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INVESTIGATING HUMAN (HOST) FACTORS ASSOCIATED WITH SUSCEPTIBILITY AND RESISTANCE TO *PLASMODIUM FALCIPARUM* INFECTION IN RUFUNSA DISTRICT: A CASE-CONTROL STUDY

BY

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A research dissertation submitted to the University of Lusaka in partial fulfilment of the requirements of a Master's Degree in Epidemiology and Biostatistics

DECLARATION

I, KELVIN CHIBWALWE, hereby declare that this dissertation is my original work and has not been submitted for any academic award at any institution other than to University of Lusaka for the award of Master's degree in Epidemiology and Biostatistics. All sources used have been properly acknowledged.

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Signature:

Date: 20th January, 2026

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Date: 20th January, 2026

DEDICATION

This work is dedicated to God Almighty, my beloved family, friends, and the Healthcare workers in Rufunsa district, whose strength and support and strength inspired me to undertake this research.

ACKNOWLEDGEMENT

I extend my heartfelt gratitude to God Almighty for the gift of life, strength, wisdom and resources that enabled me to complete this research. My supervisor, Dr Ray Handema for his guidance and support. My wife **Hellen** for her unwavering support, my children **Kelvin**, **Faith** and **Gracious** for their understanding. The district Health Director for Rufunsa, fellow laboratory personnel in the district and facility In-charges in areas where this study was conducted for their support. I also want to thank the study participants, healthcare workers, and staff at Rufunsa District Hospital for their invaluable contributions. Special thanks to my colleagues and friends who supported me throughout this journey.

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ABBREVIATIONS

RDT: Rapid Diagnostic Tool

Pf: Plasmodium Falciparum

Pv: Plasmodium Vivax

Pm: Plasmodium Malariae

Po: Plasmodium Ovale

RBC: Red Blood Cells

G6PD: Glucose-6-phosphate dehydrogenase

HbA: Heamoglobin A

HbS: Haemoglobin S

HbC: Haemoglobin C

RH: Rhesus Factor

PfRh5:

BSG: Basigin Receptor

ABSTRACT

Background: Malaria is a significant public health burden in Zambia, with Rufunsa District being particularly affected. Understanding host factors influencing susceptibility and resistance to Plasmodium falciparum malaria is crucial for developing effective prevention and treatment strategies.

Methods: A total of 319 participants were included in the study. Malaria testing was done using first response Malaria RDT brands. Blood Grouping (ABO and Rh Typing) and Sickle SCAN test kit were used to screen for genetic traits. Host factors were collected using a structured questionnaire. Descriptive statistics and Logistic regression analysis were performed using Stata version 14.

Results: The study found that male participants were significantly more likely to test positive for malaria compared to females (aOR = 7.26; 95% CI: 2.77-19.03; p = 0.001). Malaria risk varied significantly by health facility, with participants from Chimusanya, Kanyongoloka, and Mpanshya having substantially higher odds of malaria. Prior malaria infection (aOR = 8.49; 95% CI: 3.07-23.50; p = 0.001) and inconsistent mosquito net use (aOR = 5.00; 95% CI: 1.46-17.09; p = 0.010) were also significantly associated with malaria susceptibility.

Conclusion: The study highlights the need for strengthening malaria prevention strategies, including promoting consistent use of insecticide-treated nets and improving coverage of indoor residual spraying, particularly in high-transmission areas.

CHAPTER ONE: INTRODUCTION

1.1. Background

Malaria is caused by a parasite called Plasmodium. The parasite infects and destroys the person's red blood cells. There are five malaria plasmodium species which infect humans and include *Plasmodium Falciparum*, *Plasmodium Vivax*, *Plasmodium Malariae (PM)*, *Plasmodium Ovale (PO)* and *Plasmodium Knowlesi (PK)* but only *Plasmodium Falciparum* (PF) is considered dangerous and responsible for most severe malaria cases resulting in deaths in most African countries Zambia inclusive. *Plasmodium Falciparum* can cause severe malaria which may result into death. Malaria infection is spread from person to person through the bites of an infected female Anopheles mosquitoes which usually bite at night with peak hours being between 22:00 to 02:00hrs.

The malaria life cycle involves a female Anopheles mosquito transmiting sporozoites to a human during a bite, and in a human, sporozoites multiply in the liver, then merozoites invade red blood cells and reproduce, leading to illness. Some parasites develop into gametocytes, which are then ingested by the mosquito to start the sexual cycle, producing sporozoites that are transmitted to a new human. Therefore, an infected female Anopheles mosquito injects sporozoites into the human during a blood meal. The Sporozoites then travel to the liver and infect liver cells, where they mature into schizonts. The schizonts rupture, releasing thousands of merozoites, which then invade red blood cells. Inside the red blood cells, merozoites multiply, causing the cells to burst and release more merozoites, hence the fever. Some parasites differentiate into male (microgametocytes) and female (macrogametocytes) gametocytes. Female anopheles' mosquito bites a malaria-infected person and sucks blood containing the plasmodium parasites. The parasite multiplies (grows and increases) inside the mosquito, and after 10-14 days, the parasites mature into sporozoites. As the infected mosquito bites a healthy person, the malaria parasites enter the bloodstream and travel to the liver where the parasites again multiply and mature into schizont and merozoites in the liver for 10-14 days, then released into the bloodstream. In the bloodstream, the merozoites infect and destroy the red blood cells (RBC, responsible for carrying oxygen from the lungs to the tissues) and make an individual feel sick. In the case of *Plasmodium Vivax*, the parasite can remain dormant (asleep) in the liver, reactivate later and flow in the bloodstream, which often results in relapse or repeat attacks of malaria. Malaria parasite can also be passed from a pregnant mother to the foetus (unborn baby) through the placenta which can result in stillbirth or death of the baby while still in the mother's womb. Malaria parasites can also be passed to another person through blood transfusion.

There are a number of factors that influence malaria infection and the course of the disease. Basigin (BSG) also known as extracellular matrix metalloproteinase inducer (EMMPRIN) or cluster of differentiation 147 (CD147) is a protein that in humans is encoded by the *BSG* gene and plays a critical role in malaria infection. This protein other than being a determinant for the Ok blood group system has also been shown to be an essential receptor on red blood cells for the human malaria parasite, *Plasmodium falciparum*. G6PD deficiency protects against malaria in that lack of functional G6PD enzyme creates a hostile, oxidative environment within red blood cells that is detrimental to the malaria parasite's survival and replication. This environment makes it harder for the parasite to invade, grow, and mature, while damaged G6PD-deficient red blood cells are also more likely to be cleared by the immune system, reducing parasite development. Beta thalassemia puts affected individuals at a lower risk of *P. falciparum* infection due to the persistence of hemoglobin F (fetal hemoglobin) which isn't as easily broken down by the malaria parasite, making these cells somewhat resistant to infection. Sickle cell trait which is the most common genetic defense against malaria in individuals with one abnormal allele of the ß-globin gene. It causes some RBCs to take on an abnormal sickle shape, making the environment hostile for malaria parasite survival hence reducing susceptibility to *P. falciparum* infection. Also hemoglobin AC affects the shape and structure of RBCs, protecting against *P. falciparum* infection. Hereditary ovalocytosis creates elliptically shaped RBCs which interferes with the parasite's ability to adhere to, invade, and grow within the cells. Red blood cells with abnormalities: sickle cell, hemoglobin AC, hereditary ovalocytosis.

Despite the availability of this information regarding the role of the host genetic diversity of ABO blood, Glucose-6-phosphate dehydrogenase (G6PD) deficiency, Sickle Cell traits and Basigin receptor in the Plasmodium Falciparum malaria pathogenesis, no study has been carried out in Zambia where malaria is endemic to affirm to their role in predisposing or protecting individuals from *Plasmodium falciparum* malaria.

1.2. Statement of the problem

Malaria is the commonest cause of morbidity and major cause of mortality mostly in children and pregnant women globally, in Africa and in Zambia. In Zambia, high Malaria incidences are recorded mostly during the rain seasons from October to March every year caused mostly by *Plasmodium falciparum.* Different interventions such as indoor residue spraying and distribution of insecticide treated nets (ITNs) have been implemented extensively at least every year. With these interventions coupled with test and treat, it is anticipated that malaria cases would reduce and also that every individual in a household and community where these interventions have been implemented would have equal chances of not getting Malaria.

Surprisingly, despite these interventions being cross-cutting, there seems to be geographical areas, communities, families and/or individuals who are more prone to malaria than others. Malaria seems to affect certain individuals more frequently than others in certain households and communities despite all of them having equal exposure in the presence of common preventive and control strategies including indoor residual spraying and use of insecticide treated bed nets. Some individuals have never suffered from Malaria; others have had only one episode of confirmed malaria while others have had several episodes of confirmed malaria in their lifetime. With these reported experiences, it is evident enough that there could be other factors playing a role in the susceptibility and resistance to falciparum malaria. These factors include human host social, biological and genetic factors.

Lastly, despite the availability of this information regarding the role of the genetic diversity of ABO blood, Glucose-6-phosphate dehydrogenase (G6PD) deficiency, Sickle Cell diseases and Basigin receptor in the Plasmodium Falciparum malaria pathogenesis, no study has been carried out in the populations of Malaria endemic areas in Rufunsa district to affirm to their role in protecting or predisposing individuals from or to malaria causing agents.

1.3 Justification of the Study

Malaria remains a significant public health burden globally, particularly in tropical and subtropical regions. Despite similar exposure to biting by the female parasite carrying Anopheles mosquitoes, individuals exhibit varying degrees of susceptibility and resistance to the disease. Understanding host factors that influence susceptibility and resistance is crucial for developing effective prevention and treatment strategies. Malaria is very prevalent in Zambia and particularly in Rufunsa and if there is delay in seeking medical services it can lead to death particularly in children and pregnant women. Different prevention and control methods have been used but malaria cases are still high.

There are individuals who are more susceptible to malaria than others, therefore understanding their biological and genetic make-up would not only help in appreciating factors associated with susceptibility to malaria but also design appropriate prevention and control interventions for malaria towards elimination

Identifying host genetic factors that contribute to malaria susceptibility and resistance holds the promise of enhancing the diagnosis and treatment of malaria. Such knowledge has the potential to add to the existing body of knowledge as well as pave way for more targeted and effective strategies in elimination of malaria.

This study aims to contribute to the understanding of host factors influencing susceptibility and resistance to *Plasmodium falciparum* malaria, informing the development of targeted interventions and personalized treatment strategies to reduce malaria morbidity and mortality

1.4 Main Objective

To investigate the human host factors associated with susceptibility to and resistance against *Plasmodium falciparum* infection among individuals residing in malaria endemic areas of Rufunsa district.

1.5 Specific Objectives

1.5.1 To identify genetic polymorphisms associated with increased susceptibility or resistance to *Plasmodium falciparum* malaria among cases and controls

1.5.2 To examine the use of mosquito nets in modulating host susceptibility and resistance to *Plasmodium falciparum* infection.

1.5.3 To explore potential interactions between genetic and socioeconomic factors in influencing malaria susceptibility and resistance.

1.6 Research Questions or Hypothesis

1.6.1 What is the frequency of specific genetic polymorphisms (e.g., HbS, HbE, and G6PD) among cases (individuals with confirmed *Plasmodium falciparum* infection) and controls (individuals without malaria)?

1.6.2 Are socioeconomic status levels associated with increased susceptibility or resistance to *Plasmodium falciparum* infection?

1.6.3 Do interactions between genetic and socioeconomic factors influence *Plasmodium falciparum* infection susceptibility and resistance?

1.7 Scope of the study

The scope of this study is to investigate human host factors associated with susceptibility and resistance to Plasmodium falciparum malaria in Rufunsa District, Zambia. The study will focus on identifying demographic, behavioral, and genetic factors that contribute to the risk of *Plasmodium falciparum* infection.

1.7.1 Specific Areas of Focus:

1.7.1.1 Demographic factors: age, sex, occupation

1.7.1.2 Malaria history and prevention practices: history of malaria, mosquito net use, indoor residual spraying (IRS)

1.7.1.3 Genetic factors: blood group, Rhesus factor, sickle cell trait

1.7.2 Study Boundaries:

1.7.2.1 Geographical: Rufunsa District, Zambia

1.7.2.2 Population: Residents of Rufunsa District, age ≥ 1 year

1.7.3 Timeframe: 3 months

The study will provide insights into the human host factors associated with Plasmodium falciparum malaria, informing targeted interventions for malaria control

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The purpose of this section is to review the previous work and findings of researchers on the influence of basigin receptor (CD147), G6PD, sickle cell diseases and ABO blood types in susceptibility and resistance to *Plasmodium falciparum* infection. This is important to provide a foundation of knowledge on a topic, establish the context for new work, identify gaps in current work and justify the need for further study.

In this subsequent section therefore, we review previous research findings associated to Basigin receptor and its influence on the *Plasmodium falciparum* invasion. Muramatsu T. (2015) defines Basigin receptor as a multifunctional cell surface receptor protein, also known as EMMPRIN or CD147, found on red blood cells and other cells. It acts as a receptor for several ligands, including the malaria parasite *Plasmodium falciparum* reticulocyte binding-like homogue 5 (PfRh5) protein. Henrietta *et al* (2015) analyzed the erythrocyte invasion mechanisms of *Plasmodium falciparum* in clinical isolates of blood samples collected from children aged 2–14 years across the three malaria endemic areas of Ghana. Findings were that expression of the Basigin ligand PfRh5 was the best predictor of host malaria parasitaemia.

In another study by Luyi *et al* (2018) blood samples were collected to detect the expression of OK/BSG using enzyme-linked immunosorbent erythrocyte binding assay to measure the recombinant PfRh5 (rPfRh5) binding of RBCs with different OK/BSG expressions. The findings also were that the OK/BSG expression levels varied among blood donors and were strongly associated with rPfRh5 binding. The study by Crosnier (2011) focused on identifying a receptor–ligand pair that is essential for erythrocyte invasion in all tested *Plasmodium falciparum* strains by systematically screening a library of erythrocyte proteins. The findings were that the Ok blood group antigen also known as Basigin, a receptor for PfRh5, is a parasite ligand that is essential for blood stage *Plasmodium falciparum* attachment and growth. Izumi *et al* (2014) investigated the role of six single nucleotide polymorphisms (SNPs) in the *BSG* gene and association between *BSG* SNPs and cerebral malaria in 312 adult patients with *Plasmodium falciparum* of which 109 had cerebral malaria and 203 mild malaria patients living in northwest Thailand. With the allele and haplotype frequencies compared in cerebral and mild malaria patients, the findings were that common *BSG* single nucleotide polymorphisms and haplotypes were not significantly associated with cerebral malaria.

In this section, we now review previous research findings associated to G6PD deficiency and its influence on *Plasmodium falciparum* invasion. Richardson S.R (2022) refers to Glucose-6-phosphate dehydrogenase (G6PD) deficiency as an X-linked genetic disorder that results in impaired enzyme activity in red blood cells. Also reported that the growth of *Plasmodium falciparum* is impaired in glucose-6-phosphate dehydrogenase (G6PD) deficient red blood cells (RBCs) and *Plasmodium falciparum* was implicated in the spreading of deficient variants of G6PD in malaria endemic areas. The study by Kotepui *et al* (2016) in the analysis of prevalence and hematological indicators of G6PD deficiency in malaria- infected patients, venous blood samples were collected at the time of admission to hospital to determine G6PD deficiency by fluorescence spot test and detect malaria parasites by thick and thin film examination. The findings showed that among the patients with a G6PD deficiency, only 11.8 % were infected with *Plasmodium falciparum*, while the remaining were infected with *Plasmodium vivax*. Also found that malaria patients with a G6PD deficiency have high monocyte counts.

In the systematic review by Chibunna *et al* (2017) on association of glucose-6-phosphate dehydrogenase deficiency and malaria, results showed absence of negative association between G6PD deficiency and uncomplicated *Plasmodium falciparum* malaria and that this negative association happened in Africa, in the heterozygotes but not the homo/hemizygous individuals. The study also found no association between G6PD deficiency and severe malaria disease as well as with other plasmodium species, hence concluded that G6PD deficiency can potentially protect against uncomplicated malaria but not severe malaria.

Amoah *et al* (2016) studied the prevalence of G6PD deficiency and *Plasmodium falciparum* parasites in asymptomatic school children living in southern Ghana. Blood samples were obtained once a month from 170 healthy Ghanaian school children aged between five and twelve years from basic schools in two communities Obom and Abura with similar rainfall patterns and malaria peak seasons. G6PD enzyme activity was assessed using the qualitative G6PD RDT kit (Access BIO). The findings included that 10.6 % of the children were G6PD deficient while the asymptomatic carriage of *Plasmodium falciparum* by PCR was 39.1% on average hence the conclusion that G6PD deficiency was significantly associated with a lower risk of PCR estimated asymptomatic *Plasmodium falciparum* carriage in children during the off peak malaria season in Southern Ghana.

Tsegaye *et al* (2014) investigated the presence of Glucose-6-phosphate dehydrogenase deficiency among malaria suspected individuals attending Gambella hospital in southwest Ethiopia. Venous blood samples were collected from febrile patients (n = 449) attending Gambella hospital in November and December 2013. Malaria was diagnosed using blood films and G6PD deficiency screened using CareStart™ G6PDd screening test (Access Bio, New Jersey, USA) and found that 90.9% of the G6PD deficient individuals had malaria suggesting the non-protective role of the disorder at least from malaria. Amoah *et al* (2021) conducted a cross-sectional survey of G6PD deficiency genotypic variants amongst suspected malaria patients attending health care facilities across the entire country. Malaria was diagnosed using microscopy whilst G6PD deficiency was determined using restriction fragment length polymorphisms at position 376 and 202 of the G6PD gene. It was found that G6PD deficient genotypic variants, (A-A-, AA- and A-) were protective against *Plasmodium falciparum*, *Plasmodium ovale and Plasmodium malariae* infections in Ghana. Research on the association between Rhesus factor and malaria susceptibility is limited. However, a study in Ghana found that Rhesus negative individuals were more likely to be infected with malaria than Rhesus positive individuals (Afoakwa et al., 2015).

In this section now, we review previous research findings associated with sickle cell anaemia and its influence on *Plasmodium falciparum* invasion. According to National Institutes of Health (2024) defines Sickle cell anemia as a group of inherited disorders known as sickle cell disease. It affects the shape of red blood cells, which carry oxygen to all parts of the body. According to the study by Sophie *et al* (2022) in the analysis of Sickle cell anaemia (SCA) and severe *Plasmodium falciparum* malaria, the finding was that children with SCA are innately protected against classic severe malaria. However, it also showed that even low level infections can precipitate severe anaemic crises that was fatal without rapid access to blood transfusion services.

According to the review by Morse *et al* (2021) findings indicated that people with sickle cell traits (SCT) are not as affected by malaria compared to those with normal hemoglobin. During infection, those with SCT have 50 to 90 percent fewer parasites in their blood than people with normal hemoglobin. People with SCT also get rid of the parasites faster compared to those with normal hemoglobin and with malaria. Also found that having sickle cell trait provides malarial protection than having sickle cell anemia (HbSS). A similar study of children in Kenya between 16 months and 2 years old showed that those with HbSS had the lowest chance of surviving from malaria while kids with SCT had the highest chance of survival. Survival rates for those with normal hemoglobin were between those with sickle cell trait and HbSS.

Sickle cell trait has been shown to provide protection against severe malaria (Williams et al., 2005). A study in Kenya found that individuals with sickle cell trait were less likely to be infected with malaria than those without the trait (Ndungu et al., 2015).

Ngou *et al.* (2023) studied the influence of sickle cell trait on *Plasmodium falciparum* infectivity from naturally infected gametocyte carriers among asymptomatic children living in the locality of Mfou, Cameroon. Blood samples were collected from asymptomatic children to perform malaria diagnosis by microscopy. *Plasmodium* species were differentiated by PCR and hemoglobin typing by RFLP. The study concluded that *Plasmodium falciparum* transmission stages were more prevalent in individuals with SCT. This may reflect the parasite's enhanced investment in the sexual stage to increase their survival rate when asexual replication is impeded.

Finally, in this section, we review previous research findings associated to ABO blood types and its influence on *Plasmodium falciparum* invasion. Abraham *et al* (2018) conducted a systematic review and meta-analysis on the effect of the ABO blood group on susceptibility to severe malaria. A total number of 1923 articles were retrieved from five databases and showed an increased odds of severe *Plasmodium falciparum* infection among individuals with blood group type A, B, AB or simply non O blood groups compared to blood group O. However, the difference in the level of *Plasmodium falciparum* parasitaemia was not significant among individuals with blood group A or non-O compared to blood group O. The difference in hemoglobin level among *Plasmodium falciparum* infected individuals was also not significant between those with blood group A, B or AB *versus* those with blood group O. Further studies have found associations between blood group and malaria susceptibility, with individuals with blood group O being more susceptible to infection (Cserti & Dzik, 2007). A study in Nigeria found that individuals with blood group A were more likely to be infected with malaria than those with blood group O (Okoroiwu et al., 2015).

Opi *et al* (2023) conducted a case control study of the association between *Plasmodium falciparum* rosetting and severe malaria in Kenyan children. Findings were that ABO genotype influences *Plasmodium falciparum* rosetting hence supporting the hypothesis that double dose non-*O* genotypes confer a greater risk of severe malaria than *AO/BO* heterozygosity. Onanuga and Lamikanra (2016) conducted a study to determine the association of ABO blood group and *Plasmodium falciparum* malaria among children in the Federal Capital Territory of Nigeria. Blood specimens from deep finger pricks were collected from 730 children aged between 0-2 years and examined for malaria parasites using field stains method. ABO and Rhesus blood group antigens tests were performed using standard tile protocols. Findings indicated that there was no association between malaria infection and ABO blood groups but that the prevalence of malaria parasite density was significantly greater in children with blood group A.

Sirina and Clement (2013) also conducted the study to determine prevalence of malaria parasitaemia and predisposition of ABO blood groups to *Plasmodium falciparum* infection among blood donors at a Ghanaian Hospital. A total of 437 blood samples were examined following best practices with thick and thin blood films made from each sample and ABO blood groups determined by a standard tube agglutination technique. Results showed that the difference in infection percentage between the various blood groups was not statistically significant. Richmond *et al* (2016) further conducted the study to determine relative susceptibilities of ABO blood groups to *Plasmodium falciparum* malaria in Ghana. The findings showed that individuals with complicated malaria were about twice likely to be of blood groups A and B compared to group O. Blood Group O participants with complicated diseases had low parasitaemia compared to the other blood groups. Also concluded that this may give blood group O individuals a survival advantage over the other groups in complicated malaria.
Age and Malaria Susceptibility

Studies have shown that age is a significant factor in malaria susceptibility, with children under 5 years and pregnant women being more vulnerable to infection (WHO, 2020). A study in Kenya found that children aged 1-4 years had a higher risk of malaria infection compared to older children and adults (Ndungu et al., 2015). In contrast, a study in Zambia found that adults aged 15-24 years were more likely to be infected with malaria (Masaninga et al., 2018).

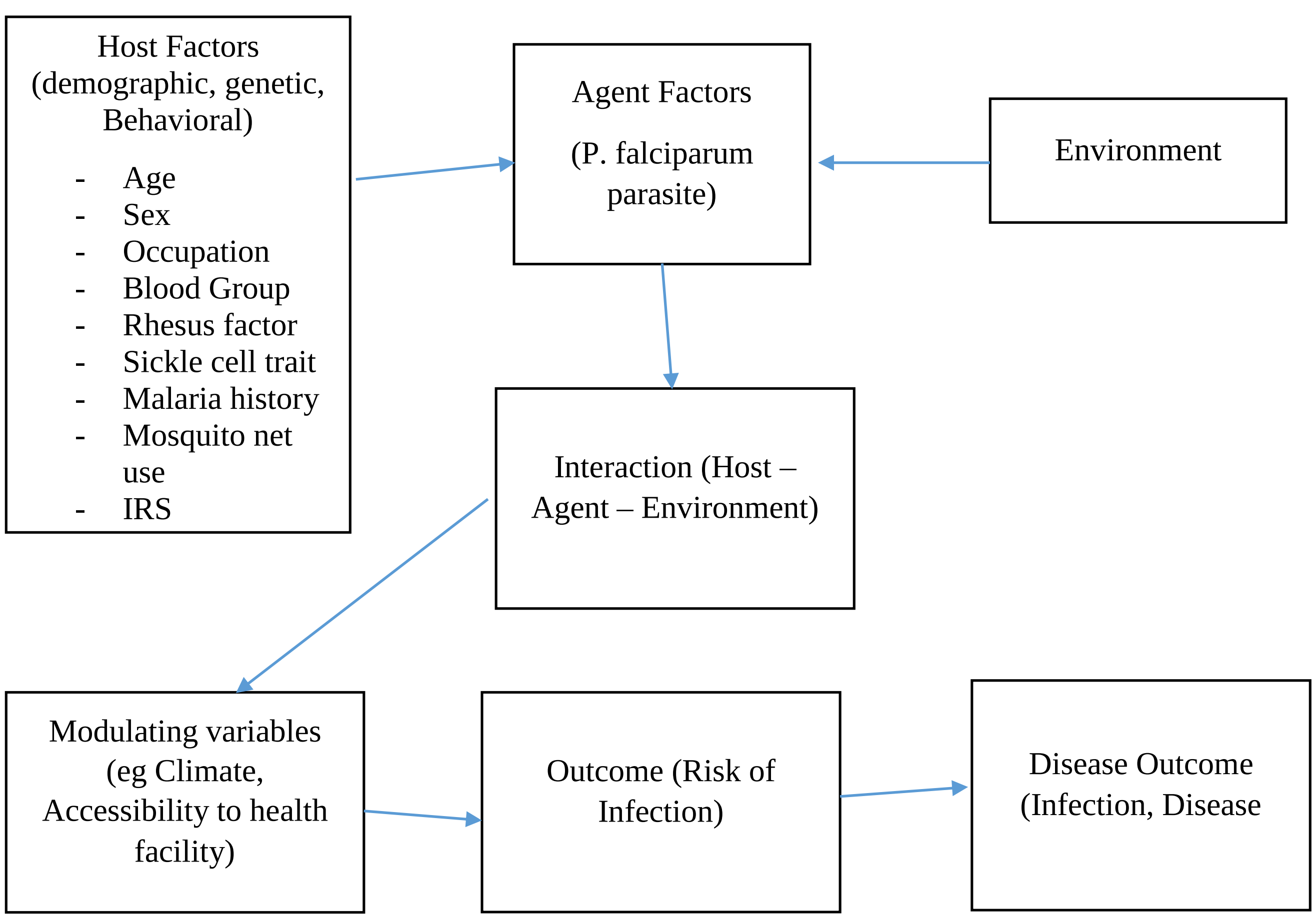
Sex has also been identified as a factor in malaria susceptibility, with males being more likely to be infected than females in some studies (Hossain et al., 2018). A study in Nigeria found that males were more likely to be infected with malaria than females, possibly due to differences in occupational exposure (Okoroiwu et al., 2015). However, other studies have found no significant association between sex and malaria susceptibility (Ndungu et al., 2015).

The use of mosquito nets has been shown to be an effective way to prevent malaria infection (WHO, 2020). A study in Zambia found that households with at least one mosquito net were less likely to have malaria cases (Masaninga et al., 2018). Another study in Kenya found that the use of insecticide-treated nets reduced malaria incidence by 44% (Lindblade et al., 2015).

IRS has been shown to be an effective way to reduce malaria transmission (WHO, 2020). A study in Mozambique found that IRS coverage was associated with a reduced risk of malaria infection (Mabote et al., 2016). Another study in Zambia found that IRS was associated with a 50% reduction in malaria cases (Masaninga et al., 2018).

Therefore, according to this review, it is evident that there are some minimal inconsistences in the findings and that all the reviewed research was not exhaustive as they considered only one host factor hence not addressing confounding. Last and not the least, these types of research have been in particular regions of Africa and it has not been in Zambia in which malaria is endemic.

2.2 Conceptual framework and /or Theoretical framework



CHAPTER THREE: METHODOLOGY

3.1. Study Design

This study was a case-control design, recruiting individuals with confirmed malaria or frequently suffering from malaria as cases and matched to those never been confirmed with malaria or unknown history of confirmed malaria within families within the community and endemic area as controls. Participants would provide blood samples for genetic and immunological analyses after head of household consents to participate in the study and a questionnaire to gather biometrical and socioeconomic data was administered.

3.2. Study Design

The study design was a case-control study

3.3. Study Population

Cases: Individuals with confirmed *Plasmodium falciparum* malaria or frequently suffer from malaria

Controls: Individuals without confirmed malaria infection or an unknown history of suffering from malaria in the life time

3.4. Inclusion Criteria

Age 2-60 years

Residing in malaria-endemic areas of Rufunsa district

3.5. Exclusion Criteria

Pregnant women

Individuals with chronic medical conditions

3.6. Variables

3.6.1. Dependent Variables

i. Malaria status

3.6.2. Independent variables

i. Age
ii. Sex

iii. ITN use
iv. IRS
v. Blood group type
vi. Sickle cell trait status

3.7. Sample size and sampling techniques

Using Cochran's Formula (for large or unknown populations):

$$n = (Z^2 * p * (1-p)) / e^2:$$

Where

n = Sample size

Z = Z-score, representing the desired confidence level (1.96 for 95% confidence).

p = Estimated population proportion at 29% average malaria prevalence in Zambia

e = Margin of error (e.g., 0.04 for 4%).

Substituting values in the formula we get:

$$n = [(1.96^2 \times (0.29 \times (1 - 0.29) / (0.04)^2$$

$$n = [3.8416 \times (0.29 \text{ x } 0.71)] / (0.04)^2$$

$$n = [3.8416 \times 0.2059] / 0.0016$$

$$n = 0.7910 / 0.0016$$

$n \approx 319$ participants per group

3.8. Data Collection

1. Malaria testing

MOH approved malaria testing rapid diagnostic tests was used in the field to test for malaria.

2. Blood Grouping (ABO and Rh Typing)

Blood grouping would identify a participant’s blood type (A, B, AB, or O) and Rh factor (positive or negative). A tube Agglutination Test method was used in which Anti-A, Anti-B, and Anti-D (for Rh) sera was mixed with the patient’s whole blood in a tube individually then centrifuged for five minutes to observe agglutination. This test was done at Mpanshya Mission Hospital Laboratory, Rufunsa district using the collected samples

3. Sickle Cell Testing

Used to diagnose sickle cell disease (SCD) or sickle cell trait (SCT), caused by the HbS mutation. RDTs like SICKLECHECK™, Sickle SCAN® test strip containing antibodies that bind specifically to HbS or other hemoglobin types was used as immunochromatographic assays to detect hemoglobin variants (HbA, HbS, and sometimes HbC) in a small blood sample. Blood is mixed with a buffer and applied to a test device. Results are visible as clear bands on the test device within 5–15 minutes. This test was done at Mpanshya Mission Hospital Laboratory, Rufunsa district using the collected samples.

6. Host socioeconomic status

The Questionnaires would include (socioeconomic status, nutrition, exposure to mosquitoes) Indoor residue spraying (IRS) and usage of mosquitoes treated nets (ITNs) malaria history and treatment outcomes

3.9. Data Analysis

A spreadsheet was generated to include all collected data parameters

i. Descriptive statistics using Stata version 14
ii. Logistic regression analysis (identifying factors associated with susceptibility and resistance)
iii. Two-way or multiway ANOVA FOR interaction analysis between host genetic and socioeconomic factors

3.10. Expected Outcomes

i. Identification of genetic variants associated with susceptibility and resistance.
ii. Characterization of immunological responses distinguishing susceptible and resistant individuals.
iii. Identification of potential biomarkers for predicting individual susceptibility or resistance.

3.11. Dissemination of findings

The results of this study was presented to the University of Lusaka annual research Committee and to other national and international scientific conferences. The results would also be published in the University of Lusaka Journal and in both national and international reputable peer-reviewed scientific journals.

3.12. Ethical Considerations

In this study both cases and control participants was required to consent or have someone consent for them to participate in the study. All participants were pricked on the second or third finger using a sterile lancet to collect about 250 - 500uL of capillary blood. In the process of pricking, the participants would feel a bit of slight pain or a discomfort. To mitigate on the slight pain, the study would use community health workers trained already in malaria rapid testing and residents of that particular community who would just be oriented on the collection of blood in the EDTA microtainer. Prior to the start of the study, authority was gotten from the district health office, health facility and notification made to the village headmen. An announcement would also be made in Churches to sensitize the community.

The benefit of participants in this study was that those found with malaria would be given the standard treatment for malaria as per Ministry of Health malaria treatment guidelines and also was an opportunity for them to know their blood group type, sickle cell and G6DP status after results are shared with each and every participant.

Ethical clearance was obtained from University of Lusaka Ethics Committee and approval and from the National Health Research Authority (NHRA). Permission to conduct this study would also be obtained from the Provincial and District Health Offices as appropriate. Written informed consent would also be obtained from every study participant. To ensure confidentiality participants information, anonymous typing was used and identification numbers of participant was used.

3.13. Personnel

i. Principal Investigator
ii. Research assistants – Trained Community Based Volunteers
iii. Laboratory technicians
iv. Statisticians

CHAPTER FOUR: RESULTS

Participant Characteristics

A total of 319 participants were included in the study. The participants were fairly evenly distributed across age groups, with the largest proportion in the 15-24 years' group (25.08%) and the smallest in the 1-4 years' group (13.48%). Slightly more participants were female (54.23%) than male (45.77%).

Regarding participant classification, the majority were controls (70.85%), while cases constituted 29.2% of the study population. Malaria status, as determined by rapid diagnostic tests (RDT), showed that most participants tested negative (71.20%), while 28.8% were positive.

Table 1. Demographic characteristics of participant

Variable	Category	Frequency(n)	Percent %
Age -group	1-4	43	13.48
	5-14	71	22.26
	15-24	80	25.08
	25-34	65	20.38
	35+	60	18.81
Gender	Male	146	45.77
	Female	173	54.23
Participant Classification	Case	93	29.15
	Control	226	70.85
	Negative	227	71.20
Total		**319**	**100**

Malaria History, Prevention, and Genetic Factors Among Participants

Participants were recruited from multiple areas in Rufunsa district, with the largest numbers from Chifundo and Nyangwena (18.81% each). Malaria in the past 12 months was reported by 52.37% of participants. Most participants (66.46%) owned a mosquito net, with 42.30% using it daily, 26.00% sometimes, and 31.70% never. Indoor residual spraying (IRS) coverage in the past 12 months was 16.00%. Based on RDT results, 28.80% tested positive for malaria, while 71.20% were negative.Regarding genetic characteristics, the most common blood group was O (44.8%), followed by A (27.90%). Most participants were Rhesus positive (96.24%). The majority had normal hemoglobin (HbAA, 93.42%), while 6.58% were carriers (HbAS).

Table 2. Malaria History, Prevention, and Genetic Characteristics of Participants

Variable	**Category**	**Frequency(n)**	**Percent %**
Area	Bunda-Bunda	34	10.66
	Chifundo	60	18.81
	Chimusanya	40	12.54
	Chinyunyu	10	3.13
	Chitemalesa	11	3.45
	Kanyongoloka	25	7.84
	Luangwa bridge health post	4	1.25
	Mpanshya	15	4.70
	Mulamba	20	6.27
	Nyangwena	60	18.81
	Rufunsa	40	12.54
Malaria in 12 Months	no	151	47.63
	yes	166	52.37
Own mosquito net	no	107	33.54
	yes	212	66.46

Use of mosquito net	Daily	135	42.30
	Never	101	31.70
	Sometimes	83	26.00
IRS in Last 12 Months	No	269	84.00
	yes	52	16.00
Malaria status	Positive	92	28.80
	Negative	227	71.20
Blood Group	A	89	27.9
	AB	23	7.2
	B	64	20.1
	0	143	44.8
Rhesus factor	Rh +ve	307	96.24
	Rh -ve	12	3.76
Sickle cell status	HbAA	298	93.42
	HbAS	21	6.58

Factors Associated with Malaria Susceptibility and Resistance

The results indicated that male participants were significantly more likely to test positive for malaria compared to females (aOR = 7.26; 95% CI: 2.77-19.03; p = 0.001). Age groups were not significantly associated with malaria after adjustment, although the 25–34 and 35+ age groups showed lower odds compared to children aged 1-4 years. Malaria risk varied significantly by health facility. Participants from Chimusanya (aOR = 17.74; 95% CI: 3.32-40.84; p = 0.001), Kanyongoloka (aOR = 8.60; 95% CI: 6.97-27.08; p = 0.001), and Mpanshya (aOR = 17.10; 95% CI: 2.70-34.21; p = 0.001) had substantially higher odds of malaria compared to those from Mulamba. Participants from Rufunsa also had increased odds (aOR = 6.60; 95% CI: 1.45-29.92; p = 0.014), while other facilities and Nyangwena did not show statistically significant differences.

A history of malaria in the past 12 months was strongly associated with current malaria infection (aOR = 8.49; 95% CI: 3.07–23.50; p = 0.001). Regarding mosquito net use, occasional users (Sometimes) had higher odds of malaria compared to daily users (aOR = 5.00; 95% CI: 1.46–17.09; p = 0.010), while mere ownership of a net was not statistically significant. IRS coverage in the past 12 months did not show a significant association with malaria.

None of the genetic factors, including blood group, Rhesus factor, or sickle cell trait, were significantly associated with malaria infection, although participants with blood group O showed a borderline increase in risk (aOR = 2.41; 95% CI: 0.90–6.46; p = 0.081).

Table 3. Analysis of Factors Associated with Malaria Susceptibility: Univariable and Multivariable Analysis

PREDICTOR	***cOR***	***aOR***	***P>|Z|***	***[95%Conf. Interval]***
Gender				
Female(reference)				
Male	2.50	7.26	0.001	2.77-19.03
Age group				
1-4 (reference)				
5-14	3.02	1.09	0.902	0.27-4.41
15-24	2.73	0.70	0.656	0.15-3.31
25-34	0.70	0 .26	0.076	0.06-1.17
35+	1.33	0 .30	0.136	0.06-1.46
Area				
Mulamba (reference)				
Chimusanya	3.11	17.73	0.001	3.32-40.84
Kanyongoloka	7 .0	8.6	0.001	6.97-27.08
Mpanshya	11.00	17.1	0.001	2.70-34.21
Nyangwena	0.52	4.17	0.145	0.61-28.40
Rufunsa	2.51	6.60	0.014	1.45-29.92

Other facilities	0.11	0.19	0.121	0.02-1.56
Malaria in Past 12 Months				
No(reference				
yes	6.79	8.49	0.001	3.07-23.50
Own mosquito net				
No (reference)				
yes	1.65	1.97	0.42	0.38-10.15
Mosquito net use				
Daily (reference)				
Never	1.19	0 .89	0.895	0 .16-4.83
Sometimes	3.38	5.00	0.010	1.46-17.09
IRS in Last 12 Months				
No (reference				
yes	0 .24	1.84	0.504	0.31-10.91
Blood group				
Reference(A)				
AB	0.93	0 .49	0.545	0.05-5.02
B	1.10	0.84	0.796	0.22-3.22
O	2.39	2.41	0.081	0.90-6.46
Rhesus factor				
Reference (Rh+ve)				
Rh-ve	0.622	0.69	0.752	0.07-6.81
Sickle cell traits				
HbAA(reference)				
HbAS	0.66	2.14	0.332	0.45-9.98

CHAPTER FIVE: DISCUSSION

The majority of participants (25.08%) are in the 15-24 age group, which is consistent with the demographic trends in many African countries, including Zambia (Central Statistical Office, 2015). The smallest age group is 1-4 years (13.48%), which might indicate that young children are underrepresented in this study or that malaria prevention strategies are targeting this age group effectively (WHO, 2020).

The slightly higher proportion of female participants (54.23%) compared to males (45.77%) is consistent with some studies in sub-Saharan Africa, which suggest that women are more likely to participate in health studies (Okoroiwu et al., 2015). However, this could also reflect the local demographic or cultural factors.

The majority of participants are controls (70.85%), which is expected in a case-control study design (Rothman, 2012). The malaria prevalence (28.8% tested positive) is relatively low, which might indicate effective prevention strategies or a low transmission setting (Masaninga et al., 2018). This study examined the demographic, behavioral, and genetic factors associated with malaria susceptibility among participants in malaria-endemic areas. The findings revealed that male participants had significantly higher odds of malaria infection than females (aOR = 7.26; 95% CI: 2.77-19.03) in contrast with previous studies suggesting that female may have greater exposure, although data on gender is often collected from patients presenting to public health facilities, routine malaria surveillance data is rarely disaggregated by gender. Given prior studies suggesting that *Plasmodium* parasite prevalence may be higher in post-adolescent males compared to females (Okiring et al., 2022).

The highest proportions of participants are from Chifundo (18.81%) and Nyangwena (18.81%), indicating these areas might be key locations for malaria transmission or have high population. About 52.37% of participants reported having malaria in the past 12 months, indicating a relatively high burden of malaria in this population (WHO, 2020). 66.46% of participants own a mosquito net, but only 42.30% use it daily. This suggests a gap in consistent use of mosquito nets, which are a key malaria prevention tool (Lindblade et al., 2015). Only 16% of participants reported IRS in the last 12 months, indicating potentially low coverage of

28.80% of participants tested positive for malaria, consistent with the reported malaria burden (WHO, 2020).

The most common blood group is O (44.8%), followed by A (27.9%). Most participants are Rhesus positive (96.24%). Some studies suggest associations between blood groups and malaria susceptibility, but evidence is mixed (Cserti & Dzik, 2007). 6.58% of participants have the HbAS trait, which is known to provide some protection against severe malaria (Williams et al., 2005).

Health facility was a significant predictor of malaria risk, with participants from Chimusanya, Kanyongoloka, and Mpanshya showing substantially higher odds of infection compared to Mulamba. This finding likely reflects fine-scale geographical heterogeneity in malaria transmission, driven by localized environmental, ecological, and service-related factors rather than individual-level risk alone. Similar evidence from spatial epidemiological studies has shown that malaria transmission often clusters within specific health facility catchment areas, even within the same district, due to variations in vector density, land use, forest proximity, and human movement patterns (Surendra et al., 2020).

A history of malaria in the past 12 months strongly predicted current infection in this study, indicating that prior malaria episodes are more likely a marker of continued exposure or gaps in prevention rather than protective immunity. This finding is consistent with evidence from other malaria-endemic settings, where individuals with a history of clinical malaria have been shown to have a significantly higher prevalence of parasitaemia than those without such history (32.8% vs. 14.1%), and parasitaemic individuals were more likely to report previous malaria compared with parasite-negative participants (18.9% compared to 7.7%) (Peto et al., 2016).

Regarding mosquito net use, participants who reported occasional use (“Sometimes”) had higher malaria risk compared to daily users (aOR = 5.00; 95% CI: 1.46-17.09), highlighting that consistent use of insecticide-treated nets (ITNs) remains crucial for malaria prevention (Badolo et al., 2025). In our study, IRS coverage in the past 12 months was not significantly associated with malaria infection (OR = 1.84, 95% CI: 0.31-10.91), likely due to the low coverage (16%) and potential inconsistencies in application. This is consistent with evidence from systematic reviews showing that IRS is most effective at high coverage levels (≥80%), whereas low coverage often fails to significantly reduce malaria risk (Zhou et al., 2022). These results suggest that the lack of association in our study reflects operational and coverage limitations rather than the inefficacy of IRS. To achieve measurable protection, high coverage with consistent application is critical, particularly in endemic settings.

No significant associations were observed with genetic factors, including blood group, Rhesus factor, or sickle cell trait, although blood group O showed a borderline increase in malaria risk. This is consistent with studies showing that genetic traits such as sickle cell trait may confer some protection, but their effects can be population-specific and may require larger sample sizes to detect genetic abnormalities. Males are significantly more likely to have malaria compared to females (aOR = 7.26, 95% CI: 2.77-19.03, p = 0.001). This is consistent with some studies suggesting males might be more exposed to mosquito bites due to occupational or behavioral factors (Hossain et al., 2018). Area: Compared to Mulamba, participants from Chimusanya (aOR = 17.73), Kanyongoloka (aOR = 8.6), Mpanshya (aOR = 17.1), and Rufunsa (aOR = 6.60) have significantly higher odds of malaria, indicating these areas might be high-transmission zones (Masaninga et al., 2018).

Malaria in Past 12 Months: Participants with a history of malaria in the past 12 months have higher odds of current malaria infection (aOR = 8.49, 95% CI: 3.07-23.50, p = 0.001), suggesting recurrent infections or ongoing exposure (WHO, 2020). Mosquito Net Use: Occasional users (Sometimes) have higher odds of malaria compared to daily users (aOR = 5.00, 95% CI: 1.46-17.09, p = 0.010), highlighting the importance of consistent net use (Lindblade et al., 2015). Blood Group, Rhesus Factor, Sickle Cell Trait: No significant associations with malaria in this study, though some trends were observed (e.g., blood group O, aOR = 2.41, p = 0.081).

Overall, these findings underscore that malaria susceptibility in endemic areas is influenced primarily by behavioral and environmental factors rather than host genetics, emphasizing the continued importance of consistent vector control measures and preventive practices (Patrick et al., 2023).

CHAPTER SIX

CONCLUSION

In this study, male gender, health facility location, prior malaria infection, and inconsistent mosquito net use were identified as key factors associated with malaria susceptibility. Genetic factors, including blood group, Rhesus factor, and sickle cell trait, were not significantly associated with malaria risk in this population. These findings emphasize the need for strengthening malaria prevention strategies, including promoting consistent use of insecticide-treated nets and improving coverage of indoor residual spraying, particularly in high-transmission areas.

LIMITATIONS

The study design limited the ability to infer causality between the identified risk factors and malaria infection. Information on mosquito net use and history of malaria relied on participant recall, which may introduce recall bias. Some genetic traits were rare in the study population (e.g., HbAS, Rh-negative), limiting statistical power to detect associations.

RECOMMENDATIONS

1. The future studies should consider using molecular tests as rapid tests have limitations
2. Future research need to include screening for Basigin receptors and Glucose-6-phosphate dehydrogenase
3. Future research should also consider different socioeconomic indicators

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APPENDICES

ANNEX 1: QUESTIONNAIRE

HOUSEHOLD MALARIA QUESTIONNAIRE FOR ASSESSING SOCIOECONOMIC AND BEHAVIORAL FACTORS TO SUSCEPTIBILITY AND RESISTANCE TO MALARIA

Hello, l am (**name of interviewer**) from **University of Lusaka**. The university is conducting a study to ascertain the host factors associated with susceptibility and resistance to *Plasmodium faciparum* infection in Rufunsa district. As you may be aware that there are certain people who frequently suffer from malaria while others despite being in the same area or travelled to high malaria risk areas never suffer from malaria or have unknown history of suffering from malaria hence the question why. A little of blood was collected after a small prick on the finger. The questionnaire would also be administered to collect the biometrical data. Are you allowing your household members to participate in this study? If yes, please sign below:

Location of Household: ____

Phone number of household head: ________________________________

Sex of household head: ____

Age of household head: ____

Occupation of head of household: _________________________________

Signature of household head: _____________________________________

Date of Survey: ____

Name, Age and Sex of household participants consented for:

1. ____ Sex: _________. Age: ______________

2.____ Sex: _________. Age: ______________

3.____ Sex: _________. Age: ______________

4.____ Sex: _________. Age: ______________

5.____ Sex: _________. Age: ______________

6.____ Sex: _________. Age: ______________

7.____ Sex: _________. Age: ______________

8.____ Sex: _________. Age: ______________

10.____ Sex: _________. Age: ______________

Section A: Household Questions

1. What is the total number of members in this household?

☐ 5 and below ☐ 6 and above

1.1 How many adults (above 15yrs)? __________

1.2 How many children (below 5yrs)? ________

2. Have you or any member of your household suffered from malaria in the last 12 months?

☐ YES ☐ NO

2.1.How many? ______________

3. How many had malaria confirmed at the heath facility? ______________

4. Classification of the household: ☐ High risk household ☐ Low risk Household

4.1 Name of the household member

4.2 Sex

4.3 Age

4.4 Occupation

4.4 Have you suffered from malaria in the last 12 months?

☐ YES ☐ NO

4.5 Was the malaria infection confirmed at the health facility?

☐ YES ☐ NO

4.6 What medication did you take? (List) ______________________________

4.7 Did you visit any high risk malaria areas prior to this confirmed malaria disease or in the last 6 months?

Section B: Household characteristics

5 What is the main source of indoor lighting in the night for this household?

☐ ZESCO ☐ Solar ☐ Others

6 What is the main source of drinking water for members of the household?

7 How far is that water source located?

8 How long does it take to go and get water and come back?

9 What kind of toilet facility do members of your household usually use?

10 Do you share this toilet facility with other households?

11 How many households use this toilet facility?

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

13 Main material of the house floor

14 Main material of the house roof

15 Main material of the house wall

16 How many rooms in this house are used for sleeping?

17 Do you undertake any agricultural activities?

18 Name of livestock kept by this household

Section C: Interventions

19 At any time in the last 12 months, has anyone come into your dwelling to spray the interior walls against mosquitoes?

20 Who sprayed the dwelling?

21 Does your household have any mosquito nets that are used while sleeping?

22 How many mosquito nets does your household have?

23 Does everyone in this household sleep under a treated mosquito net?

24 How many months ago did your household get the mosquito net(s)?

25 Since you got the net, have you washed them before?

26 How many months ago was it last washed?

27 Did anyone sleep under the mosquito nets last night?

28 How many slept or did not sleep under the mosquito net last night?

29 What are the reasons for not sleeping in the mosquito net last night?

End of the Questionnaire and thank you for your participation