1.0 CHAPTER ONE: INTRODUCTION

1.1 Background

Malaria remains a major public health problem globally. This disease has been in existence for over four thousand years and has greatly affected human populations and human history (Yotoko et al, 2006). An estimated 3.4 billion people are at risk of the disease worldwide. Each year 300-500 million acute illnesses and 600,000 to 1 million deaths are attributed to malaria (WHO report, 2012). The disease affects 109 countries worldwide with the majority of cases (90%) in Sub-Saharan Africa. The public health costs and economic implications are tremendous and an estimated decrease in economic growth of >1% is reportedly due to malaria, this has a great impact on development in developing countries. Pregnant women, children below 5 years old and the immune-suppressed are the most affected populations (UN Global malaria report, 2010).

Malaria is caused by parasitic protozoa of the genus *Plasmodium* and transmitted by a female mosquito of the *Anopheles* genus. The five species that cause malaria in humans include *Plasmodium malariae, Plasmodium vivax, Plasmodium ovale, Plasmodium knowlesi and Plasmodium falciparum.* Most infections (80%) in sub-saharan Africa are caused by *Plasmodium falciparum*. There are over 30 *anopheline* species that have been identified that can transmit the malaria parasite (Singh et al, 2004).

Malaria is both treatable and preventable, when the recommended interventions are properly implemented. Malaria prevention requires vector control in the form of Insecticide use (Indoor residual spraying and larvicide spraying), use of Insecticide treated nets (ITNs) and a controlled environment to prevent the establishment of breeding areas for the *Anopheline* mosquitoes. Chemoprevention in vulnerable groups such as infants and pregnant women further prevents infection. Prompt diagnosis and effective treatment with the appropriate anti-malarials reduces the risk of mortality due to this disease (UNICEF report, 2010).

1.2 Malaria in Pregnancy

Malaria infection in pregnancy is a major cause of maternal morbidity and may lead to poor birth outcomes. In Africa, more than 30 million women become pregnant each year and are at risk of malaria. In Sub Saharan Africa malaria prevalence is estimated at 27.6% and severe anemia (haemoglobin <8g/dl) due to malaria is estimated at 26%, this is more prevalent in women in their first pregnancy (Marchesini et al, 2010). Placental malaria which leads to even more serious complications for the unborn child is estimated at 26% while Low birth weight due to malaria is approximately 20% (Desai et al, 2007). Physiological changes during pregnancy and pathological effects of malaria adversely affect pregnancy outcome (Ansell et al, 2002). The woman is at an increased risk of high parasitaemia, severe anemia, hypoglycemia, acute pulmonary edema and death while the unborn child is at a greater risk of premature and still birth, low birth weight, congenital malaria, anemia and impaired fetal growth (Saba et al, 2008).

Differences in epidemiological settings and host immunity influence the clinical presentation of pregnancy associated malaria. In sub-Saharan Africa which comprises highly endemic areas with stable transmission, malaria is generally asymptomatic with heavy placental parasitaemia while in low transmission areas epidemic outbreaks lead to high mortality and morbidity due to low immunity (Recker et al, 2009). In stable transmission areas women in their first and second pregnancies have a greater risk of developing complications than those with successive pregnancies. However, malaria immunity maybe further compromised by HIV infection. Increased malaria prevalence in HIV positive women puts the unborn child at a higher risk irrespective of the number of previous pregnancies. In low endemic areas malaria in HIV positive pregnant women is likely to result in severe illness, complications and poor pregnancy outcomes with every pregnancy (Steketee et al, 2001).

1.3 Malaria Prevention and Control Strategies

Zambia has taken an integrated approach towards malaria control. The national malaria strategic plan 2006-2010 focused on a scale up of proven interventions: distribution of insecticide treated nets, Indoor residual spraying, program management, impact assessment and monitoring and evaluation of programs. Most recently the 2011-2015

national malaria strategic plan focuses on sustainability of impact, improved malaria diagnostics, capacity building and strengthening of delivery of key interventions and surveillance. The current policy is to provide artemisinin combination therapy as first line treatment and provide free intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP). Free insecticide treated bed nets (ITNs), early diagnosis and prompt case management using effective anti malarials and indoor residual spraying comprise the strategies for combating malaria in pregnant women. These services are available through ante natal care (ANC) at all government health facilities (Ministry of Health guidelines, 2010).

It has been reported that nearly two thirds of the country is now covered by either an insecticide treated net (ITN) or recent indoor residual spraying (IRS). Over 4.5 million ITNs have been delivered to households with distribution targeting hard to reach areas. Pregnant women seeking prenatal care can now receive preventive medicines and ITNs at public health centers nationwide. There has also been an increase in the number of women with knowledge about malaria, symptom recognition and methods of prevention which are key to effective malaria control. The first line drugs (Artemisinin combination therapy) and rapid malaria kits are available nationwide (Malaria Indicator survey, 2010).

Sulphadoxine-pyrimethamine (SP) is the recommended drug by WHO for use in IPTp. Sulphadoxine-pyrimethamine is safe in pregnancy, cost effective, easy to administer and ideal for prophylaxis as it comprises sulphadoxine which has a long half-life of approximately 140 hours. It is administered presumptively three months after quickening (16-26 weeks). The pregnant woman is given three tablets (each containing 500mg of Sulphadoxine and 25mg pyrimethamine) per dose, under direct observation of the health worker. Three (3) doses are given and should be at least one month apart, as SP has a one month prophylactic effect (McGready et al, 2011, Ministry of health guidelines, 2014). Quinine is the recommended drug for treatment for women in their first trimester; however, IPTp when given can treat infection and prevent further infections. Randomized control trials have shown that SP supplemented with Iron

decreases the risk of placental parasitaemia, maternal anemia and low birth weight (Kayentao 2005, Njagi et al 2003, Schultz et al 1994).

1.4 Drug Resistance

Drug resistance remains one of the greatest challenges of malaria control programs. It is characterized by delay or failure of a drug to clear asexual parasites from the blood which allows production of gametocytes that are responsible for the transmission of the resistant genotype. Widespread resistance to sulphadoxine-pyrimethamine and chloroquine in the general population led to the shifting of national treatment policies by many African countries to artemisinin combination therapy treatments (ACTs) which are more efficacious as first line treatment. The malaria treatment policy in Zambia was revised in 2003 and artemisinin combination therapy was introduced as first line treatment. In 2008, the country adopted a policy of universal laboratory diagnosis of suspected malaria infection before commencement of treatment. The current policy that all public health facilities offer free artemisinin based combination therapy at no cost to patients has led to increased access to malaria treatment in Zambia (Ministry of health guidelines, 2010).

However, ACTs are not recommended for prevention of malaria in pregnancy due to lack of adequate safety data (McGready et al 2009, Njagi et al 2003). Hence, while SP remains ideal for IPTp in stable transmission areas, increasing resistance has been reported. SP resistance affects both efficacy and effectiveness of IPTp (Nnaemeka et al, 2012). IPTp efficacy is defined as 'the ability of the SP to clear parasites in a clinical trial' while effectiveness is 'the performance of SP to achieve desired outcomes under program conditions'. Due to differences in acquired immunity of the host and parasite characteristics it is unknown as to what level of parasite resistance would render SP ineffective (Kuile, 2007). Sulphadoxine and pyrimethamine are folic acid antagonists that target the asexual erythrocytic stages of *Plasmodium falciparum*. Sulphadoxine inhibits production of dihydropteroate synthetase (*dhps*) while pyrimethamine inhibits dihydrofolate reductase (*dhfr*). SP resistance is linked to point mutations in the parasite genome specifically the dihydrofolate reductase (*dhfr*) and dihydrofolate synthetase

(*dhps*) genes. Hence, mutations in *dhfr* confer resistance to pyrimethamine while mutations in *dhps* confer resistance to Sulphadoxine and other sulpha drugs. There are variations in SP Mutations; single, double or triple the more mutations, the stronger the resistance. The *dhfr* triple mutant (Asn-108/Ile-51/Arg-59) and *dhps* double mutant (Gly-437/Glu-540) have been strongly associated with resistance in sub-Saharan Africa (Triglia et al 1997, Peterson et al 1988). There is a need therefore to strengthen operational research on therapeutic efficacy studies, adherence to case management protocol and studies on impact and access to interventions.

A study done in 2006 in six districts in the general population of Zambia showed variation of rates of resistance to fansidar with mutated *dhfr* frequency ranging from 71-92% and 39-71% frequency for the double mutant *dhps* respectively (Chanda et al, 2007). Data from southern Zambia showed that by 2006, the prevalence of *dhfr* and *dhps* mutants had escalated rapidly since 1988, and that the quintuple (*dhfr* triple + *dhps* double) mutant associated with highest levels of SP clinical failure was starting to set in (Mkulama et al., 2008). Studies done in Tanzania showed an emergence of the presence of the *dhps* triple mutant and suggested that IPTp may not improve overall pregnancy outcome (Gesase et al, 2009). Furthermore, conflicting results have been found in HIV positive pregnant women with some studies showing increased prevalence of *dhfr* and *dhps* mutations while still other studies have found that SP IPTp is effective (Ayisi et al 2004, Perrault et al, 2009). A study done by CDC in HIV negative pregnant women in Mansa district, Zambia showed that IPTp is still effective as shown by the failure rate of 22% despite the presence of 63% quintuple mutant resistance molecular markers. However, further highly powered studies need to be done to further establish the efficacy of SP use in IPTp (Tan et al, 2014).

1.5 Epidemiology of Malaria in Zambia

Malaria is endemic in Zambia and transmission is stable (moderate to high) in most districts with a seasonal peak associated with rains from November to April. There are epidemiological variations in malaria prevalence countrywide with an increasing malaria transmission gradient from south-west to north-east regions of the country. The

main parasite species is *Plasmodium falciparum* accounting for over 95% infections. *Anopheles gambiae* and *Anopheles funestis* are the main vectors (NMCC report, 2011).
Malaria remains one of the leading causes of morbidity and mortality in Zambia. It affects over 4 million Zambians and is responsible for over 40% hospital admissions with an estimated 8000 deaths each year. Similar to other countries, children and over 200,000 pregnant women are at most risk of infection annually. Currently, malaria accounts for 35-50% under 5 children mortality and an estimated 20% maternal mortality (UNICEF, 2010).

The country has, however, made great progress in malaria control and prevention.
Increased intervention coverage coupled with significantly strengthened capacity and infrastructure to plan and manage rapid malaria control have resulted in tremendous declines (>66% reduction) in malaria and anemia prevalence since 2002. Although the entire country is endemic for malaria with moderate to high transmission in all districts, the National population estimates have shown changes overtime in the malaria parasite prevalence across the country. Hence, the country has been stratified according to malaria epidemiological settings into three categories; the first category includes all areas where malaria control has markedly reduced transmission and parasite prevalence to <1% (Lusaka), the second category comprises areas where parasite prevalence is at under 10% (Central ,Western, North-western, Copper belt and Southern provinces) while category three (3) includes all areas where malaria control has been attained but not sustained leading to resurgence of infection and illness with parasite prevalence in young children exceeding 20% in the peak of the transmission season (NMCC report, 2011).

1.6 Statement of the Problem

The burden of Malaria in pregnant women in Zambia is unacceptably high. According to UNICEF (2010 report) over 20% maternal mortality in Zambia is due to malaria. This is despite current reported scale-ups in the implementation of prevention and control strategies. This is a foreseeable hindrance to the attainment of MDG 6 which is to halt and begin to reverse the incidence of malaria and other major diseases by 2015.

Reasons why some areas continue to experience increased malaria prevalence is unclear and suggested reasons are that malaria prevention and control strategies are not properly implemented and particularly IPTp may not be effective. There are many possible reasons why IPTp may not work: Lack of adherence, non-compliance, sub-optimal absorption (vomiting, diarrhea), sub-optimal regimen (dose, schedule), co infection with HIV and drug resistance (WHO report, 2010).

Therefore, it is of major research interest that increased prevalence of malaria in some parts of the country has been reported particularly in pregnant women despite the interventions which include Intermittent Preventive Treatment in pregnancy (IPTp) (MOH, HMIS, 2010).

1.7 Study rationale

There is an urgent need to examine the reasons for the high prevalence of malaria in pregnancy by assessing the interventions and investigating drug effectiveness. Drug resistance in pregnant women taking sulphadoxine-pyrimethamine has severe implications as it affects both maternal and child health. One tool that can be used to assess drug resistance is the use of molecular markers that confer resistance to SP. Although underutilized, this is potentially a very useful tool which can be used in research and surveillance. Regular surveillance, constant monitoring and evaluation of drug efficacy and effectiveness are needed to avoid re-emergence and increased disease burden.

This study sought to provide adequate and comparable data on malaria prevalence SP resistance markers taking into account the risk factors. The information obtained from this study can improve knowledge on epidemiology of malaria in pregnancy and potentially provide guidance on the current SP IPTp policy. The risk factors investigated may guide prioritization of interventions in the two areas so as to obtain desired results. The study is in line with millennium development goals 4, 5 and 6 which are to reduce child mortality, improve maternal health and combat HIV/AIDS, malaria and other diseases.

2.0 CHAPTER TWO: AIMS AND OBJECTIVES

2.1 Research Question

What is the prevalence of malaria, Sulphadoxine-Pyrimethamine (SP) resistance molecular markers and associated risk factors in pregnant women of Nchelenge and Choma districts?

2.2 General Objective

To determine and compare the prevalence of malaria, characterize SP molecular resistance markers and examine the associated risk factors in pregnant women of Nchelenge and Choma.

2.3 Specific Objectives

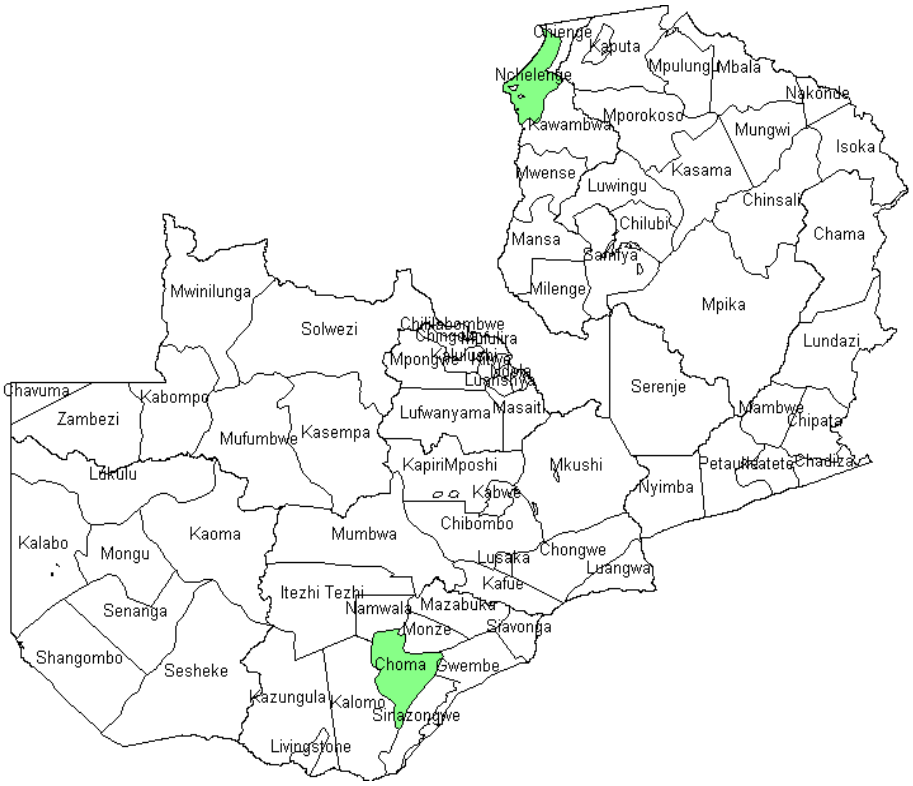
i) To determine prevalence of malaria in pregnant women in Nchelenge and Choma
ii) To characterize SP resistance in pregnant women found with malaria
iii) To examine factors that may be associated with malaria in pregnancy

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study setting and Population

The study was done in Nchelenge and Choma districts. The two districts were selected conveniently on the basis of epidemiological settings with Choma showing drastic reduction in prevalence of malaria <10% while Nchelenge district showed an increase in malaria prevalence >20% despite reportedly similar malaria control and prevention strategies undertaken since 2002 (NMCC report, 2012).

Fig 1: Map of Zambia highlighting the districts Choma and Nchelenge



Nchelenge district

Nchelenge is one of the seven districts in Luapula province, north of Zambia. It lies between -9° (latitude) and 28° (longitude) with an elevation of 919 m above sea level. As of 2010 the population in Nchelenge stood at 174, 000 (CSO, 2010). The district has a total surface area of 4,793 square kilometers of which 60% is island, 10 % swamps

and 30% is water. The district shares borders with Chienge district in the north, Kaputa in the north-east, Kawambwa district in the South east and Congo DR in the west. The Lake Mweru marks the boundary between Nchelenge and Congo DR. The inhabitants of Nchelenge are mostly fishermen and peasant farmers. The district has one first level referral hospital St Paul's Mission hospital, ten rural health centers and three health posts. Malaria prevalence in Nchelenge stood at 44.7% of all Out Patients Department cases in the RHCs as of July, 2012 and 80% of deaths recorded were attributed to malaria with the most affected being children under 5 and pregnant women (Nchelenge DMO report, 2012).

Choma district

The other study site was Choma, the provincial capital of Southern Province. Most of the district lies at 1400 metres above sea level. The study was conducted in Macha which is 80km from Choma town. The resident population is estimated at 200,000 and comprises mainly subsistent farmers of the Batonga tribe. The area has two referral hospitals- Choma district and Macha Mission hospital. The area experiences meso endemic malaria although recent evidence suggests possible transition to hypo endemic status following scale-up of malaria control and introduction of Artemisinin based drugs. The prevalence of malaria was estimated to be below 10% in the general population.

Study Population

The target population was all pregnant women in the reproductive age and resident within the catchment areas of Nchelenge and Choma districts.

Pregnant women who were resident and attended ante natal care at the health facility selected for the study were eligible to be included in the study. Parental consent and Child assent was obtained for those below 18 years old. Non pregnant women, pregnant women not resident in the study sites or those within the catchment area but had health complications or did not give consent were not included in the study.

3.2 Research Design and Sampling

This was a comparative cross sectional study. Multi stage sampling was employed. The districts were conveniently selected while Simple Random sampling using Microsoft excel was used to select the rural health centers that were included in the study. The rural health centers (RHC) selected were specifically those within the catchment area of St Paul's Mission Hospital in Nchelenge and Macha Mission hospital in Choma. In Nchelenge district the rural health centers selected were Kabuta RHC, Nchelenge and Kashikishi RHC, while in Choma district; Macha, Mang'unza, Lupata and Nalituba RHC were selected. Consenting individuals were included in a census survey from the rural health centers. Hence, all pregnant women who came to the center regardless of either for revisits or first attendance and were eligible were included in the study.

The sample size was calculated using the formula:

$$n = \frac{Z^2 P (1-P)}{D^2}$$

N=sample size Z=statistic for 95% 1.96 P=expected prevalence D=0.05 desired level of precision.

The prevalence of malaria in pregnant women was unknown in both areas so we assumed 50% as the estimated prevalence for the general population in Nchelenge and 10% for Choma. Hence, the calculated Total sample size was (Choma 138 and Nchelenge 384) 522 plus 10% of the total is 574 individuals as total sample size at 95% significance.

3.3 Data Collection

Facility Survey data

The data collection tool used in this study was a structured questionnaire (appendix 4). This tool was used to document demographic data, basic malaria knowledge, health seeking behavior, access to health care (distance from health facility), ITN ownership and usage, ITN condition, Social economic status and anemia as possible risk factors of malaria. The ITN condition was categorized into poor, good, very good, fair and very

poor according to the number of holes in the net and description by the participant. The participants were interviewed face to face in a private room. This enhanced collection of data quality and reduced information biases.

The data selection tool (questionnaire) was modified from the Malaria Indicator survey (2010) questionnaire. To validate the data collection tool, pre-testing of the questionnaire was done twofold: First in Lusaka, the tool was pre-tested to ensure clarity of questions in English. Secondly, the questionnaire was pre-tested in Choma and Nchelenge districts to ensure consistency, precision, clarity of questions and correct translations in both Tonga and Bemba. Necessary adjustments and revisions were made based on the pre-test results. Some questions were redundant and had to be dropped while an option of "Other" was included to minimize bias. In addition the pre-test was used to determine the average time it would take to interview a participant. Hence a modified and reliable questionnaire was obtained at the end of the pre-test of the questionnaire.

Laboratory Methods

The eligible pregnant women were screened for malaria and anaemia on the scheduled ante-natal care day. Approximately 100 microlitres of venous blood was collected from each participant by a single finger prick. A rapid diagnostic test and Microscopy was used to test for Malaria. The Rapid diagnostic test used detects the antigen histidine rich protein II (HRP II) found on the parasite surface, the test kits brand was the Standard Diagnostics test (SD-p.f™) manufactured by Standard diagnostics Inc., Korea. The test results were ready within 15 minutes after the test. Thick blood smears were collected on microscope slides. The slides were dried, packed and transported for reading in the laboratory.

Anaemia was measured using a hemocue (digital instrument) and defined using the WHO standard definition as hemoglobin less than 8 g/dl (Hb<8 g/dl) for severe anemia and Hb 8-11.5g/dl as moderate anemia, mild anemia Hb; 11.6-12.5g/dl and normal Hb was that greater than 12.5g/dl.

Dried blood spots were collected on Whatman 3mm filter paper. The dried blood spots (DBS) were packed in sealable bags, labeled with an Identification number and date and stored at room temperature. *Plasmodium falciparum* DNA was extracted from the DBS by a chelex protocol and amplified by polymerase chain reaction (PCR). Molecular techniques employed were those described by Duraisingh et al, 1998. Markers for SP resistance investigated were specifically mutations at *dhfr* 108, 59, 51 and 16 and *dhps* codon 436, 437, 540 and 581. Durasingh et al, (1998) developed a technique of nested PCR where the region of interest is amplified by one set of primary PCR primers followed by nested PCR with two sets of primers and then restriction digestion. The primers used and regions of interest amplified are as shown below:

Table1: Primer sequences used for detection of polymorphisms in *dhfr* genes

dhfr Primers	PCR Nest I	Nucleotide no.	
MI	5' TTTATGATGGAACAAGTCTGC 3'	-3	…..18
M5	5' AGTATATACATCGCTAACAGA 3'	625	….645
	PCR Nest II		
M3	5' TTTATGATGGAACAAGTCTGCGACGTT 3'	-3	…..24
F/	5' AAATTCTTGATAAACAACGGAACGGAACCTttTA 3'	491	...519
F	5' GAAATGTAATTCCCTAGATATGgAATATT 3'	144	…172
M4	5' TTAATTTCCCAAGTAAAACTATTAGAgCTTC 3'	439	….469

Restriction digestion was done with enzymes BStN1, Alu1 and Bsr1, NlaIII, XmnI, MnlI, MwoI, HhaI, HindIII, MspAI, and FokI, Tsp509I and AvaII at the required temperatures. The number of mutations determined the genotype category whether they were sensitive or ‘wild type’, ‘single mutation’ ‘double mutation’ or ‘mixed mutation’.

Quality assurance was done at Tulane University, School of Public health and Tropical medicine in the Kumar Laboratory. Positive samples were randomly selected and PCR was done followed by restriction digestion with enzymes BStNI, AluI and BSrI. The results were similar to those initially obtained hence results were reproducible and reliable.

3.4 Data Analysis

Data Analysis for this study was done as follows:

All data collected was pre-coded, entered into Excel, exported and analysed using Stata version 12.2. Variables were defined, descriptive statistics; means, medians standard deviations and odds ratios were used to assess the data. Chi square was used to determine the association between malaria prevalence and each of the associated factors, similarly this was done for ITN ownership. Significance (p-value) was set at 5% with a confidence interval at 95%. Multivariate analysis using logistic regression was done for adjustments, to control for confounding and to examine the odds ratios in relation to each of the risk factors.

A composite variable analysis was done to determine malaria prevalence from the Microscopy, RDT and PCR results, this was to have one variable for analysis and PCR was used as the gold standard.

Hence, multivariate analysis was done as follows:

Dependent variable: Malaria prevalence

Independent variables: age, education, knowledge, access to health care (distance from health facility), ITN ownership, ITN usage, ITN condition, Anemia and social economic status

Further Multivariate analysis was done with ITN ownership as a dependent variable to determine and compare the Adjusted odds ratios of the risk factors in the two sites. The Social economic status was categorized as poor, moderately poor and not poor based on a combination of assets owned by the household of the study participant.

3.5 Ethical Approval

Protocol approval was sought from the University of Zambia, School of Medicine Postgraduate committee and obtained in November, 2012. The Protocol was submitted for ethical approval to The University of Zambia Biomedical Research Ethics Committee (UNZABREC) and approved in February, 2013 (IRB004-11-12). Permission to carry out the study in the districts was granted by the Ministry of health. Good Clinical Practices (GCP) guidelines were used in the data and sample collection procedures. Only consenting and assenting participants were included in the study. Parental consent was obtained for those below 18 years old.

4.0 CHAPTER FOUR: RESULTS

4.1 Overall Demographic Characteristics

A Total number of 520 participants were enrolled in this study with a response rate of 95%. The mean age of the participants was 25, mode was 20 and median was 23 years old. The Overall demographic characteristics were as shown in the table below:

Table 2: General characteristics of study participants from Choma and Nchelenge

General Characteristics	Sample Characteristic	Choma (n=145)	Nchelenge (n=375)
Age(years)	14-18	16.55%	14.40%
	19-24	33.79%	39.20%
	25-30	21.38%	21.60%
	31-34	13.79%	13.87%
	>35	14.48%	10.93%
Marital Status	Single	38.62%	9.09%
	Marrried	57.93%	84.76%
	Divorced/Seperated	1.38%	4.28%
Gravidity	Primigravidae	26.90%	24.00%
	Secundigravidae	13.10%	21.87%
	Multigravidae	60.00%	54.13 %
Doses of SP	None	14.58%	14.88%
	One	40.28%	31.13%
	Two	23.61%	14.88%
	Three	21.52%	39.12%

4.2 Malaria Infection

Malaria infection was tested using the rapid diagnostic test (RDT), microscopy and polymerase chain reaction (PCR).

Nchelenge

In Nchelenge district the prevalence of malaria by rapid diagnostic test was 29%, by microscopy prevalence was 14% and 22% by PCR. It is important to note that the rapid diagnostic test is an antigen based test and may remain positive two weeks after treatment hence may result in false positives. A composite variable analysis was done to have one variable as 'malaria prevalence' and this was found to be 22%.

Choma

The prevalence of malaria by all tests: rapid diagnostic test, microscopy and PCR was 0% in Choma district.

4.3 Resistance to Sulphadoxine Pyrimethamine

The PCR positives (n=83) of these 72 were genotyped using restriction fragment length polymorphism (RFLP) for the presence of mutations at the different loci : in the *dhfr* gene specifically at 108, 59, 51 and 16 loci and *dhps* 436, 437, 540 and 581. Mixed infections were those that showed both sensitive and resistant parasite strains while sensitive strains were parasites that showed no polymorphisms in the region of interest after restriction digestion.

The figures 2(a) and 2(b) below show the results of mutations obtained at each loci:

Fig 2(a): *Plasmodium falciparum* DHFR mutations (n=72) in pregnant women of Nchelenge district, Zambia, 2013.

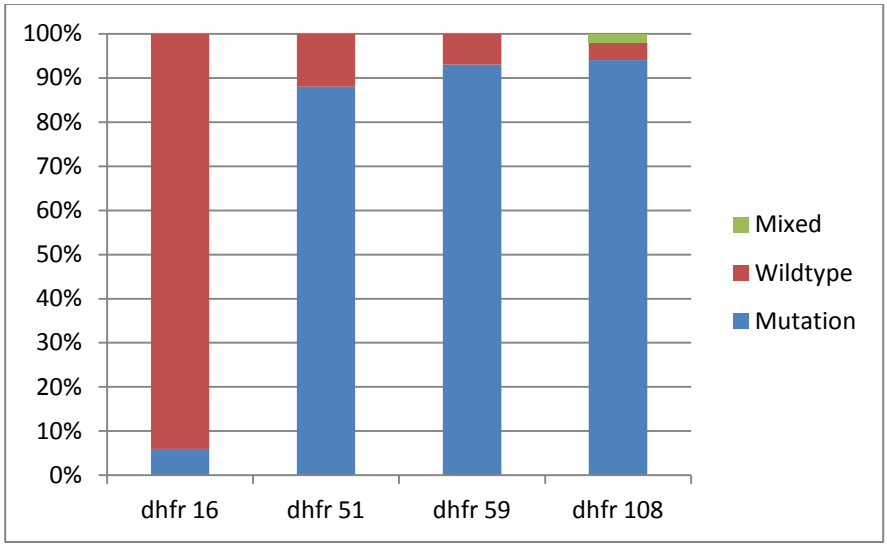
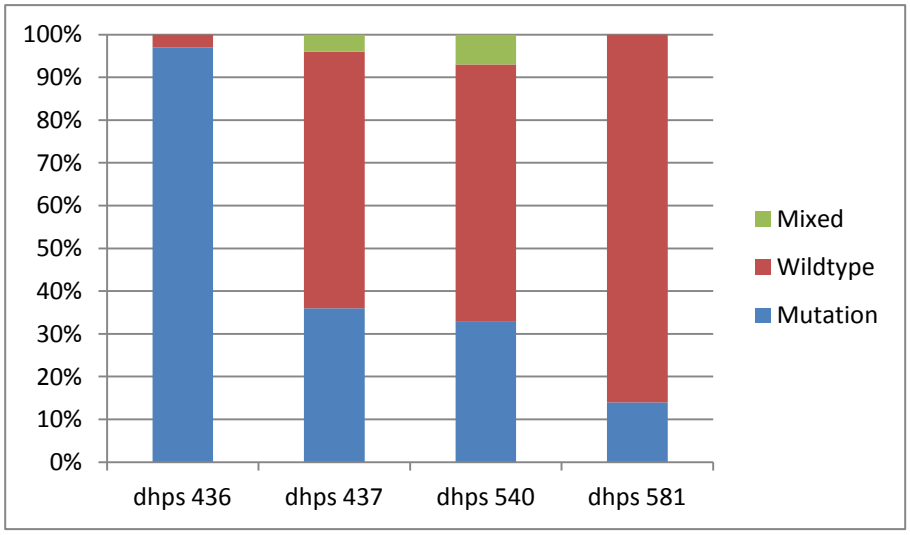


Fig 2(b): *Plasmodium falciparum* DHFR mutations (n=72) in pregnant women of Nchelenge district, Zambia, 2013.



The figure shows that at most of the parasites loci were either wild type (sensitive) or mutated at most loci.

Factors associated with Malaria prevalence

The table below shows results obtained using Chi-square test. Significant results ($p<0.05$) were obtained for the medication taken in the last malaria infection, SP doses taken and Malaria symptom knowledge, as shown in the table below:

Table 3: Factors associated with Malaria prevalence in pregnant women in Nchelenge district, Zambia, 2013.

		Malaria Prevalence				
Factor		**Negative**		**Positive**		**P - value (Chi2)**
		n	**%**	**n**	**%**	
Anaemia						
Severe anaemia(HB <8g/dl)		5	1.75	2	2.41	0.067
Moderate anaemia(8-11.5g/dl)		144	50.35	55	66.27	
Mild anaemia(11.5-12g/dl)		52	18.18	10	12.05	
Normal Hb		85	29.72	16	19.28	
Malaria History						
Ever had Malaria:	No	15	5.24	7	8.43	0.353
	Yes	271	94.41	76	90.36	
Medication Taken in last Malaria infection						
	Fansidar	62	21.68	26	31.33	0.004
	Chloroquine	0	0	1	1.2	
	Coartem	144	50.35	27	32.53	
	Quinine	25	8.79	9	10.84	
	Other	28	9.79	16	19.28	
	None	25	8.74	4	4.81	
SP Doses taken						
	None	62	21.79	27	33.33	0.033
One or More doses		223	78.21	56	66.67	
Malaria Symptom Knowledge						
Headache:						
	No	241	84.27	61	73.49	0.025
	Yes	45	15.73	22	26.51	
Body weakness:						
	No	252	87.76	64	77.11	0.016
	Yes	34	12.24	19	22.89	
Sweating:						
	No	272	95.45	83	100	0.048
	Yes	14	4.55	0	0.00	

Predictors of malaria prevalence adjusted odds ratios

The table below shows results obtained after multivariate analysis.

Table 4: Predictors of Malaria Prevalence-Adjusted odds ratios

Risk Factor	Proportions (%)	Adj. OR(95% CI)
Age (years)		
14-18	20.48	Reference
19-24	46.99	0.94 (0.47 - 1.90)
25-29	15.66	0.47 (0.21 - 1.10)
30-34	8.43	0.36 (0.13- 0.99)
35-40	7.23	0.41 (0.14 - 1.20)
>40	1.2	4.27 (0.21 - 86.7)
ITN ownership		
No	25.30	Reference
Yes	74.70	0.32 (0.04 -2.70)
ITN Use		
Never	25.30	Reference
Sometimes	7.23	3.40 (0.42 - 27.9)
Most times	12.05	3.20 (0.42 - 24.3)
All the time	55.42	2.80 (0.42 - 18.5)
ITN condition		
None	24.10	Reference
Poor	7.23	1.01 (0.08 - 12.3)
Good	16.87	0.66 (0.07 - 6.60)
Very good	22.89	0.53 (0.05 - 5.40)
Fair	14.46	0.94 (0.09 - 9.70)
Very poor	14.46	1.12 (0.11 - 11.6)
Accessibility		
<5km	92.77	Reference
5-10km	6.02	0.49 (0.18 - 1.30)
>10km	1.20	0.18 (0.02 - 1.50)
Level of Education		
None	10.84	Reference
Primary	56.63	0.61 (0.25 - 1.50)
Secondary	32.53	0.77 (0.30 - 1.99)
Malaria knowledge		
Symptom knowledge-Fever		
No	27.71	Reference
Yes	72.29	1.10 (0.62 – 2.0)
Cause of Malaria		
No	78.31	Reference
Yes	21.69	1.20 (0.59 - 2.40)

Table 5 (a): Predictors of ITN ownership- Adjusted odds ratios-Choma 2013.

Risk Factor		Proportions	*Adj. OR (95% CI)	
Age				
	14-18	16.18		Reference
	19-24	33.82	1.7	(0.09-31.4)
	25-29	20.59	0.91	(0.05-17.5)
	>30	29.41	0.55	(0.12-24.0)
Level of Education				
	None	2.94		Reference
	Primary	57.35	16.4	(0.25-1059)
	Secondary	39.71	57.4	(0.63-5249)
Symptom Knowledge				
	No	38.97		Reference
	Yes	61.03	0.39	(0.03-5.20)
Anaemia				
	Severe anaemia (<8g/dl)	2.21		Reference
	Moderate anaemia (8-11.5g/dl)	55.15	59.9	(0.82-4208)
	Mild Anaemia (11.6-12.5g/dl)	25	27.4	(0.27-2737)
	Normal Hb (>12.5g/dl)	17.65	32	(0.29-3585)
Months Pregnant				
	3months or less	0.75		Reference
	3-6 months	30.83	0.24	(0.00-14.1)
	>7 months	68.42	0.037	(0.00-3.18)

*Adj.OR (95%CI) = Adjusted odds ratio at 95% Confidence interval

The table shows that there was no association between ITN ownership and any of the risk factors in Choma district. Proportion of women with Primary education was 57.35% and Secondary education was 39.71% respectively.

Table 5 (b): Predictors of ITN ownership in Nchelenge,2013- Adjusted odds ratios

Risk Factor	Proportions	*Adj. OR	(95%CI)
Age			
14-18	12.75		Reference
19-24	39.6	1.69	(0.80-3.65)
25-29	22.15	1.88	(0.85-4.19)
30-34	14.43	1.94	(0.80-4.71)
35-40	10.74	1.18	(0.48- 2.91)
>40	0.34	0.73	(0.05-10.0)
ITN Use			
Never	2.36		Reference
Sometimes	7.07	0.71	(0.13-3.80)
Most times	16.84	2.13	(0.43-10.6)
All the time	73.74	1.17	(0.26-5.15)
Level of Education			
None	11.07		Reference
Primary	58.39	0.25	(0.08-0.81)
Secondary	26.51	0.19	(0.06-0.65)
Tertiary	4.03	0.48	(0.08-2.87)
Symptom Knowledge			
No	30.87		Reference
Yes	69.13	1.35	(0.82-2.22)
Anaemia			
Severe anaemia (<8g/dl)	1.68		Reference
Moderate anaemia (8-11.5g/dl)	50.67	1.64	(0.27-10.1)
Mild Anaemia (11.6-12.5g/dl)	17.45	1.23	(0.19-7.90)
Normal Hb (>12.5g/dl)	30.2	4.02	(0.62-25.8)
Not poor			
No	94.97		Reference
Yes	5.03	0.94	(0.34-2.61)
Months Pregnant			
3 months or less	16.8		Reference
3-6 months	42	0.053	(0.01-0.41)
>7 months	41.2	0.021	(0.00-0.17)

The table shows that there was an association between ITN ownership and level of education Primary (OR=0.25), Secondary (OR=0.19) and number of months pregnant 3 to 6 months (OR=0.053) and >7months (OR=0.021) in Nchelenge district.

5.0 CHAPTER FIVE: DISCUSSION AND CONCLUSION

This study showed that the prevalence of malaria in pregnant women is high in Nchelenge district and none in Choma. The Zero prevalence of malaria in Choma may be attributed to the concerted efforts in implementation of preventive measures such as the high distribution of insecticide treated nets, the consistent surveillance including the active and passive case detection carried out in the area by Macha research trust in collaboration with Ministry of health in Choma district for over 20 years. Data showing the general trends of malaria in children at Macha Mission hospital Paediatric ward since the 1990s shows that the introduction of artemisinin combination therapy and changes in climate (droughts) experienced in the area which led to the reduction in number of vectors may have contributed to the reduced malaria prevalence in the area (Thuma P,2013, Unpublished data). Nchelenge on the other hand has experienced failure of malaria control; despite control efforts, the malaria prevalence has continued to increase. Reasons for this are unclear, although reports of poor implementation of interventions such as the use of ITNs for fishing by the local population (Ministry of Agriculture, fisheries report 2011) and poor social economic factors may contribute to this failure.

It was evident from this study that the prevalence of SP *dhfr* and *dhps* mutations was high suggesting a positive association between malaria prevalence and resistance. This is consistent with other studies that have shown that in holo endemic areas with high rate of transmission as is the case for Nchelenge, resistant parasites have a tendency to increase and contribute to treatment failure (Chanda et al, 2007). This finding can be explained by the fact that SP has been in use for over two decades and due to drug pressure parasite resistance is expected to be high as is evident from trends analysis in populations were SP has been used for even shorter periods of time (Mkulama et al, 2008). However, a study done in Mansa district on the efficacy of SP in IPTp showed that SP may still be effective despite the high prevalence of mutations. The treatment failure recorded was 22%, although this was not a highly powered study the results suggest that the use of SP in IPTp may still be a viable option (Tan et al, 2014).

The analysis using chi-square suggested an association between malaria prevalence and medications taken in last malaria infection, the majority of women who were negative for malaria had taken coartem while those that had malaria in this study had taken SP in the last malaria infection. It is also important to note that >66% of women who were found with malaria had taken at least one dose of SP suggesting possible treatment failure. However, after adjustments with multivariate analysis there was no association between prevalence and any of the associated risk factors except age hence the association found with chi square may be due to confounding. A multivariate analysis on predictors of malaria prevalence suggests an association between malaria prevalence and women aged 30-34 years compared to those women aged 15-19 years old. One reason for this is that women in this age group have likely had previous pregnancies (multigravidae) compared to those in the reference age group 15-19 years resulting in a protective effect, this is in line with other literature which has shown that malaria prevalence is higher in primigravidae than mutigravidae and strongly associated with anaemia (Shulman et al, 1996, Akanbi et al, 2009).

Further analysis to determine differences in the two sites was done using ITN ownership. The results showed that there was no association between ITN ownership and any of the risk factors in Choma. In Nchelenge, the results obtained suggested an association between ITN ownership and level of education with the odds higher in those that have attained primary education compared to those with secondary education. The results also showed that women who were in the third trimester were less likely to own an ITN than those in the second or first trimester. Generally, women in Nchelenge were less likely to own a net than those in Choma, this association is supportive of studies that have shown that ITNs reduce mortality and morbidity due to malaria and may have long term community benefits (Maxwell et al, 2002).

The comparative aspects of this study were limited by the Zero (0%) prevalence of malaria in one site. Possible non-participation bias may have occurred as this study was based exclusively at the health centers hereby excluding those pregnant women who do not attend antenatal and who may have a different profile from those that do, there is no

way of knowing. Another limitation of this study was that clinical treatment failure was not assessed as no follow ups were made to determine efficacy of SP.

The findings on anaemia (proportions) showed that women in Nchelenge were less likely to have severe and moderate anaemia than those in Choma despite the fact that none of the women in Choma had malaria. This suggests that there are other underlying causes of anaemia other than malaria in this population. In addition, though the results were not significant the multivariate analysis showed that the women in Nchelenge were poorer compared to those in Choma which is consistent with what has been found that Luapula province is one of the poorest provinces in Zambia (CSO, 2012). The social economic status may partially explain the high prevalence of malaria in this district as infectious diseases generally thrive in poverty stricken conditions (Worral et al 2005, Yusuf et al, 2010). In terms of education, it was found that Nchelenge women were less likely to have a primary and secondary education but more likely to have college education compared to Choma. However, women who had been to college were less than 5% of the population. Although, Nchelenge seemed to have a significantly higher college attainment it had significantly less women who have attained a primary or secondary education. A study done in Ghana showed that formal levels of education may not affect ones knowledge about malaria and also that one may have knowledge about the disease but this may not result in actions towards prevention and control (Appiah-Darkwah et al, 2011). There were more women who were in the late stages of pregnancy in Nchelenge compared to those in Choma district, this maybe that there were more re-visits than first antenatal visit attendants in Nchelenge than in Choma, but from observation women in Nchelenge had their first antenatal visit much later in the pregnancy. Hence, late attendance of ante-natal care deemed as poor health seeking behavior maybe a contributing factor to the results obtained.

The results obtained in this study clearly show the differences in the distribution of malaria associated factors, this cannot however, be generalized or assumed to be the reason for the resulting differences in the prevalence in the two sites.

Conclusion

In sharp contrast to Choma, we found a high burden of malaria in pregnant women in Nchelenge. This high prevalence of Malaria in pregnant women of Nchelenge district despite IPTp with Sulphadoxine-Pyrimethamine may be due to the high parasite resistance to the drug as was evident in this study. However, the reasons for the differential prevalence states in the two districts is unclear and beyond the scope of this study. It is suggested that differences in environmental settings, as well as local responses to available interventions in the two sites maybe a major contributing factor. This may further indicate historical limitations in efforts to prevent and control malaria in Nchelenge, calling for a need to learn from the Choma site and the need to carry out further investigations in Nchelenge so as to obtain more epidemiologically desired results.

6.0 CHAPTER SIX: RECOMMENDATIONS

The findings in this research have research and policy implications. The following are the recommendations in this regard:

a) Research implications are that more studies need to be undertaken to further examine the reasons for the high prevalence of malaria in pregnant women in Nchelenge so as to reduce the burden of disease. In addition there is need to conduct efficacy studies to examine the efficacy of sulphadoxine-pyrimethamine
b) It was evident from this study that the prevalence of malaria is high despite IPTp hence the need to explore alternative avenues for drugs to be used in IPTp if it is to be beneficial to this population. This would pave way for the revision of the current policy to use SP in IPTp.
c) Finally, a multi-sectoral approach is recommended for the containment of this disease including all the line ministries such as The Ministry of Commerce and Agriculture, to improve economic conditions. The Ministry of Health and The Ministry of Community development mother and child health and all stakeholders to ultimately improve access to interventions and improved service delivery
d) Furthermore, there is need for constant surveillance even in areas such as Choma were the prevalence of malaria has drastically reduced so as to prevent resurgence of disease. Hence, in areas of low transmission active case detection is necessary to further control and prevent malaria and sustain current progress made towards the elimination of this disease.

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APPENDIX 2: INFORMATION SHEET

My name is Mwiche Siame, a Master of Science-Epidemiology student from The University of Zambia, School Of Medicine. I am undertaking a study: **Prevalence of malaria and resistance to fansidar in pregnant women in Nchelenge and Choma districts**. I am working with a team comprising two nurses, two lab technicians and one data entry clerk. We want to know the prevalence of malaria in pregnant women in this area and if current interventions to prevent malaria in pregnancy are working.

Purpose of the study

This study will examine the reasons for malaria in pregnancy despite the interventions. Also to improve knowledge on malaria in pregnancy and potentially provide information to policy makers on the subject and recommend how interventions can be prioritized.

Procedures

If you agree to take part in this study, you will be asked a few questions and a nurse will take a small amount of blood from your finger. We will ask you questions about your household, education, general malaria knowledge, bed net use and your health. This should only take 20minutes.

We will take about 6 drops of blood. This will be for malaria testing using the rapid test and microscope slide and testing for low levels of blood (anemia).The remaining blood will be put on a paper for further analysis of malaria. The results for low levels of blood and anemia will be given to you within 15minutes of taking the test. There is no charge to you and your family for this. The rest of the results will be ready after 2 weeks.

Risks and Benefits

You will feel a pinch of pain when pricked which will last only a few seconds as we take the blood tests. For any malaria or anemia found the nurse will give you treatment according to The Ministry of health guidelines.

Voluntariness

It is your choice to be in this study. If you agree to take part your answers will be confidential and kept private to the extent that the law allows. If you decide not to take part it will not affect the nurse will give you should you wish to receive it. Even, when you agree to take part in the study you can decide to not answer some questions, it is up to you. If you have any questions or concerns ask the study team members or contact the Principal Investigator - Ms.

Mwiche Siame- P.O Box 50110 UNZA or UNZABREC , tel.260-1-256067 P.O Box 50110 Ridgeway campus, Lusaka.

Thank you for your time.

APPENDIX 3: CONSENT FORM

You are invited to participate in this study. If you agree to take part, your answers will be confidential and kept private. You can decide at any time if you wish to discontinue your participation and you can decide to not answer some questions, it is up to you. If you decide not to participate in the study it will not in any way affect the care you get from the nurse at the clinic as you wish to receive it. It is your choice to be or not to be in this study.

If you have any questions or need clarifications please feel free to ask any of the study team members. You may also contact Ms. Mwiche Siame, Study Principal Investigator, The University of Zambia, Lusaka. Tel: 0976-506616.

Would you like to participate in this study?

Consent from Participant

By signing my name below, I confirm the following:

- The study purpose, procedure ,risks and benefits have been clearly explained to me
- All my questions have been answered to my satisfaction
- I voluntarily agree to participate in this research study. I agree to follow the study procedure as directed and have been told I can stop at any time.

Participant's Name /Thumb print

Participant's Signature

Date……

Person obtaining consent-Name

Person obtaining Signature

Date:……..

Witness Name……Signature……

Date …….

APPENDIX 4: QUESTIONNAIRE

A comparative cross-sectional study to determine Prevalence of *Plasmodium falciparum* malaria and Resistance molecular markers to Fansidar in Pregnant women in Choma and Nchelenge district, Zambia

Introduction and Consent

Informed consent

Hello. My name is _____and I'm working on this study as part of the team from The University of Zambia. I'm speaking with you because we are working on a study to know the prevalence of malaria in pregnant women in this area and the risk factors, so we can recommend how to prevent additional and future illness in the community. I would like to ask you some questions about your health, education, use of mosquitoe nets and general malaria knowledge that will help us in this work. We will greatly appreciate your participation in this study. No one except the study team will know that it was you who provided the answers. Do you have any questions?

Participation in this study is voluntary and you can choose not to answer any individual question or questions.

Are you willing to participate? May I begin the interview now?

_____ ___/___/_____

Signature of the interviewer Date

(Tick (√) appropriately, some questions may have more than one answer)

Demographic Characteristics

1. Name/ID: ……

2. Sex	Male		Female	

3. Date of Birth: dd-mm-				year

4. Age		Years

5. Marital status ☐1.Single ☐2. Married ☐3.Widowed ☐4. Divorced/separated

6. Address/village: ……..

7. Town ☐1.Nchelenge ☐ 2.Choma

Education

8. What is the highest level of education you have attained?

☐1.Nil ☐ 2.Primary ☐ 3.Secondary ☐4.Tertiary/College

9. Years spent in school

☐ (1)0-3 ☐ (2) 4-7 ☐ (3) 8-9 ☐ (4) 10-12 ☐ (5) more than 12 years

10. What is the highest level of education attained by spouse/partner?

☐1.Nil ☐2.Primary ☐ 3.Secondary ☐ 4. Tertiary/College

11. Years spent in school by spouse

☐(1) 0-3 ☐ (2) 4-7 ☐(3) 8-9 ☐(4) 10-12 ☐(5) more than 12 years

General Malaria Knowledge

12. Have you ever heard of an illness called malaria? ☐1.Yes ☐ 2. No

13. If yes, what are the general symptoms?

☐1. Diarrhea ☐2. Vomiting and nausea ☐3.headache ☐4.joint pain

☐5. Body weakness

☐ 6. Fever ☐ 7. Feeling cold ☐ 8.Dizziness ☐ 9.Loss of appetite

☐ 10. Other (specify)……. ☐ 11.Sweating

14. In your opinion what causes malaria? ☐1.Eating immature sugarcane

☐2. Eating cold nsima ☐ 3.Mosquitoe bites ☐4.Eating dirty food

☐ 5. Getting soaked in the rain ☐ 6.Witchcraft ☐ 7.Weather changes

☐8. Other……..

15. How can someone protect themselves against malaria?

☐1. Sleep under a mosquito net ☐ 2.Not eat dirty food ☐3. Cut the grass/bushes

Near the house ☐Use repellants ☐ill in puddles ☐pray with insecticide

☐ 7. Other (specify)……..

Medical history

16. Have you been ill with malaria? (*If no move to next section*) ☐ 1. Yes ☐ 2. No

17. If yes, specify symptoms

☐1. Diarrhea☐ 2.vomiting and nausea☐ 3.headache☐4.joint pain ☐ 5.body weakness

☐6. Fever ☐7.Feeling cold ☐ 8.Dizziness ☐9. Loss of appetite

☐10. Other (specify)…… ☐11.Sweating

18. When was the last time you had malaria?

☐ (1) Less than 3months ago ☐(2) 3-6months ago ☐(3) 6-12months ago

☐(4) 1yr -2yrs ago ☐ (5) 2-5yrs ago☐ (6) >5yrs ago

19. Did you receive any treatment at home before reporting to the clinic

☐1. Yes ☐ 2. No

20. If Yes, specify ……..

21. Treatment received at a clinic ☐ 1.Fansidar ☐ 2. Chloroquine ☐ 3.Coartem

☐4. Quinine ☐ 5. Other……

Insecticide Treated Nets

22. Does your household have any mosquito nets? ☐1. Yes ☐ 2.No

23. How many? .....

24. How often do you sleep under a mosquito net?

☐ 1. Never ☐ 2.sometimes ☐ 3.often ☐ 4.every time

25. How would you describe the condition of your mosquito net?

☐ 1. Poor ☐ 2.good ☐ 3.very good ☐ 4.fair ☐ 5.very poor

26. What is the brand of your mosquito net?

☐ 1. Safenite ☐ 2. Permanet ☐ 3.KO net ☐ 4.Other ……

27. How did you obtain the mosquito net? ☐ 1. Purchased ☐ 2. Given

☐ 3. Other……

28.How much was the mosquito net if purchased? ……

29. Where was it obtained from? ☐ 1. GRZ clinic/hospital ☐ 2.Shop

☐ 3. Pharmacy ☐ 4. Workplace ☐ 5. Other ……

Accessibility to health care

30. How far is the health facility from your home?

☐ (1)Less than 1km ☐ (2) 1-3km ☐ (3) 3-5km ☐ (4) 5-10km

☐ (5) 10-15km ☐ (6) 15-20km ☐ (7) More than 20km

31. What means of transport do you use to get to the health facility

☐ 1. Walking ☐ 2. Bicycle ☐ 3.car ☐ 4.taxi ☐ 5.bus ☐ 6.other……

Social Economic Status

32. What is your occupation? ……..

33. Where does your household get its water?

☐ 1. Piped water into dwelling ☐ 2.river ☐ 3. Well ☐ 4.borehole

☐ 5. Stream ☐ 6.Other …….

34. What toilet facilities are available?

☐ 1. Flushing toilet ☐ 2.Pit latrine with slab ☐ 3.Modern Pit latrine

☐ 6. Other……..

35. Do you have ☐ 1.electricity ☐ 2.a TV ☐ 3. A radio ☐ 4.a refrigerator

☐ 5. Mobile phone

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

☐ 1. Electricity ☐ 2.Natural gas ☐ 3.Charcoal ☐ 4.Dung ☐ 5. Firewood

☐ 6. Straw ☐ 7. Other …….

37. Do you have livestock? ☐ 1. Cattle ☐ 2.goats ☐ 3.sheep ☐ 4.chickens

☐ 5. Ducks ☐ 6. Donkeys ☐ 7.None ☐ 8.Other……

38. Does any member of your household own ☐ 1.a bicycle ☐ 2.a motorcycle/scooter

☐ 3. A car ☐ 4.a bus/truck? ☐ 5. none

Reproduction

39. Have you ever given birth? ☐ 1. Yes ☐ 2.No

40. How many children do you have? …….

41. When you were pregnant previously did you see anyone about ante natal care?

☐ 1. Yes ☐ 2. No

42. Whom did you see? ☐ 1. Traditional birth attendant ☐ 2.nurse

☐ 3. Clinical officer ☐ 4. Doctor ☐ 5.Other ……..

43. During the last pregnancy did you take any drugs to prevent you from getting malaria?

☐ 1. Yes ☐ 2. No

44. Which drugs did you take? ☐ 1. Chloroquine ☐ 2. Fansidar ☐ 3. Coartem

☐ 4. Quinine ☐ 5.Other……..

45. How many times did you take SP/Fansidar during this pregnancy? (Check antenatal care form)…….

46. How many months pregnant are you? ……..

47. How many times have you taken fansidar during this pregnancy?

☐ 1. Once ☐ 2. Twice ☐ 3.three times ☐ 4.Other ……..

48. Which other antimalarials have you taken during this pregnancy?

☐ 1. Chloroquine ☐ 2.Fansida ☐ 3.Coartem ☐ 4.Quinine ☐ 5.Other……

Laboratory results (To be filled in by Lab technician)

Malaria result Rapid Diagnostic Test (RDT): ……

Microscopy: ……..

PCR: ……..

Anemia Hb ……