SP resistance markers is important, particularly in pregnant women where this information is limited [180]. Furthermore this information may support the need for finding alternative drugs for IPTp that may be effective against malaria and common STIs/RTIs.

Therefore this study has the potential to contribute to policy revision in the interest of reducing the public health impact of malaria and STIs/RTIs in pregnancy.

1.9.2 Objectives

The primary objective of this thesis was to estimate the prevalence of malaria, curable STIs/RTIs, and their coinfection among pregnant Zambian women.

Secondary objectives were as follows:

1. To identify risk factors for malaria, curable STIs/RTIs and their coinfection among pregnant Zambia women.
2. To determine the prevalence of DHFR and DHPS mutations associated with SP resistance among pregnant women.
3. To measure the *in vivo* efficacy and therapeutic failure of SP in pregnant women over a period of 28 days post-IPT.
4. To estimate the prevalence of ABO and identify their risk factors among pregnant Zambian women.

2 Methods

2.1 Chapter introduction

This chapter presents all the methods that were used in the study including design, sample size calculation, inclusion and exclusion criteria, enrolment procedure, data and sample collection, laboratory procedures and analyses.

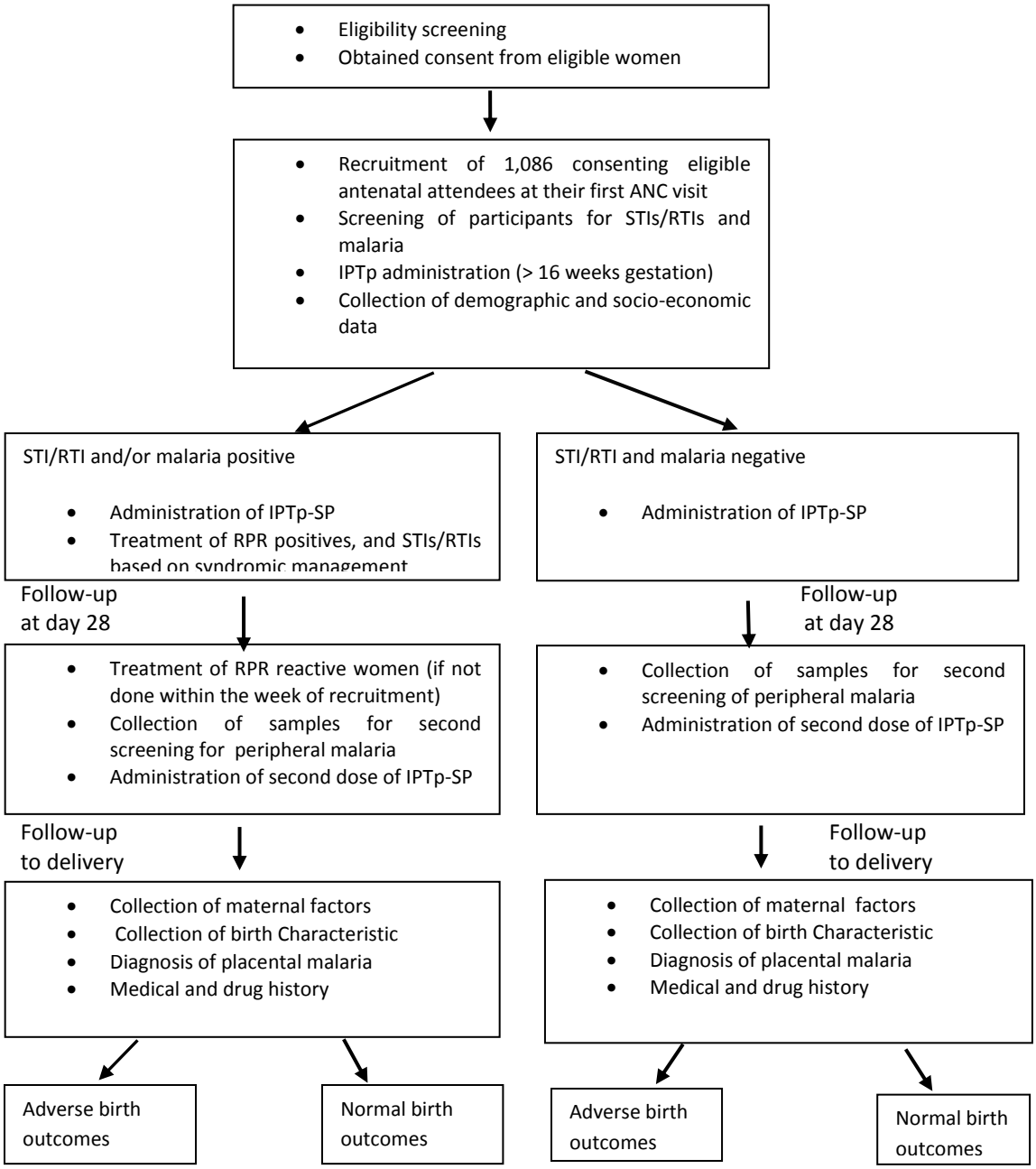
2.1.1 Study design and sample size

2.1.2 Study design

This was a prospective cohort study of antenatal attendees. Women were enrolled and observed alongside routine ANC activities. The study involved the following: (i) Screening of women for malaria and common curable STIs/RTIs at first antenatal visit; (ii) screening of women for malaria at day 28 post IPTp-SP, and (iii) following them up to delivery to capture delivery characteristics.

Figure 2.1 shows the flow of research activities that were conducted.

Figure 2. 1 Flow-chart of research activities



Consenting eligible pregnant women were recruited at their first ANC visit. Samples were collected for screening of curable STIs, bacterial vaginosis and malaria. Participants (at >16 weeks gestation) were given their first dose of SP for intermittent preventive treatment and demographic and socio-economic data was collected. Women were followed up after 28 days and screened for malaria and given a second dose of SP. At delivery placental tissue was collected for the diagnosis of placental malaria and birth characteristics, maternal factors and medical and drug histories were recorded.

2.1.3 Sample size calculation

The systematic review and meta-analysis by Chico *et al.* reported the following pooled prevalence estimates of curable STIs/RTIs and malaria from East and Southern Africa : syphilis 4.5%; gonorrhoea 3.7%; Chlamydia 6.9%; trichomoniasis 29.1%; BV 50.8%; placental malaria 25.8%; and peripheral malaria 32.0% [6]. As there were no data on the prevalence of malaria and curable STIs/RTIs coinfection from previous studies, it was assumed that the prevalence of malaria and any one curable STI/RTI is 10%. To measure a 10% prevalence with a 95% confidence limit around a +/-3% precision, a sample size of 843 women is required to estimate the proportion of pregnant women coinfected with malaria and at least one of the curable STIs/RTIs.

The 2013-14 Zambia Demographic Health Survey reported that the prevalence of LBW among deliveries in Zambia was 9% [181]. A similar estimate was found in the Zambia Electronic Perinatal Record System study conducted across 25 facilities in the capital city (Lusaka) public health sector from June 2007 to January, 2010. Records of 115,552 pregnant women showed that the proportion of LBW was 10.8%, PD was 34%, and the incidence of stillbirth was at 24 per 1000 deliveries [177]. Thus, to explore the determinants of ABO, it was assumed that 10% of the women would experience at least one ABO (PD, LBW, IUGR and stillbirth).

The incidence of ABO among women detected to have curable STIs/RTIs or malaria during antenatal period was expected to be higher than that among uninfected women. Thus the incidence of ABO in the unexposed women was assumed to be 7.5%. A study with 80% power and 95% confidence to detect risk factors that have an OR > 2 requires 984 women. To account for losses to follow up and withdrawals from the study, the sample size was increased by 10% [182]. Therefore, 1,086 women were recruited. Table 2-1 shows different assumptions and sample size calculations for the determination of risk factors for ABO.

Table 2- 1: Sample size calculation for determination of risk factors for adverse birth outcomes

Power	Proportion of unexposed with ABO	Odds ratios								
		1.5			2.0			2.5		
		Unexp.	exp	total	Unexp.	exp	total	Unexp.	exp	Total
70%	5	2688	896	3584	825	275	1100	438	148	584
	7.5	1866	622	2480	579	193	722	309	103	412
	10	1458	486	1944	459	153	612	249	83	322
80%	5	3423	1141	4564	1053	351	1404	558	186	744
	7.5	2373	791	3164	738	246	984	393	131	524
	10	1854	618	2472	582	194	776	315	105	420
90%	5	4599	1533	6132	1416	472	1888	750	250	1000
	7.5	3186	1062	4248	990	330	1320	528	176	704
	10	2487	829	3316	780	260	1,040	420	140	560

Unexp: Unexposed

Exp: Exposed

Sample size was calculated with 95% confidence and a 3:1 ratio of unexposed to expose. Exposure was infection with malaria, any STI/RTI or both.

To estimate the prevalence of SP resistance markers among pregnant women the sample size was estimated assuming that the prevalence of the quintuple mutation among pregnant women was 50% due to limited data. To detect a prevalence of 50% with 95% CIs of +/-10%, a sample size of 96 malaria positive women was needed.

The sample size needed to measure therapeutic outcomes in pregnant women receiving IPTp-SP was calculated based on the WHO treatment efficacy protocol which calculates sample size based on the expected proportion of treatment failures [183]. The proportion of treatment failure among pregnant Zambian

women is unknown. It was assumed that the proportion of therapeutic failures was 10%. To detect a 10% failure with 95% CIs of +/-5%, 138 asymptomatic malaria positive women were required.

2.2 Study area and sampling

2.2.1 Study area

Nchelenge is a rural district located in the Northern part of Zambia in the Luapula Province on the shores of Lake Mweru (Figure 2.2A) and has a population of 173,680 [184]. The district has a tropical climate with a rainy season (November to April) and a dry season (May to October).

Nchelenge District has 10 health centres, three health posts and one first level hospital [185]. The district was selected on the basis that it has a high prevalence of malaria and the prevalence of the individual STIs/RTIs of interest was expected to be moderate to high based on indications from RPR seropositives and HIV-infected women [186]. Luapula province has the highest prevalence of malaria in children under the age of five years with a prevalence of 38% by microscopy and 56% by rapid diagnostic test [187]. In a study conducted in March to April, 2012 in Nchelenge District among 782 children less than 10 years of age parasite prevalence by microscopy was reported at 30.2% (n = 236) [188]. Between 1990 and 2015, only two published studies both conducted in Luapula Province, have estimated the prevalence of malaria among pregnant women. One is a study conducted in Mansa in 2009 to 2010 and placental tissue samples of 37% (162/435) of delivering women showed histological evidence for malaria infection during pregnancy [90]. In another study conducted in 2013 in Nchelenge District, 22% of ANC attendees were positive for peripheral malaria by PCR [115].The prevalence of RPR-positive women at first ANC attendance was estimated at 10.0% [186]. The prevalence of HIV among ANC attendees at Kashikishi and Nchelenge health centres, in 2013 was 13.0% and 13.3%, respectively [186]. These estimates combined new cases and already known HIV positive cases.

Although other economic activities exist in Nchelenge District, the inhabitants are mainly fishermen and/or farmers. The population focuses on farming during periods of the fishing ban which is in effect from 1st December to 1st March each year.

The study was conducted in a catchment area of two health centres, Kashikishi and Nchelenge Health centres, with a total catchment population of 44,115 [185]. The community in the catchment area of two health centres, which served as recruitment sites, was selected due to the close location of the two health centres to the only hospital in the district. Figure 2.2B shows the location and the catchment area of the two health centres.

According to the 2013-14 Zambia Demographic and Health Survey, 94.6% of women aged between 15 and 49 in Luapula Province reported having received ANC from a skilled health care provider during pregnancy for their most recent birth in the preceding five-year period [167]. The coverage of IPTp-SP reported in the MIS at Provincial level was as follows; 89.7%, 76.6% and 57.6 % of women took at least one dose, two or more doses, and 3 or more doses of IPTp-SP, respectively [189]. At the time of the study, Kashikishi health centre offered laboratory, ANC and maternity services while Nchelenge Health Centre offered ANC services but not maternity services and depended on Kashikishi Health Centre for laboratory services.

The only hospital in the area is Saint Paul's Missionary Hospital. In 2013, 2620 deliveries were recorded at the hospital, 306 (11.7%) by caesarean section and 2314 were normal births. Of the recorded births, 2556 were live births and 64 (2.4%) were stillbirths, 29 (1.1%) macerated and 35 (1.3%) fresh stillbirths. Of the 2556 live births 564 (21.5%) were born with a LBW (≤ 2500). The system does not capture the number of preterm deliveries.

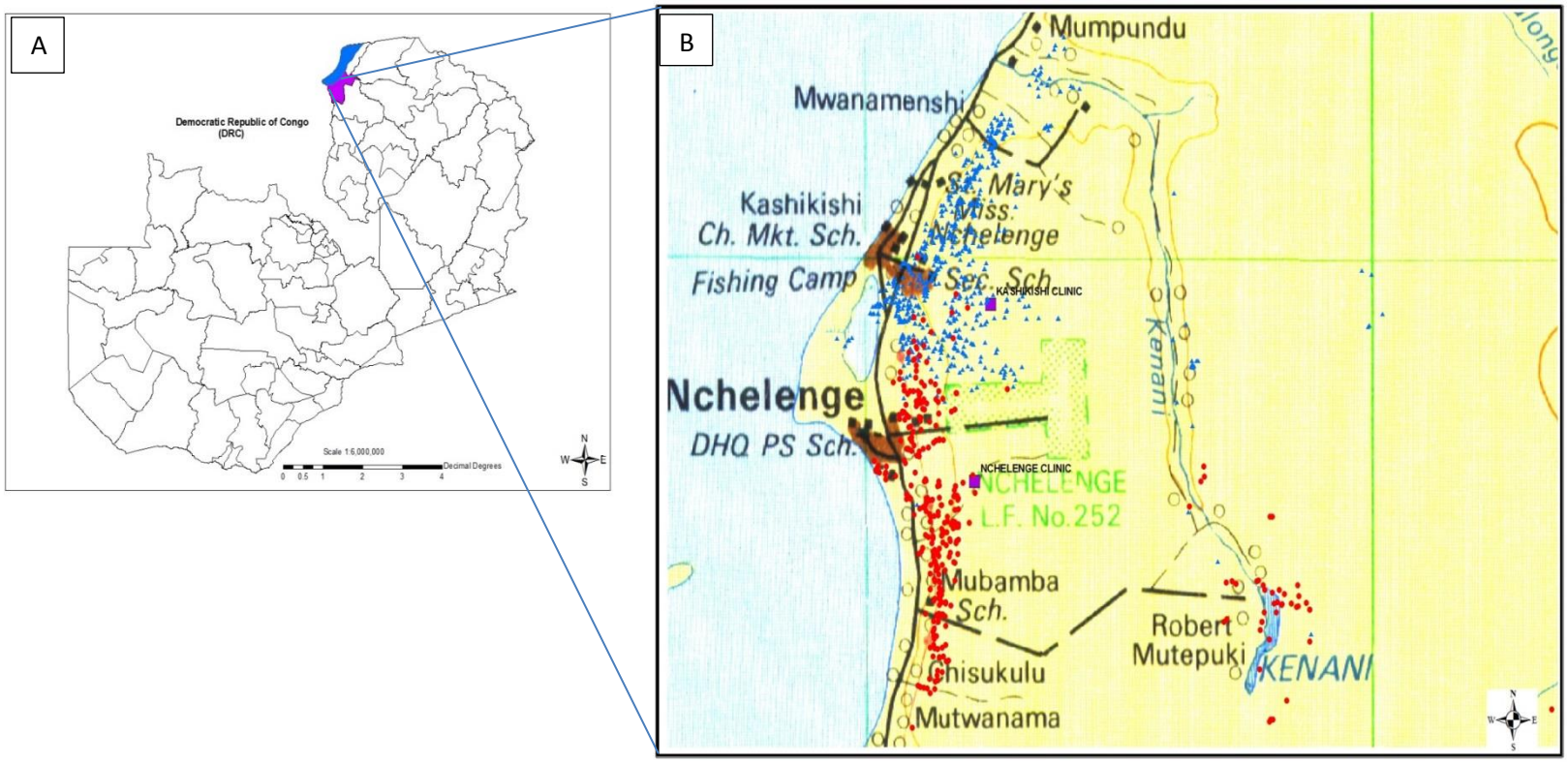


Figure 2. 2: Map showing the position of Nchelenge district in Zambia and the study area

Key: A-Blue area indicates the area within the borders of Zambia covered by Lake Mweru. Purple area indicates the location of Nchelenge District. B-Blue triangles indicates homes of women recruited at Kashikishi Health Centre, red dots indicate homes of women recruited at Nchelenge Health Centre, the two purple squares indicate the location of Kashikishi and Nchelenge health centres. Locations of participant homes and health centres were plotted using global positioning system coordinates. Maps: Courtesy of Ministry of Lands, Lusaka, Zambia

2.2.2 Sampling

Women visiting the two selected Health Centres for their first antenatal care visit between November 2013 and April 2014 were invited to join the study and enrolled consecutively until the desired sample size was reached. The participants who took IPTp-SP at enrolment were followed up at day 28 and screened for peripheral malaria. All women enrolled in the cohort were then followed to delivery.

2.3 Inclusion and exclusion criteria

2.3.1 Inclusion criteria

- Women of all ages attending the ANC for their first visit
- Gestational age ≤ 32 weeks
- Willing to take part in the study and give consent
- Agree to have their HIV test result from routine counselling and testing recorded by a member of the research team

2.3.2 Exclusion criteria

- Women reporting to have taken antimalarial and/or antibiotic treatment within the previous 4 weeks
- Women aged below 18 years without a guardian present to give written consent

2.4 Enrolment and antenatal care package

Enrolment and biological sample collections were conducted alongside the provision of routine ANC. Booking and ANC revisits were done once each on different days of the week at each health centre. Each week the ANC clinic for booking started with a health talk which covered various aspects of ANC including nutrition, curable STIs, HIV and malaria. At the end of the health talk the study was introduced to the whole group of first ANC attendees and their partners. It is worth noting that in the community where this study was conducted, attending ANC booking with spouses/partners has been encouraged over the years as a way of improving maternal health especially when dealing with issues relating to HIV and this was

generally accepted by community members. Therefore, women and their partners commonly undergo counselling and testing together.

Height, weight and blood pressure of each participant were measured and recorded on the ANC cards. HIV testing was routinely conducted using finger-prick blood with two rapid test kits (Determine® HIV-1/2 [Abbott Diagnostic Division, Hoofddorp, The Netherlands] and Uni-Gold™ Recombigen® HIV-1/2 [Trinity Biotech USA Inc. New York, USA]). Women were generally tested together with their spouses/partners. Individuals found to be seropositive using Determine® HIV-1/2 were then tested with Uni-Gold™; HIV status was concluded based on the result of the second test. Before determination of gestational age, ANC attendees were individually given copies of the information sheet which had been translated into Bemba (Appendix 2.1). Upon determination of a gestational age ≤ 32 weeks by last menstrual period and fundal height assessment, eligibility screening was completed by filling out the eligibility screening form (Appendix 2.2). Eligible women were then requested to give written consent, which they did after their assent, by signing two copies of the consent form (Appendix 2.3) or appending a thumbprint. In the case of women aged below 18 years, written consent was sort from their guardian if present after their assent. One copy was kept by the research team and a second copy was given to the participant together with a copy of the information sheet. Participants were at this point assigned an identification number which was used to anonymize all the paperwork and samples collected from a particular participant. A record of the participants' names and Identification number was kept separately by the study team. All study materials were kept in a private office and were only accessible to authorised research team members.

As part of the antenatal care package, all ANC attendees were given iron and folic acid supplements to last them up to their next ANC visit. First ANC attendees who had complaints indicative of STI/RTI were further assessed by a clinician for decisions on treatment. Women and/or their partners who tested positive for HIV for the first time were also assessed for eligibility to receive antiretroviral therapy by their regular clinician. All women with ≥ 16 weeks gestation were given IPTp-SP except those on cotrimoxazole prophylaxis due to toxicity concerns.

2.5 Data and sample collection

2.5.1 Data and sample collection at recruitment

One vaginal swab and one cervico-vaginal swab were collected from each participant by a trained midwife. Non-lubricated vaginal specula were used to collect the samples. The vaginal swab was collected using a dry cotton swab. The swab was placed in the vaginal cavity for 10 seconds and immediately rolled onto a slide for diagnosis of BV. A Dacron® (Medical Wire & Equipment, Wiltshire, England, United Kingdom) was used to collect a cervico-vaginal sample for the detection of *C. trachomatis*, *N. gonorrhoea* and *T. vaginalis* by PCR. The cervico-vaginal swabs for PCR were placed in cryo vials (Narang Medical Limited, New Delhi, India), labelled with each participant's ID and date of collection. The swabs from each participant were transported and stored at -20^{0}C for a few hours pending extraction which was conducted within 24 hours.

A questionnaire (Appendix 2.4) was administered to each of the enrolled women by a trained field worker in a private room. Gestational age was then measured by ultrasound using crown-rump length in first trimester pregnancies and a combination of biometric parameters (biparietal diameter, head circumference, abdominal circumference, and femur length) in the second and third trimester pregnancies. Women with a gestational age > 32 weeks were excluded at this point and counted among those who were ineligible. Peripheral and venous blood (approximately 4ml) was collected from each participant for malaria and syphilis screening, respectively. Peripheral blood was collected via finger pricking and venous blood was collected from the arm. Approximately 160µl of peripheral blood was placed on four circles of Whatman® filter paper and labelled with the participant ID number. The thick smear and blood spots were later used for the diagnosis of malaria by microscopy and polymerase chain reaction (PCR), respectively.

2.5.2 Collection of global position system coordinates

Landmarks near the homes of participating women and home addresses of all the participants were collected at enrolment. Members of the research team visited the homes of participants guided by the information collected at recruitment. Permission to visit a participant's home was obtained at recruitment. The reason for

the visit was to collect global positioning system coordinates of participants' homes for the purpose of locating women at the time of delivery if they required transportation to the hospital.

2.5.3 Follow-up at day 28

This part of the study required 138 asymptomatic malaria-positive women in order to determine prophylactic and therapeutic failure of IPTp-SP over a period of 28 days after SP administration under direct observation. Only women who were eligible to receive SP i.e., those who presented with a gestational age ≥ 16 weeks and were not currently receiving cotrimoxazole were included in this part of the study. At the follow up visit at day 28, peripheral blood for a thick smear and for PCR diagnosis of malaria was collected from participants who had taken SP at day 0. Blood sample collection was only done after verbal confirmation that a participant had not received anti-malarial treatment besides the IPTp-SP given at the time of enrolment. The second dose of SP was administered and efforts to collect samples from participants who did not show up 28 days post-IPT were made through home visits on the same day. Participants who developed malaria symptoms before 28 days were tested and treated by the health centre staff following national guidelines as routinely done. Such cases were not followed up at day 28 post-IPTp-SP.

2.5.4 Data and sample collection at delivery

Participants were followed to delivery and placental blood and tissue were collected by midwives. The midwives at the Kashikishi Health centre and Saint Paul's Missionary Hospital were informed of the study from the start and were trained on how to identify participants from their ANC cards/records and collect placental samples and delivery characteristics. Instructions on sample collection were also made available to the maternity ward staff. Placental blood for the measurement of haemoglobin was collected by making an incision in placental tissue using a blade and blood was drawn using a syringe and immediately placed in Ethylenediaminetetraacetic acid tubes for determination of haemoglobin level. Haemoglobin was read using an ABX Micros ES60 (HORIBA ABX SAS). Placental tissue samples were collected as follows: A 2cm x 2cm x 1cm specimen was taken from the

maternal side, and placed in 10% formalin filled bottles labelled with the patient's ID number and date of collection. Delivery characteristics and drug history were collected at delivery using the second part of the questionnaire (Appendix 2.4).

New born babies were weighed and birth characteristics recorded. Stillbirth was defined as foetal death at ≥ 28 weeks gestation [190], PD as delivery at < 37 weeks, LBW as birthweight < 2500 grams and IUGR was defined as an infant with LBW born at ≥ 37 weeks gestation [36, 61]. Gestational age after birth was recorded based on earlier assessment at enrolment by ultrasound.

To ensure that major outcomes were captured in most of the deliveries, two mobile phone numbers belonging to team members were given to participants to call at the time they went into labour and required transport to the hospital/health centre. Those who called for transport were located with the help of global positioning system coordinates collected earlier in the study. Participants were also urged to call if they experienced a delivery, late miscarriage or stillbirth at home. A driver and a fieldworker followed-up such cases to collect samples and data at delivery and/or provide transport to the hospital where the mothers were attended to and samples and data were collected. A study completion form was filled out for each participants and their completion of the study or loss to follow-up was documented (Appendix 2.5)

2.6 Laboratory testing and quality control

2.6.1 Syphilis testing and treatment of seropositives

Diagnosis of syphilis by rapid plasma reagin (RPR) for samples collected at both Nchelenge and Kashikishi Health centres was conducted at Kashikishi Health Centre laboratory. Syphilis testing was done using rapid plasma reagin (RPR) (Omega Diagnostics Limited, Alva, Scotland, United Kingdom). Since it was not possible to have RPR test results on the same day, results for syphilis test were ready the day after sample collection. Seropositive women were followed up (incorporated within the global positioning coordinate collection exercise explained above) within three days and presented with a note to return to the health centre with their partner for treatment with benzathine penicillin G (2.4 million units IM weekly x 3 doses) according to the national STI policy. Those who did not show up were started on

treatment at the day 28 follow-up if they returned to the health centre. A second note was delivered to those or the homes of those who did not return at day 28 for them to return to the health centre with their partner for treatment.

For quality control 55/1077 (5%) serum samples were taken to the national STI reference laboratory at the University Teaching Hospital (UTH). Testing of all RPR seropositive samples with *Treponema pallidum* haemagglutination assay (TPHA) for confirmation of syphilis and RPR reactivity at 1:8 titre were also conducted at the national STI laboratory.

2.6.2 Malaria diagnosis by microscopy

Thick blood films were stained using 10% Giemsa at Kashikishi Health Centre Laboratory. Slides were there after transported to the National Malaria Control Centre for reading by two independent microscopists. Parasite density was determined by assuming 8000 white blood cells (WBCs) per µl and counting the number of parasites per 200 WBCs. In cases where fewer than nine parasites were counted against 200WBCs, parasites were counted against 500WBCs. Two-hundred high power fields were read before declaring a slide negative. In the event that discrepant results were observed between the two microscopists, a third observer read the slide to reach an agreement on diagnosis and parasite count.

2.6.3 Diagnosis of placental malaria by histology

Formalin fixed placental tissue specimens were sent to the UTH histopathology laboratory for processing. Fixed placental biopsies were wax embedded, and two sections were stained with Gurr's modified Giemsa and another two with haematoxylin and eosin. Slides were examined by a qualified pathologist for the presence of parasites, pigment or both. Placental histology was classified as described by Rogerson *et al*, 2003 [191]. The classes were as follows: (1) Parasites, no pigment in monocytes or fibrin; (2) Parasites, pigment in monocytes +/- fibrin Parasites; (3) Parasites, pigment in fibrin, (4) No parasites, pigment only (past infection) and (5) No parasites or pigment (no infection). For quality control, the

reading of 6.5% (46/702) randomly selected slides was repeated by a second observer. Any disagreements were resolved by a third observer.

2.6.4 Diagnosis of bacterial vaginosis by microscopy

The vaginal smear samples for the diagnosis of BV were air dried and Gram stained using safranin as a counter stain. Gram staining of slides was conducted at the St. Paul's Mission Hospital laboratory and thereafter slides were transported to the University of Zambia for reading. Results were classified based on the Nugent criteria [120] and recorded in the appropriate result record form (Appendix 2.6). For quality control (5%) 55/1085 randomly selected slides were transported to the UTH Microbiology laboratory for repeat reading.

2.6.5 Nucleic acid amplification

In this study, in-house standard end-point PCR assays were employed to detect the presence of the *C. trachomatis, N. gonorrhoea* and *T. vaginalis* in cervico-vaginal swabs; and *P. falciparum* in dried peripheral blood spots.

DNA extraction from cervico-vaginal swabs was conducted within 24 hours of collection and the extracts from each sample were stored at -20^{0}C pending molecular detection by PCR. Samples were then transported on ice to the Tropical Gastroenterology and Nutrition Group laboratory at UTH where molecular detection of *C. trachomatis*, *N. gonorrhoea* and *T. vaginalis* was conducted. In the case of *P. falciparum,* dried blood spots were transported to the Tropical Gastroenterology and Nutrition Group laboratory for extraction and molecular detection by PCR.

2.6.5.1 PCR primers

Plasmodium falciparum was detected using the Snounou primers [94]. For the detection of *N. gonorrhoea*, primers targeting the *orf1* gene (*Ngu1* and *Ngu2*) were employed to amplify a 260 base pair (bp) product specific for *N. gonorrhoea* [192]. Primers KL1 and KL2 were used to amplify a Chlamydial plasmid 241 bp fragment for the detection of *C. trachomatis* [193]. Plasmid based PCR reactions for the detection of *C. trachomatis* have been shown to be 10 to 1000 more sensitive than procedures

which use the major outer membrane protein and ribosomal DNA as templates for PCR amplification [194, 195] and have been used for the detection of *C. trachomatis* in clinical samples [196]

Primers based on *T. vaginalis* repeated DNA target for PCR identification have been used for the molecular detection of *T. vaginalis* [197, 198]. The primer pair, TV3 and TV7, was used to amplify a 300bp piece of the genome. This primer set has been demonstrated to have higher sensitivity than other primer sets [199]. The primer names and sequences for these four organisms are as shown in Appendix 2.7.

2.6.5.2 DNA extraction and PCR for malaria parasite detection

Filter paper containing blood spots were air dried and individually stored in envelops at room temperature pending DNA extraction for the diagnosis of malaria by PCR. Parasite DNA extraction was carried out using the chelex method as described previously [200]. The extract was transferred in approximately 100 µl aliquots to storage vials and were stored -20 ^{0}C pending PCR.

The detection of *P. falciparum* was carried out using a nested PCR method as described by Snounou *et al*., [201] with modifications to the PCR parameters [202]. Briefly, all PCR reactions were carried out in total volumes of 25µl using Thermo Scientific® Dream Taq PCR Master Mix (2X) and 0.5µM of each primer and 2µl of template. A 2µl of the primary amplicon was used as a template in the secondary reaction. The PCR parameters used for both the primary and secondary reactions were as shown in Appendix 2.8.

2.6.5.3 Detection of SP resistance markers

To estimate the prevalence of SP resistance among first ANC attendees, a random sample of 96 was selected from among the *P. falciparum* malaria positive samples detected by PCR. Ninety-six was the required sample size for the characterisation of SP resistance markers in the study area. Therefore, only 96 samples were processed for this part of the study.

Parasite DNA template extracted earlier for malaria detection was used for this part of the study. Nested PCR and enzyme restriction digestion as previously described [203] were employed to detect antifolate polymorphisms in the DHFR and DHPS

genes. Names and sequences of the primers that were used are shown in Appendix 2.9. The names of the enzymes that were employed and their expected activity depending on the whether the amplicon carried the wild type or mutant strain were as shown in the Appendix 2.10.

Negative and positive controls were included in every batch and put through the entire process from amplification electrophoresis. Secondary PCR amplicons were analysed by gel electrophoresis to confirm amplification and band intensity before enzyme digestion. Amplicon and restriction fragments were analysed on ethidium bromide agarose gels and visualised under UV light on a Biosens (Genescope V1.76) digital imaging system.

2.6.5.4 Determination of *in vivo* efficacy and prophylactic effect of sulphadoxine-pyrimethamine

The pair of blood samples collected at recruitment and 28 days post-IPT visit were used to determine the *in vivo* efficacy to clear parasitaemia and the post-IPT prophylactic effect of SP. The sample size required for this part of the study was 138 asymptomatic malaria positive pregnant women. A woman with a malaria positive blood smear at first ANC visit and found to have a malaria positive blood slide at day 28 post-IPT visit, was classified as a case of therapeutic failure of SP. It was assumed that after a participant with parasitaemia initially receives SP, subsequent detection of parasitaemia 28 days post-IPT was caused by either parasite strains present before SP administration (recrudescence) or parasite strains acquired after treatment (reinfection). Therefore, therapeutic failure cases were further classified as re-infection or recrudescence by PCR analysis. To differentiate between recrudescence and re-infection in women who tested positive at both the initial (day 0) and second screening (day 28), genotyping was done to examine genes coding for the merozoite surface protein 2 (MSP2) [204, 205]. Women who had a negative blood slide at visit one and became slide positive at day 28 post-IPT were classified as cases of prophylactic failure of IPTp-SP.

Polymorphic regions of MSP2 were amplified by nested PCR. The primary PCR primers corresponding to the conserved sequence flanking this region and the secondary PCR primers were used to amplify the IC3D7 and FC27 allelic families of

msp-2. For controls DNA from HB3 and 3D7 laboratory strains was used. Appendix 2.11 presents the sequences of the primers that were used for this part of the study.

The secondary amplicon from each sample was analysed using electrophoresis on 2% ethidium bromide stained agarose gels. Samples from an individual participant were loaded in adjacent lanes. In cases where there was no amplification, PCR was repeated using 3 times the quantity of template DNA. In cases where no amplicon was detected after the second reaction, amplification was classified unsuccessful. Alleles were considered the same for day 0 and day 28 within 10 base pairs. Reinfection was defined as having completely different alleles between parasites from day 0 and day 28. If a similar allele was found between parasites at 0 and at day 28 this was classified as recrudescence. Comparisons of band sizes were done using a manual method by two different qualified molecular biologists and any discrepancies were settled by a third person.

2.6.5.5 DNA Extraction and detection of curable STIs/RTIs by PCR

Extraction of DNA from cervico-vaginal swabs was done within 24 hours of sample collection using the Quick-gDNA™ (Zymo Research Corp, California, USA) Miniprep kit following the manufacturer's instructions. The extract was stored at -20^{0}C awaiting nucleic acid amplification.

PCR reactions for the detection of the three organisms, *C trachomatis*, *N. gonorrhoea* and *T. vaginalis* were set up separately for each sample. All PCR reactions were carried out in total volumes of 25µl using Thermo Scientific® Dream Taq PCR Master Mix (2X) and 1µM of each primer and 4µl of template. Amplicon was resolved on 2% ethidium bromide agarose gels and visualised under UV light.

All the PCR parameters that were employed in this study are as indicated in Appendix 2.8.

2.6.6 Quality control of PCR methods

All the PCR assays were tried on known positive samples before they were used on research samples. Positive and negative controls were included at the extraction, amplification and electrophoresis stages. A negative and a positive control were

included in every batch of 46 samples. Five percent (55 samples) of the samples were randomly selected and processed using the Seeplex® STI Master Panel 1 (V2.0) [Seegene Technologies Inc. California, USA]. The Seeplex® STI Master Panel 1 by Seegene is a multiplex conventional PCR system for the detection of seven organisms including *C. trachomatis*, *N. gonorrhoea* and *T. vaginalis* from urine, vaginal swabs and liquid based cytology specimens.

2.6.7 Quality assurance of data and sample collection

The questionnaire was translated into Bemba, the language that is commonly spoken in the study district. The questionnaire was pretested among 30 eligible participants at an ANC booking to determine pattern of response and clarity of the questions. Questions were rephrased, removed or added where necessary.

To ensure that good quality data was collected, research assistants were trained in data collection. Assistants were urged to ensure that questionnaires were checked for missing data before releasing the participant. The collection of cervico-vaginal samples was restricted to midwives after training on how samples were to be collected for diagnosis of BV and other STIs/RTIs. Other biological samples were collected by trained laboratory technicians who routinely carry out procedures such as venous and peripheral blood collection and slide preparation.

2.7 Ethical consideration

The study protocol was reviewed and approved by the University of Zambia Biomedical Research Ethics Committee, reference number 004-02-13 (Appendix 2.12) and the London School of Hygiene and Tropical Medicine Observational/Interventions Research Ethics Committee (reference number 6292) before the research was carried out. In addition, the Ministry of Health (MoH) in Zambia also reviewed the research proposal after the ethical approval had been granted. The research project was then discussed with relevant local authorities in the study district such as the district health management team, community leaders and the in-charge personnel at the two health centres.

At the beginning of each ANC booking session an explanation of the project was given to the group of first ANC attendees and their spouses/guardians/partners in

addition to the routine weekly health talk. The information included the aims of the study, the methods of data and sample collection, number of participants required, potential advantages and disadvantages of participation and the expected benefits of carrying out the research. The study population was also assured of confidentiality. After the details of the project had been explained potential participants were informed that participation was voluntary and participants could withdraw at any time. There was no age cut off for exclusion because inclusion of younger pregnant women was considered to be important in order to have better representation of pregnant women. Written consent was sort from guardians of women aged below 18 years if present after their assent. To ensure confidentiality interviews were conducted privately and each participant was assigned a unique identity number that was used to anonymise biological samples, questionnaires, and result record forms.

Participating women received everything that the ANC package routinely offered at the time of the study including iron and folic acid tablets. Women who were found RPR reactive were given a note to return to the health centre with their sexual partner for treatment. Pregnant women who had complaints of symptoms associated with STI/RTI were assessed by the regular clinician and managed based on their symptoms as part of the recommended ANC package. Women and/or their partners who tested HIV positive for the first time underwent assessment for eligibility to receive antiretroviral therapy and cotrimoxazole prophylaxis as per routine procedures. All women, except those who were receiving cotrimoxazole prophylaxis at the time**,** were provided IPTp-SP according to national guidelines. In the rare event that a woman showed up for ANC booking with symptomatic malaria, routine laboratory testing was done using a rapid diagnostic test kit (SD Bioline Malaria Ag P.f/Pan brand; Standard Diagnostics, Inc.) and treatment was administered based on national policy guidelines. Molecular diagnosis of Chlamydia, trichomoniasis and gonorrhoea was done in the latter part of the project and results were only available after the participants had delivered. Results were sent back to the two health centres and participants were who were traced were given a note to return to the health centres with their partners for treatment.

2.8 Data Processing and Analysis

Data were double-entered in EpiData version 3.1 software [206] , cleaned, processed and analysed using Stata Software version 13 [207]. Cleaning involved visual checks of entered data for consistence and valid values. Frequencies of all variables were generated to check for missing data. Variables were recoded and composite variables were generated. Data on sources of income, level of education and fixed and durable assets were used to create an index of household wealth using principal components analysis [208]. The malaria and STI/RTI coinfection variable was generated from a combination of six outcomes, five curable STIs/RTIs and malaria. Coinfection was defined as infection with malaria and at least one of the five STIs/RTIs. High blood pressure (BP) was defined as systolic blood pressure ≥ 140mm Hg and/or diastolic BP ≥ 90 mm Hg. Hypertension was defined as two high BP readings [209]. A variable was created from BP readings collected at recruitment and before delivery but during labour. Adverse birth outcomes that were captured in this study were LBW, IUGR, stillbirth and PD. A new variable for composite ABO was generated from the four outcome variables and was defined as experiencing at least one of the four birth outcomes mentioned above. New variables were generated for the DHFR triple (Ile-51 + Arg-59 + Asn-108), DHPS double mutant (Gly-437 + Glu-540), quintuple (DHFR triple + DHPS double) and sextuple (quintuple + Gly-581) mutants.

Therapeutic efficacy was defined as aparasitaemia in a previously parasitaemic woman 28 days post-IPTp-SP without recrudescence. Women who were parasitaemic upon enrolment and were screened for malaria at day 28 were included in this analysis. Women who tested positive at day 0 and at day 28 were further separated into cases of recrudescence and reinfection. Therapeutic failure was defined as testing positive at day 0 and at day 28 due to recrudescence.

Prophylactic failure was defined as parasitaemia in a previously aparasitaemic woman 28 days after IPTp-SP administration. Women found aparasitaemic at recruitment and were tested for malaria at day 28 and those who tested positive at day 0 and were re-infected by day 28 were included in this analysis.

Quantitative variables (age and parasite count) were summarised using means and median. Characteristics of study participants were described using percentages and the Chi-squared test was used to assess differences in proportions between women

recruited at the two sites and between women followed up to delivery and those who were lost to follow-up. Standard statistical tests (i.e. Mann-Whitney *U* test, kruskal-wallis test, t-tests and analysis of variance were used to assess significance ($P < 0.05$) of differences in the distribution of continuous variables and comparison of means. Non-normally distributed variables were log-transformed before conducting t-tests or analyses of variance. The prevalence of malaria infection, determined by microscopy or by PCR, and their 95% CIs were estimated. The prevalence of the main outcomes (malaria, curable STIs/RTIs, malaria and curable STI/RTI coinfection, and ABO) and their 95% CIs were estimated. The prevalence of these outcomes was also estimated according to HIV status. The prevalence of malaria and coinfection was also estimated according to parity.

For malaria, the pre-specified outcome was malaria prevalence as determined by PCR. Results obtained from PCR diagnosis were used to assess potential risk factors for malaria infection. Potential predictors identified from literature were used to determine risk factors for infection in this study. Univariate analyses of potential predictors of: (i) Malaria infection; (ii) Infection with at least one STI; (iii) infection with BV, and (iv) coinfection with malaria and at least one STI/RTI, were conducted using logistic regression. Crude odds ratios were estimated. Potential predictors that showed significance at 10% were entered into a multivariable model and assessed using a likelihood ratio test. Factors that were found to be independently associated with infection at $P < 0.05$ were entered in a final model and adjusted odds ratios were obtained. Independent variables in final models were checked for interaction using a likelihood ratio test. The criterion for interaction was $P < 0.01$.

Mean birth weight, mean Hb and median gestational age were compared between the uninfected class and each of the other three pathology classes. Proportions of different ABO were estimated among the different placental pathology Chi-squared test was used to check for association between pathology class and ABO.

The prevalence of individual ABO and composite ABO among delivering participants and their 95%CIs were estimated. Risk factors for composite ABO were explored using univariate and multivariate logistic regression as described above. The variables for recruitment site, malaria, STIs/RTIs and their coinfection were included in the final model to assess whether their individual effect on birth outcome was

modified by the site of recruitment. Risk factors for individual ABO (PD, LBW and IUGR) were also explored using univariate and multivariate logistic regression as described above.

The prevalence estimates of the DHFR triple, DHPS double; DHFR + DHPS quintuple and sextuple mutants and their 95% CIs were calculated. Proportions of women in whom parasitaemia at day 0 was cleared by day 28 and those who experienced therapeutic and prophylactic failure were quantified.

3 Results (1): Maternal characteristics and prevalence estimates of malaria, STIs/RTIs and their coinfection

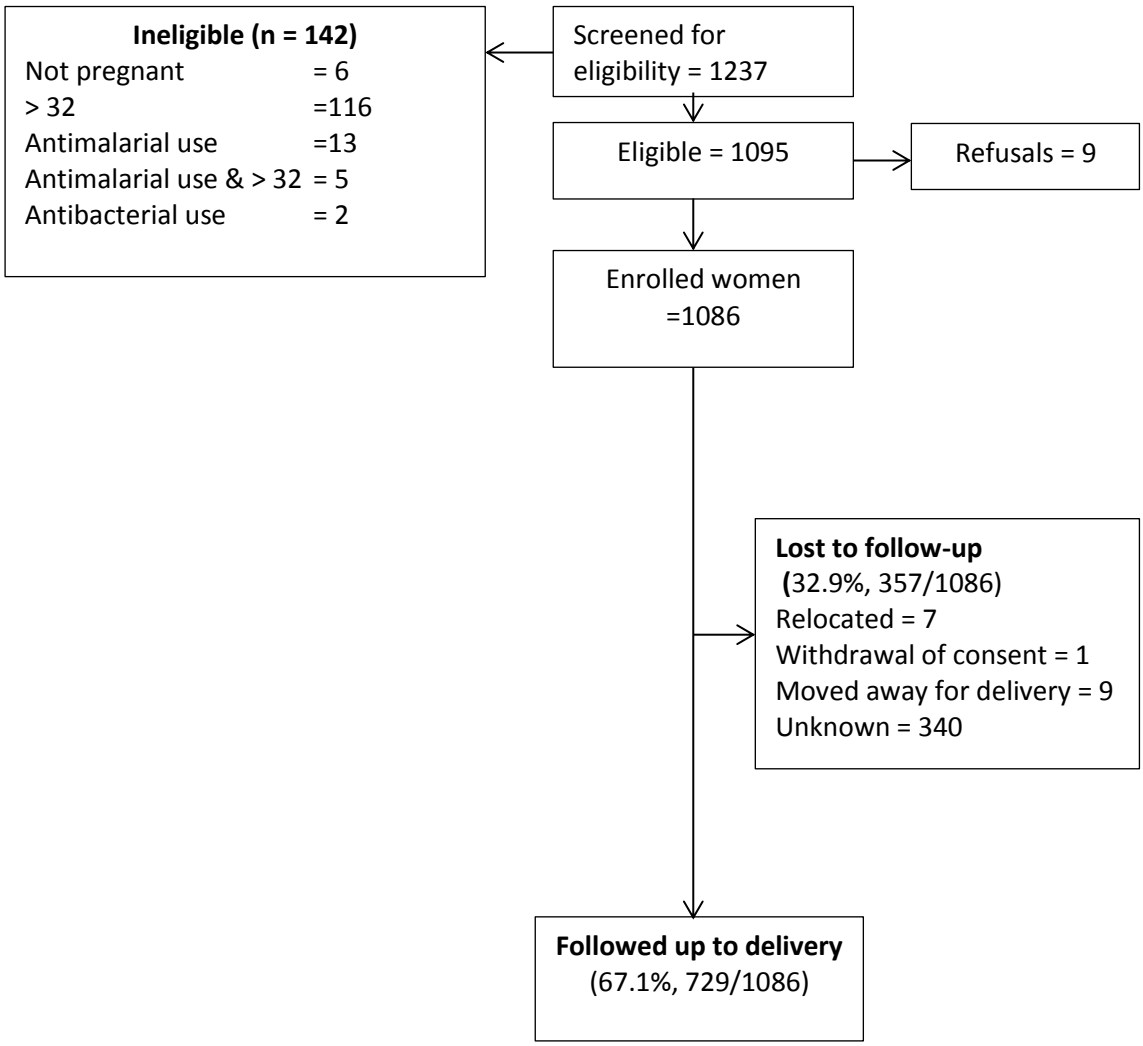
3.1 Chapter introduction

This chapter presents the participation distribution and socio-demographic characteristics of study participants. Characteristics are presented according to recruitment sites for assessment of any differences in proportions between the two sites. The chapter also describes prevalence of malaria, STIs/RTIs and their coinfection and HIV infection. The prevalence of malaria only and malaria and STI/RTI coinfection are presented according HIV infection as well as gravidity. STIs/RTIs are presented according to HIV status. Results are presented in this manner for the assessment of whether these factors modulate the prevalence malaria, STIs/RTIs and their coinfection in this study population.

3.2 Participation distribution

A total of 1086 women were recruited and 67.1% (n = 729) were successfully followed up to delivery. Details of the distribution of participants at recruitment and follow-up to delivery are presented in Figure 3.1.

Figure 3. 1: Participation flow-chart



Note: The loss to follow-up on the actual sample size (984) was 24%.

3.3 Socio-demographic characteristics of participants

Table 3-1 presents characteristics of study participants and their respective frequencies. The youngest participant was 14 and the oldest was 47 years old. The median age of participants was 25 years (Interquartile range [IQR], 20-30) and the median gestational age was 22 weeks (IQR, 19-25.5). Almost a quarter (24.1%) of women who were enrolled were primigravidae, 15.2% were secundigravidae and more than half (60.7%) were multigravidae.

The majority of these women, 80.6% (n = 874) were married and 40.7% (n = 426) reported six or fewer years of schooling. Nearly half of the 49.2% (n = 534) of the women reported ownership of a bed net (treated or untreated). Of 531 bed net owners, 78.3% (n = 416) reported to have slept under a bed net the previous night. Overall only 38.5% (416/1082) of participants slept under a bed net on the previous night. Indoor residual spraying had been applied to the homes of 20.9% (n = 226) of women at least once in the previous 12 months.

There were no significant differences in the socio-demographic characteristics (age, marital status, years of schooling, bed net use and wealth index) between women recruited at Kashikishi and at Nchelenge health centre. The pregnancy related factors (gravidity, past experience of miscarriage, past history of stillbirth or PD) and clinical symptoms and treatments (symptoms of STI/RTI, HIV status, and use of antimalarial drugs prior to ANC visit) also did not differ between the two sites. However, Nchelenge site had a higher prevalence of peripheral malaria parasitaemia than Kashikishi (PCR positive malaria 67.4% versus 53.5%, $P < 0.001$; microscopy positive malaria 37.6% versus 29.1%, $P = 0.006$).

Table 3- 1: Baseline characteristics of first ANC attendees of Nchelenge District, Zambia, according to recruitment site

variable	All (N = 1085) n (%) or median (IQR)	Kashikishi (n = 747) n (%) or median (IQR)	Nchelenge (n = 338) n (%) or median (IQR)	*P*-value
Age (median)	25 (20-30)	24 (20-30)	25 (20-31)	0.133
Marital status				
Single	203 (18.7)	134 (17.9)	69 (20.4)	0.591
Married	874 (80.6)	607 (81.3)	267 (80.0)	
Widowed/divorced	8 (0.7)	6 (0.8)	2 (0.6)	
Years of schooling				
None to 6	426 (39.3)	287 (38.4)	139 (41.1)	0.398
7 and above	659 (60.7)	460 (61.6)	199 (58.9)	
Gravidity				
Primigravidae	261 (24.1)	183 (24.5)	78 (23.1)	0.657
Secundigravidae	165 (15.2)	117 (15.6)	48 (14.2)	
Multigravidae	659 (60.7)	447 (59.8)	212 (62.7)	
Bed net ownership				
No	551 (50.8)	367 (49.1)	184 (54.4)	0.105
Yes	534 (49.2)	380 (50.9)	154 (45.6)	
Bed net usage				
No	666 (61.6)	451 (60.6)	215 (63.6)	0.349
Yes	416 (38.4)	293 (39.4)	123 (36.4)	
Missing*	3	3	0	
IRS in previous 12 months	808 (78.1)	563 (75.7)	245 (72.7)	0.851
No	226 (21.9)	156 (21.0)	70 (20.8)	
Yes	51	28	26	
Missing*				
Wealth quintile				
Lowest	217 (20.0)	135 (18.1)	82 (24.3)	0.089
Second	221 (20.4)	151 (20.3)	70 (20.7)	
Middle	214 (19.8)	155 (20.8)	59 (17.5)	
Fourth	215 (19.9)	158 (21.20	57 (16.9)	
Highest	216 (19.9)	146 (19.6)	70 (20.7)	
Missing*	2	2	0	
Past experience of miscarriage				
No	708 (85.1)	482 (85.3)	226 (87.3)	0.455
Yes	116 (14.1)	83 (14.7)	33 (12.7)	
NA (Primigravidae)*	261	182	79	
Past experience of PD				
No	784 (95.3)	538 (95.4)	246 (95.0)	0.797
Yes	39 (4.7)	26 (4.6)	13 (5.0)	
NA (Primigravidae)*	261	182	79	
Missing*	1	1	0	

Table 3-1: Baseline characteristics of first ANC attendees of Nchelenge District, Zambia, according to recruitment site continued

Variable	All (N = 1085) n (%)	Kashikishi (n = 747) n (%)	Nchelenge (n =338) n (%)	*P*-value
Prior delivery of a still born No Yes NA (Primigravidae)*	755 (91.6) 69 (8.4) 261	521 (92.2) 44 (7.8) 182	234 (90.4) 25 (9.7) 79	0.370
Prior delivery of a still born No Yes NA (Primigravidae)*	755 (91.6) 69 (8.4) 261	521 (92.2) 44 (7.8) 182	234 (90.4) 25 (9.7) 79	0.370
Prior delivery of a Preterm baby No Yes NA (Primigravidae)* Missing*	784 (95.3) 39 (4.7) 261 1	538 (95.4) 26 (4.6) 182 1	246 (95.0) 13 (5.0) 79 0	0.797
Antimalarial treatment ‡ No Yes Missing*	1005 (93.1) 74 (6.9) 6	693 (93.2) 51 (6.9) 3	312 (93.1) 23 (6.9) 3	0.995
STI/RTI symptoms complaints among all No Yes	951 (87.7) 134 (12.4)	650 (87.0) 97 (13.0)	301 (89.1) 37 (10.9)	0.345
STI/RTI symptoms complaints among infected No Yes NA (Negative)*	602 (88.0) 98 (14.0) 384	415 (85.4) 57 (14.6) 260	187 (87.4) 27 (12.6) 124	0.487
HIV status Negative Positive Missing*	941 (86.8) 143 (13.2) 1	643 (86.2) 103 (13.8) 1	298 (88.2) 40 (11.8) 0	0.374
ART among HIV Positives No Yes NA (HIV-negative)*	82 (57.7) 60 (42.7) 941	58 (56.9) 44 (43.1) 643	24 (60.0) 16 (40.0) 298	0.734

Table 3-1: Baseline characteristics of first ANC attendees of Nchelenge District, Zambia, according to recruitment site continued

Variable	All (N = 1085) n (%)	Kashikishi (n = 747) n (%)	Nchelenge (n =338) n (%)	*P*-value
Microscopy				
Negative	736 (68.2)	525 (70.9)	211 (62.4)	0.006
Positive	343 (31.8)	216 (29.1)	127 (37.6)	
Missing*	6	6	0	
STI/RTI				
Negative	384 (35.4)	260 (34.9)	124 (36.7)	0.599
Positive	700 (64.6)	486 (65.1)	214 (63.3)	
Missing	1	1	0	
Malaria & STI/RTI Coinfection[†]				
No infection	177 (16.5)	128 (17.4)	49 (14.6)	0.071
Single infection[¥]	480 (44.8)	339 (46.2)	141 (41.8)	
Coinfection	414 (38.7)	267 (36.4)	147 (43.6)	
Missing*	14	13	1	

NA: Not applicable, IQR: interquartile range, IRS: Indoor residual spraying

* Missing and NA values are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association

[†]Infection with at least one STI/RTI and malaria

[¥]Single infection: Infection with at least one STI/RTI or malaria

[‡]Antimalarial treatment during pregnancy taken more than four weeks before recruitment

Less than 5 women took antibacterial agents during pregnancy but more than a month before recruitment

3.4 Malaria and HIV Burden

3.4.1 The Prevalence of HIV infection

The prevalence of HIV infection among participants was 13.2% (95% CI, 11.3–15.3) and the highest burden was observed in multigravidae (15.9%) followed by secundigravidae (13.9%) and was lowest in primigravidae (8.0%), *P* = 0.018. A higher proportion of infection was noted among women aged above 25 years than women aged ≤ 25 years (17.9% versus 9.4%, P < 0.001).

3.4.2 The prevalence of peripheral malaria

The prevalence of malaria infection at first ANC attendance measured by microscopy and by PCR was 31.8% (95% CI, 29.1-34.6) and 57.8% (95% CI, 54.9-60.8) respectively. Parasite density ranged from 64–24,760 parasites per µl of blood with a geometric mean of 1,082 (95% CI, 962-1,217) asexual parasites per µl of blood. Parasite density was highest among primigravidae with the geometric mean being significantly different across gravidae (*P* < 0.001). Of the 343 malaria positive samples detected by microscopy, seven were negative for *P. falciparum* by PCR (Table 3-2).

Of the 1,074 women whose results were available for malaria detected by PCR, 8.4% (n = 90, 95% CI, 6.7-10.2) were coinfected with HIV. The prevalence of malaria among HIV-infected and HIV-uninfected women detected by PCR was 65.2% (90/138) and 56.7% (531/936), respectively (*P* = 0.059). 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 proportions of malaria infection detected by PCR among HIV-infected women on ARV treatment and those who were not on ARV treatment were 71.8% (56/78) and 56.7% (34/60) respectively, *P* = 0.064.

In the case of microscopy, 31.3% (294/939) of HIV-uninfected had parasitaemia compared to 35.0% (49/140) among HIV-infected women (*P* = 0.382). HIV-uninfected women had significantly lower parasite densities than HIV-infected women, 990 (95% CI, 876-1120) versus 1836 (95% CI, 1306-2580), *P* < 0.001. A lower proportion of malaria infection diagnosed by microscopy was observed among HIV-infected

women on ARV therapy than among those who were not on therapy, 24.6% (15/61) versus 43.0% (34/79) respectively, *P* = 0.023. Parasite density among HIV-infected women receiving ARV therapy was lower than that observed among those who were not on therapy at recruitment, 1,310 (95% CI, 693-2,476, n = 15) versus 2,131 (95% CI, 1,408-3,224, n = 34), respectively but this was not statistically significant (*P* = 0.189).

3.4.3 Placental malaria infection

Of the 729 women who were followed to delivery, placental tissue was successfully processed for 96% (n = 702) participants and 40.9% (n=287) of these had histopathological evidence of placental malaria (Table 3-3). The highest proportion (30.4%) was past infections detected by the presence of the pigment without the presence of parasites. The frequency of placental infection, past or active or both, was higher in primigravidae than multigravidae and this difference was statistically significant (52.7% versus 33.6%; *P* < 0.001). Further results on placental malaria infection and histopathology classes are presented in chapter six (section 6.2).

Table 3- 2: Prevalence of HIV and *Plasmodium falciparum* malaria infection at first antenatal attendance and at delivery among women of Nchelenge district according to gravidity/parity

Infection Diagnostic method	**All gravidae N = 1085 n(%) or n, geometric mean**	**95% Confidence Interval**	**Primigravidae n=261 n(%) or n, geometric mean (95% CI)**	**Secundigravidae n=165 n(%) or n, geometric mean (95% CI)**	**Multigravidae n=659 n (%) or n, geometric mean (95% CI)**	***P-value***
HIV (serology)						
Negative	941 (86.8)	86.8-84.8	240 (92.0)	14286.1)	559 (84.9)	0.018
Positive	143 (13.2)	11.3-15.3	21 (8.0)	23 (13.9)	99 (15.1)	
Missing*	1		0	0	1	
Malaria (*PCR)*						
Negative	453 (42.2)	39.2-45.1	81 (31.3)	63 (38.2)	309 (47.5)	< 0.001
Positive	621 (57.8)	54.8-60.7	I78 (68.7)	102 (61.8)	341 (52.5)	
Missing*	11		2		9	
Microscopy						
Negative	736 (68.2)	65.4-71.0	127 (49.3)	104 (63.0)	505 (77.1)	< 0.001
Positive	343 (31.8)	29.1-34.6	132 (51.0)	61 (37.0)	150 (22.9)	
Missing*	6		2	1	3	
Geometric mean of Parasite density	343, 1082	962-1217	132, 1378 (1133-1677)	61, 1164 (897-1509)	150, 848 (714-1010)	< 0.001
Placental malaria						
Negative	415 (59.1)	55.5-62.8	89 (47.3)	46 (50.0)	280 (66.4)	< 0.001
Positive	287 (40.9)	37.3-44.6	199 (52.7)	46 (50.0)	142 (33.6)	
Missing*	27		6	6	15	
Lost to follow-up*	356		67	67	222	

Of the 343 malaria positive samples detected by microscopy 7 were found negative by PCR specific for *P. falciparum* detection

The overall prevalence (40.9%) is a combination of different classes of pathology as follows: Presence of parasites only - 2.4%; Presence of parasites and pigment - 8.1%; Presence of pigment only – 30.4%.

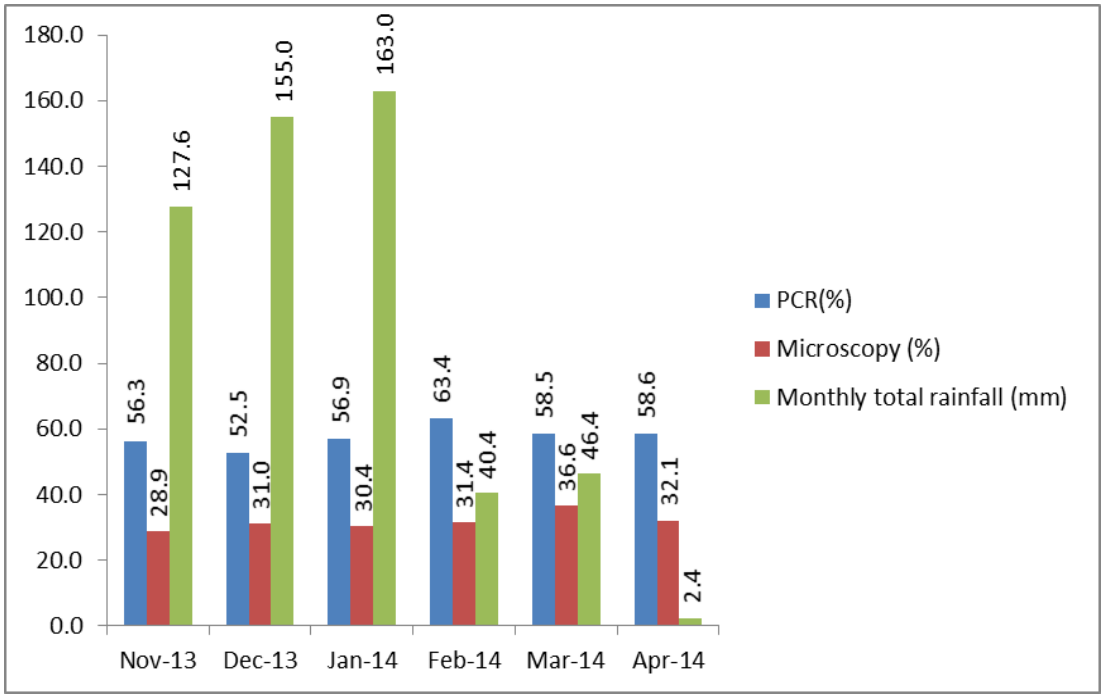
*Missing and lost to follow-up. *P*-values are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association

Of the expected samples from 1085 participants, 4 dry blood spots and 4 slides were not collected. In addition to this 5 dry blood spots were misplaced

3.4.4 Monthly peripheral malaria infection among first ANC attendees

Figure 3.2 shows the prevalence of malaria infection among study participants each calendar month during the six-month recruitment period from November 2013 to April 2014. The malaria prevalence was slightly higher between February and April compared to the period between November and January.

Figure 3. 2: Prevalence of malaria in pregnant women of Nchelenge District and total monthly rainfall during the study recruitment period.



PCR: Polymerase chain reaction

Mm: Millimetres

3.5 Reproductive tract and curable sexually transmitted infections

The prevalence of STIs/RTIs among pregnant women in this study was high. Overall, infection with at least one STI/RTI was observed in 64.8% (95% CI, 61-67.4) of the participating women. The most prevalent infection was BV (48.3%) and the least prevalent was gonorrhoea (3.1%). With the exclusion of BV the prevalence of infection with at least one curable STI was 34.5% (95% CI, 31.7-37.4).

Contrary to what was observed in the case of HIV infection, a higher prevalence of STIs was observed among women aged ≤ 25 years than women aged above 25 years (38.7% versus 29.2%, *P* = 0.001). The prevalence of BV was higher among women aged ≤ 25 years than among older women, but this was not statistically significant (50.1 versus 45.8%, *P* = 0.130).

Table 3-4 presents the prevalence of individual and composite STIs/RTIs among participants according to HIV status. The prevalence of curable STIs/RTIs was higher among HIV-infected women than among HIV-uninfected women. The prevalence of BV, Chlamydia, gonorrhoea and syphilis was higher among HIV-infected women than among HIV-uninfected women. This was statistically significant in the cases of gonorrhoea, BV and syphilis but not in the case of Chlamydia. There was no statistically significant difference in the prevalence of trichomoniasis between HIV-uninfected women and HIV-infected women, 25.0% verse 23.8% (*P* = 0.752). With the exclusion of BV, prevalence of any STI was higher among HIV-infected than HIV uninfected women but this was not statistically significant (41.3 versus 33.5%, *P* = 0.069).

Of the 700 women who were infected with at least one STI/RTI at first ANC visit 14.0% (n = 98) complained of at least one symptom associated with curable STIs/RTIs (itching, discharge, burning session during urination or presence of sores/ulcers). About 9.4% (36/384) had similar complaints among uninfected women. A similar proportion was observed when only curable STIs were considered. Of the 374 women who tested positive to at least one STI, 14.2% (n = 53) indicated that they were experiencing at least one symptom indicative of infection with an STI at the time of recruitment.

Table 3- 3: Prevalence of curable STIs/RTIs among first ANC attendees of Nchelenge district according to HIV status

Infection	All (N = 1084) n (%)	95% Confidence interval‡	HIV-uninfected (n = 941) n (%)	HIV-infected (n = 143) n (%)	*P*-value
Bacterial vaginosis					
Normal	375 (34.7)	31.8-37.5	342 (36.5)	33 (23.1)	< 0.001
Intermediate	184 (17.0)	14.9-19.4	169 (18.0)	15 (10.5)	
BV	524 (48.3)	45.2-51.2	429 (45.8)	95 (66.4)	
Missing*	4		4	0	
Trichomoniasis					
Negative	814 (75.2)	72.6-77.8	705 (75.0)	109 (76.2)	0.752
Positive	269 (24.8)	22.3-27.5	235 (25.0)	34 (23.8)	
Missing*	1		1	0	
Chlamydia					
Negative	1027 (94.8)	93.5-96.2	894 (95.1)	133 (93.0)	0.291
Positive	56 (5.2)	3.9-6.7	46 (4.9)	10 (7.0)	
Missing*	1		1	0	
Gonorrhoea					
Negative	1049 (96.9)	95.8-97.9	915 (97.3)	134 (93.7)	0.020
Positive	34 (3.1)	2.2-4.4	25 (2.7)	9 (6.3)	
Missing*	1		1	0	
Confirmed syphilis					
Negative	1001 (92.9)	91.4-94.5	884 (94.2)	117 (84.2)	< 0.001
Positive[a]	76 (7.1)	5.6-8.7	54 (5.8)	22 (15.8)	
Missing*	7		3	4	
Composite STI					
Negative	710 (65.5)	62.7-68.3	625(66.5)	84 (58.7)	0.069
Positive	374 (34.5)	31.7-37.4	315 (33.5)	59 (41.3)	
Missing*	1		1	0	
Composite STI/RTI					
Negative	381 (35.2)	32.6-38.3	348 (37.0)	33 (23.1)	0.001
Positive	702 (64.8)	61.7-67.4	592 (63.0)	110 (76.9)	
Missing*	1		1	0	

HIV: Human Immunodeficiency Virus

Composite STI/RTI: sexually transmitted and reproductive tract infections (included bacterial vaginosis, Chlamydia, gonorrhoea, trichomoniasis and TPHA confirmed syphilis)

Composite STI: included Chlamydia, gonorrhoea, trichomoniasis and TPHA confirmed syphilis

* Missing values are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association

[a]The figure represents the number of samples that were TPHA reactive among those who were seropositive by RPR calculated out of all the participants who were tested with RPR (1077).

Note: Among the participants who were RPR reactive, 65% (76/117) were found reactive with TPHA

Overall the total number was 1084 instead of 1085 because the HIV status of one participant was not determined.

3.6 Malaria and curable STI/RTI coinfection

Out of the 1071 women tested for malaria and all the five curable STI/RTI, 38.7% (n= 414) were coinfected with malaria and STI/RTI (Table 3-5). The prevalence of coinfection was lower in the case of malaria diagnosed by microscopy (21.4%, 230/1076).

3.6.1 Malaria and STI/RTI coinfection according to parity

Mono-infection with malaria diagnosed by PCR did not differ significantly among the three gravidity groups (primigravidae, secundigravidae and multigravidae) but infection with at least one curable STI/RTI increased slightly with increasing gravidity (19.4%, 25.5% and 29.0%, respectively, $P = 0.012$). However, the prevalence of coinfection decreased with increasing gravidity (49.6%, 44.2% and 32.9% respectively, $P < 0.001$. This trend in the frequency of coinfection across gravidity groups persisted even when the malaria diagnosis was done by microscopy (33.7%, 28.5% and 14.7% respectively, $P < 0.001$).

The median ages of women in the three groups of infection were as follows; coinfection 22.5 years (IQR, 19-28), mono-infection (at least one STI/RTI or malaria) 25 years (IQR, 21-30) and uninfected 27 years (IQR, 21-31) ($P < 0.001$, kruskal-wallis equality-of-population rank test).

Table 3- 4: Prevalence of malaria and STI/RTI coinfection according to gravidity

Coinfection[¥] based on malaria diagnosed by PCR						
Category of infection	**All (N =1085) n (%)**	**All 95% CI**	**Primigravidae n = 261 n (%)**	**Secundigravidae n = 165 n (%)**	**Multigravidae (n = 648) n (%)**	***P*-value**
Coinfection[¥]	414 (38.7)	35.7 - 41.6	128 (49.6)	73 (44.2)	213 (32.9)	< 0.001
Malaria only	202 (18.8)	16.5 - 21.2	47 (18.2)	29 (17.5)	126 (19.4)	0.822
STI/RTI only	278 (26.0)	23.5 - 28.8	50 (19.4)	42 (25.5)	188 (29.0)	0.012
No infection	177 (16.5)	14.1 - 18.6	33 (12.8)	21 (12.7)	121 (18.7)	0.038
Missing*	14		3	0	11	
Coinfection[¥] based on malaria diagnosed by microscopy						
Category of infection	**All (N =1085) n (%)**	**All 95% CI**	**Primigravidae n = 261 n (%)**	**Secundigravidae n = 165 n (%)**	**Multigravidae (n = 648) n (%)**	***P*-value**
Coinfection[¥]	230 (21.4)	18.9 - 23.8	87 (33.7)	47 (28.5)	96 (14.7)	< 0.001
Malaria only	93 (8.6)	6.9 -10.3	39 (15.1)	13 (7.7)	41 (6.3)	< 0.001
STI/RTI only	469 (43.5)	40.6 - 46.5	91 (35.3)	69 (41.8)	309 (47.3)	0.004
No infection	285 (26.4)	23.8 - 29.1	41 (15.9)	36 (21.8)	208 (31.8)	< 0.001
Missing*	9		3	0	6	

[¥]Coinfection: Infection with malaria and at least one STI/RTI

*Missing values are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association
Missing values were due missing samples, 14 samples could not be classified due to some missing results.

3.5.2 Malaria and STI/RTI coinfection according to HIV status

Table 3-5 presents the prevalence of individual STI/RTI and malaria coinfection as well as composite STI/RTI and malaria coinfection according to HIV status. The prevalence of coinfection with malaria and individual STIs/RTIs was higher among HIV-infected than HIV-uninfected women but this was only statistically significant in the case of syphilis and BV regardless of the diagnostic tool used for malaria detection. In the case of malaria diagnosed by PCR, coinfection with syphilis among HIV-infected and HIV-uninfected women was 8.8% versus 3.3%, respectively (P = 0.002). Coinfection with BV and malaria was 45.7% versus 26.8% among HIV-infected and HIV-uninfected women respectively ($P < 0.001$). Coinfection with malaria and BV was the most prevalent (29.2%; 95% CI 26.5-32.0) and coinfection with malaria and gonorrhoea was the least prevalent (2.1%; 95% CI 1.3-3.1). STI/RTI among the 621 women who tested positive to malaria by PCR was as follows: 50.9% were infected with BV; 26.4% were infected with trichomoniasis; 6.0% were infected with Chlamydia; 3.5% were infected with gonorrhoea and 7.0% had syphilis. Composite STI/RTI and malaria coinfection was higher among HIV-infected than HIV-uninfected women, 50.0% versus 37%, P = 0.003 in case of Malaria diagnosed by PCR and 27.1% versus 20.5%, P = 0.073 in the case of malaria diagnosed by microscopy.

Table 3- 5: Prevalence of individual curable STI/RTI and malaria coinfection among first antenatal care attendees of Nchelenge District, Zambia

Malaria diagnosed by PCR					
Coinfection	**N = 1084 n (%)**	**95% CI**	**HIV-uninfected n = 941 n (%)**	**HIV-infected n = 143 n (%)**	**P-Value**
Malaria and Bacterial vaginosis					
No	757 (70.8)	68.0-73.5	682 (73.2)	75 (54.4)	< 0.001
Yes	313 (29.2)	26.5-32.0	250 (26.8)	63 (45.7)	
Missing*	14		9	5	
Malaria and *Trichomonas vaginalis*					
No	909 (84.7)	82.6-86.9	793 (84.8)	116 (84.1)	0.818
Yes	164 (15.3)	13.3-17.6	142 (15.2)	22 (15.9)	
Missing*	11		6	5	
Malaria and *Chlamydia trachomatis*					
No	1036 (96.6)	95.5-97.6	905 (96.8)	131 (94.9)	0.263
Yes	37 (3.4)	2.4-4.5	30 (3.2)	7 (5.1)	
Missing*	11		6	5	
Malaria and *Neisseria gonorrhoeae*					
No	1051 (97.9)	97.1-98.8	918 (98.2)	133 (96.4)	0.162
Yes	22 (2.1)	1.2-2.9	17 (1.8)	5 (3.6)	
Missing*	11		6	5	
Malaria and *Treponema Pallidum*					
No	1029 (96.0)	94.8-97.2	904 (96.7)	125 (91.2)	0.002
Yes	43 (4.0)	2.8-5.2	31 (3.3)	12 (8.8)	
Missing*	12		6	5	
Malaria and STI/RTI coinfection[$]					
Coinfection	414 (38.7)	35.8-41.6	345 (37.0)	69 (50.0)	0.003
Malaria only	202 (18.9)	16.6-21.3	182 (19.5)	20 (14.5)	0.154
STI/RTI only	278 (26.0)	23.4-28.7	237 (25.5)	41 (29.7)	0.286
No infection	177 (16.5)	14.3-18.8	169 (18.1)	8 (5.8)	< 0.001
Missing*	13		8	5	

Table 3-5: Prevalence of individual curable STI/RTI and malaria coinfection among first antenatal care attendees of Nchelenge District, Zambia continued

Malaria diagnosed by microscopy					
Coinfection	**N = 1084 n (%)**	**95% CI**	**HIV-uninfected n = 941 n (%)**	**HIV-infected n = 143 n (%)**	**P-Value**
Malaria and Bacterial vaginosis					
No	896 (83.1)	80.8-85.3	789 (84.4)	104 (74.3)	0.003
Yes	182 (16.9)	14.7-19.2	146 (15.6)	36 (25.7)	
Missing*	9		6	3	
Malaria and *Trichomonas vaginalis*					
No	983 (91.2)	89.5-92.9	851 (90.7)	132 (94.3)	0.166
Yes	95 (8.8)	7.1-10.5	87 (9.3)	8 (5.7)	
Missing*	6		3	3	
Malaria and *Chlamydia trachomatis*					
No	1055 (97.9)	97.0-98.7	919 (98.0)	136 (97.1)	0.527
Yes	23 (2.1)	1.2-3.0	19 (2.0)	4 (2.9)	
Missing*	6		3	3	
Malaria and *Neisseria gonorrhoeae*					
No	1069 (99.2)	98.6-97.7	931 (99.2)	138 (98.6)	0.408
Yes	9 (0.83)	034-1.4	7 (0.8)	2 (1.42)	
Missing*	6		3	3	
Malaria and *Treponema Pallidum*					
No	1051 (97.8)	96.9-98.7	922 (98.4)	129 (93.5)	< 0.001
Yes	24 (2.2)	1.3-3.1	15 (1.6)	9 (6.5)	
Missing*	9		4	5	
Malaria and STI/RTI coinfection[$]					
Coinfection	230 (21.4)	18.9-23.8	192 (20.5)	38 (27.1)	0.073
Malaria only	93 (8.6)	7.0-10.3	84 (9.0)	9 (6.5)	0.322
STI/RTI only	467 (43.4)	40.4-46.4	395 (42.2)	72 (51.4)	0.039
No infection	286 (26.6)	23.9-29.3	265 (28.3)	21 (15.0)	< 0.001
Missing*	9				

*Missing values are only presented as numbers and were not included in the calculation of percentages
$ Coinfection: infection with malaria and at least one STI/RTI

3.7 Quality control assessment results

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. Repeat reading of slides for placental malarial diagnosis by histology was 95.7% (44/46) concordant.

4 Results (2): Risk factors for malaria, STI/RTI and their coinfection

4.1 Chapter introduction

This chapter presents risk factors for (i) malaria (ii) STI, (iii) BV and (iv) malaria and STI/RTI coinfection as identified by logistic regression models. The variables included in the analyses were guided by risk factors for infection as identified from literature in section 1.5.1. The tables presented include both univariate and multivariate analyses of risk factors for infection.

4.2 Risk factors for malaria infection

Table 4-1 presents risk factors for malaria infection. In the univariate analyses the association between malaria infection and the following factors was statistically significant ($P < 0.1$); recruitment site, age group, marital status, gravidity, bed net ownership, bed net usage, wealth quintile and HIV status. However, in the multivariate analysis, only three factors i.e. HIV status, site of recruitment and wealth quintile were independently associated with malaria infection ($P < 0.05$). Wealth quintile was not associated with malaria infection in a model with HIV infection and site of recruitment and was therefore excluded from the final model. In the final model, the risk of malaria infection was higher among women recruited at Nchelenge health centre (aOR = 1.81; 95% CI, 1.38-2.37, $P < 0.001$) and HIV-infected women across both health centres (aOR = 1.46; 95% CI 1.00-2.13, $P = 0.045$). There was no interaction between HIV status and site of recruitment ($P = 0.751$).

Table 4- 1: Predictors of malaria infection in pregnant women of Nchelenge District

Potential risk factor	**Number**	**%**	**Unadjusted OR**	**Adjusted OR**	**Adjusted OR for final model**	***P*-value**
	1074	**100**	**-**	**N = 1068**	**N = 1072**	**< 0.001¥**
Recruitment site			P < 0.001	**P < 0.001**		
Kashikishi	737	68.6	1.00	**1.00**	**1.00**	
Nchelenge	337	31.4	1.80 (1.37-2.35)	**1.89 (1.43-2.50)**	**1.81 (1.38-2.37)**	**< 0.001**
Age group			*P* < 0.001	*P* = 0.153	-	-
≤ 20	295	27.5	1.00	1.00		
21-25	304	28.3	0.51 (0.37-0.72)	0.63 (0.41-0.98)		
26-30	244	22.7	0.51 (0.36-0.73)	0.67 (0.41-1.09)		
≥ 30	231	21.5	0.45 (0.32-0.64)	0.58 (0.34 -0.96)		
Marital status			*P* = 0.114	-	-	-
Single	201	18.7	1.00			
Married	865	80.5	0.72 (0.53-0.99)			
Divorced/separated/ widowed	8	0.8	0.56 (0.14-2.30)			
Gravidity			P < 0.001	P = 0.077	-	-
Primigravidae	259	24.1	1.00	1.00		
secundigravidae	165	15.4	0.74 (0.48-1.11)	0.80 (0.48-1.33)		
Multigravidae	650	60.5	0.50 (0.37-0.68)	0.58 (0.35-0.97)		
Bed net ownership			*P* = 0.015	*P* = 0.589	-	-
No	547	50.9	1.00	1.00		
Yes	527	49.1	0.74 (0.58-0.94)	1.13 (0.73-1.75)		
Bed net use on previous night			P = 0.006	*P* = 0.188	-	-
No	659	61.5	1.00	1.00		
Yes	412	38.5	0.75 (0.48-0.91)	0.74 (0.48-1.16)		
Missing*	3					

Table 4-1: Predictors of malaria infection in pregnant women of Nchelenge District continued

Potential risk factor	Number	%	Unadjusted OR	Adjusted OR for multivariate Analysis	Adjusted OR for final model	*P-value*
	1074	**100**	**-**	**N = 1068**	**N = 1072**	**< 0.001[¥]**
IRS in past 12 months			*P* = 0.386	-	-	-
No	799	78.0	1.00			
Yes	225	22.0	1.19 (0.88-1.61)			
Unknown*	46					
Wealth quintile			*P* = 0.072	*P* = 0.031	-	-
Lowest	216	20.1	1.00	1.00		
Second	219	20.4	0.80 (0.54-1.17)	0.74 (0.50-1.09)		
Middle	209	19.5	0.91 (0.61-1.34)	0.77 (0.51-1.16)		
Fourth	214	20.0	1.08 (0.73-1.60)	0.89 (0.58-1.36)		
Highest	214	20.0	0.64 (0.44-0.95)	0.53 (0.35-0.81)		
Missing *	2					
HIV status			*P* = 0.057	1.00	**1.00**	
Negative	941	86.8	1.00	1.56 (1.05-2.32)	**1.46 (1.00-2.13)**	**0.045**
Positive	143	13.2	1.30 (0.91-1.88)			

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[¥]*P*- value for overall final model

*P-value*s appearing with unadjusted odds ratios are for each univariate model indicating associations of the variable with the outcome at P < 0.1.

*P-value*s appearing with adjusted odds ratios indicate the independent association of each variable with the outcome at *P* < 0.05

Bold type- statistically significant outcome for multivariate analysis and final model.

Number of participants less than 1074 (available malaria test results by PCR) were due to missing values in individual variables.

*Missing/unknown values are only presented as numbers and were not included in the calculation of percentages and in the models

When wealth quintile was put in the model with HIV and site of recruitment it was not significantly associated with malaria infection and was therefore excluded from the final model.

There was no interaction between HIV infection and site of recruitment in the final model (*P* =0.751)

4.3 Risk factors for curable STIs/RTIs

Risk factors for STIs and BV which is not sexually transmitted were analysed separately and are presented in Tables 4-2 and Table 4-3.

4.3.1 Risk factors for STIs

Table 4-2 shows univariate and multivariate analyses of risk factors for composite STIs (Chlamydia, gonorrhoea, trichomoniasis and TPHA confirmed syphilis). In the univariate analysis infection with at least one STI was associated with age group, marital status, gravidity, number of life-time sexual partners, wealth quintile, HIV status and BV at $P < 0.1$. In multivariate analysis infection with at least one STI was associated with wealth quintile and infection with BV. Wealth quintile was not independently associated with infection with at least one STI in a model with BV and was therefore excluded from the final model. In the final model, infection with at least one STI was associated with BV. In comparison to uninfected women, women infected with BV were at a higher risk of being infected with at least one STI (aOR 1.44; 95% CI, 1.08-1.92, $P = 0.012$).

4.3.2 Risk factors for BV

Table 4-3 shows univariate and multivariate analyses of risk factors for BV. In the univariate analysis bacterial vaginosis was associated with age group, marital status, gravidity, HIV and infection with at least one STI. In the multivariate analysis infection with HIV and infection with at least one STI were independently associated with BV. In the final model HIV-infected women had a higher risk of infection with BV than HIV-uninfected women (aOR 1.87; 95% CI, 1.24-2.83, $P = 0.003$) and women infected with at least one STI had a higher risk of BV (aOR 1.40; 95% CI (1.07 -1.84, $P = 0.01$).

Table 4- 2: Univariate and multivariate analyses of risk factors for STIs

Variable	**Number**	**%**	**Unadjusted OR**	**Adjusted OR**	**Final model**	***P*-value**
Category	**1084**	**100**	**-**	**N = 1067**	**N = 1078**	**0.007[¥]**
Age group			*P* < 0.001	*P* = 0.101	-	-
≤ 20	296	27.3	1.00	1.00		
21-25	309	28.5	0.79 (0.56-1.09)	0.89 (0.58 -1.35)		
26-30	245	22.6	0.72 (0.51-1.02)	0.82 (0.51-1.35)		
≥ 30	234	21.6	0.45 (0.31-0.66)	0.56 (0.33-0.95)		
Maternal education			*P* = 0.893	-	-	-
0-6 years	426	39.3	1.00			
≥ 7 years	658	60.7	0.98 (0.76-1.27)			
Marital status			*P* = 0.022	*P* = 0.768	-	-
Single	203	18.7	1.00	1.00		
Married	873	80.4	0.64 (0.47-0.87)	0.87 (0.58-1.29)		
Separated/widowed/divorced	8	0.7	0.80 (0.19-3.4)	0.76 (0.13-4.40)		
Gravidity			*P* < 0.001	*P* = 0.151	-	-
Primigravidae	261	24.1	1.00	1.00		
secundigravidae	165	15.2	1.03 (0.69-1.52)	1.12 (0.69-1.81)		
Multigravidae	658	60.7	0.59 (0.43-0.79)	0.76 (0.46-1.24)		
No. of life time sexual			*P* = 0.037		-	-
partners	797	74.2	1.35 (1.02-1.79)	*P* = 0.05		
1 or 2	277	25.8	0.49 (0.42-0.56)	1.00		
3 or more	10			1.34 (1.00-1.80)		
Unknown						
Age at sexual debut			*P* = 0.771	-	-	-
≤15	245	26.3	1.00			
16-17	282	30.3	0.88 (0.61-1.26)			
≥ 18	404	43.4	0.93 (0.67 -1.31)			
Unknown*	153					

Table 4-2: Univariate and multivariate analyses of risk factors for STIs

Variable	**Number**	**%**	**Unadjusted OR**	**Adjusted OR**	**Final model**	***P*-value**
Category	**1084**	**100**	**-**	**N = 1067**	**N = 1078**	**0.007[¥]**
Wealth quintile			*P* = 0.018	***P* = 0.008**	-	-
Lowest	217	20.0	1.00	**1.00**		
Second	221	20.4	1.13 (0.76-1.68)	**1.00 (0.67-1.51)**		
Middle	213	19.7	1.03 (0.69-1.54)	**0.79 (0.51-1.20)**		
Fourth	215	19.9	1.44 (0.97-2.13)	**1.02 (0.67-1.55)**		
Highest	216	20.0	0.71 (0.47-1.08)	**0.52 (0.33-0.82)**		
Missing*	2					
HIV status			*P* = 0.072	*P* = 0.064	-	-
Negative	940	86.8	1.00	1.00		
Positive	143	13.2	1.39 (0.97-1.99)	1.44 (0.98-2.10)		
Missing*	1					
Bacterial vaginosis			*P* = 0.034	***P* = 0.037**		
Negative	375	34.7	1.00	**1.00**	**1.00**	
Intermediate	184	17.0	1.37 (0.95-2.00)	**1.52 (1.04-2.23)**	**1.38 (0.95-2.00)**	**0.091**
Positive	521	48.3	1.44 (1.08-1.91)	**1.40 (1.04-1.87)**	**1.44 (1.08-1.92)**	**0.012**
	4					

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[¥]*P*-value for overall final model

P-values appearing with unadjusted odds ratios are for each univariate model indicating associations of the variable with the outcome at *P* < 0.1.

P-values appearing with adjusted odds ratios indicate the independent association of each variable with the outcome at *P* < 0.05

Bold type- statistically significant outcome for multivariate analysis and final model.

When wealth quintile was put in the model with BV, it was not independently associated with infection with at least one STI and was therefore excluded from the final model.

*Missing/unknown values are only presented as numbers and were not included in the calculation of percentages and in the models

Table 4- 3: Univariate and multivariate analyses of risk factors for BV

Potential risk factor	**Number**	**%**	**Unadjusted OR**	**Adjusted OR**	**Final model**	***P*-value**
	1081	**100**	**-**	**N= 1079**	**N = 1079**	**0.001¥**
Age group			***P* = 0.050**	*P* = 0.120	-	-
≤ 20	294	27.3	1.00	1.00		
21-25	307	28.4	1.46 (1.04-2.03)	1.43 (0.93-2.20)		
26-30	246	22.8	1.43 (1.00-2.06)	1.45 (0.89-2.39)		
≥ 30	234	21.6	1.02 (0.72-1.46)	1.04 (0.63-1.72)		
Maternal education			*P* = 0.550	-	-	-
0-6 years	425	39.3	1.00			
≥ 7 years	656	60.7	1.08 (0.84-1.39)			
Marital status			*P* = 0.066	*P* = 0.127	-	-
Single	201	18.6	1.00	1.00		
Married	872	80.7	1.46 (1.06-1.99)	1.50 (1.01-2.24)		
Separated/widowed/divorced	8	0.7	1.20 (0.27-5.14)	1.19 (0.26-5.39)		
Gravidity			*P* = 0.191	*P* = 0.261	-	-
Primigravidae	259	23.9	1.00	1.00		
secundigravidae	164	15.2	1.47 (0.97-2.24)	0.99 (0.60-1.64)		
Multigravidae	658	60.9	1.14 (0.84-1.54)	0.73 (0.44-1.20)		
No. of life time sexual partners			*P* = 0.570	-	-	-
1 or 2	795	74.2	1.00			
3 or more	276	25.8	1.08 (0.81-1.45)			
Unknown*	10					
Age at sexual debut			*P* = 0.977	-	-	-
≤15	244	26.3	1.00			
16-17	282	30.4	1.04 (0.72-1.49)			
≥ 18	402	43.3	1.03 (0.74-1.44)			
Unknown*	153					

Table 4-3: Univariate and multivariate analyses of risk factors for BV continued

Potential risk factor	Number	%	Unadjusted OR	Adjusted OR	Final model	*P*-value
	1081	**100**	**-**	**N = 1079**	**N = 1079**	**0.001¥**
Wealth quintile			*P* = 0.484	-	-	-
Lowest	217	20.0	1.00			
Second	221	20.5	1.10 (0.75-1.62)			
Middle	214	19.8	1.39 (0.93-2.03)			
Fourth	213	19.7	1.36 (0.91-2.02)			
Highest	215	19.9	1.13 (0.76-1.67)			
Missing*	2					
HIV status			*P* = 0.001	***P* = 0.002**		
Negative	937	86.8	1.00	**1.00**	**1.00**	
Positive	143	13.2	1.91 (1.27-2.89)	**1.88 (1.24-2.86)**	**1.87 (1.24-2.83)**	**0.003**
Missing*	1					
STI			*P* = 0.010	**P = 0.016**		
Negative	708	65.6	1.00	**1.00**	**1.00**	
Positive	372	34.4	1.42 (1.09-1.87)	**1.40 (1.06-1.85)**	**1.40 (1.07-1.84)**	**0.015**
Missing	1					

-: Blank

¥*P*-value for overall final model

P-values appearing with unadjusted odds ratios are for each univariate model indicating associations of the variable with the outcome at $P < 0.1$.

P-values appearing with adjusted odds ratios indicate the independent association of each variable with the outcome at $P < 0.05$

Bold type- statistically significant outcome for multivariate analysis and final model.

*Missing/unknown values are only presented as numbers and were not included in the calculation of percentages and in the models

4.4 Risk factors for malaria and curable STI/RTI coinfection

In the univariate analysis the following factors were associated with malaria and STI/RTI coinfection ($P < 0.1$); age group, gravidity, bed net ownership, bed net usage and HIV status (Table 4-3). None of the factors tested using the likelihood ratio test were independently associated with coinfection except HIV infection. HIV-infected women were at a higher risk of being coinfected with malaria and at least one curable STI/RTI than HIV-uninfected women [OR; 3.59 (95% CI, 1.73-7.48), $P < 0.001$].

Table 4- 4: Risk factors for malaria and curable STI/RTI coinfection in pregnant women of Nchelenge District

Potential risk factor	Number	%	Unadjusted OR	Adjusted OR	Final model	*P*-value
	1071	**100**	**-**	**N = 931**	**N = 1071**	**< 0.001¥**
Recruitment site			*P* = 0.273	-	**-**	**-**
Kashikishi	734	68.5	1.00			
Nchelenge	337	31.5	1.22(0.85-1.74)			
Age group			*P* = 0.026	*P* = 0.149	-	-
≤ 20	295	27.5	1.00	1.00		
21-25	304	28.3	0.84 (0.53-1.34)	0.95 (0.52-1.72)		
26-30	244	22.7	0.71 (0.44-1.13)	0.83 (0.43-1.61)		
≥ 30	231	21.5	0.51 (0.32-0.81)	0.58 (0.30-1.14)		
Marital status			*P* = 0.751	-	-	-
Single	201	18.8	1.00			
Married	862	80.5	0.91 (0.60-1.39)			
Divorced/separated/widowed	8	0.7	0.55 (0.11-2.84)			
Gravidity			*P* = 0.039	*P* = 0.681	-	-
Primigravidae	258	24.1	1.00	1.00		
Secundigravidae	165	15.4	1.95 (0.53-1.70)	0.96 (0.52-1.88)		
Multigravidae	648	60.5	0.63 (0.42-0.95)	0.77 (0.41-1.46)		
Bed net ownership			*P* = 0.064	*P* = 0.937	-	-
No	734	68.5	1.00	1.00		
Yes	337	31.5	0.74 (0.53-1.02)	0.83 (0.47-1.47)		
Bed net use on previous night			*P* = 0.097	*P* = 0.386	-	-
No	658	61.6	1.00	1.00		
Yes	410	38.4	0.76 (0.55-1.05)	0.98 (0.56-1.74)		
Missing*	3					

Table 4-4: Risk factors for malaria and curable STI/RTI coinfection in pregnant women of Nchelenge District continued

Potential risk factor	Number	%	Unadjusted OR	Adjusted OR	Final model	*P*-value
	1071	**100**	**-**	**N = 931**	**N = 1071**	**< 0.001[¥]**
IRS in past 12 months			*P* = 0.193	-	-	-
No	798	74.7	1.00			
Yes	224	21.0	0.78 (0.53-1.15)			
Unknown*	46					
Missing*	3					
No. of life time sexual partners			*P* = 0.793	-	-	-
1 or 2	788	74.2	1.00			
3 or more	274	25.8	1.47(1.06-2.04)			
Unknown*	9					
Age at sex debut			*P* = 0.593	-	-	-
>14 years	97	10.4	1.00			
14 years & above	821	89.6	1.88 (0.49-1.60)			
Unknown*	153					
Wealth quintile			*P* = 0.166	-	-	-
Lowest	215	20.1	1.00			
Second	219	20.5	0.77 (0.46-1.26)			
Middle	208	19.5	1.07 (0.63-1.82)			
Fourth	214	19.9	1.35 (0.77-2.34)			
Highest	214	20.0	0.76 (0.46-1.26)			
Missing*	2					
HIV status			*P* < 0.001	**P < 0.001**		
Negative	933	87.1	1.00	**1.00**	**1.00**	
Positive	138	12.9	3.59 (1.73-7.48)	**3.93 (1.87-8.27)**	**3.59 (1.73-7.48)**	**< 0.001**
Missing*	1					

-: Blank; [¥]*P*- value for overall final model

P-values appearing with unadjusted odds ratios are for each univariate model indicating associations of the variable with the outcome at *P* < 0.1.

P-values appearing with adjusted odds ratios indicate the independent association of each variable with the outcome at *P* < 0.05.

Bold type- statistically significant outcome for multivariate analysis and final model.

*Missing/unknown values are only presented as numbers and were not included in the calculation of percentages and in the models

Only HIV infection was found to be associated with malaria and STI/RTI coinfection therefore was not adjusted for any other variable in the final model.

5 Results (3): The prevalence of DHFR and DHPS mutations associated with SP resistance and *in vivo* efficacy, parasitological failure and prophylactic failure of SP in pregnant Zambian women

5.1 Chapter introduction

The work in this chapter was included in the thesis in order to present information on the status of parasite resistance in the study population. The chapter gives results of the prevalence of SP resistance markers in a random sample of 96 women who tested positive at first ANC visit. The chapter also presents results from the determination of *in vivo* efficacy of SP and its treatment and prophylactic failure over a period of 28 days in pregnant women who were tested for peripheral parasitaemia at both day 0 and day 28.

5.2 The prevalence of DHFR and DHPS mutations associated with SP resistance

Only 96 samples were processed based on the required sample size for the characterisation of SP resistance markers in this study population. The pyrimethamine resistant DHFR Asn-108 was high among the 96 *P. falciparum* infections that were assayed (94.8%, n = 91). Mixed infections of Asn-108 occurring with the wild type Ser-108 were observed in 3.1% (n = 3) of the samples. High levels of the DHFR Ile-51 and Arg-59 mutants (Figure 5.1) as well as the DHPS Gly-437 and Glu-540 were found (Figure 5.2).

Figure 5. 1: Prevalence of *P. falciparum* DHFR mutations in pregnant women of Nchelenge district

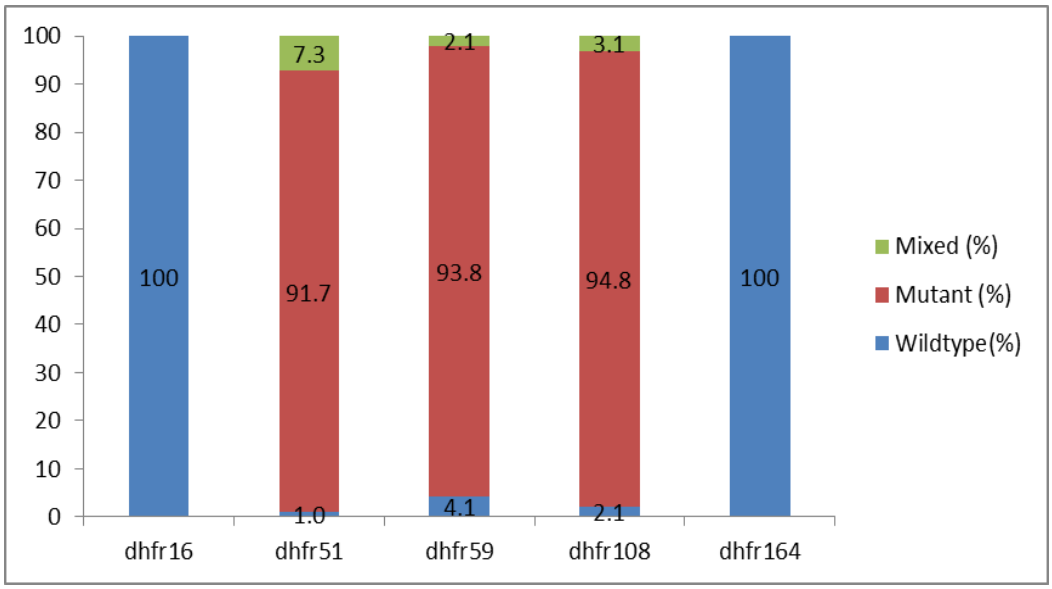


Figure 5. 2: Prevalence of *P. falciparum* DHPS point mutations in pregnant women of Nchelenge district

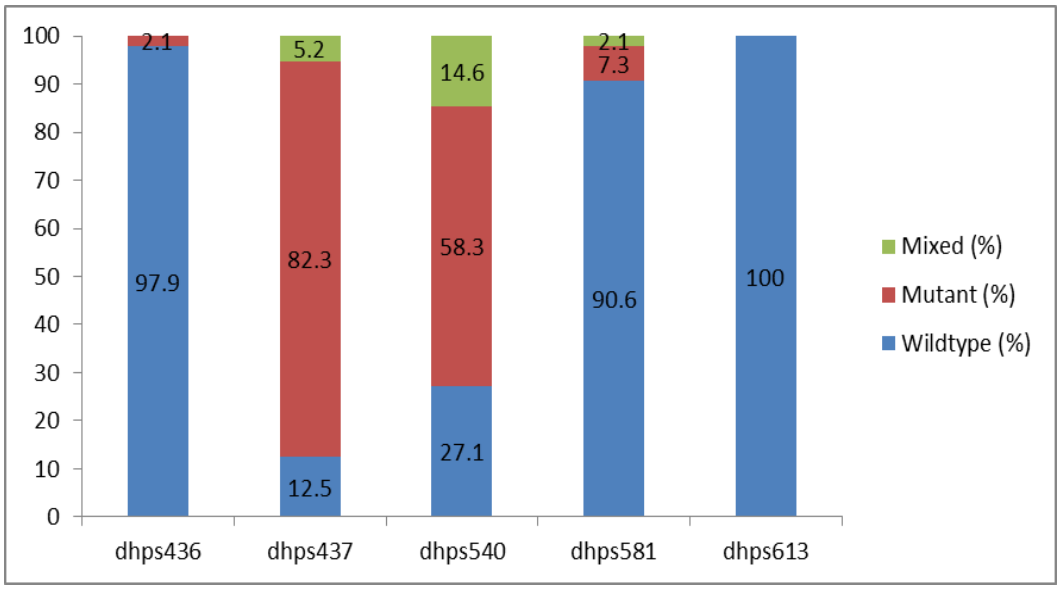


Table 5-1 presents the prevalence of the double, triple, quintuple and sextuple mutants. The DHPS double (Gly-437 + Glu-540) mutant was found in 70.8% (n = 68) and the DHFR triple (Asn-108 + Ile-51 + Arg-59) mutant in 92.7% (n = 89) of the samples that were assayed. The quintuple mutant (DHFR triple + DHPS double) was detected in 68.8% (n = 66). The sextuple mutant (DHFR triple + DHPS double + Arg-581) was observed in 9.4% (n = 9) of the overall total with 2 of these occurring as mixed infections with the wild type.

Table 5- 1: Antifolate multiple mutants among first ANC attendees of Nchelenge District, Zambia

Number of *P. falciparum* infections	Mutant							
	DHFR Triple		DHPS Double		DHFR/DHPS Quintuple		DHFR/DHPS Sextuple	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
96	89	92.7 (85.3- 96.5)	68	70.8 (60.8 -79.2)	66	68.8 (58.6 -77.34)	9	9.4 (4.2-16.0)

95% CI: 95% confidence interval
DHFR: Dihydrofolate reductase
DHPS: Dihydropteroate synthase

Table 5-2 presents results of DHFR and DHPS genotyping of 96 samples. It highlights the number of samples that carried a particular combination of point mutations associated with SP resistance.

Table 5- 2: Results of DHFR and DHPS genotyping of samples from 96 first antenatal care attendees of Nchelenge District

n	dhfr51	dhfr59	dhfr108	dhps437	dhps540	dhps581
1	-	-	-	-	-	-
2	+	-	+	-	-	-
2	+	+	+	-	+	-
1	+	-	+	+	+	-
1	+	+	-	+	+	-
7	+	+	+	-	-	-
16	+	+	+	+	-	-
57	+	+	+	+	+	-
9	+	+	+	+	+	+
- = wild type + = Mutant or mixed genotype						

5.3 Therapeutic efficacy of IPTp-SP over a 28-day period

Therapeutic efficacy of IPTp-SP was assessed based on malaria diagnosed by microscopy only. Of the 343 women who tested positive for malaria by microscopy at day 0, 60.3% (n = 207) were screened for malaria at day 28 post IPTp-SP to assess the therapeutic efficacy and prophylactic effect of SP. The number of women was higher than the 138 asymptomatic malaria positive pregnant women required for determination of therapeutic efficacy as earlier mentioned in the methods section. Of these women 14.3% (n = 49) were HIV-infected and 85.7% (n = 294) were HIV uninfected.

Sulphadoxine-pyrimethamine cleared parasitaemia in 70.0% (n = 145) of the 207 pregnant women who tested positive for malaria at enrolment and were tested at day 28. Slightly below a third of these women, 30.0% (n = 62) were positive at day 0 and at day 28 and were classified as cases of therapeutic failure before PCR correction.

Among those with a negative malaria smear test result at day 0, 52.0% (383/736) were also screened at day 28. Of the 383 women who tested negative at day 0, 12.3% (n = 47) tested positive for malaria at day 28 and 87.7% (n = 336) of those who were negative at day 0 maintained the malaria negative status by day 28. The 47 women who tested negative at day 0 and positive at day 28 were classified as cases of prophylactic failure before PCR correction.

5.4 Classification of parasitological failure cases based on MSP-2 genotyping

Sixty out of the 62 pairs of samples from the participants, who tested positive by microscopy at day 0 and day 28, were successfully processed. One dried blood spot sample among those collected at day 28 was missing and amplification of another sample from another pair was unsuccessful. Of the 60 pairs that were processed 46.7% (95% CI, 33.7-59.7, n = 28) were classified as recrudescence and the rest, 53.3% (95% CI, 40.3-66.3, n = 32) as reinfection (Figure 5.3).

After PCR correction, therapeutic efficacy without recrudescence occurred in 84.4% (173/205) of the women who were infected at day 0. Failure of SP to prevent reinfection occurred in 15.6% (32/205) of participants.

The proportion of therapeutic failure observed among primigravidae, 24.1% (21/87), and secundigravidae, 24.1% (7/29) was similar, however what was observed among multigravidae was much lower (7.9% (7/89), *P* = 0.009). Younger women were more likely to experience failure. Median ages of women who experienced therapeutic failure and those whose parasitaemia cleared were 20 (IQR, 18-22) and 22 (IQR, 19-28) years respectively and the difference in the distribution of age in the two groups was statistically significant (*P* = 0.001).

The median age of women who experienced prophylactic failure was 24 (IQR, 20-29) years and that of women who did not experience prophylactic failure was 26 (IQR, 22-32) years with the distribution of age in the two groups being statistically significant, (*P* = 0.03).

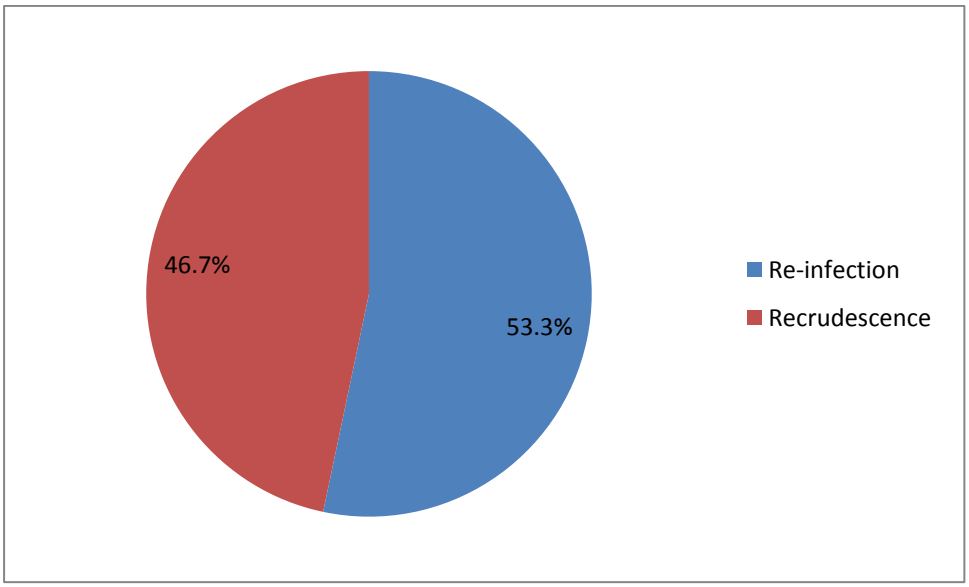
Table 5- 3: Frequency of prophylactic and treatment failure of sulphadoxine-pyrimethamine among HIV negative first ANC attendees of Nchelenge District, Zambia

Category	Total	Number (%)	95% Confidence interval
PCR-uncorrected			
Therapeutic efficacy	207	145 (70.0)	63.8 - 76.3
Therapeutic failure	207	62 (30.0)	23.7 - 36.2
Prophylactic failure	338	47 (12.3)	9.0 -15.6
PCR-corrected			
Therapeutic efficacy	205	173 (84.4)	79.4-89.4
Therapeutic failure	205	32 (15.6)	10.6-20.6

PCR: Polymerase chain reaction

Two samples could not be differentiated into recrudescence or reinfection because one dried blood sample among those collected at day 28 was missing and amplification of another sample from another pair was unsuccessful, thus the reduction in the denominator after PCR correction in the case of therapeutic efficacy and failure

Figure 5. 3: Classification of recurrent parasitaemia among participating women at day 28 post-IPTp-SP in reference to day 0.



6 Results (4) The prevalence of adverse birth outcomes among delivering participants and predictors of adverse birth outcomes

6.1 Chapter introduction

This chapter presents infant and maternal characteristics. Characteristics of delivering women are presented overall and according to recruitment site and follow-up with comparisons of proportions in the groups. The chapter also presents the prevalence of ABO among participating women according to parity and infection status. Proportions of ABO are also presented for each placental malaria histopathology class. The chapter also presents risk factors for composite ABO and for individual ABO (PD, LBW and IUGR). Analysis of risk factors for stillbirth was not conducted because the number of women who delivered still born babies was small.

6.2 Maternal and infant characteristics

6.2.1 Baseline characteristics of delivering participants

Table 6-1 shows baseline characteristics of women who were successfully followed-up to delivery and those who were not. There were no differences in proportions of characteristics among women who were followed up to delivery and those who were lost to follow-up except in the case of gravidity. The proportion of primigravidae among women who were followed up to delivery was higher than that among women who were lost to follow-up (26.6 versus 18.6%, P = 0.004). A lower proportion of secundigravidae was observed among those followed up to delivery than among women lost to follow-up (13.4 versus 18.6%, P = 0.025). In the case of multigravidae, a higher proportion was observed among women lost to follow-up than among those followed to delivery but this was not statistically significant (62.8 versus 60.0%, P = 0.375).

Table 6- 1: Baseline characteristics of study participants according to follow-up to delivery

variable	All (n = 1085) n (%) or Median (IQR)	Followed-up (n = 729) n (%) or Median (IQR)	Lost to follow-up (n = 356) n (%) or Median (IQR)	*P*-value
Age (median)	25 (20-30)	24 (20-30)	25 (20-31)	0.510
Recruitment site				
Kashikishi	747 (68.9)	508 (69.7)	239 (67.1)	0.395
Nchelenge	338 (31.2)	221 (30.3)	117 (32.9)	
Marital status				
Single	203 (18.7)	143 (19.9)	60 (16.4)	0.304
Married	874 (80.6)	569 (79.3)	305 (83.1)	
Divorced/separated/ widowed	8 (0.7)	6 (0.8)	2 (0.5)	
Years of schooling				
None to 6	426 (39.3)	273 (37.4)	153 (43.0)	0.080
7 and above	659 (60.7)	456 (62.6)	203 (57.0)	
Gravidity				
Primigravidae	261 (24.1)	194 (26.6)	67 (18.6)	0.004
Secundigravidae	165 (15.2)	98 (13.4)	67 (18.6)	0.025
Multigravidae	659 (60.7)	437 (60.0)	222 (62.8)	0.375
Bed net ownership				
No	551 (50.8)	373 (51.2)	178 (50.0)	0.718
Yes	534 (49.2)	358 (48.8)	178 (50.0)	
Bed net use				
No	666 (61.6)	452 (62.3)	214 (60.1)	
Yes	416 (38.5)	274 (37.7)	274 (37.7)	0.718
Missing*	3	0	3	
IRS in previous 12 months	808 (78.1)	553 (78.8)	255 (76.8)	0.475
No	226 (21.9)	149 (21.2)	77 (23.2)	
Yes	51	27	24	
Unknown*				
Wealth quintile				
Lowest	217 (20.0)	137 (18.8)	80 (22.6)	0.174
Second	221 (20.4)	148 (20.3)	73 (20.6)	
Middle	214 (19.8)	148 (20.3)	66 (18.6)	
Fourth	215 (19.9)	138 (18.9)	77 (21.8)	
Highest	216 (19.9)	158 (21.7)	58 (16.4)	
HIV status				
Negative	941 (86.8)	635 (87.1)	306 (86.2)	0.678
Positive	143 (13.2)	94 (12.9)	49 (13.8)	
Missing*	1	0	1	
Past experience of miscarriage				
No	708 (85.1)	463 (86.5)	245 (84.8)	0.486
Yes	116 (14.1)	72 (13.5)	44 (15.2)	
NA (primigravidae)*	261	194	67	

Table 6-1: Baseline characteristics of study participants according to follow-up to delivery continued

variable	All (n = 1085) n (%) or Median (IQR)	Followed-up (n = 729) n (%) or Median (IQR)	Lost to follow-up (n = 356) n (%) or Median (IQR)	P-value
Prior delivery of stillborn				
No	755 (91.6)	489 (91.4)	266 (92.0)	0.752
Yes	69 (8.4)	46 (8.6)	23 (8.0)	
NA (primigravidae)*	261	194	67	
Prior delivery of a Preterm baby				0.110
No	784 (95.3)	505 (94.4)	279 (96.9)	
Yes	39 (4.7)	30 (5.6)	9 (3.1)	
Missing*	1	0	1	
NA (primigravidae)	261	194	67	
Malaria infection *PCR*				
Negative	453 (42.2)	304 (42.1)	149 (42.3)	0.944
Positive	621 (57.8)	418 (57.9)	203 (57.7)	
Missing*	11	4	7	
Microscopy				
Negative	736 (68.2)	491 (67.6)	245 (69.4)	0.557
Positive	343 (31.8)	235 (32.4)	108 (30.6)	
Missing*	6	3	3	
STI/RTI				
Negative	384 (35.4)	262 (35.9)	122 (34.4)	0.611
Positive	700 (64.6)	467 (64.1)	233 (65.6)	
Missing*	1	0	1	
Malaria & STI/RTI Coinfection				
No infection	177 (16.5)	119 (16.5)	58 (16.6)	0.782
Malaria	202 (18.8)	142 (19.7)	60 (17.1)	
STI/RTI	278 (26.0)	186 (25.8)	92 (26.3)	
Coinfection	414 (38.7)	274 (38.0)	140 (414)	
Missing*	14	8	6	

IRS: Indoor residual spraying
*Missing values/NA primigravidae are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association

6.2.2 Characteristics of delivering women and infants

There were no known maternal deaths among participants. Table 6-2 presents characteristics of the 729 participants followed up to delivery by recruitment site. There was no statistically significant difference in the place of delivery or type of delivery between the two sites. Slightly more deliveries were conducted by TBAs among women recruited at Nchelenge than those recruited at Kashikishi.

The clinical factors (level of Hb, treatment of RPR positives women, IPTp-SP doses taken, and use of cotrimoxazole and ART, and antimalarial treatment) did not differ between the two sites.

The median gestational age at delivery was 38 (IQR, 37-39) weeks and the mean birth weight was 2784g (95% CI, 2752-2816). Among the 729 participants who were followed-up to delivery, 98.4% (n = 717) had a singleton delivery and the rest delivered twins. Of the 717 singleton deliveries 50.9% (n = 365) were female and 49.1% (n = 352) were male babies. The mean birth weight of female babies was lower than that of male babies (2738g versus 2831g) and this difference was statistically significant *(P* = 0.004).

With the exclusion of BV, 18.8% (n = 46) of the 248 STI-infected women received antibacterial treatment during the pregnancy duration. Of the 467 women who were infected with at least one STI/RTI only 12.8% (n = 60) received antibacterial treatment during the pregnancy duration. Of the 60 women who received antibacterial treatment only eight received drugs other than benzathine penicillin G and 10 of the remaining 52 received both benzathine penicillin G and a different antibiotic during the pregnancy duration based on syndromic management assessment. Antibacterial agents that were used were metronidazole, erythromycin and spectinomycin. Some of the STI/RTI-infected women (n = 40) had been treated with antifungal agents by the time of delivery and only two of these also received an antibiotic. Antifungal agents that were recorded were nystatin and fluconazole.

Coverage of IPTp-SP among participants was high with 99.2% of delivering women receiving at least one dose of IPTp-SP during the pregnancy duration; 0.8% (n = 6), 17.0% (n = 124), 43.0% (n = 314), 38.3% (n = 279) and 0.7% (n = 5) of participants received, none, one, two, three and four doses respectively.

Among the 92 HIV-infected delivering women, 37.0% (n = 34) were on cotrimoxazole prophylaxis. Of the 88 HIV-infected women who took at least one dose of IPTp-SP, 36.4% (n = 32) also took cotrimoxazole during the pregnancy duration.

Table 6- 2: Characteristics of delivering participants according to recruitment site

Variable	All (N = 729) n (%)	Kashikishi (n = 508) n (%)	Nchelenge (n =221) n (%)	P-value
Number of babies				
One	717 (98.4)	501 (98.6)	216 (97.7)	0.388
Two	12 (1.6)	7 (1.4)	5 (2.3)	
Sex of baby				
Female	365 (50.9)	248 (49.5)	117 (54.2)	0.252
Male	352 (49.1)	253 (50.5)	99 (45.8)	
Twins	12	7	5	
Place of delivery				
Hospital	683 (93.8)	479 (94.4)	204 (92.3)	0.320
Health Centre	20 (2.8)	14 (2.8)	6 (2.7)	
Home	25 (3.4)	14 (2.8)	11 (5.0)	
Delivered by				
Family member	22 (3.0)	13 (2.5)	9 (4.1)	0.015
TBA	14 (1.9)	5 (1.0)	9 (4.1)	
Midwife	651 (89.3)	457 (90.0)	194 (87.7)	
Doctor	42 (5.7)	33 (6.5)	9 (4.1)	
Type of delivery				
Vaginal	687 (94.2)	475 (93.5)	212 (95.9)	0.197
C-section	42 (5.8)	33 (6.5)	9 (4.1)	
Haemoglobin level				
Normal	585 (84.0)	406 (83.9)	179 (84.4)	0.855
Anaemia	111 (16.0)	78 (16.1)	33 (15.6)	
Missing*	33	24	9	
Antimalarial treatment besides IPTp-SP				0.649
No	635 (87.5)	440 (87.1)	195 (88.2)	
Yes	91 (12.5)	65 (12.9)	26 (11.8)	
Missing*	3	3	0	
Iron supplementation				
No	15 (2.1)	13 (2.6)	2 (0.9)	0.254
Yes	714 (97.9)	495 (97.4)	219 (99.1)	
IPTp-SP doses taken				
0 to 1	130 (17.9)	97 (19.1)	33 (14.9)	0.174
2 or more	598 (82.1)	410 (80.9)	188 (85.1)	
Missing*	1	1	0	
Cotrimoxazole use among HIV+				0.547
No	58 (63.0)	41 (61.2)	17 (68.0)	
Yes	34 (37.0)	26 (38.8)	18 (32.0)	
Missing*	2	1	1	
NA (HIV negative)*	635	440	195	
ART among HIV positive women				0.333
No	36 (37.2)	24 (35.3)	12 (46.2)	
Yes	58 (61.7)	44 (64.7)	14 (53.8)	
NA (HIV negative)*	635	440	195	

Table 6-2: Characteristics of delivering participants according to recruitment site continued

Variable	All (N = 729) n (%)	Kashikishi (n = 508) n (%)	Nchelenge (n = 221) n (%)	P-value
Treatment among RPR Reactive				0.413
No	29 (35.8)	21 (38.9)	8 (29.6)	
Yes	52 (64.2)	33 (61.1)	21 (70.4)	
Non-reactive	648	454	194	
Anti-bacterial‡ treatment among all STI/RTI positives				0.972
No	407 (87.2)	284 (87.1)	123 (87.2)	
Yes	60 (12.8)	42 (12.9)	18 (12.8)	
NA (Negative)*	262	182	80	

* Missing and NA (not applicable) values are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association
IPTp-SP: Intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine
ART: Antiretroviral therapy. Anaemia was defined as haemoglobin level < 11grams/decilitre
‡Antibacterial treatment included benzathine penicillin G, metronidazole, erythromycin and spectinomycin.

6.3 Prevalence of adverse birth outcomes

Overall the prevalence of ABO among the 717 women with singleton deliveries was 35.0%. Women who experienced at least one ABO were younger than women who had normal birth outcome, median age 22 (IQR, 19-28) verses 25 (IQR, 21-31) years, with the age distribution being significantly different in the two groups, $P < 0.001$, Mann-Whitney *U* test. Preterm delivery and LBW were the most common ABOs, 22.1% and 21.9% respectively.

6.3.1 Prevalence of adverse birth outcome according to parity

The prevalence of composite ABO was 53.4% among primiparae, 31.2% among para II and 27.8% among multiparous women, $P < 0.001$. The prevalence of LBW, PD and IUGR were highest among primiparae followed by para II and lowest among multiparae as follows; PD 29.5%, 20.8% and 18.9%, $P = 0.013$; LBW 41.1%, 19.8% and 13.8%, $P < 0.001$; IUGR 31.6%, 13.2% and 9.3%, $P < 0.001$. None of the para II women experienced stillbirth. The prevalence of stillbirth was higher among primiparae than multiparae but the difference was not statistically significant, 2.6% versus 1.6%, $P = 0.309$ (Table 6-3).

Table 6- 3: Frequency of adverse birth outcomes among participants with singleton deliveries according to parity

Adverse outcome	**All N = 717 n (%)**	**95% Confidence interval[‡]**	**Primiparae n = 193 n (%)**	**Para II n = 96 n (%)**	**Multiparae n = 428 n (%)**	***P-value***
PD						
No	558 (77.9)	74.9-81.0	136 (70.5)	76 (79.2)	346 (81.0)	0.013
Yes	158 (22.1)	19.2-25.3	57 (29.5)	20 (20.8)	81 (18.9)	
Missing*	1					
LBW						
No	559 (78.1)	75.0-81.1	113 (58.9)	77 (80.2)	369 (86.2)	< 0.001
Yes	157 (21.9)	19.0-25.0	79 (41.1)	19 (19.8)	59 (13.8)	
IUGR						
No	473 (84.8)	81.8-87.8	93 (68.4)	66 (86.8)	314 (90.8)	< 0.001
Yes	85 (15.2)	12.5-18.5	43 (31.6)	10 (13.2)	32 (9.3)	
Na (preterm)*	158					
Missing*	1					
Stillbirth						
No	705 (98.3)	97.4-99.3	188 (97.4)	96 (100)	421 (98.4)	0.309
Yes	12 (1.7) [†]	1.0-2.9	5 (2.6)	0 (0.0)	7 (1.64)	
Composite ABO						
No	466 (65.0)	61.5-68.5	90 (46.6)	66 (68.8)	310 (71.4)	< 0.001
Yes	251 (35.0)	31.6-38.6	103 (53.4)	30 (31.2)	118 (27.6)	

[†]The percentage of stillbirths (1.7%) consisted of 1.0% macerated and 0.7% fresh stillbirths. One woman among those with twin deliveries had spontaneous abortion at 26 weeks.
*Missing values are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association
LBW: low birth weight
PD: preterm delivery
IUGR: Intrauterine growth retardation
ABO: Adverse birth outcome

6.3.2 Prevalence of adverse birth outcome among infected and uninfected women

Table 6-4 presents the prevalence of PD, LBW, IUGR and stillbirth) among women classified as infected (infected with at least one STI/RTI or malaria) or uninfected at first ANC attendance. Although not statistically significant, the prevalence of PD, LBW and IUGR was slightly higher among infected women than among uninfected women. The frequency of stillbirth was slightly higher among uninfected than infected women, 1.7% versus 1.5% but this could be due to chance (P = 0.880). The mean birth weight of babies born to uninfected women was higher than those born to infected women but this difference was not statistically significant (2821g versus 2774g, P = 0.285).

Table 6- 4: Prevalence of preterm delivery, low birth weight, intra-uterine growth retardation and stillbirth among women classified as infected and uninfected at first ANC visit

Adverse birth outcome	**All** **N = 709** **n (%)**	**Infected** **N = 117** **n (%)**	**Uninfected** **N = 592** **n (%)**	***P*-Value**
PD				
No	550 (77.7)	93 (79.5)	457 (77.3)	0.608
Yes	158 (22.3)	24 (20.5)	134 (22.7)	
Missing*	1	0	1	
LBW				
No	551 (77.8)	95 (81.2)	456 (77.2)	0.337
Yes	157 (22.2)	22 (18.8)	135 (22.8)	
Missing*	1	0	1	
Stillbirth				
No	698 (98.5)	15 (98.3)	583 (98.5)	0.880
Yes	11(1.5)	2 (1.7)	9 (1.5)	
IUGR				
No	465 (84.5)	82 (88.2)	383 (83.8)	0.288
Yes	85 (15.5)	11 (11.8)	74 (16.2)	
NA (Pre-term)*	158			
Missing*	1			
All				
No	459 (64.7)	81 (69.2)	378 (63.9)	0.266
Yes	250 (35.3)	36 (30.8)	214 (36.1)	

N: Number, was equal to 709, 8 of the 717 participants with singleton deliveries could not be classified as infected or uninfected at recruitment due to missing results and negative outcomes in those available

LBW: low birth weight

PD: preterm delivery

IUGR: Intrauterine growth retardation

NA: Not applicable

* Missing values and NA(pre-term) are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association

6.4 Placental malaria burden

Of the 702 placental tissue samples, placental malaria was identified in 40.9% (n = 287) by histopathology, the majority of these, 30.3% (n = 213) were classified as past infections (class 4). Placental histopathology identified 8.1% (n = 57) chronic infections (class 2) and 2.4% (n = 17) active infections.

The median gestational age of women with placental malaria infection regardless of the pathology class was 38 weeks (IQR, 36-39) and that of uninfected women was 38 weeks (IQR, 37-39) with a statistically significant difference in the distribution of gestational age in the two groups, $P = 0.017$.

The mean birthweight, mean Hb, and median gestational age in the different placental pathology classes and comparison of each class with class 5 (the uninfected) are presented in Table 6-5.

The median gestational age of women with chronic placental malaria infection was lower than that of uninfected women and the distribution of gestational age was significantly different in the two groups 37 (IQR, 35-39) weeks and 38 (IQR, 37-39) weeks, $P = 0.001$. The mean birth weight of babies born to mothers with chronic placental malaria infection was significantly lower than the mean birthweight of babies born to uninfected mothers, 2537 grams versus 2834 grams, $P = 0.001$. Furthermore, the mean Hb of women with chronic placental malaria infection was significantly lower than that of uninfected women, 12.2g/dl versus 13.0g/dl, $P = 0.024$.

Table 6- 5: Association between placental pathology class and birthweight, maternal haemoglobin and gestational age among study participants with singleton deliveries

Pathology class (Number)	Description	Mean Birth weight in grams (95% CI)	Probability compared with uninfected (class 5)	Maternal Haemoglobin Geometric Mean (95% CI)	Probability compared with uninfected (class 5)	Median gestational age (IQR)	Probability compared with uninfected (class 5)
1 (17) Active infection	Parasites, No pigment in monocytes or fibrin	2765 (2561-2969)	0.5022	13.2 (12.2-14.4)	0.731	38 (37-38)	0.284
2 (57) Chronic infection	Parasites, pigment in monocytes +/- fibrin	2537 (2408-2665)	< 0.001	12.2 (11.2-13.2)	0.026	37 (35-39)	0.001
3 (0)* Chronic infection	Parasites, pigment in Fibrin	-	-	-	-	-	-
4 (213) Past infection	No parasites, pigment only	2767 (2706-2828)	0.066	12.8 (12.5-13.3)	0.521	38 (37-39)	0.212
5 (415) No infection	No parasite or pigment (No infection)	2834 (2793-2874)	Reference group	13.0 (12.8-13.3)	Reference group	38 (37-39)	Reference group
-: Blank, no specimen was classified under class 3							

6.4.1 Frequency of adverse birth outcome in placental malaria histopathology classes

Table 6-6 presents the prevalence of LBW, PD, IUGR and stillbirth among 691 women with singleton deliveries and placental histology results. The highest proportion of each of these four ABO was observed among babies born to mothers with placental infection classified under pathology class 2 as follows: PD among women with chronic infection was 37.5% versus 23.5%, 24.0% and 18.1%, in women with active infection, past infection and no infection respectively, *P* = 0.007; LBW in women with chronic infection was 38.5% versus 17.7%, 24.0% and 18.1% in women with active infection, past infection and no infection respectively, *P* = 0.004; IUGR was 17.7% among women with chronic infection against 7.7% 16.3% and 14.9% in women with active infection, past infection and no infection respectively, *P* = 0.827 and the prevalence of stillbirth in women with chronic infection was 5.3% versus 0%, 1.9% and 1.6% in women with active infection, past infection and no infection respectively, *P* = 0.093. The overall prevalence of ABO was also highest among women with chronic placental malaria infection, 50.9%; and in the other classes the prevalence was 29.4% among women with active infection; 38.0% among women with past infection and 31.0% among uninfected women, *P* = 0.017.

Table 6- 6: Prevalence of low birth weight, preterm delivery, intrauterine growth retardation and stillbirth according to pathology class among participants with singleton deliveries

Pathology class	All	1	2	3	4	5	
Adverse birth outcome	**All N = 691 n (%)**	**Parasites, No pigment in monocytes or fibrin (active infection) n (%)**	**Parasites, pigment in monocytes +/- fibrin (Chronic infection) n (%)**	**Parasites, pigment in Fibrin (Chronic infection) n (%)**	**No parasites, pigment only (past infection) n (%)**	**No parasites, no pigment (No infection) n (%)**	**P-Value**
PD							
No	542 (78.4)	13 (76.5)	35 (62.5)	-	158 (76.0)	335 (81.9)	0.007
Yes	149 (21.6)	4 (23.5)	21 (37.5)		50 (24.0)	74 (18.1)	
Missing*	1	0	1		0	0	
LBW							
No	542 (78.4)	14 (82.3)	35 (61.4)	-	158 (76.0)	81.9 (335)	0.004
Yes	149 (21.6)	3 (17.7)	22 (38.6)		50 (24.0)	74 (18.1)	
IUGR							
No	458 (84.7)	12 (92.3)	28 (82.3)	-	133 (83.7)	285 (85.1)	0.827
Yes	83 (15.4)	1 (7.7)	6 (17.7)		26 (16.3)	50 (14.9)	
NA (preterm)*	150						
Stillbirth							
No	680 (98.4)	17 (100)	54 (94.7)	-	204 (98.1)	405 (99.4)	0.098
Yes	11 (1.6)	0 (0)	3 (5.3)		4 (1.9)	4 (1.6)	
Composite ABO							
No	51 (65.3)	12 (70.6)	28 (49.1)		129 (62.0)	282 (69.0)	0.017
Yes	240 (34.7)	5 (29.4)	29 (50.9)		79 (38.0)	127 (31.0)	

LBW: low birth weight, PD: preterm delivery, IUGR: Intrauterine growth retardation, NA: Not applicable
-: Blank, no specimen was classified under pathology class 3
*Missing and NA (preterm) values are only presented as numbers and were not included in the calculation of percentages and in the Chi-squared test for association

6.5 Prevalence of adverse birth outcome according to antimalarial and antibiotic treatment

Table 6-7 shows proportions of ABO in different categories of STIs/RTIs and malaria treatment taken during the pregnancy duration. The proportion of LBW babies delivered among those who were uninfected, infected and treated and infected and untreated women was 20.9%, 10.0% and 24.4% (P = 0.039) respectively. The proportion of LBW babies delivered among women who received antimalarial treatment was higher than that observed among women who were not treated for malaria during pregnancy (34.4 versus 20.2, P = 0.002).

Table 6- 7: Proportions of adverse birth outcome in antimalarial and antibacterial treatment categories

Factor	**Number in category**	**Composite Adverse birth outcome**	**Preterm delivery**	**Low birth weight**	**Intra-uterine birth retardation**
		Number (%)	Number (%)	Number (%)	Number (%)
Syphilis		*P* = 0.840	*P* = 0.352	*P* = 0.449	*P* = 0.423
No	637	223 (35.0)	136 (21.4)	142 (22.3)	80 (16.0)
Yes-treated	52	17 (32.7)	13 (25.0)	8 (15.4)	3 (7.7)
No-untreated	28	11 (39.3)	9 (32.1)	7 (25.9)	2 (10.5)
STI/RTI including syphilis		*P* = 0.566	*P* = 0.912	***P* = 0.037**	*P* = 0.261
No	259	88 (34.0)	50 (19.3)	**54 (20.9)**	33 (15.8)
Yes-treated	60	18 (30.0)	15 (25.0)	**6 (10.0)**	3 (6.7)
Yes- untreated	398	145 (36.4)	93 (23.4)	**94 (24.4)**	49 (16.2)
Antimalarial treatment*		*P* = 0.195	*P* = 0.912	***P* = 0.002**	*P* = 0.217
No	464	213 (34.1)	137 (22.0)	**126 (20.2)**	71 (14.6)
Yes	250	37 (41.1)	20 (22.5)	**31 (34.4)**	14 (20.3)

*Antimalarial treatment (not prophylaxis) during the pregnancy duration
Bold type: Significant outcome (Chi-square test)

6.6 Protective and risk factors for composite adverse birth outcomes

In the univariate analyses the following factors were associated with ABO; height, age, parity, sex of the baby, placental malaria, hypertension and the number of doses of IPTp-SP taken during pregnancy. In the univariate analysis women who were mono-infected (malaria or at least one STI/RTI) or coinfection had 24% and 31% increased risk of experiencing an ABO, respectively compared to uninfected women but this was not statistically significant. None of the STIs/RTIs or malaria diagnosed at first ANC were associated with composite ABO. Women infected with STI/RTI and treated had 17% reduced risk and STI/RTI-infected and untreated women had 11% increased risk of experiencing an ABO than uninfected but this was not statistically significant in univariate analysis.

In the multivariate analysis short stature (< 1.5 metres), parity and the number of doses of IPTp-SP were associated with ABO (Table 6-8). Women with a height < 1.5 metres had a higher risk of experiencing at least one ABO after adjusting for the number of doses of IPTp-SP taken during the pregnancy duration and parity (aOR 1.55; 95% CI, 1.10-2.18, $P = 0.013$). The risk of having an ABO among para II was less than half of the risk observed in primiparous (aOR 0.42; 95% CI, 0.28-0.63, $P < 0.001$) women and this was even much lower among multiparous women (aOR 0.32; 95% CI, 0.22-0.48, $P < 0.001$). After taking account of parity and height, women who had taken 2 or more doses of SP during their pregnancy had a lower risk of experiencing an adverse delivery outcome than women who had taken one dose or none (aOR 0.51; 95% CI, 0.34-0.76, $P = 0.001$). There was no interaction between parity and height ($P = 0.036$), parity and doses of IPTp-SP ($P = 0.067$), height and doses of IPTp-SP ($P = 0.096$).

To check if the effects of malaria, STI/RTIs and their coinfection on birth outcome were modified by the site of recruitment, these variables were individually included in the final model with the variable for recruitment site. There was no interaction between malaria and site of recruitment ($P = 0.672$), STI/RTI and recruitment site ($P = 0.864$) and coinfection and the site of recruitment ($P = 0.809$).

Table 6- 8: Risk factors for composite adverse birth outcomes among study participants with singleton deliveries

Potential risk factor	**Number**	**%**	**Unadjusted OR**	**Adjusted OR**	**Adjusted OR (Final model)**	***P-value***
	717	**100**	**-**	**N = 595**	**N = 715**	**< 0.001[¥]**
Recruitment site			*P* = 0.329	-	-	-
Kashikishi	501	69.9	1.00			
Nchelenge	216	30.1	0.84 (0.59-1.19)			
Age group			*P* < 0.001	*P* = 0.716	-	-
≤ 20	204	28.5	1.00	1.00		
21-25	205	28.6	0.52 (0.35-0.77)	1.26 (0.69-2.29)		
26-30	148	20.6	0.43 (0.27-0.66)	1.14 (0.56-2.35)		
≥ 30	160	22.3	0.34 (0.22-0.54)	1.01(0.48-2.10)		
Years of schooling			*P* = 0.247	-	-	-
0-6 years	266	37.1	1.00			
7 years and above	451	62.9	1.21 (0.88-1.66)			
Maternal height			*P* = 0.002	***P* = 0.013**		
≥ 1.5m	503	70.2	1.00	**1.00**	**1.00**	
< 1.5m	213	29.8	1.69 (1.21-2.35)	**1.57 (1.06-2.32)**	**1.55 (1.10-2.18)**	**0.013**
Missing*	1					
Marital status			*P* = 0.03	*P* = 0.512	-	-
Single	142	19.8	1.00	1.00		
Married	569	79.4	0.57 (0.39- 0.82)	1.14 (0.66-1.97)		
Separated/widowed/divorced	6	0.8	0.24 (0.03-2.03)	-		
Parity			*P* < 0.001	***P* < 0.001**		
Primiparae	193	27.0	1.00	**1.00**	**1.00**	
Para II	96	13.4	0.39 (0.24-0.67)	**0.27 (0.13-0.57)**	**0.41 (0.28-0.63)**	**< 0.001**
Multigravidae	428	59.6	0.33 (0.23-0.47)	**0.31 (0.15-0.61)**	**0.32 (0.20-0.43)**	**< 0.001**
Past experience of miscarriage			P = 0.023	-	-	-
No	457	87.2	1.00			
Yes	67	12.8	1.87 (1.10-3.19)			
Primigravidae*	193					

Table 6-8: Risk factors for composite adverse birth outcomes among study participants with singleton deliveries continued

Potential risk factor	Number	%	Unadjusted OR	Adjusted OR	Adjusted OR (Final model)	*P-value*
	717	**100**	**-**	**N = 595**	**N = 715**	**< 0.001¥**
Prior delivery of still born			P = 0.184	-	-	-
No	481	91.8	1.00			
Yes	43	8.2	1.57 (0.82-3.00)			
Primigravidae*	193					
Prior delivery of a Preterm baby			*P* = 0.733	-	-	-
No	495	94.5	1.00			
Yes	29	5.5	1.15 (0.51-2.59)			
Primigravidae*	193					
Peripheral malaria infection[a]			*P* = 0.181	-	-	-
Negative	301	42.4	1.00			
Positive	409	57.6	1.24 (0.90-1.69)			
Missing*	7					
Placental malaria infection			*P* = 0.015	*P* = 0.149	-	-
Negative	409	59.3	1.00	1.00		
Positive	282	40.7	1.48 (1.08-2.03)	1.28 (0.89-1.84)		
Missing*	26					
Syphilis			*P* = 0.842	-	-	-
Negative	637	88.8	1.00			
Positive-Treated	52	7.3	0.90 (0.49-1.65)			
Positive-Untreated	28	3.9	1.20 (0.55-2.61)			
Chlamydia trachomatis			*P* = 0.971	-	-	-
Negative	683	95.3	1.00			
Positive	34	4.7	1.01 (0.49-2.08)			
Neisseria gonorrhoeae			*P* = 0.893	-	-	-
Negative	695	36.9	1.00			
Positive	22	3.1	1.06 (0.44-2.57)			

Table 6-8: Risk factors for composite adverse birth outcomes among study participants with singleton deliveries continued

Potential risk factor	Number	%	Unadjusted OR	Adjusted OR	Adjusted OR (Final model)	*P*-value
	717	**100**	**-**	**N = 595**	**N = 715**	**< 0.001[¥]**
Trichomonas vaginalis			*P* = 0. 121	-	-	-
Negative	547	76.3	1.00			
Positive	170	23.7	1.33 (0.93-1.89)			
Bacterial vaginosis			*P* = 0.539	-	-	-
Normal	246	34.4	1.00			
Intermediate	130	18.2	1.01 (0.65-1.58)			
Positive	339	47.4	0.84 (0.60-1.19)			
Missing*	2					
STI/RTI			*P* = 0.562	-	-	-
Uninfected[‡]	259	36.1	1.00			
Infected & treated	60	8.4	0.83 (0.45-1.53)			
Infected & untreated	398	55.5	1.11 (0.80-1.55)			
Malaria and STI/ RTI coinfection[b]			*P* = 0.507	-	-	-
No infection	117	16.5	1.00			
Single infection[c]	326	46.1	1.24 (0.79-1.96)			
Coinfection[b]	266	37.5	1.31 (0.82-2.09)			
Missing*	8					
Anaemia[$]			*P* = 0.255	-	-	-
Normal	574	83.8	1.00			
Anaemic	111	16.2	1.28 (0.83-1.96)			
Missing*	32					
Hypertension			*P* = 0.027		-	-
Normal	608	97.8	1.00	1.00		
Hypertensive	14	2.2	3.36 (1.11-10.2)	0.73 (0.50-1.05)		
Missing*	95					
Sex of baby			*P* = 0.055	*P* = 0.103	-	-
Female	365	50.9	1.00	1.00		
Male	352	49.1	0.74 (0.54-1.00)	0.73 (0.50-1.05)		

Table 6-8: Risk factors for composite adverse birth outcomes among study participants with singleton deliveries continued

Potential risk factor	Number	%	Unadjusted OR	Adjusted OR	Final model	*P*-value
	717	**100**	-	**N = 595**	**N = 715**	**< 0.001[¥]**
Antimalarial treatment			*P* = 0.199	-	-	-
No	464	65.0	1.00			
Yes	250	35.0	1.35 (0.86-2.11)			
Missing*	3					
Number of doses of IPTp-SP			*P* < 0.001	***P* = 0.015**		
0 or 1	126	17.6	1.00	**1.00**	**1.00**	
2 or more	590	82.4	0.57 (0.38-0.84)	**0.56 (0.36-0.89)**	**0.51 (0.34-0.76)**	**0.001**
Missing*	1					

[a]Malaria infection at first ANC attendance; [b]Infection with at least one STI/RTI and malaria; [c]Infection with at least one STI/RTI or malaria
IPTp-SP – Intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine
[¥]*P-value* for overall final model
P-values appearing with unadjusted odds ratios are for each univariate model indicating associations of the variable with the outcome at *P* < 0.1.
P-values appearing with adjusted odds ratios indicate the independent association of each variable with the outcome at *P* < 0.05.
Bold print: statistically significant outcome for multivariate analysis and final model
[$] Normal haemoglobin level (Hb) was defined as Hb ≥ 11grams per decilitre and anaemia as Hb < 11 grams per decilitre.
Number of participants less than 715) (Number of singleton deliveries) are indicated in the first column. These were due to missing values in individual variables
*Missing/primigravidae values are only presented as numbers and were not included in the calculation of percentages and in the models
When placental malaria was put in a model with maternal height, parity and number of doses of IPTP-SP, it was not independently associated with adverse birth outcomes.
[†]Antibacterial treatment included benzathine penicillin, metronidazole, erythromycin and spectinomycin.
[‡] Uninfected in relation to STI/RTI among those who were followed-up to delivery (malaria was not considered)
Past experience of a miscarriage was associated with adverse birth outcome in the univariate analysis but was not included in the multivariable model because that would have excluded all primiparous women from the model.

6.7 Risk factors for PD, LBW and IUGR

Univariate and multivariate analyses of factors associated with individual ABO (PD, LBW and IUGR) are reported in Table 6-9. The number of women who experienced stillbirth was small (12) and was not amenable to statistical analyses. None of the individual STIs/RTIs or malaria diagnosed at first ANC or placental malaria diagnosed at delivery were associated with any one of the individual ABO.

Potential risk factors for ABO that were associated with individual ABO at $P < 0.1$ and were included in the multivariate models for each outcome were as follows: PD was associated with maternal age group, maternal height, parity, placental malaria, anaemia, and the number of doses of IPTp-SP taken during pregnancy; LBW was associated with maternal age group, maternal height, parity, infection with trichomoniasis, HIV status, placental malaria and antimalarial treatment during pregnancy; IUGR was associated with maternal age group, maternal height, parity, and peripheral malaria diagnosed at first ANC attendance.

In the multivariate analysis only the number of doses of SP taken during the pregnancy duration was independently associated with PD; LBW was associated with marital status, maternal height, parity, HIV and antimalarial treatment during the pregnancy duration; and IUGR was associated with parity and height.

The final models for factors associated with PD, LBW and IUGR are shown in Table 6-10. In the final model women who took 2 or more doses of SP had a reduced risk of experiencing PD (OR 0.34; 95% CI, 0.23-0.52) than women who took one dose or none.

Marital status was not independently associated with LBW in a model with height, parity, HIV and antimalarial treatment during the pregnancy duration hence it was excluded from the final model. Compared to primiparae, Para II women had a reduced risk of delivering LBW babies (aOR 0.32; 95% CI; 0.18-0.58) and the risk was slightly lower among multiparous women (aOR 0.22; 95% CI, 0.15-0.34). Women with height < 1.5 metres had increased risk of delivering a LBW baby than women whose height was ≥ 1.5 metres (aOR 2.10, 95% CI, 1.43-3.08). Women who had been treated for malaria were at increased risk of experiencing LBW than women who had not been treated for malaria during the pregnancy duration (aOR 2.01; 95%

CI, 1.20-3.5). Women infected with HIV had twice the risk of delivering LBW babies than HIV-uninfected (aOR 2.10; 95% CI, 1.23-3.55).

A reduced risk of IUGR was observed among para II (aOR 0.33; 95% CI, 0.16-0.71) and multiparae (aOR 0.23; 95% CI, 0.13-0.37) in comparison to primiparae and babies born to shorter women were at increased risk of experiencing IUGR (aOR 1.71; 95% CI, 1.04-2.83) than those born to women whose height was ≥ 1.5 metres.

Table 6- 9: Univariate and multivariate analyses of factors associated with PD, LBW and IUGR

Potential risk factor	Preterm delivery		Low birth weight		Intra-uterine growth retardation	
	Unadjusted OR	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**
	-	**N = 659**	**-**	**N = 659**	**-**	**N = 540**
Recruitment site Kashikishi Nchelenge	*P* = 0.648 1.00 1.09 (0.74-1.60)	-	*P* = 0.900 1.00 1.02 (0.69-1.51)	-	*P* = 0.910 1.00 0.97(0.58-1.16)	-
Age group ≤ 20 21-25 26-30 ≥ 30	*P* = 0.045 1.00 0.61 (0.38-0.97) 0.63 (0.38-1.04) 0.54 (0.31- 0.87)	*P* = 0.812 1.00 0.85 (0.44-1.65) 0.88 (0.39-1.96) 0.69 (0.31-1.56)	*P* < 0.001 1.00 0.48 (0.31-0.74) 0.31 (0.18-0.53) 0.24 (0.13-0.42)	*P* = 0.677 1.00 1.42 (0.74-2.71) 1.20 (0.51-2.78) 1.02 (0.41-2.55)	*P* < 0.001 1.00 0.48 (0.27-0.85) 0.28 (0.13-0.58) 0.22 (0.11-0.47)	*P* = 0.918 1.00 1.34 (0.60-2.88) 0.95 (0.33-2.72) 0.86 (0.29-2.52)
Height ≥ 1.5m < 1.5m	*P* = 0.053 1.00 1.45 (0.99-2.11)	*P* = 0.262 1.00 1.28 (0.83-1.98)	*P* < 0.001 1.00 2.25 (1.56-3.26)	***P* = 0.002** **1.00** **1.90 (1.24-2.91)**	*P* = 0.019 1.00 1.80 (1.11-2.91)	***P* = 0.026** **1.00** **1.81 (1.07-3.06)**
Parity Primiparae Para II Multiparae	*P* = 0.015 1.00 0.63 (0.35-1.12) 0.56 (0.38-0.83)	P = 0.150 1.00 0.47 (0.21-1.02) 0.69 (0.34-1.39)	*P* < 0.001 1.00 0.35 (0.20-0.63) 0.22 (0.15-0.34)	***P* < 0.001** **1.00** **0.18 (0.09-0.37)** **0.15 (0.06-0.34)**	*P* < 0.001 1.00 0.33 (0.15-0.70) 0.22 (0.13-0.37)	***P* = 0.005** **1.00** **0.33 (0.13-0.86)** **0.27 (0.11-0.66)**
Bacterial vaginosis Negative Intermediate Positive	*P* =0.783 1.00 0.92 (0.54-1.55) 1.08 (0.73-1.62)	-	*P* = 0.404 1.00 1.05 (0.64-1.73) 0.80 (0.53-1.19)	-	P = 0.175 1.00 1.05 (0.57-1.96) 0.65 (0.39-1.10)	-
Trichomoniasis Negative Positive	*P* = 0.435 1.00 1.18 (0.78-1.77)	-	*P* = 0.069 1.00 1.45 (0.97-2.16)	*P* = 0.722 1.00 0.50 (0.10-2.49)	*P* = 0.132 1.00 1.49 (0.89-2.5)	-

Table 6-9: Univariate and multivariate analyses of factors associated with PD, LBW and IUGR continued

Potential risk factor	Preterm delivery		Low birth weight		Intra-uterine growth retardation	
	Unadjusted OR	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**
	-	**N = 659**	**-**	**N = 659**	**-**	**N = 540**
Chlamydia Negative Positive	P = 0.834 1.00 1.09 (0.48-2.46)	-	P = 0.818 1.00 1.08 (048-2.48)	-	P = 0.982 1.00 1.01 (0.34-3.01)	-
Gonorrhoea Negative Positive	P = 0.559 1.00 1.34 (0.51-3.47)	-	P = 0.659 1.00 0.78 (0.26-2.35)	-	P = 0.752 1.00 0.79 (0.18-3.54)	-
Confirmed Syphilis Negative Positive-treated Positive-untreated	*P* = 0.380 1.00 1.02 (0.46-2.29) 1.53 (0.58-4.07)	-	*P* = 0.292 1.00 0.57 (0.22-1.49) 1.63 (0.61-4.36)	-	*P* = 0.270 1.00 0.41 (0.09-1.74) 0.62 (0.05-3.15)	-
STIs/RTIs No Yes-treated Yes- untreated	*P* = 0.387 1.00 1.39 (0.72-2.70) 1.28 (0.87-1.88)	-	*P* = 0.023 1.00 0.42 (0.17-1.03) 1.23 (0.84-1.79)	*P* = 0.127 1.00 0.39 (0.15-1.04) 0.91 (0.56-1.47)	*P* = 0.186 1.00 0.38 (0.11-1.30) 1.02 (0.63-1.66)	-
HIV status Negative Positive	*P* = 0.193 1.00 1.40 (0.85-2.31)	-	P = 0.035 1.00 1.71 (1.05-2.77)	***P* = 0.029** **1.00** **1.91 (1.04-3.49)**	P = 0.48 1.00 1.27 (0.65-2.50)	-
Peripheral Malaria at first ANC visit Negative Positive	*P* = 0.989 1.00 1.00 (0.70-1.43)	-	*P* = 0.160 1.00 1.30 (0.90-1.87)	-	*P* = 0.081 1.00 1.53 (0.94-2.48)	*P* = 0.208 1.00 1.38 (0.82-2.34)
Placental malaria Negative Positive	*P* = 0.007 1.00 1.64 (1.14-2.37)	*P* = 0.069 1.00 1.46 (0.97-2.20)	*P* = 0.008 1.00 1.64 (1.14-2.36)	*P* = 0.157 1.00 1.37 (0.90-2.07)	*P* = 0.732 1.00 1.08 (0.67-1.75)	-

Table 6-9: Univariate and multivariate analyses of factors associated with PD, LBW and IUGR continued

Potential risk factor	Preterm delivery		Low birth weight		Intra-uterine growth retardation	
	Unadjusted OR	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**	**Unadjusted OR**	**Adjusted OR**
	-	**N = 659**	**-**	**N = 659**	**-**	**N = 540**
Anaemia[¥] Normal Anaemic	*P* = 0.051 1.00 1.60 (1.01-2.52)	*P* = 0.177 1.00 1.44 (0.85-2.46)	*P* = 0.610 1.00 1.13 (0.70-1.84)	-	*P* = 0.413 1.00 1.75 (0.37-1.52)	-
Hypertension Normal Hypertensive	*P* = 0.097 1.00 2.57 (0.87-7.54)	*P* = 0.245 1.00 1.99 (0.63-6.20)	*P* = 0.573 1.00 1.41 (0.44-4.58)	-	*P* = 0.112 1.00 3.55 (0.83-15.2)	-
Sex of baby Female Male	*P* = 0.035 1.00 0.68 (0.48-0.98)	*P* = 0.083 1.00 0.69 (0.46-1.05)	*P* = 0.349 1.00 0.84 (0.59-1.20)	-	*P* = 0.739 1.00 0.92 (0.58-1.47)	-
Antimalarial treatment No Yes	P = 0.912 1.00 1.03 (0.60-1.76)	-	*P* = 0.004 1.00 2.08 (1.29-3.34)	***P* = 0.016** **1.00** **2.00 (1.15-3.47)**	*P* = 0.233 1.00 1.49 (0.79-2.82)	-
Number of doses of IPTp-SP 0 or 1 2 or more	*P* < 0.001 1.00 0.34 (0.23-0.52)	***P* < 0.001** **1.00** **0.35 (0.22-0.56)**	*P* = 0.260 1.00 0.77 (0.49-1.21)	-	*P* = 0.104 1.00 1.87 (0.83-4.24)	-

Bold type indicates statistically significant outcome

[¥] Normal haemoglobin level (Hb) was defined as Hb ≥ 11grams per decilitre and anaemia as Hb < 11 grams per decilitre.

Only 12 women experienced stillbirth and were not amenable to statistical analysis because of the small number.

Table 6- 10: Final logistic regression models of factors associated with PD, LBW and IUGR

Risk factor	**Preterm delivery**	***P*-value**	**Low birth weight**	***P*-value**	**Intra-uterine growth retardation**	***P*-value**
	N = 715	**< 0.001¥**	**N = 713**	**< 0.001¥**	**N = 557**	**< 0.001¥**
Parity Primiparae Para II Multigravidae	-	**-**	 **1.00** **0.32 (0.18-0.58)** **0.22 (0.15-0.34)**	 **< 0.001** **< 0.001**	 **1.00** **0.33 (0.16-0.71)** **0.23 (0.13-0.37)**	 **0.005** **< 0.001**
Maternal Height ≥ 1.5m < 1.5m	-	**-**	 **1.00** **2.10 (1.42-3.10)**	 **< 0.001**	 **1.00** **1.71 (1.04-2.83)**	 **0.035**
HIV status Negative Positive	-	-	 **1.00** **2.10 (1.23-3.55)**	 **0.006**	-	-
Antimalarial Other than IPTp-SP No Yes	-	-	 **1.00** **2.01 (1.20-3.35)**	 **0.008**	-	-
Number of doses of IPTp-SP 0 or 1 2 or more	 **1.00** **0.34 (0.23-0.52)**	 **< 0.001**	-	-	-	-

¥*P* value for overall final model

-: Blank-only variables independent associated with each adverse outcome in multivariate analysis are shown

Bold type indicates statistically significant outcome

When marital and HIV status were put in a model with parity and height in the analysis of risk factors for LBW, there were not independently associated with LBW therefore they were excluded from the final model.

7 Discussion

7.1 Losses to follow-up

The loss to follow in this study was much higher (33%) than was expected (10%) and provided for in the sample size mainly due to interruptions in the provision of transport that were experienced as a result of delays in the disbursement of project funds during the follow-up to delivery period. Monthly efforts to supplement the budget during the waiting period were not sufficient because provision of transport was only possible for about a week in each month. The extended periods of lack of funds for fuel resulted in high losses to follow-up as it was impossible to provide transport to some delivering women who called members of the team during the period when funds were unavailable. Long distances to the health facilities and lack of transport have been cited as some of the reasons why women deliver at home in Zambia [174, 210]. During this period, team members who were still involved in the study went without pay and this may have negatively influenced their attitude towards work although they were assured of backdated payment when funds became available.

Furthermore, before the unavailability of funds became a problem, the main field vehicle that was used to transport participants to and from the hospital was involved in an accident and was destroyed by an act of arson which resulted in a period of transport shortage before alternative transport arrangements could be made.

In the current study refusals were low (< 1%) and only one participant withdrew from the study, however a high proportion of participants (30%) were lost to follow-up. A higher proportion of loss to follow-up than that observed in the current study was noted in another study conducted in 2010-2011 in Mansa District by Tan *et al*., in the same province where the current study was carried out. The study was designed to examine the efficacy of IPTp-SP and presence of quintuple mutant in pregnant women [114]. Participants were followed up weekly over a period of 5 weeks. Among 109 pregnant women who were recruited, 53 (58%) completed the study, 34 (31%) had incomplete follow-up and 17 (16%) were lost after day 0 despite the fact that there were no refusals and withdrawals [114]. The study by Tan *et al*., differs from the current study in that the number of follow-up points was higher (weekly) over a shorter period of time (5 weeks) while in the current study follow-up was scheduled at day 28 (for some participants) and at delivery for all participants.

Weekly follow-up over a period of 5 weeks may be difficult to endure in comparison to follow-up at day 28 and at delivery although follow-up after longer periods of time may be difficult to remember. However in the current study global positioning system mapping of homes locations and checking on some participants via phone calls helped to reduce incidence of loss to follow-up although contact via phone was only possible in the case of participants with active phone lines over the study period. Factors associated with high losses to follow-up in this region are not clear but understanding these factors might be helpful to future studies. It is highly likely that the proportion of women lost to follow-up to delivery was increased by myths and traditional beliefs discussed in Section 1.8 that influence birthing practices in Zambia and cause women to prefer delivery at home in seclusion or away from hospital and their home villages.

7.2 Prevalence of malaria, STI/RTI and their coinfection

7.2.1 The malaria burden

This study provides a robust estimate of the prevalence of peripheral malaria infection among pregnant women of Nchelenge District. A high burden of *P. falciparum* malaria diagnosed by PCR was observed in this population. The prevalence of malaria detected by microscopy was much lower than that detected by PCR suggesting that a good proportion of this study population had sub-microscopic infections (smear negative but PCR positive). Sub-microscopic infections have been associated with placental malaria and decreased maternal Hb [211]. Although no association was found between sub-microscopic infections and adverse maternal and foetal outcomes, the importance of these infections in pregnancy cannot be ignored in malaria policy formulation because of their role in maternal morbidity and malaria transmission [211].

Seven samples that were positive by microscopy in this study were found negative by PCR. Because PCR diagnostic method is more precise than standard microscopy [212], we would expect that a malaria sample found positive by microscopy would also be positive by PCR. The observation above could be attributed to a number of factors including malaria infection due to other *Plasmodium* species and insufficient DNA extraction. A negative PCR test result may be an outcome if the amount of

blood collected on filter paper was less than 25µl, coupled with low parasitaemia. Negative PCR results for microscopic positive samples have been noted in other studies [98, 99].

The prevalence of malaria infection by microscopy was very similar to the prevalence of parasitaemia observed in children less than five-years-old in 2012 (32.1%, 114/356) in the MIS in Luapula Province [189]. Another study conducted in the same area among children less than 10 years-of-age reported a similar parasite prevalence, 30.2% (236/782) by microscopy [188]. The prevalence of placental malaria in the current study, 40.9% (95% CI, 37.3-44.6), is comparable to what was found in another study, 37% (95% CI: 32.5-41.5), conducted within Luapula province in Mansa District [90].

Analysis of monthly-reported district-level malaria cases among pregnant women showed that MiP in Zambia decreased between 2010 and 2013, although persistent hot spots were reported in the southeast and northeast of the country [213].

The prevalence of malaria among 375 pregnant women in the study conducted in Nchelenge District between February and April 2013 by Siame *et al.* was much lower than what was observed in the current study, 15.0% by microscopy and 22.0% by PCR [115]. The observed difference in malaria prevalence in the two studies could be attributed to a number of factors. Firstly, in the current study women attending the Nchelenge and Kashikishi health centres were recruited, whereas in the study by Siame *et al.* a third health centre, Kabuta, was included in addition to these two. Secondly, in the study by Siame *et al.* pregnant women who sought ANC services were included, regardless of visit number; in this study, only first ANC attendees who had not taken antimalarial treatment in the previous month before recruitment were enrolled. Some of the participants who were included in the study by Siame *et al.* had received IPTp-SP during their earlier ANC visit(s) as well as antimalarial treatment before enrolment, and it is unclear what time period may have elapsed between treatment and subsequent screening. The prevalence of malaria is likely lower in a group of women exposed to any IPTp-SP because even one dose of IPTp-SP has been shown to protect against maternal parasitaemia [154]. Furthermore, the last IRS exercise before the current study was conducted in November 2012 and no IRS was conducted in the community by the district health office between October

2012 and April 2014 [186]. The study by Siame *et al.,* was conducted in April 2013 a period during which the insecticide residue in the houses in the community would be expected to be more effective against mosquitoes in comparison to recruitment period of the current study which commenced about 11 months after the IRS exercise (November 2013 to April 2014).

The coverage of bed nets in this study was lower than that observed in the study by Siame *et al.,* (74% versus 49.2%). In the study by Siame *et al.,* bed net ownership among pregnant women was used to characterise the percentage of households with at least one bed net while in the current study bed net ownership describes the percentage of pregnant women who slept in a protected space. It is therefore expected that ownership of at least one bed net in a household would be higher than that observed when bed net ownership is limited to the participant's sleeping spaces. No association between malaria infection and bed net ownership was observed in the study by Siame *et al*., just like in the current study.

There was a difference in malaria infection and parasite densities among HIV-uninfected and HIV-infected women, a finding similar to other studies [214-220]. The proportion of malaria infection detected by microscopy and by PCR in this study was consistently higher in HIV-infected than HIV-uninfected women. HIV-infected women had a 46% increased risk of malaria infection detected by PCR at first ANC visit (P = 0.045). However, the association between HIV and malaria infection was not statistically significant.

Previous studies have reported stronger association between HIV and peripheral malaria detected by microscopy in pregnant women. A hospital based study in Zimbabwe in 2000-2001 reported a high odds of malaria parasitaemia in HIV-infected women compared to HIV-uninfected women (OR 3.96; 95% CI, 2.42-6.46) [219]; a study in Rwanda conducted between 1992 and 1993 reported that the risk of parasitaemia was moderately higher in HIV-infected compared to HIV-uninfected women (RR 1.4; 95% CI, 1.1-1.6, P = 0.016) [216]; a similar finding was reported from Kenya in a study conducted between 1996 and 1999 among HIV-infected women in the third trimester (RR 1.70; 95% CI, 1.52-1.90) and at delivery (RR 1.56; 95% CI, 1.34-1.81) [220]. The prevalence of malaria parasitaemia among HIV-infected

women at first ANC visit was higher than among HIV-uninfected women in a study in Malawi conducted between 1987 and 1990 (56.6% versus 43.6%, $P < 0.001$) [218].

The use of antiretroviral therapy was not stated in all the studies above and, considering the years in which these studies were conducted, it can be assumed that coverage of antiretroviral therapy among pregnant women should have been very low if not non-existent. In the current study, however, the coverage of antiretroviral therapy at first ANC visit was 42.7% among all HIV-positive women, newly-tested and known HIV-positive cases. Some of the women who were screened in the current study were receiving cotrimoxazole prophylaxis although coverage data of cotrimoxazole at recruitment was not collected. However, data from delivery showed that 37% of HIV-infected study participants had received cotrimoxazole prophylaxis during pregnancy. Thus, the relatively a weaker association between HIV-infected status and malaria parasitaemia observed in the current study compared to the previously reported studies could be due to the fact that most HIV-infected women were on ARV therapy and/or cotrimoxazole prophylaxis.

There was no difference in the distribution of parasite counts in HIV-infected women on antiretroviral therapy and women who were not on treatment. This could be because HIV in women who were not on antiretroviral therapy had not progressed to a state of highly compromised immunity since some women tested positive for the first time. Furthermore, eligibility to receive antiretroviral drugs in this community was linked to CD4 count. Therefore, if women known to be HIV-positive were not on therapy, it was likely that their CD4 count was above 500 cells per cubic millimetre of blood.

7.2.2 Prevalence of curable STIs/RTIs

The combined prevalence of STI/RTIs (64.6%) was higher than that of malaria as was suggested in the review by Chico *et al*., [6]. Estimates of individual STIs/RTIs in the current study fall within the 95% upper and lower limits of pooled estimates for East and Southern Africa reported in the study by Chico *et al.,* except in the case of syphilis which was estimated at syphilis 4.5% (95% CI, 3.9-5.1). Although the prevalence of syphilis (7.1%) obtained in this study is higher than the estimated 95% CI upper limit for East and Southern Africa (3.9-5.1), it falls within the range of

estimates (lowest to highest prevalence, 0-14.6) obtained from studies conducted in Zambia and neighbouring countries.

There are a few published studies on STIs/RTI in Zambia. Two papers were published from the HIV Preventions Trials Network (HPTN) 024 study, one reported on syphilis and the other was on the rest curable STI/RTI [135, 140]. The HPTN-024 study (conducted between 2001 and 2003) showed a high prevalence of individual STIs/RTIs in HIV-infected pregnant women (N = 642) of Lusaka. The prevalence estimates of the same STIs/RTIs infections among HIV-infected pregnant women (n = 143) of Nchelenge district in this study were higher than those found in the HPTN-024 study. Comparison of the prevalence of infection in pregnant HIV-infected women of Lusaka in the HPTN-024 study versus what was observed in HIV-infected pregnant women in the current study are as follows; Chlamydia, 6.1% versus 7.0%; gonorrhoea, 1.6% versus 6.3%; trichomoniasis 20.9% versus 23.8%; syphilis, 13.0% versus 15.8% and BV, 40.0% versus 66.4%. The diagnostic tools that were used in the HPTN-024 study were Gram stain microscopy using the Nugent score, wet mount, an enzyme immunosorbent assay kit, culture (then gram stain appearance and positive oxidase reaction) and RPR (as well as *Treponema pallidum* particle agglutination or TPHA confirmation) for diagnosis of BV, trichomoniasis, Chlamydia, gonorrhoea and syphilis respectively. The methods that were used for BV and syphilis diagnosis in the current study were the same as in the HTPN-024 study and the other infections (Chlamydia, gonorrhoea and trichomoniasis) were detected using PCR. It is likely that the prevalence of STIs/RTIs was higher in the current study population than in the HTPN-024 study population but the differences can partially be attributed to the diagnostic tool (PCR) with higher sensitivity that was used for the detection of some of the infections in the current study.

The fact that high levels of asymptomatic STIs/RTIs exist in this population is not surprising because literature shows that high proportions of STIs/RTIs in women are asymptomatic [166, 221]. The number of women who complained of at least one symptom associated with STIs/RTIs among women with at least one curable STI/RTI was low (14.0%). The proportion remained unchanged when only curable STIs (excluding BV) were considered. Among 374 women who tested positive for at least one STI, only 14.2% (n = 53) indicated that they were experiencing at least one STI

symptom at recruitment. This implies that a large proportion of (86.8%) of curable STI-infected women were asymptomatic in agreement with what has been observed from literature. High proportions of gonococcal (80%) and Chlamydial (70-75%) infections have been shown to be asymptomatic [164] and approximately 60-70% of asymptomatic *T. vaginalis* infection and BV have been reported [165, 166].

The high burden of STI/RTI that are asymptomatic in this study population is a clear demonstration of missed treatment opportunities. Although the current ANC package for the management of STI/RTIs is partially effective, it is clear that a huge burden of STI/RTI remains untreated. In the interest of reducing STI/RTI morbidity, there is need to devise alternative strategies for the management of STIs/RTIs in pregnant women.

Provision of point of care tests for STI/RTIs would be beneficial for the reduction of STI/RTI morbidity in this setting. This is crucial especially when the role of STI/RTI in HIV transmission is considered. Different STIs/RTIs, BV [222-226], trichomoniasis [227-232], Chlamydia [233, 234], gonorrhoea [233, 235] and syphilis [233, 235, 236] have been individually identified as risk factors for HIV acquisition and/or transmission. Furthermore, studies have shown that treatment of Chlamydia, gonorrhoea and trichomoniasis reduced genital shedding of HIV in women [237, 238]. In this context screening and treatment of STI/RTI would reduce HIV transmission. In the current study higher proportions of syphilis, Chlamydia, gonorrhoea and BV were found among HIV-infected women than HIV-uninfected women although this was only statistically significant in the case of BV, gonorrhoea and syphilis. A statistically significant higher proportion of composite STI/RTI was also observed among HIV-infected women. In this context good diagnostics and appropriate treatment of STIs/RTIs have the potential to impact the incidence of HIV. The fact that the prevalence of STIs/RTIs was high in this resource poor setting supports calls for the development of a vaccine against five common STI pathogens (herpes simplex virus, *C. trachomatis, N. gonorrhoea, T. vaginalis* and *T. pallidum)* [239].

7.2.3 Prevalence of malaria and STI/RTI coinfection

This is the first study to report malaria and curable STI/RTI coinfection in pregnancy. The estimated prevalence of malaria and STI/RTI coinfection in this study was considerable, irrespective of whether malaria was diagnosed by PCR or microscopy, 38.7% and 21.4% respectively. Before this the only report of coinfection was from a sub-analysis of results from a study in Tanzania which showed that 48.3% of RPR-positive women had placental malaria [6, 36].

The prevalence of STIs/RTIs and their coinfection with malaria represent an important finding in the context of the ongoing search for alternative drugs to replace SP for use in IPTp necessitated by the emergence of SP resistant parasites in some settings. The 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 poor resource settings such as this where STIs/RTIs are highly prevalent and routine screening may not be sustainable.

7.3 Risk factors for malaria, curable STIs/RTIs and their coinfection

7.3.1 Risk factors for malaria infection

In the univariate analysis, the risk of infection was strongly associated with gravidity and age, but this was not observed in the multivariate analysis. Studies have shown that in malaria-endemic areas, the prevalence of malaria, both clinical and asymptomatic, is highest in young women and primigravidae and paucigravidae (primigravidae and secundigravidae combined) [47, 240, 241]. This is due to acquisition of semi-immunity that is gravidity-dependent such that malaria infection tends to be less prevalent and less severe among multigravidae [42, 47, 240]. However, HIV infection has been shown to impair the ability of multigravidae to manage malaria infection [214, 220]. The fact that HIV prevalence was highest among multigravidae in this study population is a plausible explanation for the fact that there was no association between parity and malaria infection in multivariate analysis.

The low coverage of IRS and bed net use in an area of intense transmission may partially explain the high prevalence of malaria infection. Despite the progress that has been made in scaling-up malaria control interventions, a high burden of malaria still remains in Nchelenge District [108, 242]. Several reasons may underlie this observation including population movements from high endemic areas (internally and across borders), increasing parasite resistance to insecticides [188, 242], as well as homes having been built close to water sources that serve as breeding grounds for *Anopheles* mosquitoes.

The risk of malaria infection was higher among women at Nchelenge health centre compared to Kashikishi health centre. Since there was no difference in socio-demographic characteristics and HIV infection at the two sites, this difference in the risk of malaria infection may be attributable to the intensity of malaria transmission within the catchment areas of Kashikishi and Nchelenge health centres.

Apart from providing health services to the people living close to the Lake Mweru, Nchelenge health centre also provides health services to people in villages that are close to a rubber plantation, the Kenani River, and other in-land water bodies in the Robert Mutepuka village area which provide more breeding sites for mosquitoes.

Malaria transmission intensity is known to decrease during the winter months, April to August, in Nchelenge District [18]. Thus, the estimated prevalence of malaria in pregnancy from this study conducted during the hot rainy season from November to April is probably higher than the prevalence at other times of the year.

Zambia has different malaria transmission zones ranging from very low, to low-moderate and moderate-high [109]. Thus, the prevalence of malaria estimated in this study can only be cautiously extended to areas with similar transmission patterns.

7.3.2 Risk factors for curable STIs/RTIs infection

In the current study infection with any STI was associated with BV but not with HIV. Several studies have associated STIs with HIV [230, 235, 243]. Trichomoniasis[223], Chlamydia [223], gonorrhoea [223, 235, 244] and syphilis [156, 244] have been individually associated with HIV infection. In contrast to this, other studies have

found no such association between trichomoniasis [245], or Chlamydia [246], or gonorrhoea [246] or syphilis [226, 246] or composite STIs [247] and HIV. The lack of association between STIs and HIV in the current study can be partially explained by the fact that infection with any STI was more common in younger women (≤ 25 years) while HIV was concentrated in women aged above 25 years.

Bacterial vaginosis was associated with STIs and HIV infection in the current study. The association of BV with Chlamydia [248, 249], gonorrhoea [248, 249], trichomoniasis [250, 251] and HIV [222, 223, 252, 253] has been observed in other studies. Furthermore, HIV-infected women may have more severe or persistent BV [254, 255].

The number of life-time sexual partners, younger age at sexual debut and parity have been associated with STIs [130, 181, 256], however, these associations were not observed in the current study. Factors that relate to sexual behaviour were also not associated with STIs. This lack of association can be explained by the fact that in this study the number of sexual partners that was queried was that of a life time count rather than for a limited period of time (e.g. past one year or during pregnancy).

7.3.3 Risk factors for malaria and STI/RTI coinfection

Malaria and STIs/RTIs have both been associated with HIV infection [214-217, 219, 230, 235, 243, 257]. Consequently, it is not surprising that HIV was a risk factor for malaria and STI/RTI coinfection in this study population. Although the sample size was not large enough to conclude that the risk of malaria infection among HIV-infected women was statistically significant, HIV infection was associated with malaria and STI/RTI coinfection.

The fact that HIV was strongly associated with malaria and STI/RTI coinfection suggests that management and prevention of these infections in pregnant HIV-infected women should be prioritised in this setting through provision of point of care tests and aggressive treatment of infected women. This is particular true for syphilis as RPR results were not available on the same day as the ANC visit. 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 [258]. The facilities relied on pregnant women returning to the facility for treatment with their partners, prompted by a note with their test results. However, among 729 delivering women only 52 of the 81 women who were RPR reactive at enrolment actually returned to the facility for care. It is likely that a higher proportion of these women could have received timely treatment if point of care tests for syphilis, now recommended by the WHO [259] for use in the ANC package, had been used. Overall, clinicians and staff involved in the provision of ANC services in this setting need to be proactive in the management of STIs/RTIs, especially in HIV-infected women. Furthermore, community education on the ways that infection with malaria, HIV and STIs/RTIs can be prevented cannot be over emphasised.

7.4 Prevalence of point mutations associated with parasite resistance to SP

Point mutations associated with resistance to SP in this study were highly prevalent. The prevalence of both the quintuple and the sextuple mutants was moderate but higher than what has been recorded in earlier studies both in Luapula Province and around Zambia [114]. In a study conducted in 2006 in the southern part of Zambia, the prevalence of the quintuple mutant was 6.5% in the general population. In another study conducted by Siame *et al*., in 2013 in Nchelenge District the prevalence of the quintuple and sextuple mutant among pregnant women was 17.0% and 3.0% respectively [115]. Another study, which documented the sextuple mutant for the first time in Zambia, conducted among pregnant women at two health centres in Mansa District (which is also located in Luapula province) between January 2010 and May 2011 reported the prevalence of the quintuple and sextuple mutant at 63.0% and 2.0%, respectively [114]. In the current study the prevalence of the quintuple and sextuple mutant was 68.8% and 9.4%, respectively. This suggests that there has been an increase in the prevalence of the 581G mutation in this population which has the potential to compromise the efficacy of IPTp-SP in this population. A systematic review and meta-analysis of IPTp-SP found that $\geq$ 2 doses of IPTp-SP no longer protected against the incidence of LBW among multigravidae where the prevalence of 581G was > 10.1% among children [260]. It is therefore

important to monitor the prevalence of SP-resistance markers and the *in vivo* efficacy in Zambia especially in high transmission areas.

7.5 Therapeutic and prophylactic failure of sulphadoxine-pyrimethamine

There was 14.6% treatment failure among pregnant women of Nchelenge district 28 days post-IPTp-SP in this area with a moderate prevalence of the highly resistant quintuple mutant (68.8%) and presence of the sextuple mutant (9.4%). Sulphadoxine-pyrimethamine retained some efficacy in parasite clearance among pregnant women with asymptomatic malaria in this study population. Similar observations have been has been made elsewhere i.e. SP retaining some efficacy despite a moderate to high prevalence of the quintuple mutant and presence of the sextuple mutant [114, 261].

The risk of having parasitaemia at both day 0 and day 28 was higher among primigravidae than secundigravidae and multigravidae. This can be explained by the fact that malaria prevalence is highest in younger women and primigravidae [47, 240, 241].

Prophylactic failure could not be estimated accurately because it is impossible to know the number of women who tested negative by microscopy at day 0 and were later exposed to the malaria parasite after IPTp-SP administration. Some women may have been infected and SP cleared the parasitaemia and others may never have been infected. Nonetheless, estimating prophylactic failure based on the number of malaria positive women among those who tested negative at day 0 is a good proxy for the estimate of prophylactic failure.

Less than half of the cases (47%) with positive malaria outcome on both day 0 and day 28 were due to recrudescence by MSP2 genotyping. Only MSP2 genotyping was carried out to distinguish recrudescence from reinfection in the current study. Although genotyping for the three genes (MSP1, MSP2 and GLURP) was not done in this study, the results from MSP2 genotyping are reliable since MSP2 provides a more accurate measure of treatment outcomes in comparison to MSP1 and GLURP [262].

7.6 Characteristics of delivering women, Prevalence of adverse birth outcomes and risk factors for adverse birth outcomes

7.6.1 Characteristics of women according to follow-up

The distribution of characteristics between women who were followed up to delivery and those who were lost were similar except in the case of gravidity. The proportion of primigravidae was higher among women followed to delivery than among those lost to follow-up. This is contrary to what has been observed in other studies where women lost to follow-up to delivery were more likely to be primigravidae [36, 263, 264]. The observation of a higher proportion of primigravidae among women followed up than among those who were lost to follow-up in the current study can partially be explained by the fact that younger Zambian women prefer to deliver at a health facility due to lack of birthing experience and for fear of labour complications [175].

7.6.2 Prevalence of adverse birth outcomes

There was considerable prevalence of ABO (35.0%) in this study population. The prevalence of stillbirth and LBW in the current study was 1.7% (95% CI, 1.0-2.9) and 21.9% (95% CI, 19.2-25.3), respectively. This is similar to what was recorded in 2013 at St Paul's Hospital, the only hospital in the district where prevalence estimates of stillbirth and LBW were 2.4% (95% CI, 1.8-3.0%) and 21.5% (19.9-25.3%), respectively. The routine data system at the hospital does not contain estimates of PD nor IUGR. The rate of stillbirths observed in this study (1.7%) was also comparable to what was observed in the ZEPRS (24 per 1000 births). Differences in individual ABO between results from this study and others conducted in Zambia were noted in the prevalence of LBW and PD. In this study the prevalence of LBW was 21.9% which was much higher than what was reported in the 2013-14 ZDHS and the ZEPRS, both of which reported 10% prevalence. And the prevalence of PD recorded in the ZEPRS was 54% higher than what was recorded in this study (34% versus 22.1%). The estimate for the prevalence of LBW reported in the ZDHS survey was obtained from Lusaka, Copperbelt and Southern Provinces while the ZEPRS was conducted in 25 health facilities across Lusaka. The results seem to suggest

differences in the prevalence of these ABO in different regions of Zambia. Understanding factors that are associated with ABO in different regions is required in order to target the most affected populations with efforts aimed at reducing the prevalence of ABO.

7.6.3 Risk factors for adverse birth outcome

There was no difference in the proportions of women who delivered in the different places (hospital, health centre or home) between the two recruitment sites, but a higher proportion of women recruited at Nchelenge were assisted by TBAs than women at recruited at Kashikishi health centre. The simple explanation for this is that at the time of the study Nchelenge Health Centre did not offer maternity services and the alternative place of delivery besides St. Paul's Missionary Hospital for women who attend ANC at Nchelenge was Kabwali Health Centre, which was the other centre where some of the study participants delivered. In comparison to Kashikishi Health Centre, Kabwali was under staffed (six midwives versus one) and relied on TBAs to a greater extent to perform some of the deliveries at the health centre. In the current study adverse ABO was associated with short stature and primiparity. Both short stature [36, 265, 266] and primiparity [36, 265-268] have been associated with ABO in other studies.

In the current study peripheral malaria was diagnosed at first ANC attendance and placental malaria was diagnosed at delivery. No association between malaria (both peripheral and placental), STIs/RTIs or their coinfection and ABO was observed in the current study. Studies have associated malaria [36, 49, 50, 55-58] and individual STIs/RTIs of interest in the current study i.e BV [12, 23, 24, 37-41, 65, 66], trichomoniasis [19-21, 33], Chlamydia [11, 12, 28-31, 33-35, 62-64], gonorrhoea[17, 18, 32, 33] and syphilis[9, 10, 17, 18, 26, 69] with ABO. Interestingly, women who indicated that they had received antimalarial treatment besides IPTp-SP at the time of delivery were at a higher risk of experiencing LBW. It is assumed that women who were treated with antimalarial drugs experienced clinical malaria at some point during pregnancy which may have had an impact on the weight of the babies.

In this study no association was found between composite STIs/RTIs (BV, trichomoniasis, Chlamydia, gonorrhoea and syphilis) and composite ABO (PD, LBW, IUGR and stillbirth). Similarly, no association was found between individual STIs/RTIs or malaria and PD or LBW or IUGR. The similarity in these finding underscores the observation that there was no association between STIs/RTIs or malaria or their coinfection and ABO in this study population.

Although the prevalence of ABO was high in the study population (35%), the difference in the proportions of ABO between unexposed and exposed (infected with at least one curable STI/RTI and/or malaria diagnosed at first ANC) women was minimal (31.8% versus 36.2%).; a larger sample size of 4,469 pregnant women would be required to detect a statistically significant difference between unexposed and exposed women under the prevailing conditions. This study was underpowered for the detection of such a small difference in the outcome between infected and uninfected women.

Various ANC activities contributed to the reduction of ABO attributable to curable STIs/RTIs and malaria in pregnancy. The lack of association between infections and ABO 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 malaria in pregnancy including third-trimester maternal anaemia, placental parasitaemia and the incidence of LBW [150, 153, 217, 261]. In the current study IPTp-SP coverage of at least one dose was high with 99% among women who were followed to delivery. The coverage for at least one dose of IPTp-SP was higher than the recorded provincial coverage of 89.7%. The increase in the coverage of IPTp-SP among participants can be attributed to the fact that one of the objectives of the study was to assess the *in vivo* efficacy of SP and, thus, the administration of SP at the first and second visit as well as attendance of ANC may have influenced the IPTp-SP coverage.

It was noted that 36.4% of the 88 HIV-infected women who took IPTp-SP also took cotrimoxazole during the pregnancy duration. It is unclear whether these women took both cotrimoxazole and SP at the same point in time. However, clinicians in this setting are aware of toxicity concerns of administration of both drugs at the same time.

Treatment of BV [23, 68, 73-76], trichomoniasis [75], Chlamydia [70, 71], syphilis [60, 61] and composite STIs/RTIs[67] has been shown to improve birth outcome. Conflicting results have been observed in the case of treatment of BV [23, 73, 74, 76]. Studies in the US showed that treatment of BV reduced PD [23, 73, 74] and similar results were found in a study in the UK [76]. However, no benefit of treating asymptomatic BV was observed in a review [77]. Results from two studies looking at treatment of trichomoniasis and BV in pregnancy showed no benefit in terms of reducing ABO [75, 78]. Others found increased risk of ABO in treated women compared to untreated women [79, 80]. In contrast to this, a sub-analysis of data from another study showed that women randomised to the antibiotic arm were not more likely to deliver a preterm infant or to deliver an infant with a lower mean birth weight [84].

The coverage of syphilis treatment among RPR reactive women (64%) was quite low. All the participants were followed up within three days of screening and given a slip to return to the health centre together with their sexual partner for treatment. Counsellors were involved in the note delivery process to ensure that those who had doubts were given adequate information on the benefits of seeking treatment. However, some of the participants did not return for treatment after being given the first note or at day 28 follow-up or after a second note was delivered to them/their homes inviting them to return to the health centre for treatment. The success of the treatment exercise was partially dependent on the cooperation of individual participants. Unfortunately, the reasons for choosing to stay away are unknown but establishing this would be useful in future. 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 [258], therefore the Zambian Ministry of Health should consider amending the practise of syphilis screening during ANC.

In this study the prevalence of STIs/RTIs was high and treatment of participants based on syndromic management was low due the fact that a high proportion of infections (86%) were asymptomatic. Furthermore, only a small proportion (12.8%) of the 467 who tested positive for at least one STI/RTI and 18.6% of 248 STI-infected women received antibacterial treatment during the pregnancy duration. Some of the

women who were treated based on syndromic management received antifungal drugs. It is important to note that the assessment of participants who presented with one or more symptoms indicative of an STI/RTI for the decision on treatment was not part of this study. In this study all the participants were asked via the questionnaire if they were experiencing any signs or symptoms associated with STIs/RTIs at the time of recruitment. Those who responded positively were directed to their regular clinician for further assessment and decisions on treatment. The drugs that were given were recorded on the ANC cards and for the purpose of the study drugs taken throughout the pregnancy duration were recorded at delivery. As a result of this arrangement, it is difficult to comment on the performance of the syndromic management since the study did not assess how this was done or the competence of the clinicians who were attending to the participants.

The administration of antifungal drugs to women who tested positive for at least one STI/RTI suggests that some of the women may have had fungal infections which may have been much more obvious than the STI/RTI in terms of signs and/or symptoms.

It was surprising that infection with at least one STI/RTI, which was highly prevalent in this study population, was not associated with ABO despite a low proportion of infected women being treated. One possible explanation is the fact that the uptake of SP was high in the study population. Studies have suggested that SP could offer additional protection against infections other than malaria resulting in the protective effect against ABO which becomes more apparent in areas where parasites have lost sensitivity [260, 269].

Aside from being underpowered, this study differs from other studies that have associated STIs/RTIs with ABO in several ways. Firstly, some of the prior studies were conducted in the year 1990 or earlier [11-13, 20, 24]. Under the circumstances, one might expect that STIs/RTIs in pregnancy were not managed as well at that time compared to more recent years as interventions and implementation have evolved. Therefore, differences in proportions of ABO among infected and uninfected women may have been greater historically and, consequently, more easily be detected. Secondly, among the prospective studies involving screening of women and follow-up to delivery [20, 24, 30, 37] or record based retrospective studies, much larger

groups of women/records ranging between 3540 and 354200 were enrolled/included in the studies. Thirdly, studies from both the developing and developed world used the case-control design [17, 19, 33, 35].

Syphilis was associated with ABO in Kenya at a time when screening and treatment was policy in many countries but was not implemented in poor settings [60]. In a study in Tanzania by Watson-Jones *et al*., syphilis was strongly associated with stillbirth, PD and LBW [10]. Notable differences in relation to syphilis between the study by Watson-Jones *et al*. and the current study include; (i) the women in the study in Tanzania had not been previously screened and treated for syphilis while in the current study screening was done and about 64% of delivering participants who tested positive to RPR received treatment, (ii) although the prevalence of RPR-positives in the study by Watson-Jones *et al*., was slightly lower than that observed in the current study (8% versus 10.9%), the proportions of high-titre active syphilis among RPR-seropositive women in the study by Watson-Jones *et al*., was higher than that observed in the current study (52.9% versus 36.7%). High-titre active syphilis was shown to pose the greatest risk to the foetus in the same study in Tanzania [10].

Malaria was strongly associated with LBW, PD and IUGR but not stillbirth in a study conducted between 1997 and 2000 in Mwanza, Tanzania [36]. The Mwanza study was conducted before the WHO recommended IPTp-SP and therefore participants did not receive IPTp-SP unlike in the current study. Another study, conducted in November to December 2006 in Ghana, found an association between malaria and stillbirth [50]. The coverage of IPTp-SP among 746 delivering women was low in comparison to what was observed in this study; 47.3% of delivering women had taken two or more SP doses during the pregnancy duration, while in the current study 82.1% of participants had received two or more doses of SP. In another study in Sudan carried out between November 2006 and February 2008, malaria was associated with stillbirth, however, the coverage of IPTp-SP was not mentioned [49].

Although placental malaria diagnosed by histology at delivery was not associated with ABO, the proportions of PD and LBW were significantly higher among women with chronic placental malaria infection (pathology class 2) than in active, past and

no infection groups. Furthermore, in comparison to the uninfected women, those with chronic placental malaria infection had the lower mean maternal Hb, a lower median gestational age and the mean birthweight of the babies was lower as observed previously [191].

Infection with HIV indirectly affects birth outcomes as it is a risk factor for maternal anaemia [270]. Infection with HIV was associated with LBW in the current study. No association was found between HIV and composite ABO or the other individual ABO (IUGR and PD). Other studies also conducted in sub-Saharan Africa have associated HIV with ABO [271, 272] in contrast to others [36]. In the current study 42.7% of participants were on ARV therapy at recruitment and the coverage increased to 61.7% by the time of delivery. Studies have associated highly active antiretroviral therapy with higher rates of ABO [273-276], others found no association [271, 277, 278] or recorded favourable pregnancy outcome [279].

Having taken two or more doses of IPTp-SP was protective against ABO in the current study demonstrating the effectiveness of this strategy in this setting with moderate prevalence of the quintuple mutant. Previous studies in areas of high prevalence of the quintuple mutant have shown declining protective effect of IPTp-SP or that it is harmful [2, 280, 281]. However, in areas of low and moderate levels of SP resistance, IPTp-SP has been shown to be effective in reducing the incidence of ABO [90, 282]. In the same province where the current study was conducted, in Mansa District, an area of moderate prevalence of the quintuple mutant [114], each dose of IPTp-SP contributed to a 46% and 37% decrease in the frequency of LBW among paucigravidae and multigravidae women presenting for delivery at a rural health clinic between December 2009 and December 2010 [90].

In the univariate analysis hypertension was strongly associated with ABO (OR 3.36, 95% CI 1.1-10). Blood pressure readings were taken at recruitment and before delivery and hypertension was defined as two high BP readings. It was not always possible to take BP readings before delivery due to some deliveries occurring at home or in cases where women made it to the hospital so close to delivery resulting in a large number of missing values. However, the risk factors for ABO that were identified when hypertension was included were the same as when hypertension

was excluded in the multivariate model. Urine testing was not conducted and therefore preeclampsia was not adjusted for in this study.

This study demonstrates the existence of high prevalence of malaria and curable STIs/RTIs as well as their coinfection in a sub-Saharan setting. In this setting two or more doses of IPTp-SP was associated with a decrease in the risk of ABO. Although IPTp-SP continues to offer protection against the effects of malaria in some settings, the emergence of parasites resistant to SP has undermined the efficacy of SP to protect against the malaria attributable fraction of LBW [157]. Given the existence of malaria and curable STIs/RTIs confection, the high levels of asymptomatic curable STIs/RTIs, the limited accuracy of syndromic management and emerging parasite resistance warrants trials to explore antibacterial and antimalarial combinations for the replacement IPTp-SP. A review of studies on the efficacy of azithromycin against curable STIs/RTIs concluded that azithromycin-based combination therapy therapies are among leading potential candidates to replace SP for IPTp owing to the effectiveness of azithromycin against *T. pallidum*, *N. gonorrhoea* and *C. trachomatis* [283]. In a study in Malawi, adding azithromycin to monthly SP regimen appeared to offer further benefit to foetal and neonatal growth [284]. Such a regimen is likely to benefit pregnancy outcomes as well as reduce STI/RTI morbidity.

7.7 Potential study biases, limitations, Conclusions and recommendations

7.7.1 Potential study biases and limitations

Non-attendance of ANC may be a potential source of bias if the women who stayed away from ANC had a different profile from those that attended ANC. However, it is unlikely the results would differ much because in this part of Zambia ANC coverage is about 95% and refusal rate among eligible women was less than 1%.

The general follow-up of study participants was lower than was expected and this is a source of concern when bias resulting from losses to follow-up is considered. However, women who were followed-up to delivery were generally similar to those who were lost to follow-up with respect to socio-demographic characteristics and exposure to infection and thus were representative of the study population.

Although malaria transmission occurs all year-round, higher numbers of malaria cases are recorded in the rainy season, which was the season in which this study was conducted. Therefore, the estimated prevalence observed of malaria alone and malaria and STI/RTI coinfection may be higher than it would have been had the study been carried out at another time of the year.

Classification of participants as infected or uninfected was based on results for malaria diagnosed by PCR and all the STIs/RTIs at first ANC visit. Repeat reading of a proportion of slides for BV and RPR/TPHA testing were conducted for quality control. In the case of Chlamydia, gonorrhoea and trichomoniasis PCR was repeated using a different assay (Seeplex® STI Master Panel 1 by Seegene). The results obtained from repeat diagnosis/reading were virtually reproducible (> 95%). Therefore, it is expected that bias due to misclassification was minimal in this context. On the other hand, misclassification of some of the infected participants as uninfected was reduced by diagnosis of malaria and some curable STIs/RTIs by PCR methods which generally have higher sensitivities than traditional methods.

Diagnosis of STIs/RTIs 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 of the association between infection and ABO towards the null. A second screening for STIs/RTIs later in pregnancy is recommended in future studies although this would be much more expensive and labour intensive.

Gestation age was determined using ultrasound which is a reliable method for establishing the length of pregnancy. One of the inclusion criteria in the current study was women with gestation ≤ 32 weeks. The measurements of gestational age by ultrasound in the second part of pregnancy are less reliable than in the first trimester due to normal biological variation [285]. Under/over estimation of gestational age at recruitment would result in misclassification of participants in terms of outcomes that are dependent on gestational age i.e. PD and IUGR. Although the recruitment period would be substantially increased, setting a lower gestation

age as an exclusion criterion would minimise the level of misclassification in this respect and is therefore suggested for future studies.

Another study limitation is in the kind of information that was required for the study. Some of the questions that were posed to the women especially those on sexual behaviour required sharing of information that can be considered confidential and as a result the responses to these questions may not be reflective of the truth. Furthermore some of the responses to questions on socio-economic status, utilisation of malaria interventions, sexual behaviour etc. may have been influenced by social desirability.

The study was under powered to detect risk factors with ORs > 2 and was therefore limited in this respect.

7.7.2 Conclusions

The prevalence of *P. falciparum* malaria detected by PCR in pregnant women of Nchelenge District was high. HIV infection increased the risk of malaria infection, although the sample size was not large enough to conclude this risk was statistically significant. The high prevalence of malaria infection that was observed in this population suggests that past prevention efforts have had limited impact in pregnant women.

The combined prevalence of STIs/RTIs was higher than that of malaria and was concentrated in younger and HIV-infected women. The prevalence of malaria and STI/RTI coinfection was considerable in this study population and the risk of infection was higher in HIV-infected women.

Both quintuple and sextuple mutants were observed in this study population. Sulphadoxine-pyrimethamine retains partial efficacy in clearing parasites in pregnant women in this area with moderate prevalence of the highly resistant quintuple mutant.

The prevalence of ABO was considerable and was higher in shorter and primiparous women. However, there was no association found between infection with malaria, STI/RTI or both and ABO in this study. Two or more doses of IPTp-SP was protective against ABO, therefore IPTp-SP remains a viable option for Zambia in this context.

However, there is need to investigate alternative drugs that are effective against malaria and common STI/RTIs to replace SP for IPTp.

7.7.3 Recommendations

The findings from this study are not enough to influence change of policy; however they can be used to recommend better coverage of the current policy.

The prevalence of malaria and STIs/RTIs in this study group was generally high and ownership of bed nets and usage were also observed to be low. Malaria control policies should therefore focus on providing uninterrupted interventions such as robust vector control, distribution of bed nets, yearly IRS spraying, IPTp-SP provision and sensitising of the community about the importance of using available interventions which are vital to reducing the malaria burden in pregnancy. Health service provision administrators should ensure uninterrupted availability of test kits for syphilis and treatment drugs. Further sensitisation of community members on the effects of syphilis in pregnancy could improve the numbers of women and their partners who seek appropriate treatment for syphilis since effective treatment requires commitment from infected pregnant women. Provision of point of care tests for syphilis and prompt treatment of infected women and their partners should be considered in this setting especially among HIV positive women. Provision of point of care tests for syphilis has the potential to increase the proportion of women receiving treatment among those testing positive. Increased frequency of community education on the prevention of infection with STIs via safe sex practises and behaviour change such as the use of condoms and maintaining one sexual partner can help reduce the burden of STIs/RTIs. Dissemination of information to the community can be enhanced via other channels such as the community radio and in schools.

7. 8 Future work

The results in the current thesis show that the prevalence of malaria and STI/RTI coinfection is considerable. Although this has only been demonstrated in one setting, the fact that malaria and STI/RTI have overlapping prevalence in sub-Saharan Africa

implies that a number of settings have cases of malaria and STI/RTI coinfection. Evidence suggesting that IPTp-SP may not be beneficial and that it may be harmful in areas where parasites express the 581G DPHS mutation has emerged in Muheza, Tanzania [2], but this has not observed elsewhere [286]. It has been reported that IPTp-SP was not associated with decreased odds of placental malaria or with increased mean maternal haemoglobin or mean birthweight. Furthermore IPTp-SP was associated with decreased cord haemoglobin level and increased foetal anaemia. Given the compromised efficacy of IPTp-SP [2, 13, 157, 280] coupled with and the limitations of syndromic management in the detection of STIs/RTIs, there is need for trials for antibiotic and antimalarial combinations for IPTp. Therefore, future work will involve evaluation of the effect of an antibiotic and antimalarial combination on birth outcomes in an environment with high prevalence of SP resistance markers in comparison to the current strategy of IPTp-SP.

A possible combination of an antimalarial and antibiotic is that of azithromycin and dihydroartemisinin–piperaquine. This is because azithromycin has been shown to efficacious against *T. pallidum*, *C. trachomatis* and *N. gonorrhoea* and azithromycin combination therapies are among the leading candidates to replace SP for IPTp and may offer the added benefit of reducing morbidity and the effects of STIs/RTIs on pregnancy outcome [283]. In the case of an antimalarial, research has shown that dihydroartemisinin-piperaquine could be a good replacement for SP as IPTp. Two trials have evaluated the use of dihydroartemisinin–piperaquine for the prevention of malaria in pregnancy. One was conducted in an area of western Kenya with high levels of resistance to SP. In this trial, HIV-uninfected pregnant women were randomly assigned to receive intermittent screening and treatment with dihydroartemisinin–piperaquine or IPTp with a median of three doses of either dihydroartemisinin–piperaquine or SP [269]. Intermittent screening and treatment with dihydroartemisinin–piperaquine was not found to be a suitable alternative to IPTp-SP. However, IPTp with dihydroartemisinin–piperaquine was associated with a lower prevalence of malaria infection at delivery (15 [3%] of 457 versus 47 [10%] of 459 of women; relative risk 0·32, 95% CI 0·18-0·56; $P < 0·0001$); a lower incidence of malaria infection (19.2 versus 54·4 events per 100 person-years; incidence rate ratio

0·28, 95% CI, 0·22-0·36; *P* < 0·0001) and clinical malaria during pregnancy (37·9 versus 6·1 events; 95% CI, 0·16, 0·08-0·33; P < 0·0001) than was for SP.

The second trial conducted in Tororo, Uganda, an area of high malaria-transmission and intensity, was a double-blind, randomised, three-group controlled trial comparing SP, three-dose dihydroartemisinin–piperaquine, and monthly dihydroartemisinin–piperaquine as IPT for malaria in pregnancy [287]. Participants were HIV-uninfected pregnant adolescents or women at least 16 years of age (primigravid or multigravid), who were between 12 and 20 weeks of gestation. In this trial the prevalence of histopathologically confirmed placental malaria was significantly higher in the SP group (50.0%) than in the three-dose dihydroartemisinin–piperaquine group (34.1%, P = 0.03), or the monthly dihydroartemisinin–piperaquine group (27.1%, *P* = 0.001). The prevalence of composite ABO was marginally lower in the monthly dihydroartemisinin–piperaquine group (9.2%) than in the SP group (18.6%, *P* = 0.05) or the three-dose dihydroartemisinin–piperaquine group (21.3%, *P* = 0.02).

Adding an antibiotic to dihydroartemisinin–piperaquine is likely to be more beneficial than using this antimalarial alone. As earlier mentioned, studies have suggested that SP could offer additional protection against infections other than malaria resulting in the protective effect against ABO [260, 269]. In view of this, it is likely that if SP is withdrawn and replaced by an efficacious antimalarial without any antibacterial activities, it may not be as effective as IPTp-SP has been noted to be in reducing ABO.

Given that malaria and STI/RTI co-infections are very common and both infections are associated with ABOs, future interventions to reduce ABOs should be effective against both infections. As mentioned earlier a combination of azithromycin and an antimalarial could be considered. A clinical trial to assess the effect of SP alone, SP + azithromycin or dihydroartemisinin-piperaquine + azithromycin on ABOs is needed. In addition, as the most common RTI is BV a trial to evaluate the effects of adding metronidazole to SP or SP is also needed to inform the future guidelines to control adverse birth outcomes attributable to malaria and STI/RTIs.

7.9 Reflexivity

According to socially constructed knowledge claims, personal, cultural and historical experiences of the researcher influence the interpretation hence the need for the researcher to acknowledge this and account for it in their study [288-290]. Specifically, there is need for reflexivity from the researcher on how their socio-cultural and historical contexts shape their theories and research practises. Although this was not qualitative research, this section was included to give the reader some understanding of my background which shapes the way in which the research was conducted and my interpretation of the results. Reflexivity helps "researchers to recognise that their own background shapes their interpretation and 'position themselves' in research to acknowledge how their interpretation flows from their own personal, cultural and historical background" [291].

This section identifies the role of my personal experiences in the study which may be in the design, conduct, analysis, interpretation of results and writing of the research results [292]. I have written this section in first person to show my perspectives, experiences and roles in certain aspects of the research project.

I know that my personal experience influenced the design of this study in one way or another. A good example is in how my decision on the study design was influenced by my personal experience as a mother. I was faced with deciding whether to conduct a case-control study in which samples were to be collected from delivering women or find an alternative design. I knew that obtaining cervico-vaginal samples from delivering women might be inappropriate although I had come across a study in which vaginal and cervical samples were collected within 24 hours of delivery and at 7 days postpartum for identification of organisms including *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by the culture method [17]. This was because I strongly felt that this would be too much to ask from a participant during such an intimate time and that I would personally find it difficult to agree to such a thing having gone through the birthing process. I also thought about collection of cervico-vaginal samples a week after delivery which I dismissed for the same reason. Due to my experience in molecular diagnostic methods (which I acquired during my

Master of Science training in Medical Parasitology), I was aware of possible challenges I would have faced if I had opted to isolate genetic material from cervico-vaginal swabs stained with blood. As a result of this background I settled for a cohort study in which samples were collected at first antenatal care attendance and participants were followed up to delivery. My past training and experience also made other aspects of the study manageable. For instance my undergraduate training in Microbiology made the processing, reading of slides for diagnosis of BV and interpretation easy for me.

Having grown up in both the rural and urban settings of Zambia, I understood the importance of recognising community leaders which was cardinal to the success of the study. As a result of this I made courtesy calls on the chief and the village head men in the areas where the two recruitment sites were located. I also recognised the authority of the administrators at the district health office and the district commissioner who appreciated this and were supportive to the project throughout the period that I worked in the area.

Furthermore, I decided to engage the local people as much as possible in the research activities. The regular ANC staff, midwives and TBAs were trained and involved in the project because I understood that the local people were likely to co-operate with a research team that included people within their community based on information I had gathered prior to the commencement of the project. I was also aware of the fact that involving members of community who were familiar with participants might result in participants withholding certain information during one-on-one interviews while completing the study questionnaire. To deal with this, research team members administering questionnaires were instructed to declare personal knowledge of or familiarity with the participants and pass on such participants to another member of the team.

Some of the community members thought I was linked to the Tropical Disease Research Centre which has been conducting research in the area for several years and has a good reputation in the community. I believe this also influenced the willingness of women to take part in the study to some extent.

My gender and the fact that I had a few months old baby at the time I started my field research may also have influenced the attitude of expecting mothers towards me. Women seemed comfortable talking to me and seeking clarification on certain aspects of the study. Based on some of their questions and comments, it was clear that some of the community members thought that I am a powerful person or someone connected to politically powerful people. This may have influenced some of their responses to questions bordering on economic status or bed net ownership and usage to some extent as some may have wanted to portray a slightly different picture with the hope of getting help or may have wanted to be desirable.

The field work also had a personal impact on me as I leant to relate to and engage the community. Visiting the homes of participants was made much easier by the warmth of community members who were generally welcoming. Co-ordinating the day to day running of project activities also helped me to build my interpersonal skills. A good example of a time when I had to closely deal with the community is when the project vehicle was involved in an accident involving a drunken cyclist and two children. Our driver tried to avoid a drunken cyclist who hit his bicycle on the front passenger's door by swerving to the opposite side of the road into an area with tall grass along the road. Sadly, two little girls were playing in the grass and both were hit. One sustained injuries and the other died instantly. The people who witnessed the accident understood what happened. After about an hour while waiting for the police some youths who were not at the scene at the time of the accidents got excited and set the vehicle ablaze. This brought the project to a temporal halt. As the principle investigator I had to deal with the police, the insurance company, the community leaders, the district commissioner's office, the family of the deceased and the injured girl and cyclist. However, in dealing with all these people, I generally received support from the community including the community radio station staff that contacted me and gave me an opportunity to explain our side of the story as well as inform the community about the project progress and the way forward. I had some community leaders who came to apologise for the behaviour of the youths who took the law into their own hands and they also offered to help the police to bring the perpetuators to justice. In no time

the project was up and running and I found alternative transport under very difficult circumstances. This whole experience taught me the importance of recognition of authority when working with communities. Managing the field activities and team members financially was also crucial to the project. Despite the limited budget that I had to work with, I managed to achieve most of the objectives for the study. Fieldwork did not always run as smoothly as I would have liked but I did everything to the best of my ability under the circumstances, I believe that a good job was done.

8.0 References

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9.0 Appendices

Appendix 1.1a: Search terms for studies reporting malaria and/or STIs/RTIs and birth outcomes

Organism/infection	Medical subject heading terms	Free test terms
Treponema pallidum	*Treponema pallidum* OR syphilis AND " birth outcome"	*Treponema pallidum* and birth outcome *Treponema pallidum* and pregnancy outcome Syphilis and birth outcome Syphilis and pregnancy outcome *Treponema pallidum* and stillbirth *Treponema pallidum* and IUGR *Treponema pallidum* and Preterm delivery *Treponema pallidum* and low birth weight *Treponema pallidum* and PROM Syphilis and stillbirth Syphilis and IUGR Syphilis and Preterm delivery Syphilis and low birth weight
Neisseria gonorrhoea	*Neisseria gonorrhoeae* OR Gonorrhoea OR Gonorrhoea AND " birth outcome"	*Neisseria gonorrhoeae* and birth outcome Gonorrhoea and birth outcome Gonorrhea and birth outcome *Neisseria gonorrhoeae* and pregnancy outcome Gonorrhoea and pregnancy outcome Gonorrhea and pregnancy outcome *Neisseria gonorrhoeae* and stillbirth Gonorrhoea and stillbirth Gonorrhea and stillbirth *Neisseria gonorrhoeae* and IUGR Gonorrhoea and IUGR *Neisseria gonorrhoeae* and Preterm delivery Gonorrhoea and Preterm delivery *Neisseria gonorrhoeae* and low birth weight Gonorrhoea and low birth weight Gonorrhea and low birth weight *Neisseria gonorrhoeae* and PROM Gonorrhoea and PROM Gonorrhea and PROM
Chlamydia trachomatis	*Chlamydia trachomatis* AND " birth outcome"	*Chlamydia trachomatis* and birth outcome *Chlamydia trachomatis* and pregnancy outcome *Chlamydia trachomatis* and stillbirth *Chlamydia trachomatis* and IUGR *Chlamydia trachomatis* and Preterm delivery *Chlamydia trachomatis* and low birth weight *Chlamydia trachomatis* and stillbirth *Chlamydia trachomatis* and PROM

Appendix 1.1a: Search terms for studies reporting malaria and/or STIs/RTIs and birth outcomes continued

Organism/infection	Medical subject heading terms	Free text terms
Trichomonas vaginalis	*Trichomonas vaginalis* and birth outcome	*Trichomonas vaginalis* and birth outcome *Trichomonas vaginalis* and pregnancy outcome *Trichomonas vaginalis* and stillbirth *Trichomonas vaginalis* and IUGR *Trichomonas vaginalis* and Preterm delivery *Trichomonas vaginalis* and low birth weight *Trichomonas vaginalis* and stillbirth *Trichomonas vaginalis* and PROM Trichomoniasis and birth outcome Trichomoniasis and pregnancy outcome Trichomoniasis and stillbirth Trichomoniasis and IUGR Trichomoniasis and Preterm delivery Trichomoniasis and low birth weight Trichomoniasis and PROM
Bacterial vaginosis	Bacterial vaginosis and birth outcome	Bacterial vaginosis and birth outcome Bacterial vaginosis and pregnancy outcome Bacterial vaginosis and stillbirth Bacterial vaginosis and IUGR Bacterial vaginosis and Preterm delivery Bacterial vaginosis and low birth weight Bacterial vaginosis and PROM
Malaria	Malaria and " birth outcome"	Malaria and birth outcome Malaria and pregnancy outcome Malaria and stillbirth Malaria and IUGR Malaria and Preterm delivery Malaria and low birth weight

Appendix 1.1b: Search terms for studies reporting point prevalence for malaria and STIs/RTIs

Organism/infection	Medical subject heading terms	Free text terms
Treponema pallidum	*Treponema pallidum* OR syphilis AND "pregnant women" AND Africa	*Treponema pallidum* and pregnant and Africa *Treponema pallidum* and pregnancy and Africa Syphilis and pregnant and Africa Syphilis and pregnancy and Africa Screen and syphilis and pregnant and Africa Screen and syphilis and pregnancy and Africa Test and syphilis and pregnant and Africa Test and syphilis and pregnancy and Africa
Neisseria gonorrhoea	*Neisseria gonorrhoeae* OR Gonorrhoea OR Gonorrhoea AND Pregnant women AND Africa	*Neisseria gonorrhoea* and pregnant and Africa *Neisseria gonorrhoea* and pregnancy and Africa Gonorrhoea and pregnant and Africa Gonorrhea and pregnancy and Africa Screen and gonorrhoea and pregnant and Africa Screen and gonorrhea and pregnant and Africa Test and gonorrhoea and pregnant and Africa Test and gonorrhea and pregnant and Africa
Chlamydia trachomatis	*Chlamydia trachomatis* and Pregnant women AND Africa	*Chlamydia trachomatis* and pregnant and Africa *Chlamydia trachomatis* and pregnancy and Africa *Screen and Chlamydia* and pregnant and Africa *Screen and Chlamydia* and pregnancy and Africa *Test and Chlamydia* and pregnant and Africa *Test and Chlamydia* and pregnancy and Africa
Trichomonas vaginalis	*Trichomonas vaginalis* and Pregnant women AND Africa	*Trichomonas vaginalis* and pregnant and Africa *Trichomonas vaginalis* and pregnancy and Africa Trichomoniasis and pregnant and Africa Trichomoniasis and pregnancy and Africa Screen and Trichomoniasis and pregnant and Africa Screen and Trichomoniasis and pregnancy and Africa Test and Trichomoniasis and pregnant and Africa Test and Trichomoniasis and pregnancy and Africa
Bacterial vaginosis	Bacterial vaginosis and Pregnant women AND Africa	Bacterial vaginosis and pregnant and Africa Bacterial vaginosis and pregnancy and Africa Screen and Bacterial vaginosis and pregnant and Africa Screen and Bacterial vaginosis and pregnancy and Africa Test and Bacterial vaginosis and pregnant and Africa Test and Bacterial vaginosis and pregnancy and Africa
Malaria	Malaria and Pregnant women AND Africa	Malaria and pregnant and Africa Malaria and pregnancy and Africa Screen and malaria and pregnant and Africa Screen and malaria and pregnancy and Africa Test and malaria and pregnant and Africa Test and malaria and pregnancy and Africa

Appendix 1.2a: Peripheral malaria point prevalence estimates among pregnant women in Zambia and surrounding countries

Site Year study conducted	Reference	No. women positive	No. tested	Prevalence	95% CI	Diagnostic method
Chikwawa, Malawi (1993-94)	Verhoeff, 1998 [293]	109	575	18.9	15.7-22.1	Giemsa stain microscopy
Chikwawa, Malawi (1993-95)	Verhoeff, 1999 [294]	743	3913	19.0	17.8-20.2	Giemsa stain microscopy
Blantyre, Malawi (1997-98)	Rogerson, 2000 [141]	2034	4764	42.7	41.3-44.1	PCR
Blantyre, Malawi (1997-98)	Rogerson, 2000 [88]	85	339	25.1	20.5-29.7	Giemsa stain microscopy
Kigoma, Tanzania (NR)	Mnyika, 2000[295]	66	705	9.4	7.3-11.6	Giemsa stain microscopy
Manhica, Mozambique (1998)	Saute 2002 [296]	158	686	23.0	19.8-26.2	Giemsa stain microscopy
Blantyre, Malawi (2000)	Mankhambo, 2002[297]	70	135	51.9¥¥	43.5-60.3	PCR
Sofala, Mozambique (2003-04)	Montoya, 2006 [298]	78	587	13.3	10.6-16.1	Giemsa stain microscopy
Morogoro, Tanzania (2001-02)	Wort,2006 [142]	493	1684	29.3	27.1-31.5	Giemsa stain microscopy
Multicenter, Mozambique (2003-04)	Brentlinger,2007 [299]	255	684	37.3	33.7-40.9	Giemsa stain microscopy
Multicentre, Mozambique (2004)	Lujan,2008 [300]	52	1117	4.7¥	3.5-5.9	Giemsa stain microscopy
Southern Malawi (2004-05)	Van Den Broek, 2009[301]	571	2297	24.9	23.1-26.7	Giemsa stain microscopy
Mongochi, Malawi (2003-04)	Rantala, 2010 [95]	51	475	10.7	7.9-13.5	PCR
Blantyre, Malawi (1997-2001)	Feng, 2010 [1]*	798	7671	10.4	9.7-11.1	Giemsa stain microscopy
300 DHS geographic clusters, DRC	Taylor, 2011 [144]*	193	520	37.2	33.3-41.7	PCR
Sambizanga and Ingombota, Angola (2006-2007)	valente, 2011[89]*	49	567	8.6	6.3-10.9	PCR
Manhica, Mozambique (2003-04)	Serra-Casas, 2011 [143]	120	399	30.0	25.5-34.5	PCR
Manhica, Mozambique (2003-2005)	Mayor, 2012 [302]*	26	272	9.5	6.0-13.0	Giemsa stain microscopy
Luanda, Angola (2008)	Campos,2012 [145] *	74	679	10.9	8.6-13.2	Giemsa stain microscopy
Nchelenge, Zambia (2013)	Siame, 2015[115]*	83	375	22	17.8-26.2	PCR
Mansa , Zambia (2009-2010)	Mace, 2012 [90]*	21	419	5.0	2.9-7.1	Giemsa stain microscopy
Machinga, Malawi (2010-2011)	Gutman, 2015 [261]*	760	2,566	29.6	27.8-31.2	RDT

PCR: polymerase chain reaction
*Studies that did not contribute to the pooled prevalence estimates in the meta-analysis by Chico *et al*. 2012 [6]
¥¥ Highest prevalence estimate
¥Lowest prevalence estimate
Rapid diagnostic test

Appendix 1.2b: Placental malaria point prevalence estimates among pregnant women in Zambia and surrounding countries

Site Year study conducted	Reference	No. women positive	No. tested	Prevalence	95% CI	Diagnostic method
North Kivu, DRC (NR)	Meuris, 1993[303]	242	461	52.5	47.9-57.1	Placental histology
Blantyre, Malawi (1997-99)	Rogerson, 2000[88]	77	232	33.2	27.1-39.3	Placental impression and blood smear with microscopy
Blantyre, Malawi (NR)	Rogerson, 2003 [104]	124	464	26.7	22.6-30.7	Placental histology, impressions, smear with microscopy
Kinshasa, DRC (2004)	Lukuka, 2006 [304]	41	196	21.0	15.3-26.7	Placental impression and blood smear with microscopy
Chikwakwa, Malawi (2004-05)	Senga, 2007[305]	124	636	19.5	16.4-22.6	Placental histology and blood smear with microscopy
Muheza, Tanzania (2002-05)	Mwangoka, 2008 [306]	100	1,022	9.8	8.0-11.6	Placental blood microscopy
Muheza, Tanzania (2002-05)	Kabyemela, 2008 [146]	55	445	12.4	9.3-15.5	Placental impression and blood smear with microscopy
Blantyre, Malawi (1997-2001)	Feng, 2010 [1]	1058	7671	13.8	13.0-14.4	Placental blood microscopy
Luanda,Angola	Valente, 2011 [89]*	99	567	17.5	14.4-20.6	PCR
Manhica, Mozambique (2003-05)	Serra-Casas, 2011 [143]	110	342	32.1	27.2-37.1	PCR
Manhica, Mozambique (2003-05)	Mayor, 2012 [302]*	156	272	57.4¥¥	51.5-63.3	Placental histology
Mansa, Zambia (2009-2010)	Mace, 2012 [90] *	161	435	37.2	32.7-41.7	Placental histology
Rufiji, Tanzania (2012)	Mosha, 2014 [147]*	29	175	16.6	11.1-22.1	PCR
Moshi, Tanzania (2012)	Mosha, 2014 [147]	4	175	2.3¥	0.1-4.5	PCR

PCR: polymerase chain reaction
*Studies that did not contribute to the pooled prevalence estimates in the meta-analysis by Chico *et al*. 2012 [6]
¥¥ Highest prevalence estimate
¥Lowest prevalence estimate

Appendix 1.2c: Syphilis point prevalence estimates among pregnant women in Zambia and surrounding countries

Site Year study conducted	Reference	No. women positive	No. tested	Prevalence	95% CI	diagnostic method
Vilanculos, Mozambique (1991-92)	Vuylsteke, 1993[307]	31	201	14.6¥¥	9.7-19.5	RPR and TPHA
Umzigwana, Zimbabwe (1991)	Rutgers, 1993[308]	198	1433	13.8	12.0-15.6	RPR only
Zambiazai, Mozambique (1992-93)	Cossa, 1994[131]	211	1728	12.2	10.7-13.7	RPR and MHA-TP
Mwanza, Tanzania(1992-93)	Mayaud, 1995 [126]	97	964	10.1	8.2-12.0	RPR and TPHA
Dar es Salaam, Tanzania (1993)	Mwakagile, 1996 [129]	31	777	4.0	2.6-5.4	RPR and TPHA
Rwandan Camp, Tanzania (1994)	Mayaud, 1997[309]	2	100	2.0	0.7-4.7	RPR and TPHA
Mwanza, Tanzania (NR)	Mayaud, 1997 [310]	174	2380	7.3	6.2-8.4	RPR and TPHA
Mwanza, Tanzania (1994)	Mayaud, 1998[311]	45	628	7.2	5.2-9.2	RPR and TPHA
Blantyre, Malawi (1995)	Taha, 1998 [312]	98	808	12.1	9.8-14.7	RPR and TPHA
Blantyre, Malawi (1996)	Taha, 1999 [313]	1005	9309	10.8	10.2-11.4	RPR and TPHA
Murewa, Zimbabwe	Kambarami, 1998 [314]	36	366	9.8	6.8-12.9	RPR and TPHA
Murewa, Zimbabwe	Kambarami, 1998 [314]	28	308	9.2	6.0-12.4	VDRL and TPHA
Mwanza,Tanzania (2002)	Watson-Jones,2002 [10]	106	1809	5.9	4.8-7.0	RPR and TPHA
Gutu, Zimbabwe (NR)	Majoko, 2003 [315]	9	85	10.6	4.1-17.1	RPR only
Harare, Zimbabwe (NR)	Pham, 2005[132]	74	2969	2.5	1.9-3.1	RPR and TPHA
Sofala, Mozambique (2003-04)	Montoyo, 2006[298]	33	381	8.6	5.8-11.4	RPR and TPHA
Gaborone, Botswana (2000-01)	Romeron, 2006 [316]	35	703	5.0	3.4-6.6	RPR and TPHA
Multicentre, Tanzania (2003-04)	Swai, 2006 [136]	92	1,265	7.3	5.9-8.7	RPR only
ZDHS sites, Zambia	CSO, 2007 [317]	5	128	4.1	2.9-5.3	RPR and TPHA
Blantyre, Malawi (2000-04)	Kwiek, 2008 [137]	199	3824	5.2	4.5-5.9	RPR and TPHA
Kinshansa, DRC (2004)	Kanoshita-Moleka, 2008[318]	0	529	0.0¥	0-0	RPR and TPHA
Multicentre, Mozambique (2004)	Lujan, 2008 [300]	52	1117	4.7	3.5-5.9	RPR and TPHA
Rural Tanzania (2003-04)	Yahya-Malima 2008 [133]	21	1296	1.6	0.9-2.3	RPR and TPHA
Manhica, Mozambique	Menendez 2008 [319]	122	1030	11.8	9.8-13.8	RPR only
Moshi, Tanzania (2002-04)	Msuya, 2009[221]*	24	2654	0.9	0.5-1.3	RPR and Determine Syphilis TP
Southern Malawi (2004-05)	Van Den Broek, 2009 [301]	163	2297	7.1	6.1-8.2	VDRL only
Manhica, Mozambique (2000)	Menendez, 2010 [139]	31	258	12.0	8.0-16.0	RPR and enzyme immunoassay
Harare, Zimbabwe (2002-03)	Kurewa, 2010 [128]	8	678	1.2	0.4-2.0	RPR and TPHA
Harare, Zimbabwe (2002-04)	Mapingure, 2010 [320]	8	662	1.2	0.4-2.0	RPR and Determine Syphilis TP
Sentinel sites, Congo DR (2011)	Taylor, 2014 [321]*	742	17,669	4.2	3.9-4.5	RPR and Determine Syphilis TP
Multiple, Tanzania (2008-2010)	Chiduo, 2012 [322]*	284	25,802	1.1	1.0-1.2	RPR and TPHA
Mwanza, Tanzania (2012)	Hokororo, 2015 [130]*	21	403	5.2	3.0-7.4	RPR and TPPA
Multicentre, Tanzania (2011)	Manyahi, 2015 [138]*	973	38,920	2.5	2.3-2.7	RPR only
Kalomo, Zambia (2012)	Bonawirz, 2015 [323]*	50	1868	2.7	2.3-3.4	Rapid syphilis test

Appendix 1.2c: Syphilis point prevalence estimate among pregnant women in Zambia and surrounding countries continued

RPR: Rapid plasma reagin
TPHA: *Treponema pallidum* haemagglutination assay
TPPA: *Treponema pallidum* particle agglutination
VDRL: Venereal disease research laboratory
FTA-Abs-Fluorescent treponemal antibody absorption
MHA-TP: Micro heamagglutination assay-*Treponema pallidum*
CSO-Central statistical Office.
*Studies that did not contribute to the pooled prevalence estimates in the meta-analysis by Chico *et al*. 2012 [6]
¥¥ Highest prevalence estimate
¥Lowest prevalence estimate

Appendix 1.2d: *Chlamydia trachomatis* point prevalence estimates among pregnant women in Zambia and surrounding countries

Site Year study conducted	Reference	No. women positive	No. tested	Prevalence	95% CI	Diagnostic method
Vilancolus, Mozambique (1991-92)	Vuylsteke, 1993 [307]	11	141	7.8	3.4-12.2	Culture
Kinshasa, DRC (1990)	Vuylsteke, 1993 [324]	75	1,160	6.5	5.1-7.9	EIA
Mwanza,Tanzania (1992)	Mayaud, 1995 [126]	64	964	6.6	5.0-8.2	EIA
Mwanza, Tanzania (1994)	Mayaud, 1998 [311]	39	660	5.9	4.1-7.7	EIA
Harare, Zimbabwe	Latif, 1999 [125]*	88	1656	5.3[¥]	4.2-6.4	EIA
Gaborone, Botswana (2001-01)	Romoren, 2004 [325]	42	557	7.5	5.1-9.5	LCR cervical swab
Multicentre, Mozambique (2004)	Lujan, 2008 [300]	51	835	6.1	4.8-7.7	LCR cervical swab
Kinshasa, DRC (2004)	Kanoshita-Moleka, 2008 [318]	9	521	1.7	0.6-2.8	PCR cervical swab
Moshi, Tanzania (2002-04)	Msuya, 2009 [221]*	183	1048	17.5[¥¥]	15.2-19.8	ELISA
Manhica,Mozambique (2000)	Menendez, 2010 [139]	15	151	9.9	5.1-14.7	DNA ID-assay cervical swabs
Mwanza, Tanzania (2012)	Hokororo, 2015[130]*	48	403	11.4	8.2-14.4	PCR

PCR: polymerase chain reaction

*Studies that did not contribute to the pooled prevalence estimates in the meta-analysis by Chico *et al*. 2012 [6]

[¥¥] Highest prevalence estimate

[¥]Lowest prevalence estimate

Appendix 1.2e: *Neisseria gonorrhoeae* point prevalence estimates among pregnant women in Zambia and surrounding countries

Site Year study conducted	Reference	No. women positive	No. tested	Prevalence	95% CI	Diagnostic method
Kinshasa, DRC (1990)	Vuylsteke,1993 [324]	19	1,160	1.6	0.9-2.3	Culture
Vilanculos, Mozambique (1991-92)	Vuylsteke,1993[307]	14	201	7.0	3.5-10.5	Culture
Mwanza, Tanzania (1992-93)	Mayaud,1995 [126]	20	964	2.1	1.2-3.0	Culture
Dar es Salaam, Tanzania (1993)	Mwakagile 1996 [129]	28	777	3.6	1.9-4.3	Culture
Mwanza, Tanzania (1994)	Mayaud, 1998 [311]	15	660	2.3	1.2-3.4	Culture
Blantyre, Malawi (1993)	Taha, 1998 [312]	54	2161	2.5	1.8-3.2	Culture
Blantyre, Malawi (1995)	Taha, 1998[312]	20	808	2.5	1.4-3.6	Culture
Harare, Zimbabwe	Latif 1999 [125]*	65	1656	3.9	3.0-4.8	Culture
Blantyre, Malawi (1995)	Taha, 1999 [313]	373	9104	4.1	3.2-5.0	LCR cervical swab
Gaborone, Botswana (2000-01)	Romeron, 2007 [127]	21	703	3.0	1.7-4.3	Culture
Multicentre, Mozambique (2004)	Lujan, 2008 [300]	21	835	2.5	1.4-3.6	PCR urine
Moshi, Tanzania (2002-04)	Msuya, 2009 [221]*	13	2555	0.5[¥]	0.2-0.8	Culture and gram stain
Manhica, Mozambique (2000)	Menendez, 2010 [139]	27	145	18.6[¥¥]	12.2-24.8	Culture and gram stain
Mwanza, Tanzania (2012)	Hokororo, 2015 [130]*	27	403	6.7	4.3-9.1	PCR

PCR: polymerase chain reaction
*Studies that did not contribute to the pooled prevalence estimates in the meta-analysis by Chico *et al*. 2012 [6]
[¥¥]Highest prevalence estimate
[¥]Lowest prevalence estimate

Appendix 1.2f: *Trichomonas vaginalis* point prevalence estimates among pregnant women in Zambia and surrounding countries

Site Year study conducted	Reference	No. women positive	No. tested	Prevalence	95% CI	Diagnostic method
Kinshasa, DRC (1990)	Vuylsteke,1993 [324]	213	1,160	18.4	16.2-20.6	Wet-mount microscopy
Vilancolus, Mozambique (1991-92)	Vuylsteke 1993[307]	46	201	22.9	17.1-28.7	Wet-mount microscopy
Dar es Salaam, Tanzania (1993)	Mwakangile 1996[129]	176	777	22.7	19.8-25.7	Wet-mount microscopy
Rwadan camp, Tanzania (1994)	Mayaud, 1997[309]	31	100	31.0	21.9-40.1	Wet-mount microscopy
Mwanza, Tanzania (1994)	Mayaud, 1998 [311]	108	660	16.4	13.6-19.2	Wet-mount microscopy
Blantyre, Malawi (1993)	Taha, 1998 [312]	19	808	2.4[¥]	1.3-3.5	Wet-mount microscopy
Harare, Zimbabwe	Latif, 1999 [125]	22	1656	13.0	11.4-14.6	Wet-mount microscopy
Blantyre, Malawi (1994)	Taha, 1999 [313]	215	9137	31.1	30.1-32.1	Wet-mount microscopy
Gaborone, Botswana (2000-01)	Romoren, 2007[326]	132	703	18.8	15.9-21.7	Wet-mount microscopy
Moshi, Tanzania (2002-2004)	Msuya, 2009 [221]*	128	2555	5.0	4.2-5.8	Wet-mount microscopy
Ndola, Zambia (NR)	Crucitti, 2010 [327]*	99	307	32.2[¥¥]	27.0-37.4	PCR
Manhica, Mozambique (2000)	Menendez 2010 [139]	78	254	30.7	25.0-36.4	Wet-mount microscopy
Moshi, Tanzania (2002-04)	Mapingure, 2010 [320]	128	2555	5.0	4.2-5.9	Wet-mount microscopy
Harare, Zimbabwe (2002-04)	Mapingure, 2010 [320]	80	680	11.8	9.4-14.2	Wet-mount microscopy
Harare, Zimbabwe (2000)	Kurewa, 2010 [128]	80	678	11.8	9.4-14.3	Wet-mount microscopy
Mwanza, Tanzania (2012)	Hokororo, 2015 [130]*	54	403	13.4	10.1-16.7	Wet-mount microscopy

PCR: polymerase chain reaction

*Studies that did not contribute to the pooled prevalence estimates in the meta-analysis by Chico *et al*. 2012 [6]

[¥¥]Highest prevalence estimate

[¥]Lowest prevalence estimate

Appendix 1.2g: Bacterial vaginosis point prevalence estimates among pregnant women in Zambia and surrounding countries

Site Year study conducted	Reference	No. women positive	No. tested	Prevalence	95% CI	Diagnostic method
Rwadan camp, Tanzania (1994)	Mayaud, 1997 [309]	16	100	16.0	8.8-23.2	Clue cell count
Mwanza, Tanzania (1994)	Mayaud, 1998 [311]	159	660	24.1	20.8-27.4	Clue cell count
Harare, Zimbabwe	Latif, 1999 [125]*	71	1656	4.3[¥]	3.3-5.3	Amsel
Blantyre, Malawi (1993)	Taha, 1999 [313]	640	2077	30.8	28.8-32.8	Amsel
Multicentre, Zimbabwe (1999-2004)	Toloso, 2006 [328]	51	209	24.4	18.6-30.2	Nugent
Gaborone, Botswana (2000-01)	Romoren, 2007 [326]	264	703	37.6[¥¥]	34.0-41.2	Nugent
Tanzania (1999-2004)	Msuya, 2009 [221]*	534	2555	20.9	19.3-22.5	Amsel
Harare, Zimbabwe (2002-04)	Mapingure, 2010 [320]	195	598	32.6	28.8-36.4	Amsel
Moshi, Tanzania (2002-04)	Mapingure, 2010 [320]	534	2555	20.9	19.3-22.5	Amsel
Harare, Zimbabwe (2002-03)	Kurewa,2010 [128]	221	678	32.6	29.1-36.1	Amsel
Mwanza, Tanzania (2011)	Shayo, 2012[329]	81	284	28.5	23.2-33.6	Nugent
Mwanza, Tanzania (2012)	Hokororo, 2015 [130]*	102	403	25.3	21.1-29.5	Nugent

*Studies that did not contribute to the pooled prevalence estimates in the meta-analysis by Chico *et al*. 2012 [6]

[¥¥] Highest prevalence estimate

[¥]Lowest prevalence estimate

Appendix 1.3: Diagnostic Methods and published Sensitivity and Specificity Measures

Infection	Diagnostic method	Sensitivity	Specificity
Syphilis	*Treponema pallidum* haemagglutination assay	~ 100	~100 [330]
	Rapid plasma regain (RPR) only	86-100	93-98 [116]
	Venereal disease research laboratory (VDRL)	78-100	98 [116]
	RPR or VDRL	~ 100	~ 100 [6]
	RPR and TPHA	~ 100	~ 100 [6]
	RPR and Determine Syphilis TP	~ 100	~ 100 [6]
	RPR and Microhemagglutination assay-*Treponema pallidum* (MHA-TP)	~ 100	~ 100 [6]
	RPR and Immunoglobulin G (ELISA test)	~ 100	~ 100 [6]
Neisseria gonorrhoeae	Gram stain	50-70	95-100 [331]
	Polymerase chain reaction (PCR) with cervical swab	36-75	98-100 [332]
	Culture	~ 80	100 [117]
	Culture and Gram stain	~ 80	100 [6]
	Ligase chain reaction (LCR) with cervical swab	95-100	98-100 [117]
	PCR with cervical swab	89-97	94-100 [117]
Chlamydia trachomatis	Culture cervical swab	74-90	98-99 [117]
	Enzyme immunoassay (EIA)	71-87	97-99 [117]
	Culture cervical swab or EIA (score based on the less precise method, i.e mean of EIA)	71-87	97-99 [6]
	PCR urine	78-89	99-100 [332]
	LCR cervical culture	90-97	99-100 [117]
	PCR cervical swab	99	99-100 [117]
	Deoxyribonucleic acid identification assay-cervical swab	~ 100	~100 [6]
Trichomonas vaginalis	Wet mount microscopy	38-82	100 [117]
	In-pouch culture	96	100 [333]
	PCR	97	98 [334]
Bacterial vaginosis	Clue cells ≥ 20% of epithelium	40	97 [121]
	Amsel criteria: 3 or 4 of 4	51	98 [121]
	Nugent score ≥ 7	86-89	94-96 [122-124]
Peripheral parasitaemia	Giemsa stain microscopy	50 -90	~ 95 [91-93]
	PCR	91	91[95]
	Antigen assay	Reported as equal to microscopy [6]	
	Rapid diagnostic test	89	76
Placental parasitaemia	Placental blood microscopy	63	98-99 [104]
	PCR	93-99	73-76 [335]
	Placental histology	91	98-99 [104]
	Placental blood microscopy and tissue histology (score based on more precise method, i.e histology	91	~ 100 [6]
	Placental histology, impression, smears with microscopy (score based on most precise method i.e. histology	91	~ 100 [6]
	Placental impression and blood smear with microscopy (score based on more precise method, i.e. impression	91	~ 100 [105]

Appendix 1.4: Risk factors for malaria and STIs/RTIs

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Treponema pallidum	Vilanculos, Mozambique (1991-92)	Vuylsteke, 1993[307]	NR
	Umzigwana, Zimbabwe (1991)	Rutgers, 1993[308]	NR
	Zambiazai, Mozambique (1992-93)	Cossa, 1994[131]	The following factors were associated with infection; married status (aOR 2.5, 95% CI, 1.0-6.1), history of past STD (aOR5.6; 95% CI, 3.3-9.4), genital ulcers (aOR 3.7; 95% CI, 1.4-9.8), vaginal discharge (OR 1.5; 95% CI, 1.0- 2.1), genital warts (aOR 3.0; 95% CI, 1.1- 8.7), HIV infection (aOR 2.2; 95% CI, 1.0-5.0), and being from Nicoadala district (aOR 6.8; 95%CI, 1.7-58.1).
	Mwanza, Tanzania(1992-93)	Mayaud, 1995 [126]	NR
	Dar es Salaam, Tanzania (1993)	Mwakagile, 1996 [129]	Higher prevalence of syphilis, trichomoniasis and gonorrhoea in teenagers than older women (45.3% verses 29.2, $P < 0.001$)
	Rwandan Camp, Tanzania (1994)	Mayaud, 1997[309]	NR
	Mwanza, Tanzania (NR)	Mayaud, 1997 [310]	NR
	Mwanza, Tanzania (1994)	Mayaud, 1998[311]	NR
	Blantyre, Malawi (1995)	Taha, 1998 [312]	NR
	Blantyre, Malawi (1996)	Taha, 1999 [313]	NR
	Murewa, Zimbabwe	Kambarami, 1998 [314]	RPR positivity was not significantly associated with the women's age or parity.
	Mwanza,Tanzania (2002)	Watson-Jones,2002 [10]	NR
	Gutu, Zimbabwe (NR)	Majoko, 2003 [315]	NR
	Harare, Zimbabwe (NR)	Pham, 2005[132]	Increases in parity and gravidity were significantly associated with increased risk of syphilis infection. Prior stillbirths were associated with an increased risk of syphilis infection (odds ratio [aOR], 3.4; 95% CI, 1.61 to 7.37; $P = 0.001$).
	Sofala, Mozambique (2003-04)	Montoyo, 2006[298]	NR
	Multicentre, Tanzania (2003-04)	Swai, 2006	Women living in rural areas had higher prevalence than those in urban areas ($P < 0.0001$).Marital status did not appear to influence the prevalence of syphilis. There was a higher likelihood of having syphilis for women aged > 34 years compared to those less than 34 years. Women with no education were more likely to be infected with syphilis than were women with some education ($P < 0.0001$)

Appendix 1.4: Risk factors for malaria and STIs/RTIs continued

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Treponema palllidum	ZDHS sites, Zambia	CSO, 2007 [317]	NR
	Moshi, Tanzania (2002-04)	Msuya, 2007[336]	NR
	Blantyre, Malawi (2000-04)	Kwiek, 2008 [137]	Syphilis seroreactivity was associated with anemia (aOR: 1.4, 95% CI 1.0, 1.9), previous diagnosis of STI (aOR: 5.3, 95%CI: 3.5, 8.0), rural residence, multigravidity. Self-reported history of miscarriage or stillbirth (aOR: 2.3, 95%CI: 1.6, 3.5) Syphilis seroreactivity was inversely associated with maternal education ($\chi^2_{(trend)}$=17, *P* <0.0001). Syphilis was not associated with maternal employment, cost of housing material, being a Yao, or marital status.
	Kinshansa, DRC (2004)	Kanoshita-Moleka, 2008[318]	NR
	Multicentre, Mozambique (2004)	Lujan, 2008 [300]	No factors were independently associated with syphilis infection
	Rural Tanzania (2003-04)	Yahya-Malima 2008 [133]	Women with self-reported previous spontaneous abortion were more likely to have a positive syphilis serology (aOR 4.3, 95% CI: 1.52–12.02), infection with syphilis not associated with self-reported stillbirth (OR 2.4, 95% CI: 0.64–8.95).Odds were also higher for women who had more than one lifetime sexual partner, OR 5.4 (95% CI: 1.88–15.76).
	Manhica, Mozambique	Menendez 2008 [319]	NR
	Moshi, Tanzania (2002-04)	Msuya, 2009[221]*	NR
	Southern Malawi (2004-05)	Van Den Broek, 2009[301]	NR
	Manhica, Mozambique (2000)	Menendez, 2010 [139]**	Being divorced/widowed (aOR 3.6; 95% CI, 0.6-21.9) was associated with syphilis infection
	Harare, Zimbabwe (2002-03)	Kurewa, 2010 [128]	Risk factors for a positive serologic STI* were increasing age above 30 years (aOR 2.61; 95% CI, 1.49-4.59), polygamy (aOR 2.16; 1.06-4.39), and multigravid (aOR 3.89: 1.27-11.98), number of lifetime sexual partners (aOR 2.8; 1.2-6.2) and partner taking alcohol (aOR 1.16; 1.01-1.33).

Appendix 1.4: Risk factors for malaria and STIs/RTIs continued

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Treponema pallidum	Harare, Zimbabwe (2002-04)	Mapingure, 2010 [320]	NR
	Sentinel sites, Congo DR (2011)	Taylor, 2014 [321]	Syphilis seropositivity was significantly higher among women attending rural clinics (5.0%) as compared to urban clinics (3.0%) and those tested in antenatal care clinics in the provinces of Equateur (7.6%) and Orientale (7.7%) as compared to other provinces $P <$ 0.001).
	Mwanza, Tanzania (2012)	Hokororo, 2015 [130]	The presence of any STI$^{\alpha}$ was associated with being in a long-term (as opposed to short-term) relationship (aOR 2.6; 95% CI 1.4 - 4.9, increasing age difference between the girl and her partner (aOR 1.1; 95% CI 1.0 -1.1 per year, and history of prior pregnancy (aOR 1.6; 95% CI 1.0 - 2.6).
	Multicentre, Tanzania (2011)	Manyahi, 2015 [138]	The risk for syphilis infection was significantly higher among women attending semi-urban and rural clinics than those attending urban clinics and those having 3-4 (aOR 1.50; 95% CI, 1.17-1.92) and 5 (aOR 1.70, 95% CI, 1.27-2.27) previous pregnancies. Marital status and level of education were not statistically significant
Neisseria gonorrhoea	Kinshasa, DRC (1990)	Vuylsteke,1993 [324]	NR
	Mwanza,Tanzania (1992)	Mayaud, 1995 [126]	Chlamydia/gonorrhoea Age < 25 years (aOR=2.2, $P < 0.003$; Unmarried (aOR= 3.2, $P < 0.003$; in polygamous marriage (aOR = 2.3, $P < 0.003$; > one sexual partner over the previous year (aOR = 1.7, $P < 0.06$; previously delivered more than 5 years (OR=3.2, $P < 0.01$
	Dar es Salaam, Tanzania (1993)	Mwakagile 1996 [129]	As stated for this paper under syphilis (infections combined)
	Blantyre, Malawi (1995)	Taha, 1999 [313]	NR
	Harare, Zimbabwe	Latif 1999 [125]	Women with vaginal discharge: being separated from the partner for a month or more ($P = 0.002$), having had sex with a new partner in the previous three months ($P = 0.002$), current use of condoms ($P = 0.011$ presence of a purulent vaginal discharge ($P = 0.004$). increasing educational level was inversely associated with cervical infection ($P = 0.007$).

Appendix 1.4: Risk factors for malaria and STIs/RTIs continued

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Neisseria gonorrhoeae	Gaborone, Botswana (2000-01)	Romeron, 2007 [127]	Compared to Women aged 30 and above, women aged 20-29 (aOR 4.0; 1.60-10.10) and below 20 (aOR 10.5; 3.59-30.72) had a higher risk of infection with Chlamydia and/or gonorrhoea.
	Multicentre, Mozambique (2004)	Lujan, 2008 [300]	No factors were independently associated with infection gonorrhoea
	Moshi, Tanzania (2002-04)	Msuya, 2009 [221]*	NR
	Manhica, Mozambique (2000)	Menendez, 2010 [139]	Women with more than one lifetime sexual partner were at a higher risk of being seropositive for gonorrhoea than women with one lifetime sexual partner (aOR 2.8; 1.2-6.3)
	Mwanza, Tanzania (2012)	Hokororo, 2015 [130]	As stated for this paper under syphilis (infections combined)
Chlamydia trachomatis	Vilancolus, Mozambique (1991-92)	Vuylsteke, 1993 [307]	NR
	Kinshasa, DRC (1990)	Vuylsteke, 1993 [324]	NR
	Mwanza,Tanzania (1992)	Mayaud, 1995 [126]	As stated for this paper under gonorrhoea (the two infections were combined)
	Harare, Zimbabwe	Latif, 1999 [125]*	As stated for this paper under gonorrhoea (the two infections were combined)
	Gaborone, Botswana (2001-01)	Romoren, 2004 [325]	NR
	Multicentre, Mozambique (2004)	Lujan, 2008[300]	No factors were independently associated with infection Chlamydia
	Kinshasa, DRC (2004)	Kanoshita-Moleka, 2008 [318]	NR
	Moshi, Tanzania (2002-04)	Msuya, 2009 [221]*	NR
	Manhica,Mozambique (2000)	Menendez, 2010 [139]	Infection with chlamydia was not significantly associated with any social or behavioral risk factor
	Mwanza, Tanzania (2012)	Hokororo, 2015 [130]	As stated for this paper under syphilis (infections were combined)
Trichomonas vaginalis	Kinshasa, DRC (1990)	Vuylsteke,1993[324]	NR
	Vilancolus, Mozambique (1991-92)	Vuylsteke 1993 [307]	NR
	Dar es Salaam, Tanzania (1993)	Mwakangile 1996 [129]	Prevalence was lower in pregnant women in monogamous marriages
	Rwadan camp, Tanzania (1994)	Mayaud, 1997[309]	NR
	Mwanza, Tanzania (1994)	Mayaud, 1998 [311]	NR

Appendix 1.4: Risk factors for malaria and STIs/RTIs continued

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Trichomonas vaginalis	Blantyre, Malawi (1993)	Taha, 1999 [313]	NR
	Gaborone, Botswana (2000-01)	Romoren, 2007[326]	NR
	Ndola, Zambia (NR)	Crucitti, 2010 [327]*	NR
	Manhica, Mozambique (2000)	Menendez 2010 [139]	Women with anogenital warts were at increased risk of having trichomoniasis (aOR 7.1, 95% CI, 1.4-36.5)
	Multicentre	Mapingure, 2010 [320]	NR
	Harare, Zimbabwe (2000)	Kurewa, 2010 [128]	For vaginal infections** it was age at sexual debut; sexual debut before 20 years of age compared to whose sexual debut was after 20th birthday (aOR 1.60; 95% CI, 1.06-2.42) Being in a polygamous relationship was significantly associated with having *Trichomonas vaginalis*; (aOR 2.24, 95% CI, 1.09-4.56).
	Mwanza, Tanzania (2012)	Hokororo, 2015 [130]	As stated for this paper under syphilis (infections were combined)
Bacterial vaginosis	Rwadan camp, Tanzania (1994)	Mayaud, 1997 [309]	NR
	Mwanza, Tanzania (1994)	Mayaud, 1998 [311]	NR
	Harare, Zimbabwe	Latif, 1999 [125]	NR
	Multicentre, Zimbabwe (1999-2004)	Toloso, 2006 [328]	NR
	Harare, Zimbabwe	Taha, 1999 [313]	NR
	Gaborone, Botswana (2000-01)	Romoren, 2007 [326]	NR
	Tanzania (1999-2004)	Msuya, 2009 [221]*	NR
	Moshi, Tanzania (2002-04) and Harare, Zimbabwe (2002-04)	Mapingure, 2010 [320]	NR
	Harare, Zimbabwe (2002-03)	Kurewa,2010 [128]	As stated for this paper under *T. vaginalis* (infections were combined)
	Mwanza, Tanzania (2012)	Hokororo, 2015	As stated for this paper under syphilis (infections were combined)
Peripheral Malaria	Macha, Zambia (1989)	Larkin, 1991[337]	NR
	Chikwawa, Malawi (1993-94)	Verhoeff, 1998 [293]	NR
	Chikwawa, Malawi (1993-95)	Verhoeff, 1999 [294]	NR

Appendix 1.4: Risk factors for malaria and STIs/RTIs continued

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Peripheral malaria	Blantyre, Malawi (1997-98)	Rogerson, 2000 [141]	first attendance at a clinic in the dry season relative to the wet season (OR 0.70; 95% CI, 0.62–0.79) and a younger age, < 20 years, (OR 0.97; 95% CI, 0.95–0.99) were associated with the presence of parasitaemia but there was no significant difference in malaria prevalence with gravidity as a continuous variable (OR 0.96, 95% CI, 0.90–1.01)
	Blantyre, Malawi (1997-98)	Rogerson, 2000 [88]	Gravidity had a significant influence on malaria infection. Peripheral parasite prevalence was similar in primigravidae and secundigravidae, 24.0% and 26.1% respectively but was lower in multigravidae (17.2%)
	Kigoma, Tanzania (NR)	Mnyika, 2000[295]	NR
	Blantyre, Malawi (2000)	Mankhambo, 2002[297]	NR
	Sofala, Mozambique (2003-04)	Montoya, 2006 [298]	NR
	Morogoro, Tanzania (2001-02)	Wort,2006 [142]	Adolescent primigravidae had higher parasite prevalence than adolescent secundigravidae (41.3% versus 28.1%, $P > 0.05$) and adult primigravidae (41.3% versus 31.5%, $P = 0.007$).
	Multicenter, Mozambique (2003-04)	Brentlinger,2007 [299]	NR
	Southern Malawi (2004-05)	Van Den Broek, 2009[301]	NR
	Mongochi, Malawi (2003-04)	Rantala, 2010 [95]	NR
	Blantyre, Malawi (1997-2001)	Feng, 2010 [1]*	Bed net use protected from peripheral parasitaemia (aOR 0.47; 95% CI, 0.37, 0.60)
	300 DHS geographic clusters, DRC	Taylor, 2011 [144]*	In a multivariate model only lower wealth (all categories of wealth below the highest quintile) was significantly associated with higher prevalence of parasitaemia ($P < 0.001$). No association between age or gravidity or area of residence and malaria infection was found.
	Sambizanga and Ingombota, Angola (2006-2007)	Valente, 2011[89]*	Women ≤ 18 years old were at a higher risk of infection than women ≥ 25 years (aOR 2.1; 95% CI, 1.1-4.2). Women who had self-reported malaria infection earlier in pregnancy (aOR 2.0; 95% CI, 1.0-3.9) were at a higher risk of infection. No association was found between parity or area of residence or the level of education and placental malaria infection.

Appendix 1.4: Risk factors for malaria and STIs/RTIs continued

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Peripheral malaria	Manhica, Mozambique (2003-04)	Serra-Casas, 2011 [143]	Multiparous women were at a reduced risk of peripheral infection compared to primiparous women (aOR 0.26; 95% CI, 0.13-0.53) and women who had received SP were had reduced risk of infection compared to women who had received placebo (aOR 0.30; 95% CI, 0.14-0.63)
	Manhica, Mozambique (2003-2005)	Mayor, 2012 [302]*	Prevalence of *P. falciparum* malaria, both by microscopy and RT-PCR, was equal across age and parity groups
	Luanda, Angola (2008)	Campos,2012 [145]	Age, parity and gestational age were not associated with malaria infection. Women who had previous malaria during pregnancy also had a higher risk of current malaria (aOR 4.86; 95% CI, 1.45-16.25)
	Nchelenge, Zambia (2013)	Siame, 2015 [115]*	Ownership of at least one net in their household was not associated with malaria. Age was the only factor associated with malaria; women aged 30-34 were 64% less likely to have malaria compared to those in the reference group 14-18 years old (AOR 0.36; 95% CI, Not reported).
	Mansa , Zambia	Mace, 2012 [90]	NR
Placental malaria	North Kivu, DRC (NR)	Meuris, 1993[303]	NR
	Blantyre, Malawi (1997-99)	Rogerson, 2000[88]	Gravidity had a significant influence on malaria infection. Placental parasite prevalence was similar in primigravidae and secundigravidae, 2.5% and 36.2% respectively but was lower in multigravidae (16.0%)
	Blantyre, Malawi (NR)	Rogerson, 2003 [104]	NR
	Kinshasa, DRC (2004)	Lukuka, 2006 [304]	No association between parity or IPTp-SP and placental malaria infection
	Chikwakwa, Malawi (2004-05)	Senga, 2007[305]	NR
	Muheza, Tanzania (2002-05)	Mwangoka, 2008 [306]	NR
	Muheza, Tanzania (2002-05)	Kabyemela, 2008 [146]	Iron deficiency (aOR 0.20; 95% CI 0.11-0.36) and multigravidity (aOR 0.32; 0.15-0.66) were independently associated with a decreased risk of placental malaria. The following factors were not independently associated with placental malaria infection; Use of intermittent presumptive therapy, insecticide-treated bed nets and delivering during seasons of low malaria transmission

Appendix 1.4: Risk factors for malaria and STIs/RTIs continued

Infection	Site, country (year)	First Author, Year of publication	Risk factors for infection
Placental malaria	Blantyre, Malawi (1997-2001)	Feng, 2010 [1]	Number of sulphadoxine-pyrimethamine doses received correlated inversely with placental parasitaemia (aOR 0.79; 95% CI, 0.68, 0.91). Bednet use protected from placental parasitaemia (aOR 0.41; 95% CI, 0.31, 0.54).
	Luanda, Angola	Valente, 2011 [89]	Women ≤ 18 years old were at a higher risk of placental malaria infection than women ≥ 25 years (aOR 2.2; 95% CI, 1.1-4.5). Women who had self-reported malaria infection earlier pregnancy (aOR 1.7; 95% CI, 1.1-2.8) and those residing in the peripheral areas of Luanda (aOR 1.7; 1.0-30) were at a higher risk of infection. No association was found between parity or the level of education and placental malaria
	Manhica, Mozambique (2003-05)	Serra-Casas, 2011 [143]	Multiparous women were at a reduced risk of placental infection compared to primiparous women (aOR 0.30; 95% CI, 0.16-0.57) and women who had received SP had a lower risk of infection compared to women who had received placebo (aOR 0.42; 95% CI, 0.22-0.63)
	Manhica, Mozambique (2003-05)	Mayor, 2012 [302]	NR
	Mansa , Zambia	Mace, 2012 [90]	NR
	Rafiji and Moshi, Tanzania	Moshi, 2015	Primigravidity (aOR 2.4; 95% CI, 1.1-5.0; P = 0.025) and residing in a high transmission area (aOR 9.4; 95% C,I 3.2-27.7; P < 0.001) were significant risk factors for placental malaria. IPTp was associated with a lower risk of placental malaria (aOR 0.3; 95% C,I 0.1-1.0; P = 0.044).

aOR; Adjusted odds ration; BV: Bacterial vaginosis; HSV: herpes simplex virus; NR: Not reported
Serological STI: human immunodeficiency virus, HSV-type 2 and syphilis
Vaginal infection: Trichomoniasis, bacterial vaginosis and candidiasis
[α]Any STI: HSV-type 2, trichomoniasis, Chlamydia, gonorrhoea, syphilis and HIV

Appendix 2.1: Information Sheet

You are being invited to take part in a research study. It is important for you to understand why the research is being done and what it will involve before you decide. Please take time to read the following information carefully and discuss it with others if you wish. Feel free to ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not to take part. Please understand that participation in this study is voluntary and that you are free to withdraw at any time, without giving any reason and without your medical care or rights being affected.

About the study

The study is on the malaria and reproductive tract infections in pregnancy. The principle investigator in this study is a lecturer of Parasitology at the University of Zambia and a PhD student at the London school of Hygiene and Tropical medicine. The study will be conducted under the supervision of experienced epidemiologists from the both the London School of Hygiene and Tropical Medicine and The University of Zambia.

Malaria, curable sexually transmitted infections (STI) and non-sexually transmitted infections of the reproductive system of women put the health and lives of pregnant women and their babies at risk. All of these diseases are present in our communities. In Zambia women receive pills at antenatal care that are meant to prevent malaria. Women are also screened for syphilis and treated if necessary. This is all in the interest of protecting them and their babies. However, these are not the only diseases that are common among pregnant women. Other diseases also affect the lives and health of women and their babies. Through this study we hope to find out the burden of malaria and the other infections that affect pregnant women when they occur together.

Purpose of the project

We hope to use the information from the study to contribute to improved health policies to ensure that women receive appropriate testing and/or treatment of other infection that affect them and their babies. This study will also examine to what extent malaria and reproductive tract infections occur together among pregnant Zambian women. The Ministry of Health can use this information to make more effective recommendations for management of common infections that occur in pregnant women.

Procedure

The nurses/research assistants will collect some personal information and collect a blood sample from you by pricking your finger and another one by drawing blood from your arm. They will also collect a vaginal sample from you and you will be given the first dose of sulphadoxine-pyrimethamine for protection of you and your baby against malaria. You will be asked to return to the clinic after four weeks. It is very important that you return after four weeks for a second malaria screening and the second dose of sulphadoxine-pyrimethamine.

When you come back to have your baby, the nurses will get a small piece of tissue from your placenta (the extra tissue that comes out after the baby) and ask you a few more questions. A 2cm by 2 cm by 1 cm piece will be sampled from the placenta. The baby will be weighed only; no blood will be taken from the baby.

All blood, vaginal swabs and tissue samples will be labelled with an identification number, not your name. Your name and ID number will be recorded on a list kept only by the study coordinator. This list will be kept only to help us to report back to you on your results and to follow up with you if you are found to have any of the infections that we will test you for. After the study is over, this list will be destroyed.

Number of women required

We need about 1100 pregnant women to take part in this study and women will be recruited consecutively as they come for their first antenatal visit. We are inviting anyone who is willing to take part provided that:

1. The person is attending antenatal for the first time.
2. She has not taken antibiotics or antimalarial agents in the previous 4 weeks.
3. She is less than 32 weeks pregnant.

Risks or discomforts

We expect the risks for you being in this study to be rare. You may feel a sting when a vein is punctured or when your figure is pricked to obtain blood from you. If enough blood is not taken from one prick, a second prick may be done. We may also ask you a few personal questions and you may feel uncomfortable answering these questions. You may choose to not answer any questions that you do not feel comfortable answering.

Benefits

This study will benefit pregnant women in Zambia, their children. We intend to share the results of this study with the Ministry of Health so that they can have evidence for the need to improve their recommendations on management and control of malaria and other infections that put the lives of pregnant women and their unborn babies at risk. This study has the potential to contribute to the improvement of health care which pregnant women and babies born to them receive.

Confidentiality

Any information or samples that leave the health centre will have an identification number (ID) not your name. Your name and ID number will be recorded on a list kept only by the study coordinator. We intend to use this list to report back to you on your results so that you can get the required treatment. After the study is over, all forms and your blood samples will be destroyed. Only the final results of the study, without names, will be shared with others.

Cost/Payment:

Your participation in this study will not cost you any money. The only cost to participating in this study is your time. This should take up to an extra hour (in addition to period you will spend for the normal ANC activities) of your time today in order for us to collect samples and information about you. The project has no money to compensate you for the time you will spend for the study.

Persons to Contact

If you have any questions about this study, please contact the principle investigator at P. O. Box 33528, Lusaka or call mobile: “Mobile number for the principle investigator was inserted here”. If you feel that you have been harmed as a result of being part of this study, please contact the University of Zambia Biomedical Research Ethics Committee at the following address:

University of Zambia
Ridgeway Campus
P.O. Box 50110, Lusaka, Zambia
Telephone 260-1-250753

If you agree to take part in this study you will be asked to sign two copies of the consent form and you will keep one together with this information sheet.

Appendix 2.2: Eligibility screening form

If a potential participant's gestational age by last menstrual period and fundal height determination is less than 32 weeks please fill out this form.

1. Have you taken any antimalarial treatment in the past one month No [ ] Yes [ ]
2. Have you taken any antibacterial medication in the past one month No [ ] Yes [ ]
3. Are you willing to allow a member of our team to record your HIV test results. No [ ] Yes [ ]

Indicate to a potential participant that the results will not be recorded with their name but with an identification number that they will be given if they decide to take part in the study

If the response to question 1 and to question 2 is "NO" and that to question 3 is "Yes" please ask for consent using the consent form.

Appendix 2.3: Consent Form

Patients ID Number for study: _____ (for official use)

Title of project: The Epidemiology of malaria and sexually transmitted infections and reproductive tract infections in pregnant women in Ndola district, Zambia

Name of researcher: Mrs. Enesia Chaponda Ngulube

Please tick appropriately

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. **Yes [ ] No [ ]**

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or rights being affected. **Yes [ ] No [ ]**

3. I understand that a vaginal samples and blood form my arm and from my finger will be collected from today. **Yes [ ] No [ ]**

4. I understand that a piece of my placenta will be taken from me at delivery **Yes [ ] No [ ]**

5. I understand that sections of any of my medical notes that are of interest to the project may be read by the researchers. I give permission for these individuals to have access to my records.
   Yes [ ] No [ ]

6. I agree to take part in the above study **Yes [ ] No [ ]**

_____ _____

Participant's signature **Date**

Thumb print (if unable to sign)

_____

_____

Name of person taking consent form **Signature**
(If different from principle investigator)

1 for participant; 1 for researcher

Appendix 2.4: Questionnaire

Participant's ID Number: /___/___//___/___/

Demographics and Socio-economics

1. Date of interview:……(dd/mm/yyyy)

   Village/area of residence …….

   Name of Health centre ……..

2. HIV status
   [0] Negative [1] Positive

3. Are you on antiretroviral therapy?
   [0] No [1] Yes

4. What is your age? …….

   Measure and record weight, height and blood pressure at first antenatal visit

5. Weight: .....

6. Height: .....

7. Systolic blood pressure ……mmHg

8. Diastolic blood pressure ……mmHg

9. What is your marital status?
   [1] Single
   [2] Married
   [3] Separated/divorced
   [4] Widowed
   [5] Other, Please specify _____
   [99] Not answered

10. What is the highest level of schooling you completed?
    [Indicate number of years as 1, 2, 3… etc. count should include repeated years and years spend in tertiary education where applicable]
    _____

11. What is your main source of income?

    [1] Working for wages
    [2] Selling items
    [3] Self-employed (other than selling items)
    [4] None
    [5] Other, please specify_____
    [99] Unknown/not answered

12. What is your husband's main source of income?

[1] Working for wages
[2] Selling items
[3] Self-employed (other than selling items)
[4] None
[5] Other, please specify_____
[6] Not applicable (single/widowed)

Does your household have any of the following?

13. Television
[0] No [1] Yes

14. Electricity
[0] No [1] Yes

15. Radio
[0] No [1] Yes

16. Freezer/Fridge
[0] No [1] Yes

17. What type of fuel does your household mainly use for cooking?

[1] Firewood
[2] Charcoal
[3] Gas
[4] Electricity
[5] Other, please specify_____
[99] Not known

18. What type of floor is in the house?

[1] Earth/sand
[2] Wooden/Bamboo
[3] Vinyl/Tiles
[4] Cement
[5] Other, _____
[99=] Not known

19. What type of roof is on the main house?

[1] Plastic/tarpaulin
[2] Thatch/grass
[3] Iron sheets/tiles/Asbestos
[4] Other _____
[99] Not known

B. Indoor residual spraying and bed net ownership

20. Do you have a bed net? *(If not, go to question 23)*

[0] No [1] Yes

21. Is your net factory treated (long lasting net)? If 'yes' go to question 22

[0] No [1] Yes [99] Unknown

22. If your net is not factory treated, when was the last time it was treated?

    [1] Treated in the last 6 months
    [2] Treated 6-12 months ago
    [3] Treated more than 12 months ago
    [4] Never been treated
    [99] Unknown

23. Did you sleep under the bed net last night?

    [0] No [1] Yes

24. Has your house been sprayed for malaria control in the last 12 months?

    [0] No [1] Yes [99] Unknown

C. Medical History

25. Is this your first pregnancy? *(If yes, enter 0 in question 26 and go to question 30)*
    [0] No [1] Yes

26. If no, how many previous pregnancies have you had? *(include all pregnancies resulting in live births, stillbirths, abortions/miscarriages)*
    Number of previous pregnancies (1, 2, 3… etc) Pregnancies with twins count as 1 pregnancy.

    [ ]

27. Have you ever experienced a miscarriage (lost a baby before it could survive independently)? (Based on assessment by a medical practitioner)
    [0] No [1] Yes

28. Have you ever experienced a stillbirth (given birth to a dead child)? (Based on assessment by a medical practitioner)
    [0] No [1] Yes

29. Have you ever given birth to a premature baby? (Based on assessment by a medical practitioner)
    [0] No [1] Yes

30. Have you ever experienced itching or/and burning session when urinating and/or vaginal discharge and/or had sores on your private parts during this pregnancy? (if 'no' go to 33)
    [0] No [1] Yes

31. If your answer to 25 is 'yes', did you receive treatment?
    [0] No [1] Yes

32. Are you currently experiencing any of the symptoms mentioned in 30?
[0] No [1] Yes

33. Have you been treated for malaria during this pregnancy so far?
[0] No [1] Yes

34. Have you ever been treated for a sexually transmitted or reproductive tract infection this pregnancy
[0] No [1] Yes

D. Sexual behaviour

35. How old were you when you started your period (menstruation)?
Enter age in years. If unanswered/unknown enter 99

36. How old were you when you had your first sexual encounter?
Enter number of years. If unanswered enter 99

37. How many sexual partners have you had in your life time?

Enter number of partners. If unanswered enter 99

To be administered after delivery.

Patients ID Number: /___/___//___/___/

E. Birth characteristics and medical History

38. Have you been treated for malaria during this pregnancy?
[0] No [1] Yes [99] Not sure

39. Did you take iron supplementation for blood building?
[0] No [1] Yes [99] Not sure

40. Have you taken or used any medicines/gel for the treatment of an STI or itchiness during this pregnancy? If no go to 41
[0] No [1] Yes [99] unknown/not sure

41. If yes in which trimester[1] was the medicine taken?
[0] First Trimester
[1] Second trimester

[2] Third trimester
[99] Unknown

[1] *1st trimester = in the first 3 months of pregnancy*

2nd trimester = in the 4th to 6th month after the last menstruation

3rd trimester = in the 7th to 9th month after the last menstruation

42. Indicate the name(s) of the drug(s) taken for STI/RTI treatment as recorded on the ANC card.

.....
.....
.....

Questions 43-60 to be filled in by or with the help of the attending nurse and patients records

43. Date of delivery (dd/mm/yyyy) /___/___//___/___/___/ /___/___/____/

44. Place of delivery
    [1] Hospital
    [2] Health centre/clinic
    [3] Home
    [4] Other.....

45. Who performed delivery?
    [1] Family member
    [2] Traditional birth attendant (TBA)
    [3] Midwife
    [4] Doctor
    [5] Other

46. Delivery induced/spontaneous
    [1] Induced
    [2] Spontaneous
    [99] Not known

47. Type of delivery?
    [1] Vaginal
    [2] C-section
    [3] Assisted
    [4] Other

48. How was the baby born?
    [1] Alive
    [2] Dead
    [3] Unknown

49. Sex of baby

[0] Female [2] Male

50. Birth weight: .....grams.

51. Delivery gestational age: .....weeks [For official use, to be recorded based earlier assessment by ultrasound]

52. Number of babies delivered
[0] Singleton
[1] Twins
[2] Triplets

53. Birth outcome
[0] Live birth
[1] Stillbirth
[99] Unknown

(If live birth, go to F)

54. For stillbirths, was the baby moving at the start of labour?
[0] No
[1] Yes
[99] Unknown
If yes go to F

55. If the baby was not moving before delivery, how many days before delivery did the foetal movements stop?*(Enter number of days 1, 2, 3, 8 etc)*

F. Drug History

56. HIV status
[0] Negative [1] Positive

57. Has the participant been on ART?
[0] No [1] Yes [99] Unknown

58. Has the participants taken cotrimoxazole (commonly known as pyriton) during this pregnancy?
[0] No [1] Yes [99] unknown

59. Has the Participant taken Sulphadoxine-pyrimethamine (Fancidar) for malaria prevention during this pregnancy?

    [0] No [1] Yes [99] Unknown

60. If yes to question 59 indicate the number of doses as indicated by participant/recorded on the participants ANC card/record.

    ☐

Appendix 2.5: Study completion or drop out record form

Participant's ID number: _________________\		
1. Date of enrolment	*dd/mm/yyyy*	/__/__/__/__/__/__/___/__/
2. Date of 28 day follow-up	*dd/mm/yyyy*	/__/__/ __/___/___/___/___/___/
3. Did the participant attend the 28 day follow-up	[0] No [1] Yes	/___/
4. Was the participant lost to follow-up	[0] No [1] Yes	/___/
5. If yes give reason	*Reasons* [1] Moved [2]cannot be traced [3] withdrew consent/refused [4]Other (To give reason go to 6)	
6. If other reason	Other_____	

Appendix 2.6: Laboratory Record/Reporting forms

F1: Blood smear record form

Participant's ID number: _________________\

Date of collection: dd/mm/yy ____________\

Sample collected at day 0 ☐ Day 28 ☐ (Tick appropriately as indicated on slide)

ID number	Result	No. of parasites	No. of white blood cells	Parasites/µl of blood

F2: Record form for Chlamydia, trichomoniasis and gonorrhoea test results.

Participant's ID number: _________________\

Organism	Test result
Chlamydia trachomatis	Positive □ Negative □
Neisseria gonorrhoea	Positive □ Negative □
Trichomonas vaginalis	Positive □ Negative □

F3: Record form for bacterial vaginosis diagnosis.

Record form based on Nugent criteria

ID Number	Gram variable coccobacilli	Gram negative curved bacilli	Lactobacilli	Total score	Classification

F4: Placental Histology Results Reporting Form

ID NO	Parasite	Pigment	Inflammation	Pathology class

Appendix 2.7: Names and sequences of primers that were used for detection of chlamydia, gonorrhoea, trichomoniasis and malaria infection

Organism	Primer name		Sequence
Chlamydia trachomatis	KL1		5'-TCCGGAGCGAGTTACGAAGA-3'
	KL2		5'AATCAATGCCCGGGATTGGT 3'
Neisseria gonorrhoeae	Ngu1		5'-CAA CTA TTC CCG ATT GCG A-3'
	Ngu2		5'-GTT ATA CAG CTT CGCCTG AA-3'
Trichomonas vaginalis	TV3		5′ – ATTGTCGAACATTGGTCTTACCTC –3 ′
	TV7		5′ – TCTGTGCCGTCTTCAAGTATGC –3′
Plasmodium falciparum	1ST reaction	rPlus 5	5'-CCTGTTGTTGCCTTAAACTTC-3'
		rPlus 6	5'-TTAAAATTGTTGCAGTTAAAACG-3'
	2nd reaction	rFAL1	5'-TTAAACTGGTTTGGGAAAACCAAATATATT-3'
		rFAL2	5'-ACACAATGAACTCAATCATGACTACCCGTC-3'

Sources of primer sequences:
C. trachomatis- Schachter 1997 [193]
N. gonorrhoeae- Chaundry 2002 [192]
T. vaginalis- Kengne *et al.,* 1994 [197]
P falciparum- Snounou *et al*., 1993 [201]

Appendix 2.8: Polymerase chain reaction parameters

Organism (gene)	**Reaction**	**Initial Denaturation ^{0}C, Time**	**Denaturation ^{0}C, Time**	**Annealing ^{0}C, Time**	**Extension ^{0}C, Time**	**Final Extension ^{0}C, Time**	**No. of cycles**
Chlamydia trachomatis Chlamydial ribosomal plasmid	Single PCR reaction	95, 10 min	95, 10 min	62, 2 min	72, 1:30 min	72, 7 min	45
Neisseria gonorrhoea (Orf1)	Single PCR reaction	95, 10 min	95, 1 min	55, 2 min	72, 1:30 min	72, 7 min	45
Trichomonas vaginalis (repeat DNA sequence)	single PCR reaction	95, 10 min	95, 1min	60, 2 min	72, 1:30 min	72, 7 min	45
Plasmodium falciparum (MSP2)	Primary and secondary	94, 2 min	94 for 45 sec	61.1, 45 sec	65, 1 min	65, 2 min	25
Plasmodium falciparum (*Dhfr* and *Dhps)*	Primary and secondary	94, 2 min	94, 45 sec	43.4, 45	65,1 min	65,2 min	25
Plasmodium falciparum small subunit ribosomal RNA	primary	94,3 min	94,1 min	60, 2min	72, 2min	72, 7min	30
	secondary	94,3 min	94, 1min	55,2min	72, 2min	72,7 min	30

MSP2-Merozoite surface protein 2
DHFR-Dihydrofolate reductase
DHPS-Dihydropteroate synthase
DNA- Deoxyribonucleic acid
RNA-Ribonucleic acid

Appendix 2.9: Primer names and sequences for detection of DHFR and DHPS mutations

Polymorphism		Primer	Sequence
Pf DHFR		M1	5'-TTTATGATGGAACAAGTCTGC-3'
		M5	5'-AGTATATACATCGCTAACAGA-3'
	1st reaction		
Pf DHPS		R2	5'-AACCTAAACGTGCTGTTCAA-3'
		R1	5'-AATTGTGTGATTTGTCCACAA-3'
		M3	5'-TTTATGATGGAACAAGTCTGCGACGTT-3'
Pf DHFR	2nd reaction	F/	5'-AAATTCTTGATAAACAACGGAACCTttTA-3'
		F	5'-GAAATGTAATTCCCTAGATATGgAATATT-3'
		M4	5'-TTAATTTCCCAAGTAAAACTATTAGAgCTTC-3'
		K	5'-TGCTAGTGTTATAGATATAGGatGAGcATC-3'
Pf		K/	5'-CTATAACGAGGTATTgCATTTAATgCAAGAA-3'
DHPS		J	5'TGCTAGTGTTATAGATATAGGTGGAGAAagC-3'
		L	5'-ATAGGATACTATTTGATATTGGAccAGGATTcG-3'
		L/	5'-ATTACAACATTTTGATCATTCgcGCAAccGG-3'

Source of primer sequence: Duraisingh 1998 [203], Pf-*Plasmodium falciparum*

Appendix 2.10: Restriction fragment length polymorphisms analysis of DHFR and DHPS polymorphisms

Appendix 2-10a RFLP analysis of DHFR polymorphisms				
Substrate	Codon	Enzyme	RFLP	Residue
M3-F/	DHFR 16	NlaIII	93bp, 376bp	Ala
			146bp, 376bp	Val
	DHFR 51	Tsp509I	120bp, 154bp	Asn
			120bp, 218bp	Ile
	DHFR 108	AluI	196bp, 326bp	Ser
			522bp (no cut)	Other
		BsrI	190bp, 322bp	Asn
			522bp	Other
		BstNI	196bp, 326bp	Thr
			522bp	Other
F-M4	DHFR 59	XmnI	137bp, 189bp	Cys
			137bp, 163bp	Arg
	DHFR 108	AluI	119bp, 180bp	Ser
			299bp	Other
		BsrI	146bp, 180bp	Asn
			326bp	Other
		BstNI	145bp, 181bp	Thr
			326bp	Other
Appendix 2-10b RFLP analysis of DHPS polymorphisms				
Substrate	Codon	Enzyme	RFLP	Residue
K-K/	436	MnlI	121bp, 278bp	Ser
			121bp, 317bp	Other
		MspA1I	406bp	Ala
			438bp (no cut)	Other
	437	MwoI	387bp	Ala
			419bp	Other
		AvaII	404bp	Gly
			438bp	Other
	540	FokI	85bp, 320bp	Glu
			405bp	Other (Lys)
J-K/	436	*Hind*III	406bp	Phe
			438bp	Other
		*Hha*I	406bp	Ala
			438bp	Other
L-L/	581	BstUI	105bp	Ala
			138bp	Other
		BslI	128bp	Gly
			161bp (no cut)	Other
	613	MwoI	128bp	Ala
			161bp	Other
		BsaWI	131bp	Ser
			161bp	Other
		AgeI	128bp	Thr
			161bp	Other

Appendix 2.11: Sequences of oligonucleotide primers that were employed for the differentiation of recrudescence from re-infection

Merozoite Surface Protein 2: *msp2*		
Reaction	Primer name	Sequence
First reaction:	M2-OF	5′- ATGAAGGTAATTAAAACATTGTCTATTATA -3′
	M2-OR	5′- CTTTGTTACCATCGGTACATTCTT -3′
Second reaction:		
FC27-type	M2-FCF	5′- AATACTAAGAGTGTAGGTGCARATGCTCCA -3
	M2-FCR	5′- TTTTATTTGGTGCATTGCCAGAACTTGAAC -3′
IC3D7-type	M2-ICF	5′- AGAAGTATGGCAGAAAGTAAKCCTYCTACT -3′
	M2-ICR	5′- GATTGTAATTCGGGGGATTCAGTTTGTTCG -3
Source: Snounou et al, 1999 [205]		

Appendix 2.12: Letter of notification of Ethical Clearance

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm
Assurance No. FWA00000338
IRB00001131 of IORG0000774

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

4th September 2013

Your Ref: 004-02-13

Enesia Chaponda Ngulube,
University of Zambia
Department of Biological Sciences
P.O. Box 32379
Lusaka

Dear Ms Ngulube,

RE: APPROVAL OF RESEARCH PROPOSAL: "THE EPIDEMIOLOGY OF MALARIA, SEXUALLY TRANSMITTED AND REPRODUCTIVE TRACT INFECTIONS AND THEIR CO-INFECTION AMONG PREGNANT WOMEN IN A CATCHMENT AREA IN NCHELENGE DISTRICT, ZAMBIA."

The above research proposal was resubmitted to the University of Zambia Biomedical Research Ethics Committee on 21st June, 2013 with recommended changes. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Dr. J.C Munthali
CHAIRPERSON

Date of approval: 4th September, 2013 **Date of expiry**: 3rd September 2014