PREVALENCE AND FACTORS ASSOCIATED WITH DIFFERENTIAL MALARIA SPECIES INFECTIONS IN CHILDREN UNDER 5 YEARS IN SELECTED ZAMBIAN POPULTIONS

by

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DECLARATION

This dissertation is the original work of **LUNGOWE SITALI.** It has been done in accordance with the guidelines for dissertations for the University of Zambia. It has not been submitted elsewhere for a degree at this or another University.

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The University of Zambia approves this dissertation of Lungowe Sitali in partial fulfillment of the requirements for the award of the degree in Master of Science in Epidemiology.

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ABSTRACT

Introduction

Malaria remains one of the treatable and preventable infectious diseases, and yet still claims more than 660,000 lives every year worldwide. Malaria diagnosis in some health facilities is based on falciparum rapid diagnostic test and hence missed the other species. Furthermore, there is no data on the prevalence of non-falciparum species in Zambia. This study sought to determine the presence of differential malarial plasmodium species and examine associated factors for infection in children in selected communities in Zambia.

Methods

Data stem from the 2012 population based Zambia Malaria Indicator Survey (ZMIS) for Luapula and Northern Provinces of Zambia. The MIS took place between April and May country wide. Background, social, and behavioral information were collected from households. In addition, blood slides and dried blood spots (BDS) were collected from children below 5 years. Slides were stained using giemsa and examined under a microscope. Polymerase Chain Reaction (PCR) was used to analyse the dried blood spots for malaria plasmodia species. Multivariate logistic regression was used to examine factors associated with differential species' infection prevalence in the population

Results

Overall (n=873), prevalence of malaria by PCR was 54.3%, and the prevalence of the individual plasmodium species were *P.falciparum* 53.4%, *P.malariea* 5.0%, *P.ovale* 2.1% and *P.vivax* 0.2%. These species mostly occurred as mixed infections. The prevalence of mixed infections was 5.6%. In terms of their contribution of the species to malaria prevalence, 88% were *P. falciparum*; with 10.6% being mixed infections and 1.4% were non falciparum mono infections. Older children as compared to younger children were more likely have mixed infection AOR 2.8, CI 1.31-5.69, with a beta value of 0.09.

Conclusion

The study reveals significant high burden of malaria infection in the study population. The major factor associated with the burden of non-falciparum and falciparum malaria was the age of the child, which further supports the notion of increased exposure to environmental factors as a child grows. This might call for repackaging of additional site specific prevention and control measures grounded in local matrics. There is need to pay attention to other species but it may not be necessary to change the falciparum only RDTs as the other species mostly occur as mixed infections.

DEDICATION

This dissertation is dedicated to my lovely daughter Divine Thetiwe Njahi Zimba who had to understand that her mum was busy despite being a baby, my Husband Gerald S. Zimba who had been very supportive throughout my studies, My Dad and Mum (Dr. and Mrs. Sitali) who have always been and continue to be my source of inspiration and role models, and to my sisters and brothers Liseli, Mubita, Mwangala, Namukolo and Etambuyu, who have always and continue to encourage me in all my endeavors.

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List of Abbreviations

BCC……..Behavioural Change Communication,

CSAs…….Census Supervisory Areas

CSO……..Central Statistic Officer

DBS……..Dried Blood Spot

DNA……..Deoxyribonucleic Acid

HIV……Human Immuno-deficiency Virus

HMIS……Health Management Information System

GF……Global Funds

G6PD……Glocose-6-Phospate Dehydrogenase

HRP II……..Histidine Rich Protein II

IEC……Information, Education Communication

ITNs……..Insecticide Treated Nets

IRS ……Indoor Residual Spraying

IPTp……Intermittent Preventive Therapy in pregnancy

ACT……...Artemisinin-based combination therapy

NMCC……National Malaria Control Centre

NMCP…… National Malaria Control Programme

NMSP……National Malaria strategic plan

NPV……...Negative predictive value

MDGs…….Millennium Development Goals

OPD……..Out Patient Department

SEAs…….Standard Enumeration Areas

SP……. Sulfadoxine-Pyrimethamine

pLDH……Plasmodium Lactate Dehydrogenase

PCR……. Polymerase Chain Reaction

PPV……..Positive predictive value

RDTs…….Rapid Diagnostic Tests

WHO…….World Health Organisation

ZMIS……Zambia Malaria Indicator Survey

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Malaria is a parasitic infection caused by protozoan called plasmodia; there are five species of the Plasmodium that cause malaria in humans namely *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* (Gerald and Larry, 2000). The species, *P. knowlesi*, is typically found in animals like monkeys and had first been identified as a clinically significant pathogen in humans in 1965 (Chin W, 1965, White 2008). The burden of malaria is still a dilemma worldwide despite the intervention measures in place. Approximately 40% of the world's population lives in areas that have some risk of malaria infection. Every year more than 500 million people are infected, particularly children under 5 years of age and pregnant women who are more susceptible to severe manifestations of the disease (Carter, *et al*., 2002; World Malaria Report, 2010). Ninety percent of these cases are found in Africa, mainly in sub-Saharan Africa. In Zambia, it remains one of the serious public health problems accounting for 2.9 million Out Patient Department (OPD) cases per year in 2009, which reflects about 60% of all diseases seen at Out Patient Departments in health facilities (HMIS, 2009).

The real distribution of differential malaria parasite species worldwide including Zambia is not known. Mueller *et al.* 2007, reported that the global numbers for *P. malariae* and *P.ovale* are not known. The burdens of *P. ovale* and *P. malariae* are even more underrepresented in blood film survey since *P. ovale* can sometimes resemble *P. vivax* except for the rarer oval infected erythrocytes or number of nuclei in schizonts (Mueller *et al.* 2007). In addition, non-falciparum species are often missed on thick blood film during examination.

Differential species

Differential species refers to malaria species other than *P.falciparum*. The non-falciparum species are common in Africa, Zambia included. Species of *P.malariae* are found in Zambia and the prevalence is said to be an anectodal 2%, as there seem to be no proper documentation on the source. A case report by Blossom, *et al.*2005, describes a case of the 23 years old woman who after a trip to Zambia, presented with

a prolonged illness characterized by fevers of up to 38.1^{o}C and fatigue; malaria film and antibody tests were negative, but Polymerase Chain Reaction (PCR) showed *P.vivax.* Although scientific evidence shows that most parts of Africa have individuals with duffy negative antigen, a recent study showed that *P.vivax* was found in individuals who were duffy negative (Mendes *et al.,* 2011). This implies that the probability of finding *P.vivax* in most parts of Africa is there. The 'Duffy antigen' is one of the surface antigens found on Red Blood Cells (RBCs) that *P.vivax* exploits in order for it to enter the cells. It is one of the genetic resistance factors to malaria (Larina *et al*., 2009, Carvalho *et al*., 2009). Non-human plasmodium species have also become common in humans. In Rag-lai.Vietnam, two young children below five years were found with non-falciparum species, in one of them, *P. knowlesi* was identified again one year later, indicating that human exposure to *P. knowlesi* infection was not a rare event. *P.knowlesi* infections in humans were also found in central Vietnam in 2004 and 2006 (Eede *et al.*, 2009).

Mixed infections

Infections with more than one plasmodium species are possible, and are called mixed infections. Mixed malaria species infections are often not recognized or are underestimated. In Asia, past surveys have reported less than 2% of infections as mixed, whereas therapeutic studies in *P. vivax* or *P. falciparum* malaria have reported a high prevalence (30%) of infections with the other malaria species during convalescence, suggesting covert co-infection (Mayfong *et al*, 2004). In Manhica District of Mozambique, *P. malariae* and *P. ovale* occurred almost exclusively in mixed infections in a number of patients (Marques *et al.*, 2005). This situation may be similar to the Zambian scenario, as the geography and climate are similar. Although this has not been documented, technologists and scientists see and report a few cases of mixed infections routinely. These could have an effect on the clinical outcomes of some patients. On the contrary, Marques *et al.,* 2005, suggest that slight differences in environmental characteristics can affect transmission patterns, and may have an effect in the way different human malaria species establish in the human.

It is possible that all the species or at least three can be found in a malaria endemic country. In one study conducted in Mali, four species of malaria parasite were found

in circulation in the north-eastern region of Mali, where *P. falciparum* had a seasonal influence. (Koita, *et al*., 2012). In a southern Vietnam study, a single *Anopheles dirus* mosquito carrying sporozoites of *P. knowlesi, P. falciparum, and P. vivax* was discovered in Khanh Phu (Marchand, *et al.*, 2011). This further supports the evidence that these species can be transferred to a human being. In the same study by Marchand *et al*, 2011, *P. knowlesi* infections in humans were always associated with infections of other Plasmodium species. Mixed infections with *P. falciparum* are frequent with an interesting observation that in the dry season when *P. falciparum* densities decrease, *P. malariae* densities increase (Bousema, *et al.*, 2008, Marchand, *et al.*, 2011).

Species that were thought to be benign are now being seen to cause severe and complicated manifestations such as those associated with *P. falciparum.* Gogia *et al* 2012, highlighted that *P. vivax* malaria, although considered to be a benign entity, can have a severe and complicated course. In India, a case was found where acute pancreatitis was seen in a 17-year-old male who had *P. vivax*, and was believed to be associated *with P. vivax* (Sharma *et al.*, 2012). On the other hand, there is evidence for some cross-species immunity or a protective effect in mixed infections against some clinical outcomes of malaria. These interactions have important clinical and public health implications (Bruce, *et al.*, 2008, Maitland *et al.*, 1996), and such interactions would potentially be critical in endemic countries (Coyway, 2007). An early PCR study in Cote d'Ivoire around 1993 gave preliminary data showing that *P. malariae* infections may reduce the risk of symptomatic *P. falciparum* infection (Black, *et al.*, 1994), but since then very little has been done to follow it up in larger studies or in other African populations (Conway, 2007).

Malaria diagnosis

The laboratory diagnosis of malaria is done using microscopy by examining giemsa stained blood films. Microscopy has been known to be, and remain the 'gold standard', (MOH, Malaria training manual, 2012), while other methods used are Polymerase Chain Reaction (PCR), Rapid Diagnostic Tests (RDTs) and clinical

assessments. However, by 2007, only 19 % of health facilities in Zambia had microscopy available. Of the 19 %, only 17 % were functional (Hamer *et al.*, 2007). This implies that 83% of health facilities depended only on clinical diagnosis. While the situation might have improved slightly as microscopes were procured with the help of Global Funds (GF) and other partners, (NMCC, NMCP strategic plan 2011-2015), there are a few trained personnel to operate the microscopes and hence misdiagnosis is taking place.

In cases where there is no laboratory or RDTs, presumptive treatment based on fevers and other signs and symptoms of malaria is commenced, although this is no longer encouraged because it often contributes to misdiagnosis of the disease and irrational drug use (Amexo, *et al.,* 2004). In a study conducted in Uganda, Källander *et al* 2004, stressed the difficulty in making a presumptive diagnosis of malaria, and highlighted the urgent need for improved diagnostic tools that can be used at community and primary-care level. Furthermore, this is compounded by the presence of uncommon species. Misdiagnosis of the uncommon parasites is even more likely as seen from two studies, by Singh B *et al.*, 2004 and Cox Singh *et al.*, 2008 that showed that microscopic analysis of asexual stages of *P. knowlesi* can lead to misidentification of these parasites as *P. malariae*.

Numerous malaria RDTs have been developed and are extensively available; however, a number of challenges attributed to these products have become apparent (Murray *et al.,* 2008), some of them are to do with sensitivity and specificity. For instance, the recently discovered is the deletion of the Histidine Rich Protein II gene (Koita *et al*., 2012), which is the target for falciparum based RDTs and impacts on the RDTs' sensitivity.

The sensitivity and specificity of the diagnostic methods have been reported in literature; Coleman et al., 2006 reports that the sensitivity and specificity of PCR was 96% and 98% respectively; while Bukirwa, 2011 reported sensitivity of RDTs to be 90%, on the other hand, the sensitivity of microscopy can be as low as 65%, as reported by Relet *et al* 2010. Amexo, *et al.,* 2004 and Murray *et al*., 2008 have reported that the introduction of RDTs for malaria has changed the approach to malaria diagnosis and leads to better management of malaria cases.

Progressive strides have been made in the epidemiologic studies of malaria by increasing the utilization of diagnostic tests, there is need to greatly augment the coverage of sensitive malarial detection. Despite the achievements, a large degree of uncertainty still exists on the annual burden of malarial cases. Accurate, sensitive detection and treatment of asymptomatic reservoirs important to infections transmission are additional components necessary for future control measures (Sullivan, 2010). In addition, accurate and effective malaria diagnosis should involve a rational approach to each patient with suspected malaria employing both signs and symptoms and laboratory-based malaria diagnostic methods (Chipeta *et al.*,2009). Furthermore, diagnostic strategies need to be effective not only in resource-limited areas, where malaria has a substantial burden on society, but also in developed countries, where expertise in the diagnosis of malaria is frequently lacking (Bell *et al* 2006, Reyburn *et al* 2007)

Prevention and control strategies

Although malaria remains a serious public health problem in Zambia, different preventive and control strategies have been employed that have led to the decrease of malaria cases in the country. The control of plasmodium species is the same generally; most control and preventive measure that apply for *P. falciparum* will apply to the other species. Effective management of malaria according to WHO has focused on insecticide treated nets (ITNs), indoor residual spraying (IRS), and intermittent presumptive therapy in pregnancy (IPTp) and artemisinin-based combination therapy (ACT). Prompt and accurate treatment applies to all species. Hence, it is fundamental to improve the services and care for patients infected with malaria so they can access prompt and accurate diagnosis and treatment in order to prevent excess morbidity and mortality while avoiding unnecessary use of anti-malarial agents and minimizing the spread of resistance to anti-malarial drugs (Bell *et al.,* 2006, Reyburn *et al.,* 2007).

1.2 Challenges and gaps

In meeting the Millennium Development Goals (MDGs) on health, target 6.C - which states "to combat HIV/AIDS, malaria and other diseases; Have halted by 2015 and begun to reverse the incidence of malaria and other major diseases" *(*www.un.org/millenniumgoals). Zambia was committed to control malaria at a national level in its 2006–2011 National Malaria Strategic Plan (National Malaria

Strategic Plan 2006- 2011) and the National Malaria Control Action Plan for scaled up impact on malaria whose objective for case management was to have at least 80% of patients receiving prompt and effective diagnosis and treatment within 24hours (NMCAP, 2010).

The prevalence of malaria parasites in Zambian children is 16 % (MIS, 2010), and it has always been said that 95% or 98 % (anecdotal) of these malaria cases are caused by *P. falciparum*, the remaining 2% or 5% (anecdotal) are caused by other *Plasmodium* species, but the specific data source for this information is unavailable. Routine laboratory work and the 2008 and2010 Malaria Indicator Surveys (MIS) revealed a number of the non-falciparum species in some parts of the country, although they were not confirmed.

The current Zambia malaria drug policy recommends Coaterm™ (Artemether /Lumefantrin) as the first line treatment (Zambia treatment guideline, 2011) for malaria. In addition, WHO malaria treatment guidelines also recommend the use ACTs for treatment of all malaria species; it further recommends that treatment with primaquine should be given to patients with confirmed *P. vivax* and *P. ovale*, except in high transmission settings where the risk of re-infection is high. WHO has also indicated that *P. ovale* and *P. malariae* are susceptible to amodiaquine, mefloquine and the artemisinin derivatives and their susceptibility to Sulfadoxine-Pyrimethamine (SP), is not assured (WHO Guideline for malaria treatment, 2010).

The diagnostic services at most health facilities in the country utilize RDTs which are Histidine Rich Protein II (HRP II) based. HRP-II is a water-soluble protein produced by asexual and sexual stages of *P. falciparum* only (Rock, *et al.,* 1987) which is expressed on the Red Blood Cells (RBCs) membrane surface (Moody, 2002) and hence can only detect *P.falciparum*. This may have serious implications on patient management if non-falciparum species are present. Plasmodium lactate dehydrogenase (pLDH) based RDTs, which can detect all the species, are available, but were less reliable, as reported by Hopkins *et al*, 2007, hence not used in Zambia. Thus, currently, in Zambia, only microscopy has the capacity to detect and differentiate all plasmodium species. Unfortunately, not all health facilities have microscopes, while some facilities despite having microscopy services; the laboratory personnel may not be able to differentiate the species as it requires a lot of experience

and practice. To compound the problem, the country doesn't have enough trained laboratory staff.

1.3 Rationale

In order to reduce the impact of malaria on the population, i.e, morbidity and mortality; and to meet the Millennium Development Goals (MDGs) of reducing mortality and morbidity due to malaria *(*www.un.org/millenniumgoals), the Ministry of Health has been implementing a number of prevention and control strategies (National Malaria Strategic Plan 2006- 2011). Additionally, there is also a drive from the malaria elimination goal *(*www.rollbackmalaria.org). Unfortunately, in terms of diagnosis more needs to be done. All malaria species, if present, need to be diagnosed and treated specifically if malaria is to be eliminated.

The non-falciparum parasites are missed by the RDTs, and this may have a serious impact on treatment, management and prognosis of malaria. As a result of this, information on the prevalence of non-falciparum species is vital as it will help inform policy markers on malaria control programs and guide the distribution and allocation of resources especially in terms of building capacities of laboratories. This information will further guide malaria control programs on the distribution of malaria parasite species in the country, and also highlight the need to conduct refresher trainings in species identification and differentiation for biomedical scientists.

In addition, the results of this study will lead to studies that will profile the distribution of the species countrywide, the morbidity and mortality due to these species and it will stimulate studies on the treatment of the non-falciprum species and the effects of mixed infections.

CHAPTER TWO

2.0 AIMS AND OBJECTIONS

2.1 Research question

What is the prevalence of differential plasmodium species and what socio-demographic factors may be associated with them?

2.2 General Objective

To investigate the prevalence of differential malaria species infections and associated socio-demographic factors in children under 5 years of age in Eastern and Luapula provinces in Zambia.

2.3 Specific objectives

1. To determine the prevalence of *P. falciparum, P. vivax, P. ovale,P. malariae and mixed infections* by microscopy and PCR in the two provinces.
2. To examine socio-demographic factors associated with the presence of *mono*-plasmodium species infections.
3. To find out socio-demographic factors associated with mixed plasmodium species infections.
4. To find out socio-demographic factors associated with presence of *all* plasmodium species infections.
5. To assess the sensitivity and specificity of microscopy in detecting the malaria infection, using "PCR as the standard" in this study.

In order to achieve these objectives, a malaria proximate determinant framework was used.

2.4 Conceptual framework for determinants of malaria infections

A conceptual framework is a diagram or illustration that identifies or illustrates the relationship between all relevant systems, components and any salient factors that may affect or influence an event or programme. To understand an event or a disease a framework can be applied.

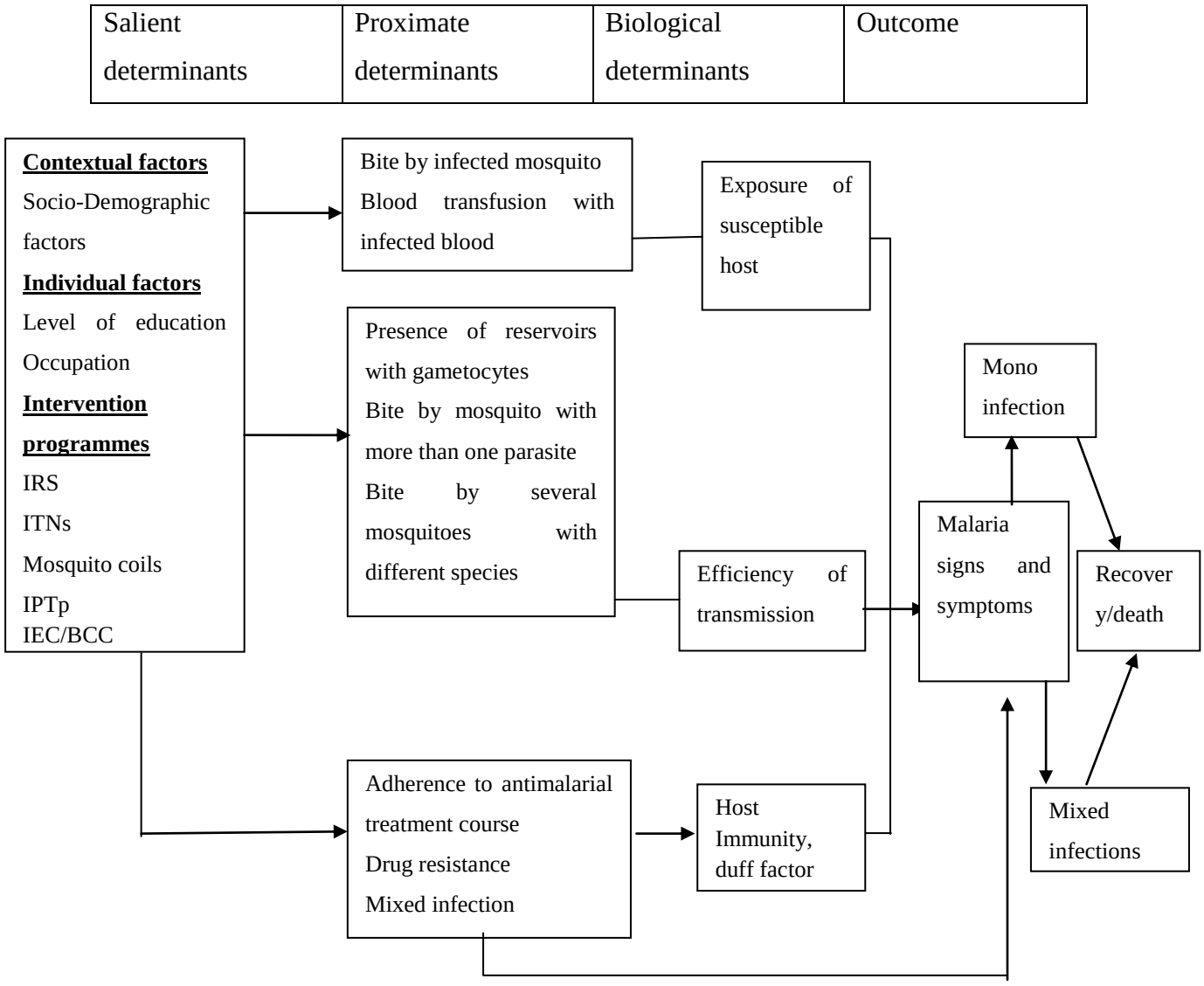
Conceptual frameworks were initially developed and used in fertility studies; later models were developed for child survival and have also been applied to HIV studies. The adapted framework has underlying or salient determinants, proximate and biological determinants. The factors directly and indirectly affect malaria transmission and acquisition in a community.

The framework shown in this document is very broad and could not be fully applied in this study. Only the underlying determinants have been assessed. Furthermore, the framework can be used for the study of malaria in general regardless of the species.

The framework was adapted from the HIV Proximate-Determinant framework by Boerma and Weir, 2006, and from a Malaria Conceptual framework used by de Castro and Fisher, 2012.

The salient determinants in this study are the main factors and demographic factors and some social factors which have an effect on any disease; and so are the proximate and biological determinants, although they are not part of the variables in this study. These factors put together determine the outcome of malaria, whether as mixed or mono-infection.

Figure 1: Conceptual framework showing Proximate- Determinant for "all plasmodium species" infections



Note:1. Figure adapted from Boerma and Weir 2006.

(IRS- Indoor Residual Spraying, IEC- Information Education Communication, BCC- Behavioral Change Communication, IPTp- Intermittent Presumptive Treatment in Pregnancy, ITNs- Insecticide Treated Nets)

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study setting and population

The study was conducted using stem data (part of main data set) from the 2012 Zambia Malaria Indicator Survey (MIS) collected from two provinces namely Eastern and Luapula Provinces, shown in figure 2. Eastern Province is located in eastern part of Zambia; it lies between Luangwa River on its western boarder and boarders Malawi on the eastern part; while Mozambique lies on its southern border; the main economic activity is agriculture. It has a population of 1,707,731, and has an area of 69,106 Km^2. Luapula Province boarders Congo DR on the west and Northern Province on the east. The major economic activity of the people in the province is fishing. It has a population of 985,976 and an area of 50,567 km^2. The two provinces have the highest malaria prevalence in the country, 50% and 22% for Luapula and Eastern Provinces respectively. From the Malaria Indicator Surveys, the prevalence in Eastern Province in the years 2006, 2008, 2010 and 2012 were 21%, 9.3%, 22% and 20.2% respectively while Luapula had 32.9%, 21.8%, 50.5% and 31.8% respectively (2012 MIS draft report). There was a reduction in malaria cases in 2008 due to an increase in donor funds which lead to the scaling up of prevention and control interventions. Unfortunately after 2009, there was a reduction in donor funds, and the Ministry of Health could not sustain the interventions well (Masaninga *et al* 2013). The funds enabled the National Malaria Control Program (NMCP) to effectively implement prevention and control strategies which include; malaria diagnostic services; prompt and effective treatment; prevention of malaria in pregnancy using IPTp; vector control; advocacy communication and social mobilization; and surveillance, monitoring and evaluation (Malaria Program Review, 2010)

Figure 2: Map of Zambia, showing the location of the two Province

3.2 Study design

This is a "Plasmodium differential species survey" which focused on data from the 2012 Zambia Malaria Indicator Survey (MIS)

3.2.1 Malaria Indicator Survey (MIS)

The MIS is conducted every two years between April and May throughout the country. Zambia is administratively divided into ten provinces and each province is in turn subdivided into districts. For statistical purposes each district is subdivided into Census Supervisory Areas (CSAs) and these are in turn subdivided into Standard Enumeration Areas (SEAs). The population of Zambia is 13,045,508 and the country has an updated list of 25,631 SEAs and 2,815,897 household (Census report 2010). The design for the survey was a representative probability sample to produce estimates for the country as a whole. The number of SEA Eastern and Luapula Province were 3524 and 2224 SEA in Luapula Province respectively (Census report 2010).

The protocol of the survey presented a plan for conducting the follow-up of the 2010 Zambia National Malaria Indicator Survey (MIS), which is a comprehensive nationally-representative household survey designed to evaluate progress toward achieving the goals and targets set forth in the NMSP 2011 – 2015. The survey was a

follow-up household survey meant to give trend information at the population level for malaria interventions and malaria disease burden. Some of the objectives were as follows: to collect up-to-date information, building on the experience of the MIS 2006, 2008, and 2010, on coverage of the core malaria interventions included in the National Malaria Strategic Plan 2011 – 2015; to assess malaria parasite prevalence and status of anaemia among the target populations (children 6 – 60 months); to implement standardized, representative household survey methods; to strengthen the capacity of the National Malaria Control Centre and local agencies involved in order to facilitate the implementation of surveys of this type in the future.

During the survey, two questionnaires were administered; one for the household head and the other for women; then blood was collected from children aged 6 months to 60 months. The collected blood was used for slides and dried blood spots preparation, as well as, malaria testing using RDTs and haemoglobin examinations were performed.

The questions in the household head questionnaires included questions on social economic status, places where medical attention is normally sought, source of drinking water, type of house and construction material used, type of malaria control strategy used (ITNs, IRS, mosquito coil) and there was a section on haemoglobin and malaria infection measurement for children under 6 years of age. On the women's questionnaire the questions addressed issues on parity, education, and knowledge about malaria. (MIS protocol 2012, unpublished).

The primary samples were from the 2012 MIS Samples that were selected using stratified two- stage cluster design with the assumption that future cross-sectional surveys would be conducted. The numbers of clusters that were selected for the MIS were calculated based on an average cluster of 25 completed interviews of all respondents. These clusters were selected systematically with probability proportional to the number of households where a sampling interval was calculated and a random number was used. Households were selected by first coming up with a sampling frame, then calculating the sampling interval and a random number was generated. There were three strata; rural, urban and IRS areas and hence there was a disproportional allocation to the three strata. Due to the disproportionate allocation of the sample to the three different strata, sampling weights were calculated at the national level. The sampling probability at first stage selection of SEAs and

probabilities of selecting the household were used to calculate the weights; the weights of the sample were equal to the inverse of the probability of selection, details in MIS report (MIS 2012).

3.2.2 Plasmodium differential species survey

A cross sectional study was carried out on some of samples from two provinces (Eastern and Luapula); that were purposely selected based on high prevalence rates. In these provinces all the samples were eligible for selection provided they met the inclusion criteria. Demographics (age, gender, education status and region), access and health seeking behavior variables were extracted from the main MIS data set; slide and Dried Blood Spot (DBS) were examined. Table 1 show the variable measured.

Table 1: Operational variable framework

Variable		Indicator	Scale of measurement
Dependent variable			
Infection with Malaria species		Presence of more than one species in a sample (*Plasmoduim malariae, ovale, vivax, falciparum)*	Nominal
Independent variables			
Health-care seeking behaviour		Good	Nominal
		Bad	Nominal
Treatment past two week		Took antimalarial in past two week (Yes/No)	Nominal
Type of drug taken		Type of antimalarial	Nominal
Ownership of bed net		Availability of ITN (Yes/ No)	Nominal
Socio-demographic: of parent	Age	Age	Interval
	Gender	Male/female	Nominal
	Region	Rural/ Urban	Nominal
	Age of mother	Age	Interval
	Level of education of the mother	Low	Ordinal
		High	

Sample size for the study: This study analyzed samples from the two provinces, in the MIS, 23 clusters from Eastern and 17 cluster from Luapula were selected giving a total of 40 clusters, out of the 3524 and 2224 respectively. This study took all the 1158 samples from the two provinces due to availability of resources and due to the fact that they were sampled for the MIS study.

Inclusion and exclusion criteria for samples: Samples from children aged 0-60 months that were collected during the survey with complete information or data were included. On the other hand, samples, whose key data for this study was not available i.e. presence or absence of fever, Hb reading, slides, dried blood spot, information of ITN and health seeking behavior of parent were excluded. The excluded 285 samples, did not have dried blood spot specimens, leaving 873 samples for analysis.

3.3 Data extraction

3.3.1 Demographic factors

A data collection tool was designed to extract socio-demographic variables from the Malaria Indicator Survey (MIS) questionnaire. The tool included the following variables: age of the children, gender, residence (rural/ urban), the province, the age of the mother, the level of education of the mother, history of taking any drug two weeks prior to study interview, the type of drug and their health seeking behavior. The data collection tool is in Appendix 2.

3.3.2 Laboratory data

Microscopy: The MIS slides were collected and processed by laboratory scientists and technologist; who were also trained for one week on data collection prior to the survey. During the training a day was dedicated to field tests of the survey instruments in Chongwe District, where children were tested. These slides are stored in special slide storage boxes, which ensure non-contact and independent standing of the slides. Staining of the slides was done at the National Malaria Control Centre (NMCC) parasitological laboratory using 3% Giemsa stain for 45 minutes.

The slides were then examined by two experienced biomedical scientists independently; slides that had discordant results were given to a third independent reader, blinded from the results to break the tie.

PCR samples: A three spotted dried blood spot (DBS) sample was collected from each child that participated in the survey. These were used for PCR analysis, which was done at the National Malaria Control Centre (NMCC) Molecular Biology Laboratory. One of the three spots was cut using scissors and placed into a 1.5 Millie litres (mls) eppendorf tubes. The scissors was sterilized with Sodium Hypochloride and flame after each sample. The DBS were then stored for future use as per MIS protocol.

Deoxyribonucleic Acid (DNA) was extracted from the blood spots using chelex boiling method. Each cut spot was boiled in a 50 micro liter of chelex solution and approximately 150 micro liter of DNA template was obtained.

PCR protocol: Identification of plasmodium species was performed using nested Polymerase Chain Reaction. The protocol, in the first reaction, amplifies a conserved region for all the four species. Then in the second reaction, different primers were run for the four species separately. The band sizes of the product were as follows: *P.falciparun* 205bp, *P. malariea* 140 bp, P *ovale* 800 bp and *P.vivax* 120 bp. These primers were provide by Harvard University for the Molecular laboratory and manufactured by a company named Integrated DNA Technologies (IDT). The Primers and protocol were adapted from (Nsobya et al., 2004) the primer sequences are listed in table 2

Primer sequences

Table 2: Primer sequences for the primers used in the study

Species	**Primer name**	**Primer sequence**	**Molecular weight**
Plasmodium	rPLUf rPLUr	5'- TTA AAA TTG TTG CAG TTA AAA CG 5'- CCT GTT GTT GCC TTA AAC TTC	
Falciparum	rFALf rFALr	5'- TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT 5'- ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC	205 bp
Vivax	rVIVf rVIVr	5'- CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC 5'- ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA	140 bp

Malariae	rMALf rMALr	5'- ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC 5'-AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA	120 bp
Ovale	rOVAf rOVAr	5'- ATC TCT TTT GCT ATT TTT TAG TAT TGG AGA 5'- GGA AAA GGA CAC ATT AAT TGT ATC CTA GTG	800 bp

Key r= reverse, f=forward

The PCR mixture for the first reaction contained a 12.5 micro litre of a 2X master mix containing Taq buffer (10mM Tris–HCl, pH 8.3, 50mM KCL, 1.5mM $MgCl_2$), 200_M of each dNTP 2.5 units of Taq DNA polymerase, the PCR primers (10 µM of rPLUf and rPLUr) and, 5 microlitres of template DNA and water in a final volume of 25 µls.

In the second reaction the same master mix was used, with different primer concentration- (10µM of each of the species primer in separate tubes), 2µl DNA template from the first reaction in a 25 µl reaction volume. The PCR was run in an Applied Biosystems thermocycler under the following conditions: first reaction: denaturation at 94 °C for 1min followed by 35 cycles at 94 °C for 1 min, 58 °C for 2 min and 72 °C for 5 min, for the first reaction. The second reaction (with four tubes for each primer) had denaturation at 94 °C for 1min, followed by 30 cycles at 94 °C for 1 min, 58 °C for 2 min and 72 °C for 5 min. The final cycle was followed by an extension time of 5 min at 72°C (Nsobya et al., 2004)

A 2% Agarose gel stained with ethidium bromide was run for visualization of the PCR product, with 50 and 100 base pairs ladders. (Nsobya et al., 2004)

Quality control: Quality control was carried out at Tulane University, School of Medicine, Department of Public Health and Tropical Medicine Kumar laboratory, New Orleans, Louisiana- USA, on the PCR analysed samples. Twenty five samples were purposefully selected, 20 of which had mixed infections or falciparum positive and 5 were negative. The data showed similar results with few discrepance: 100% agreement for *P.falciparum* and *P.vivax* cases, 75% agreement for *P.malariae*, and

83% for *P.ovale*. This showed that the results obtained from the molecular lab in Zambia could be trusted and were reliable. The primers used were different, their band size was 300bp.

3.4 Data analysis

Data analysis was performed using STATA version 12 (STATA corp., college station, Taxes USA). Prevalence rates were estimated in STATA taking into account the sampling design (clustered). Differences between groups for categorical variables were assessed using chi-squared or Fisher's exact tests depending on the numbers. Logistic regression was performed to examine the predictors of malaria infection. A *p*-value of less than 0.05 was considered significant at 95% confidence interval. Furthermore, Sensitivity and specificity was assessed using a two by two table, with PCR being the standard.

3.5 Ethical considerations

Dissertation approval to analyze the samples was sought from the University of Zambia; School of Medicine Postgraduate Committee, after which ethical clearance and permission was sought from the University of Zambia, Biomedical Research Ethics Committee (UNZABREC) and Permission was sought from the Ministry of Health, for the use of 2012 MIS data. There was no direct contact with the participants and hence there was no pain or discomfort, and there is less than minimal risk involved. Furthermore, samples did not have participants' names, but had an identification number and hence anonymity and confidentiality was guaranteed. The study did not pose any risk to the participant as samples had already been collected. The benefit of the study was that information on malaria species in Zambia would eventually improve the management of malaria as Zambia moves towards elimination of malaria.

CHAPTER FOUR

4.0 RESULTS

4.1 Overall Population description

Table 3 shows the socio-demographic factors of all 873 enrolled children, in this study, 47.0 % were males and 53.0% were females. Their ages ranged from 0 months to 5 years, with the mean age being 2.4 years, and the age of their mothers ranged from 15 years to 49 years. There were 504 children (57.3%) from Eastern and 369 children (42.7%) from Luapula Province. A larger proportion of these children were classified according to the national census as living rural clusters (95.7%), with 4.4% living in the urban clusters.

Table 3- Overall proportions of selected socio-demographic factors of the study population

Total number of children test =873		
Characteristics	**N**	**%**
Gender		
Male	410	47.0
Female	463	53.0
Age of children		
0 yrs	132	15.1
1 yr	144	16.5
2 yrs	151	17.3
3 yrs	178	20.4
4 yrs	156	17.9
5 yrs	112	12.8
Age groups		
0-1	276	31.6
2-5	597	68.4
Province		
Eastern	504	57.3
Luapula	369	42.7
Residence		
Urban	38	4.4
Rural	835	95.7
Age of Mother		
15-25	254	32.4
26-39	457	58.2
40-49	78	9.4
School level of mother		
Low	420	53.5
High	365	46.5

Population description by province

Table 4 shows the distribution of the enrolled participants by province; there wasn't much difference in terms of socio-demographic characteristic between the two provinces

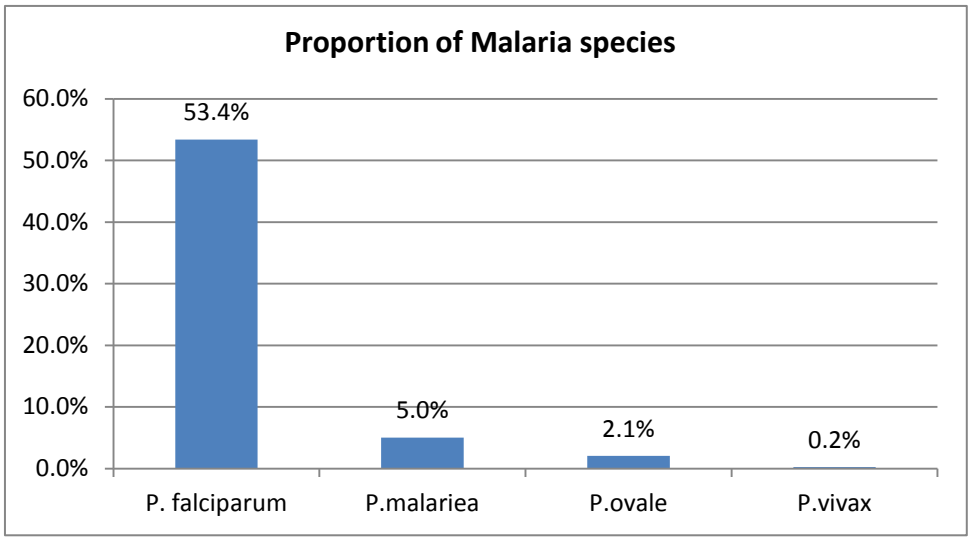
Table 4- Proportions of selected Socio-demographic factors by province

Characteristic	Eastern N (%)	Luapula N (%)
Gender		
Male	243 (48.2)	167 (45.3)
Female	261 (51.8)	202 (54.7)
Age of children		
0 yrs	82 (16.3)	50 (13.6)
1 yr	76 (15.1)	68 (18.4)
2 yrs	87 (17.3)	64 (17.3)
3 yrs	98 (19.4)	80 (21.7)
4 yrs	93 (18.5)	63 (17.1)
5 yrs	68 (13.5)	44 (11.9)
Residence		
Urban	18 (3.6)	20 (5.4)
Rural	486 (96.4)	349 (94.6)
Age of mother		
15-24 yrs	151 (33.2)	103 31.2)
25- 39 yrs	252 (55.4)	205 (62.1)
40- 50 yrs	52 (11.4)	22 (6.7)
Education level		
Low	268 (58.9)	152 (46.1)
High	187 (41.1)	178 (53.9)

4.2 Proportion of *Plasmodium* malaria species

Figure 3 shows the percentages of malaria species. The proportion of malaria infections from PCR results was found to be 54.3%, where *P.falciparum* was 53.4%, *P.malariae* 5.0 %, *P.ovale* 2.1% and *P.vivax* 0.2%. These were individual species 10results regardless of whether they occurred as mixed or mono-infections; most of the non-falciparum parasites species were found to occur as mixed infections. The gel pictures for PCR results are in appendix E.

Figure 3: Pooled province proportions of the differential malaria species



Note: 1.Overall sample size (n) 873, Eastern n=504, Luapula= 369. 2. The prevalence does not add upto the overall malaria species (54.3%), given that most of the non-falciparum species presented as mixed infections

Prevalence by province

The prevalence of *P.falciparum* in the two provinces was almost the same, 55.6% and 52.6%, for Eastern and Luapula provinces respectively, (table 4). There was no significant difference as seen from the p-value of 0.21. On the other hand, there was a significant difference between the prevalence of *P. malariae* and *P. ovale* in the two provinces; the P-values were less than 0.05.

	***P.falciparum* (%)**		***P.malariae* (%)**		***P.ovale* (%)**		***P.vivax* (%)**		**All species (%)**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Eastern	51.0	49.0	2.4	97.6	1.2	98.8	0.2	99.8	55.6	44.4
Luapula	55.2	44.8	8.7	91.3	3.3	96.8	0.3	99.7	52.6	47.4
p-value	0.21		< 0.01		0.051		0.67			0.38

Table 4: Comparative prevalence's of *Plasmodium species* by Province

Mixed infections:

Figure 4 shows the number of cases and the proportion of mixed infections and the species combinations of these mixed infections. Overall prevalence of mixed infection was 5.6%. The proportion of mono-infection was 48.7%, n=873, the total population screened.

Figure 4: Prevalence of mixed-infection and mono-infections for both provinces

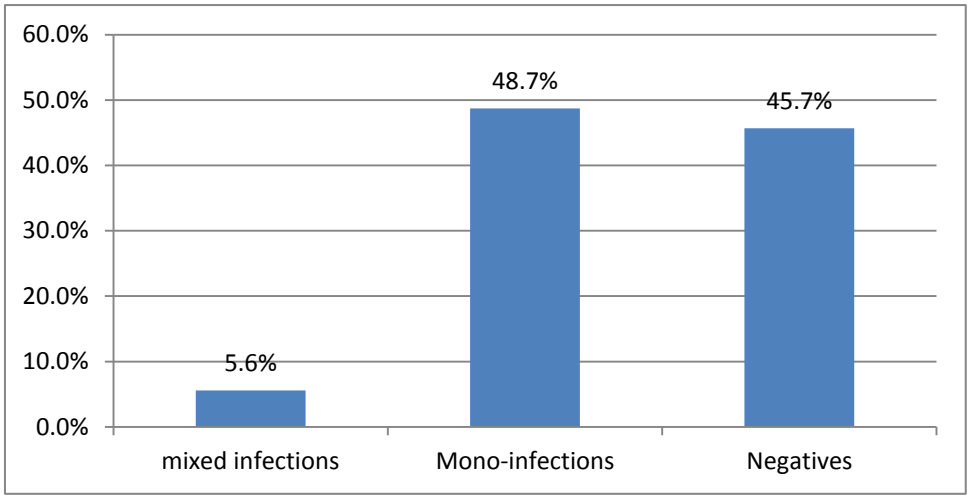
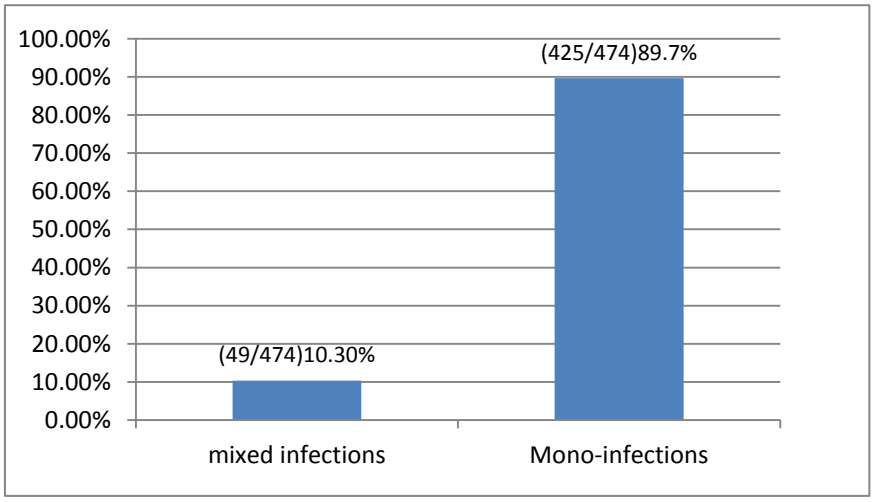


Figure 5 shows the percentage of children who had malaria with mixed infections being 10.3%, n=474 (malaria positives only), and those with mono infection as 89.7%.

Figure 5: Proportions of the mixed and mono- infections for both Provinces



Note: these proportions are based on all 474 positives (representing 54.3%, n=873)

Combinations of mixed infections

Table 5 shows different species combinations of mixed infections. Most children were found to have a combination of pf+pm, (31/ 474, 6.5 %). There were 7 children found to have a combination of three species, accounting for about 1.5%. On the other hand, there were 6 cases of mono-infections with non-falciparum species only, these accounted for 1.4%

Table 5: Overall prevalence of Mixed and mono infections in the study population (Eastern and Luapula Provinces combined)

Infection pattern	No.	Percentage n- 474	Prevalence n=873
P.f	**419**	**88**	**48**
P.m	**4**	**1**	**0.5**
P.o	**2**	**0.4**	**0.2**
Pf+Pm+Po	**6**	**1.3**	**0.7**
Pf+Pm+Pv	**1**	**0.2**	**0.1**
Pf+Pm	**31**	**6.5**	**3.6**
Pf+Po	**10**	**2.1**	**1.1**
Pf+Pv	**1**	**0.2**	**0.1**
Total	**474**	**100**	**54.3**

Notes: Proportion of Non-falciparum infections of all mono infections: 1.4%, n=474, Prevalence 0.7%, n=873 Key: P.f= *Plasmodium falciparum*, P.m =*P.malariae*, P.o= *P.ovale*, P.v= *P.vivax*

4.3 Association of factors with malaria infections

Table 6 shows the association between age of the child (P = 0.002), education level of the mother (P= 0.06) with malaria infection (PCR results) see table 3.

Table 6: Association of selected socio-demographic factors with parasiteamia

Characteristic	Presence of parasites	Absence of parasites		
	1	0	chi2	P-value
	% (n)	% (n)		
Gender				
Male	53.7 (220)	46.3 (190)	0.1	0.72
Female	54.9 (254)	45.14 (209)		
Age of children				
0 yrs	42.4 (56)	57.6 (76)	19.1	0.00
1 yr	44.4 (64)	55.6 (80)		
2 yrs	58.3 (88)	41.7 (63)		
3 yrs	59.0 (105)	41.01 (73)		
4 yrs	59.6 (93)	40.4 (63)		
5 yrs	60.7 (68)	39.3 (44)		
Age groups				
0-1	43.5 (120)	56.5 (156)	19.0	0.00
2-5	59.3 (354)	40.7 (243)		
Province				
Eastern	55.6 (280)	44.4 (224)	0.8	0.38
Luapula	52.6 (191)	47.4 (178)		
Residence				
Urban	47.4 (18)	52.6 (20)	0.8	0.38
Rural	54.6 (456)	45.4(379)		
Age of Mother in three group				
15-24	48.8 (124)	51.2 (130)	3.9	0.14
25-39	56.5 (258)	43.5 (199)		
40-50	55.4 (41)	44.6 (33)		
School level of mother				
Low	55.8 (322)	44.2 (255)	3.7	0.07
High	48.6 (101)	51.4 (107)		

Association of factors with mixed plasmodium infections

Table 7 shows the factors associated with mixed plasmodium infections, it can be suggested that there could be an association between the presence of mixed infections and age of the child, and the province, p-values less are than 0.05.

Table 7: Association of selected socio-demographic factors with mixed plasmodium infections

Characteristic	**Presence of parasites**	**Absence of parasites**		
	1	**0**	**chi2**	**P-value**
	n (%)	n (%)		
Gender				
Male	6.1 (25)	93.9 (385)	0.34	0.56
Female	5.2 (24)	94.8 (439)		
Age of children				
0 yrs	1.5 (2)	98.5 (130)	13.86	0.017
1 yr	2.8 (4)	97.2 (140)		
2 yrs	4.0 (6)	96.0 (145)		
3 yrs	7.9 (14)	92.1 (165)		
4 yrs	7.7 (12)	92.3 (144)		
5 yrs	9.8 (11)	90.2 (101)		
Age groups				
0-1	2.2 (6)	97.8 (270)	8.4	0.003
2-5	7.2 (43)	92.8 (554)		
Province				
Eastern	3.0 (15)	97.0 (489)	15.64	0.000
Luapula	9.2 (34)	90.8 (335)		
Residence				
Urban	0.00 (0)	100.0 (38)	2.4	0.12
Rural	5.9 (49)	94.1 (786)		
Age of Mother 2				
15-24	3.5 (9)	96.5 (245)	2.7	0.26
25-39	5.3 (21)	94.8 (436)		
40-50	8.1 (6)	91.9 (68)		
School level of mother				
low	5.2 (22)	94.8 (398)	0.14	0.71
High	4.7 (17)	95.3 (348)		

Association of factors with plasmodium mono infections

The table 8 shows factors associated with mono-infection, the factors are very similar to those associated with malaria infection in general as the number as almost similar

Table 8: Association of selected socio-demographic factors with plasmodium mono- infections

Characteristic	Presence of parasites	Absence of parasites		
	1	0	chi2	P-value
	n (%)	n (%)		
Gender				
Male	46.8 (192)	53.2 (218)	0.60	0.44
Female	49.5 (229)	50.5(234)		
Age of children				
0 yrs	40.9 (54)	59.1 (78)	8.00	0.16
1 yr	41.7 (60)	58.3 (84)		
2 yrs	53.0 (80)	47.0 (71)		
3 yrs	51.1 (91)	48.9 (87)		
4 yrs	51.3 (80)	48.7 (76)		
5 yrs	50.0 (56)	50.0 (56)		
Age groups				
0-1	41.3 (114)	58.7 (270)	7.74	0.00
2-5	51.4 (307)	48.6 (554)		
Province				
Eastern	52.5 (265)	47.4 (239)	9.06	0.00
Luapula	42.3 (156)	57.7 (213)		
Residence				
Urban	44.7 (17)	55.3 (21)	1.9	0.66
Rural	48.4 (404)	51.6 (431)		
Age of Mother 2				
15-24	45.3 (115)	54.7 (139)	2.2	0.34
25-39	50.8 (232)	49.2 (225)		
40-50	46.0 (34)	54.1 (40)		
School attainment of mother				
Not attended school	53.4 (117)	46.6 (102)	2.91	0.09
Have attended	46.6 (264)	53.4 (302)		
School level of mother				
low	49.3 (207)	50.7 (213)	0.20	0.65
High	47.7 (174)	52.3 (191)		

4.4 Predictors of malaria infections

Table 9 shows a logistic regression model, where gender, region, province, ITN use, and sprayed houses are controlled for, to exam the association between age and presence of malaria. The results showed an association between demographic and social factors, and malaria infection; age of the child, particularly children age 2 years as seen from the odds ratio of 5 and the confidence interval (1.05-23.89). Although, the confidence interval is wide, implying the results may not be so precise, suggesting the association is not very strong.

Table 9: Logistic regression predicting model malaria infection

Malaria parasites infection	**n=873**		
Characteristic	**Prevalence**	**aOR**	**95% CI**
Age in years			
0	42.4%	1	
1	44.4%	0.33	0.62 - 1.77
2	**58.3%**	**5.00**	**1.05 - 23.89**
3	59.0%	4.00	0.83 - 18.44
4	59.6%	1.34	0.29 - 6.16
5	60.7%	1.80	0.29 - 10.91
Gender			
Male	46.4%	1	
Female	53.6%	0.9	0.34 - 2.39
Age of the mother in years			
15-24	48.8%	1	
25- 39	61.0%	1.34	0.49 - 3.70
40-49	9.7%	1.63	0.23 - 11.10
Education level			
Low	55.2%	1	
High	48.6%	0.95	0.35 - 2.54
Residence			
Urban	47.4%	1	
Rural	54.6%	3.60	0.23 - 57.59
Province			
Eastern	55.6%	1	
Luapula	52.6%	0.27	0.10 - 0.75
Ownership of Mosquito net			
No	58.0%	1	
Yes	53.4%	0.15	0.009 - 2.32
Drug taken			
Fansidar	60.0%	1	
Quinine	71.4%	3.75	0.17 - 85.43
Coartem	56.6%	2.00	0.20 - 20.48
Place where Health Services were sought			
None	53.8%	1	
Govt/ private institution	50.5%	1.41	0.20 – 10.43
Others	69.2%	3.51	0.43 - 29.59
Houses sprayed			
No sprayed	55.5%	1	
Sprayed	49.8%	1.15	0.26 – 5.05

Notes: 1.Overall sample size (n) 873, Eastern n=504, Luapula= 369 2.Variable in the model were adjusted for age as a continuo's variable

4.5 Sensitivity and Specificity

Table 10 are 2 by 2 tables for analysis of specificity and sensitivity of the methods used in the study, these were PCR, microscopy and RDTs, and the 'standard' used in this analysis was PCR. The sensitivity and specificity of microscopy and RDTs are assessed against PCR. The table shows sensitivity of microscopy against PCR was 50% and specificity was 85%, while for RDTs it was 73% and 65% respectively.

Table 10: Sensitivity and specificity of microscopy and RDTs measured against PCR

	PCR +	**PCR -**	**TOTAL**		**PCR +**	**PCR -**	**TOTAL**
MPS +	225	44	**269**	**RDT +**	341	142	**483**
MPS -	246	358	**604**	**RDT -**	124	264	**388**
TOTAL	**454**	**419**	**873**	**TOTAL**	**465**	**406**	**871**

Microscopy/PCR			**RDTs/PCR**	
SE	50%		**SE**	73%
SP	85%		**SP**	65%
PPV	84%		**PPV**	71%
NPV	59%		**NPV**	68%

Key- MPS- Malaria parasite slides
NPV- Negative predictive value
PCR- Polymerase chain reaction
PPV- Positive predictive value
RDTs- Rapid diagnostic tests
SE – Sensitivity
SP- Specificity

CHAPTER FIVE

5.0 DISCUSSION

The findings illustrate high burden of malaria infection with an overall prevalence of 54.3%, although not significantly different by site (Eastern Province 52.6% and Luapula Province 55.6%). This might partly explain why malaria remains one of the leading causes of morbidity and mortality in Zambia, despite the preventive and control measures in place. These figures are similar to or higher than reported microscopy based results in the MIS 2010 report, where Luapula had a prevalence of 50% and Eastern Province had 22%, although different diagnostic methods were used. The reasons for this high burden are unclear (and were beyond the scope of this study), given that preventive and control measures that were available in the two provinces, were similar to the ones in the other provinces in the country with low malaria prevalence. This might suggest that additional and setting specific prevention and control measures grounded in local values need to be considered. Masaninga *et al* 2013 suggested that diagnosis of all suspected cases, prompt treatment with ACTs and strengthen surveillance in the whole country would reduce malaria cases. This high prevalence (54.3%) comprised all the four species (48.7% *P.falciparum*, 0.5% *P.malariae*, 0.2% *P.ovale* and 5.6% mixed infections). The prevalence of non-falciparum infections was found to be 6.0%, which is higher than the anecdotal 2% the country has been using notwithstanding the differential transmission patterns which are core determinants for non-falciparum infections. This may further suggest that there is need to continue monitoring non-falciparum infection burden in this population, so as to decide at which point species-specific RDTs may be introduced for diagnostic purposes especially in younger children. Age was found to be a predictor of malaria infections and mixed infection; children aged two old years and older had more infections than the other ages.

Comparing the prevalence in the two provinces, the proportions of malaria was similar 55.6% and 52.6% for Eastern and Luapula Provinces respectively, although there was a significant difference between the prevalence of two species; *P.malariae* and *P.ovale*. Eastern had a prevalence of 2.4%, and Luapula had 8.7 % for *P.malaria;* and in the case of *P.ovale,* 1.2% and 3.3% for Eastern and Luapula respectively, indicating that the prevalence of the two species is higher in Luapula than in Eastern Province. Two cases of *P.vivax* were found, one from each province, their number may not be

significant but there is need to further investigate the cases further. The reasons for these differences are beyond the scope of this study; on observation, the province with the lower prevalence of malaria has the lower prevalence of the non-falciparum species. Given this, it is reasonable to assume that, one of the contributing factors is the misuse of ITNs. Eisele et al, 2011 stated that there was documented evidence of misuse of ITNs as fishing net, wedding veils in Uganda, Kenya, Tanzania and Zambia. This could explain high burden in Luapula Province which has more water bodies, compared to Eastern Province.

Mixed malaria species infections are often unreported or under-reported; the prevalence of mixed infections was 5.6% in this study, a study by Smuthuis et al, 2010, in adults conducted in Myanmar on response to ACT regimens found 16% mixed infections. Another study by Mohapatra *et al* 2012, in Orissaon, India, on the outcome of co-infections found 13.6% malaria species mixed infections. These two studies were hospital based studies while this study was a community based survey so the results are comparable. On the other hand, Mandunda *et al*, 2008, in a community survey, found a prevalence of 2.9% in Mozambique. When stratified according to provinces, Luapula Province has a higher prevalence of mixed infection compared to Eastern Province. These mixed infections may have an impact on the development of a vaccine for malaria, as the vaccines being developed mainly target falciparum malaria and it is not clear how the vaccines can perform in individuals with mixed infections. Ohrt *et al*, 2007 state that species error could have the effect of false positives or negatives if a species-specific endpoint is defined for example in malaria vaccines. In addition, presence of these species (*P.ovale* and *P.vivax*) calls for use of Primaquine that is recommended for their treatment which can eliminate the dormant hypnozoites, but can cause haemolysis in patients who have a Glocose-6-Phospate Dehydrogenase (G6PD) deficiency (Galappaththy et al, 2008). This calls for screening of the community before treatment, but this may not be feasible as most health facilities lack laboratory facilities, hence ACTs will be used and hypnozoites will not be cleared and the control of malaria will be affected.

The prevalence of mono-infections was 48.7%, of these, 48.0% were *P. falciparum* and 0.7% were *P.malariae* and *P.ovale*, n=873. The fact that such cases are present indicate the need to have a diagnostic tool that can detect them, however, it should not necessarily be the primary diagnostic tool, but one that can be used in cases where

patients come back with symptoms of malaria and have negative Histidine Rich Protein II (HRP II) based RDTs results. An RDTs that can detect the four species should be used. With regards to health facilities where microscopy services are present, there is need to conduct refresher courses for almost all laboratory staff in the diagnosis of malaria especially on speciation despite the fact there are very few cases of non-falciparum mono-infections. Ohrt *et al*, 2007, stated that training drastically improves quality of microscopy, reducing false positives. These cases are not alarming to warrant change in the diagnostic tools as most of the non-falciparum cases occur as mixed infections, hence the use of HRP II based RDTs is still acceptable. There is need to examine the Prevalence of the species in areas with low transmission areas.

Among the factors associated with malaria infections, only age of the child seemed to have had an association with co-infections and or presence of any malaria species. Younger children aged 0-2 years were less likely to have malaria or were somehow protected from malaria compared to those aged between 3-5 years. Our study showed a gradual increase in percentage of children infected with malaria as age increased. The younger children were less likely to have malaria, but as they grow, more children tend to have malaria infections, as they reach the age of 5 years their immunity would have belt up and infections reduce. It's a known fact, that maternal acquired antibodies protect infants up to the time they are 6-9 months from a number of infections, malaria inclusive (Riley *et al*, 2001). Although recent studies suggest otherwise (Alessandro *et al*, 2012) some study finding suggest that age maybe protective. In addition, these finding could be attributed to the use of ITNs, younger children sleep under ITNs as they are breast feeding; at the age of 2-3 years they are weaned off and may not sleep under a net. The other variables which include gender, education status of the mother, residence (Urban or rural) did not show any association.

Effective, accurate and precise tools for diagnosis of any infection are vital in the management of the infection. Malaria is not an exception or a special case; an accurate and effective tool is vital to better manage it, and more so as the nation goes towards elimination of the malaria parasites. The accuracy and precision of a diagnostic tool is assessed using sensitivity and specificity analysis. The sensitivity of microscopy was found to be 50% and its specificity was 85%, implying that microscopy can detect

only 50% of the true positive results and can correctly detect 85% as the negative, thus 50% of the positives are missed. This is not very good considering the different transmission patterns in the county. The success of control hinges on effective surveillance and diagnostic methods (Kamau E *et al*, 2011). Reley *et al* 2010, reported a sensitivity of 65%, Coleman *et al*, 2006, reported sensitivity and specificity for microscopy 33.5% and 58.5% respectively. In addition Batwala V *et al*, 2010, found sensitivity and specificity of microscopy to be 41% and 97.1% respectively, and for RDT 91.0% and 86.3% respectively. These findings are similar to the ones found in this study, confirming that the shortfall of microscopy and RDTs are not unique to our study. Despite molecular techniques being available and better, microscopy remain the "gold standard" in malaria diagnosis in most countries and hence measures need to be taken to improve it.

The likelihood of people tested using microscopy having malaria is about 84% and the likelihood of them not having malaria is 59% as seen in the value of Positive Predictive Value (PPV) and Negative Predictive Value (NPV). On the hand, for patients tested using RDTs the likelihood that the test will show that they have malaria is 71%, and the likelihood that those without malaria will have a negative test is 68%. An ideal test should be easy to administer, interpret, of low cost and widely available, and hence microscopy service need to be improved, for clinicians to have confidence in it. Batwala *et al* 2010, show Positive Predictive values of 75% and 73.3% and NPV of 80.7% and 95% for microscopy and RDTs respectively.

The study had a few limitations, which were; the effect of malaria species co-infections on the outcome or clinical presentation could not be assessed as the study was conducted in a community where most children were healthy and just carrying parasites, even though some of them were found with fever and anaemia. Secondly, occupation, income, proximity to health facility and environmental factors were also not analysed, these could have given a clear picture of the associations or a better prediction model. The slides were collected under field conditions and some of them were dirty and difficult to examine. Finally, this data cannot be generalized to the whole country, as this analysis is just for two provinces.

5.1 Conclusion

This study reveals presence of a high burden of non- falciparum malaria infections in

the study population, correlating with the high overall prevalence of malaria (54.7%). The major factor associated with the burden of non-falciparum malaria was the age of the child, which further supports the notion of increased exposure to environmental factors as a child grows. This might call for repackaging of prevention and control measures to account for environmental, maternal and programme factors linked to child development. Having said this, it is further argued that the presence of non-falciparum infections which might vary with transmission patterns, demographic trends and geographical contrasts, needs to be monitored through a functional surveillance system, in order to understand its epidemiological profile. Understanding the epidemiology of non-falciparum will further help to dictate the development of more sensitive species specific RDTs for diagnostics. Given that most of the non-falciparum malaria occurred as mixed infections, the use of HRP II based RDT can still continue in health facilities without microscopy, with an additional species-specific test for patient who come back with malaria symptoms.

5.2 Recommendations

From the study, the following recommendations are made:
Firstly, a country wide study on the differential or non-falciparum species be carried out at both health facility and community level, to ascertain the prevalence of differential species. Furthermore, an active and a passive surveillance system should be put in place. Secondly, additional site specific interventions measures grounded on local malarial matrix should be introduced to eliminate the malaria. This also calls for review of treatment and diagnostic protocols. Thirdly, the laboratory staff needs to be trained on identification of species, and as such workshop and seminars should be conducted. Finally, the National Malaria Control Centre Laboratory should periodically perform PCR on randomly selected samples from the country.

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APPENDICES

Appendix A: Data collection form

PLACE NAME……

NAME OF HOUSEHOLD HEAD……

CLUSTER NUMBER.....

HOUSEHOLD NUMBER .....

REGION ..... …….URBAN/RURAL (URBAN=1, RURAL=2)

Demographic factors

Relationship of head/care giver /guardian with Child		Son……1 Daughter……2 Nephew……..3 Niece……..4 Grandchild….5 Others…..specify…..X
Age of care give/guardian		
Age of Child at last birthday		
Sex		Male 1, Female 2
Highest level of education guardian earned		NONE……0 PRIMARY .....1 SECONDARY ..... 2 HIGHER 3

Clinical and related characteristics

Child had fever in past two week		Yes, No

Child had took medicine past two week		Yes, No
Type of medicine taken		SP/FANSIDAR……A QUININE……..B COARTEM / ACT...C ASPIRIN……D PARACETAMOL....E IBUPROFEN……...F OTHER ....X DON'T KNOW…..…Z
For how many days did child take the DRUG?		Period in days
Preferred places for seeking medical attention Good= (A-L) Bad=(N,O,X)		PUBLIC SECTOR GOVT. HOSPITAL…….A GOVT. HEALTH CENTER…….B GOVT. HEALTH POST ……..C MOBILE CLINIC……D FIELD WORKER……F OTHER PUBLIC G (SPEC) PRIVATE MEDICAL SECTOR PVT. HOSPITAL/CLINIC…….H PHARMACY ……I PRIVATE DOCTOR……J MOBILE CLINIC……K FIELD WORKER……L

		OTHER PVT. MED. _____ M (SPECIY) OTHER SOURCE SHOP.....N TRAD. PRACTITIONER.....O OTHER X (SPECIFY)

ITNs and IRS

Does household have ITNs	Yes, No
Does child sleep in ITNs	Yes, No
Was the house sprayed in the last 12 months	Yes , No

Laboratory Characteristics

Parasitological characteristics

Malaria positive result by RDT				
Malaria positive by slide				
Species seen on slide				
Species seen by PCR				

Appendix B: Work plan

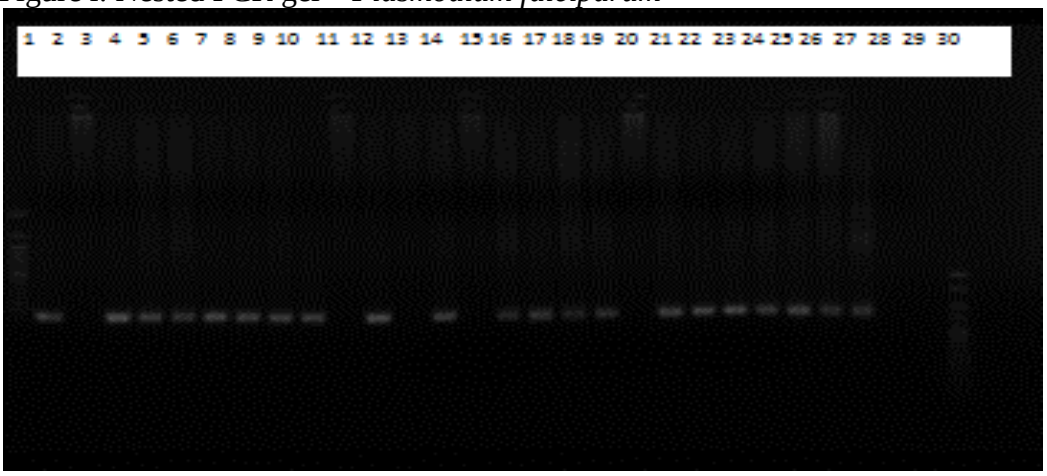
ACTIVITY	July-12	Aug-12	Sep-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	April-13	June-13
Writing of the research proposal and presentation to class											
Correction of proposal after class presentation											
Submit proposal to Assistant Dean(PG) office											
Graduate Forum Presentation											
Correction of GF comments											
Obtaining permission MoH and NMCC											
Research Ethics Committee review and approval			..								
Optimisation of PCR techniques/ Data sorting and analysis			..								
Data entry and Analysis				..							
Write dissertation					..						
Submission of draft report											
Dissemination of Findings-presentation											
Bidding and submission of final dissertation											
Graduation											

Appendix C: Budget

ACTIVITY	QUANTITY	UNIT COST	TOTAL
PCR reagent		32,000,000	32,000,000
Lab research assistant	2x 50 days	50, 000	5,000,000
Lunch allowance for data entry clerk	2x 40 days	50 000	4,000,000
A4 Ream of paper	10	30, 000	300,000
Other stationary		200,000	200,000
Toner	1	1, 000, 000	1, 000, 000
Binding of proposals	5	100, 000	500,000
The University of Zambia Research Ethics Committee fees	1	1,000,000	1,000,000
External hard disc	1	600,000	600,000
Flash disc (4GB)	1	200,000	200,000
Binding of the final report	5 copies	250,000	1,250,000
Poster	1	800,000	800,000
Transport		2,000,000	2,000,000
Communication		1 500,000	1,500,000
Publication	1	1 000,000	1,000,000
Total			51,350,000
Contigency (10%)			**5,135,000**
GRAND TOTAL			**56,485 ,000**

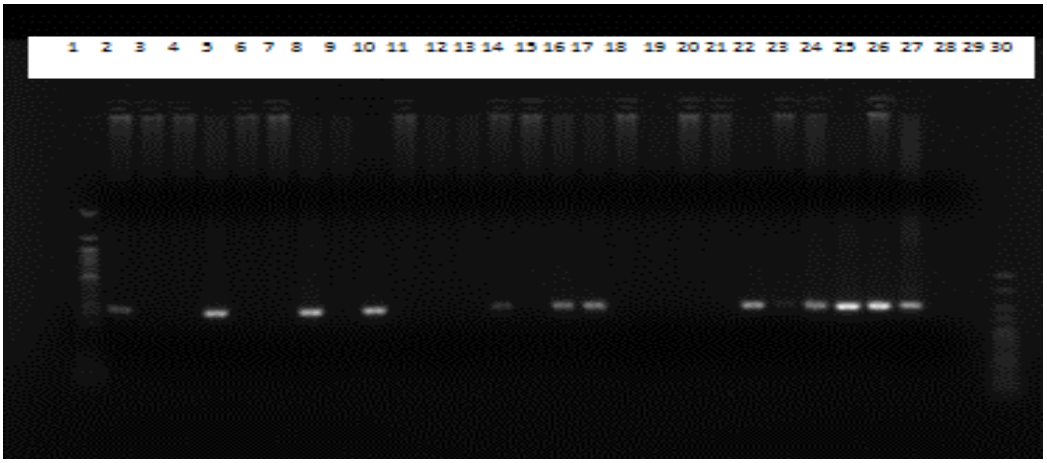
Appendix F: Gel picture from QA samples

Figure i: Nested PCR gel – *Plasmodium falciparum*



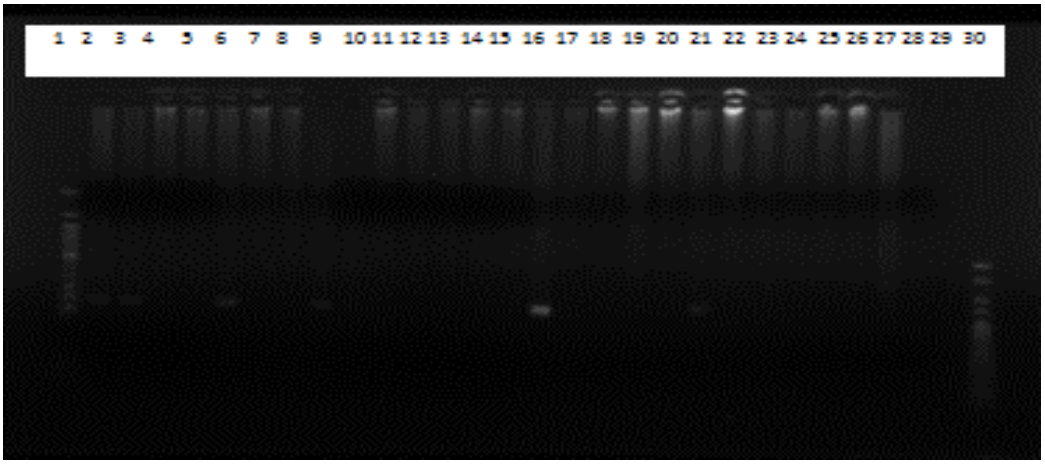
Well 1 50bp ladder, well 2-26 *Pf*, well 27 PC, well 28 NTC, well 30 1kb ladder

Figure ii: Nested PCR gel – *Plasmodium malariae*



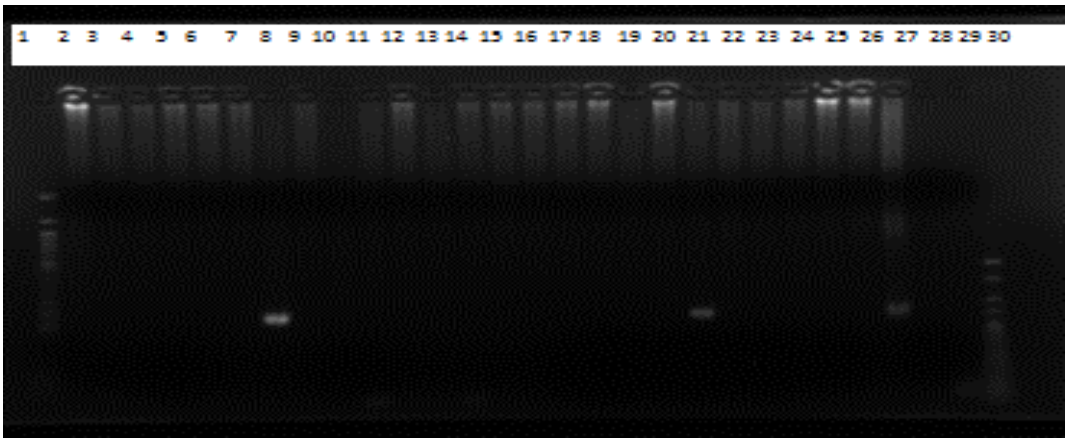
Well 1 50bp ladder, well 2-26 *Pm*, well 27 PC, well 28 NTC, well 30 1kb ladder

Figure iii: Nested PCR gel – *Plasmodium Ovale*



Well 1 50bp ladder, well 2-26 *Po*, well 27 PC, well 28 NTC, well 30 1kb ladder

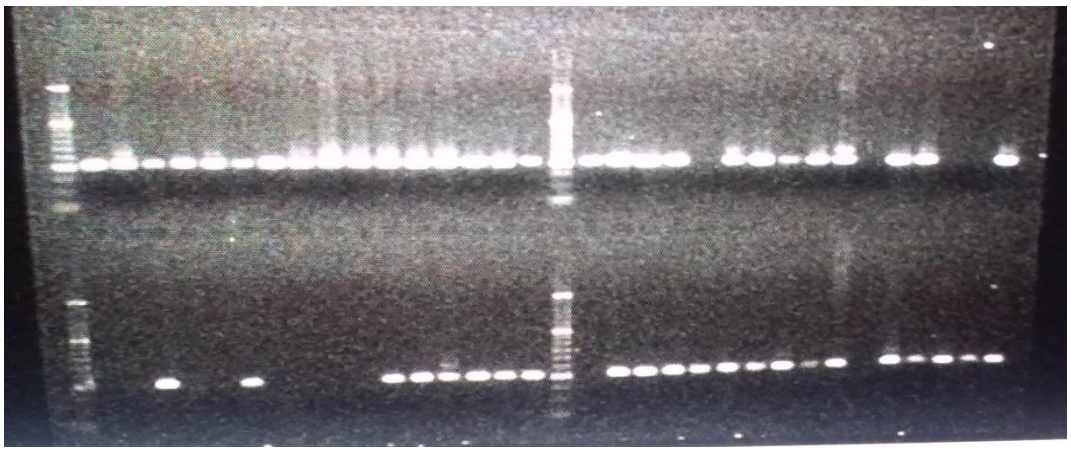
Figure iv: Nested PCR gel – *Plasmodium vivax*



Well 1 50bp ladder, well 2-26 *Po*, well 27 PC, well 28 NTC, well 30 1kb ladder

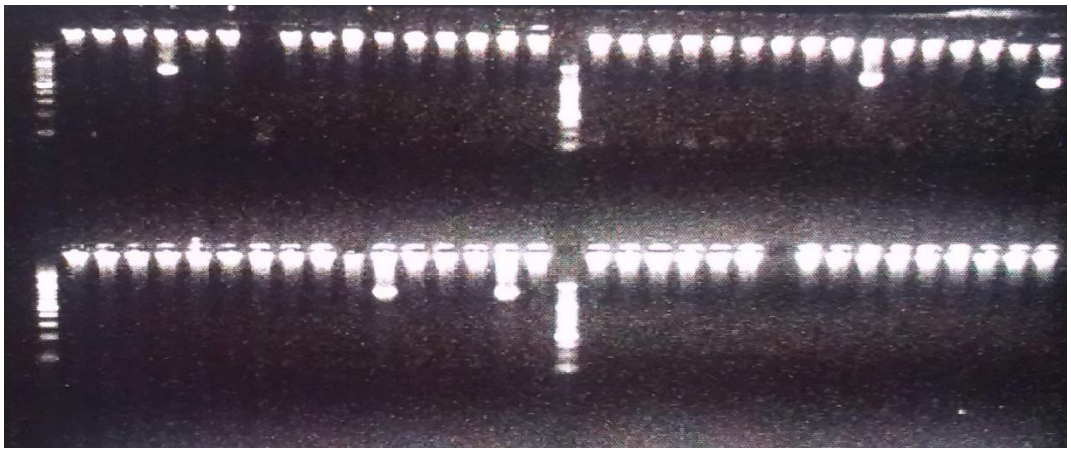
Gel picture from NMCC lab

Figure v: Plasmodium falciparum



Both rows were Pf primers, with 100bp ladders on the left and in the centre. Product band size is 205

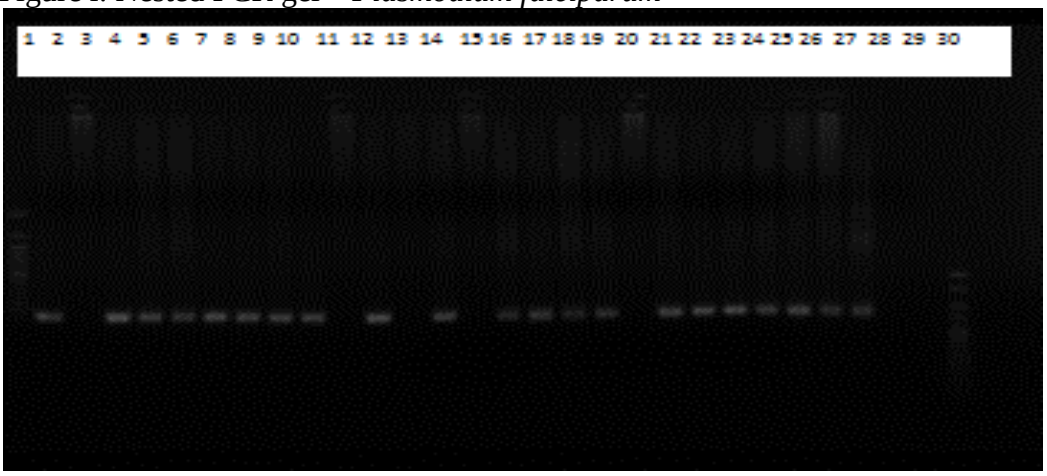
Figure vi: Plasmodium ovale



Both rows were *Po* primers, with 100bp ladders on the left and in the centre. Product band size is 800bp

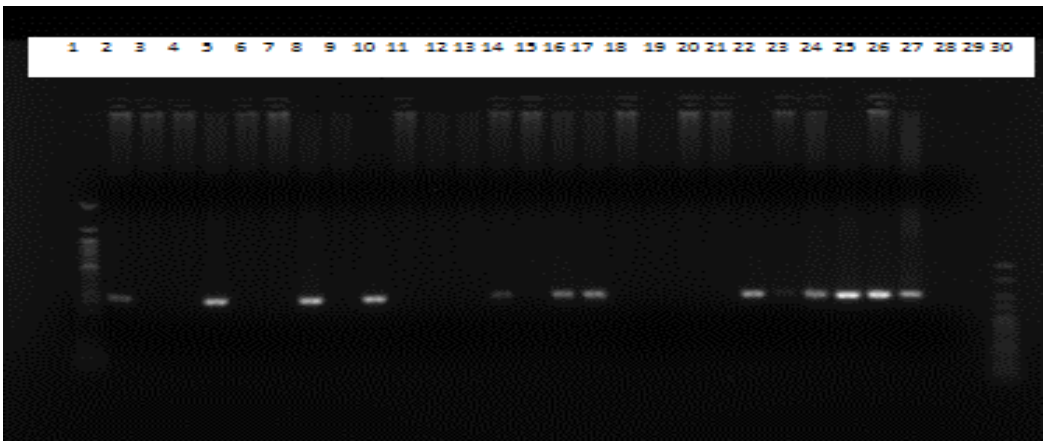
Appendix F: Gel picture from QA samples

Figure i: Nested PCR gel – *Plasmodium falciparum*

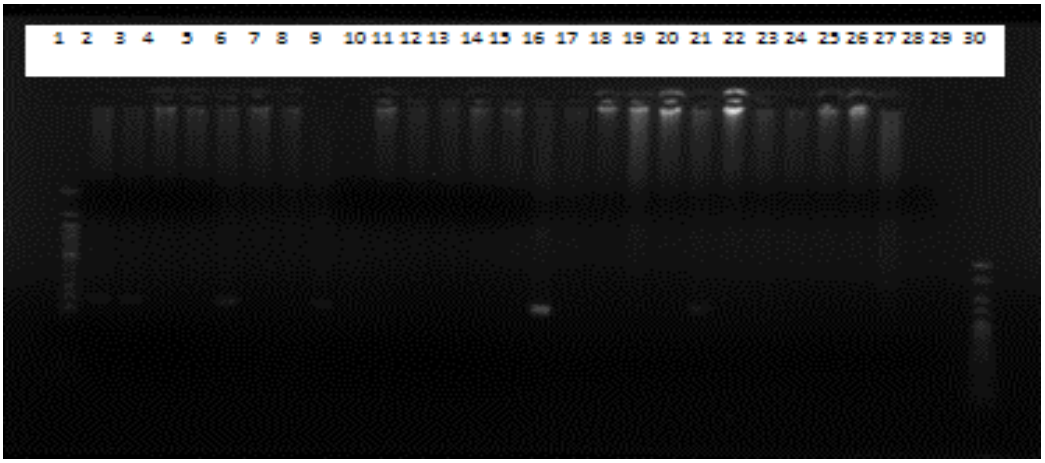


Well 1 50bp ladder, well 2-26 *Pf*, well 27 PC, well 28 NTC, well 30 1kb ladder

Figure ii: Nested PCR gel – *Plasmodium malariae*

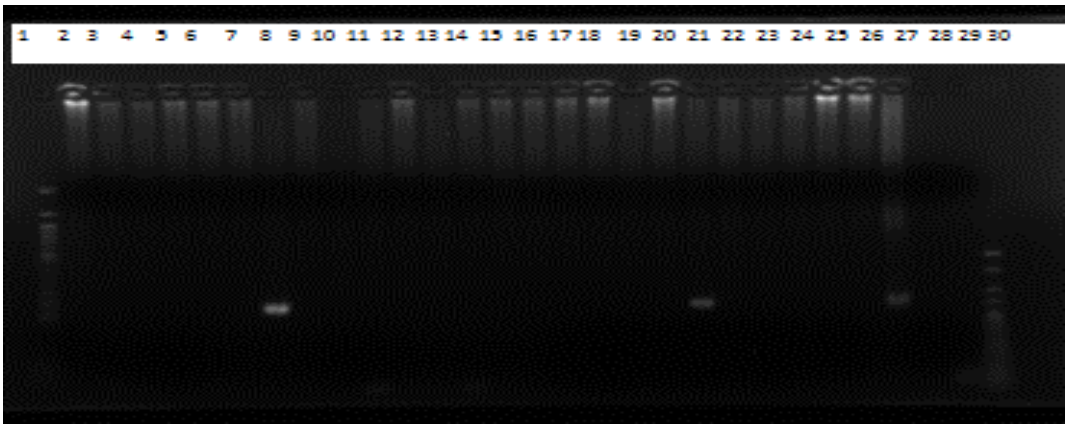


Well 1 50bp ladder, well 2-26 *Pm*, well 27 PC, well 28 NTC, well 30 1kb ladderFigure iii: Nested PCR gel – *Plasmodium Ovale*



Well 1 50bp ladder, well 2-26 *Po*, well 27 PC, well 28 NTC, well 30 1kb ladder

Figure iv: Nested PCR gel – *Plasmodium vivax*



Well 1 50bp ladder, well 2-26 *Po*, well 27 PC, well 28 NTC, well 30 1kb ladder

Gel picture from NMCC lab

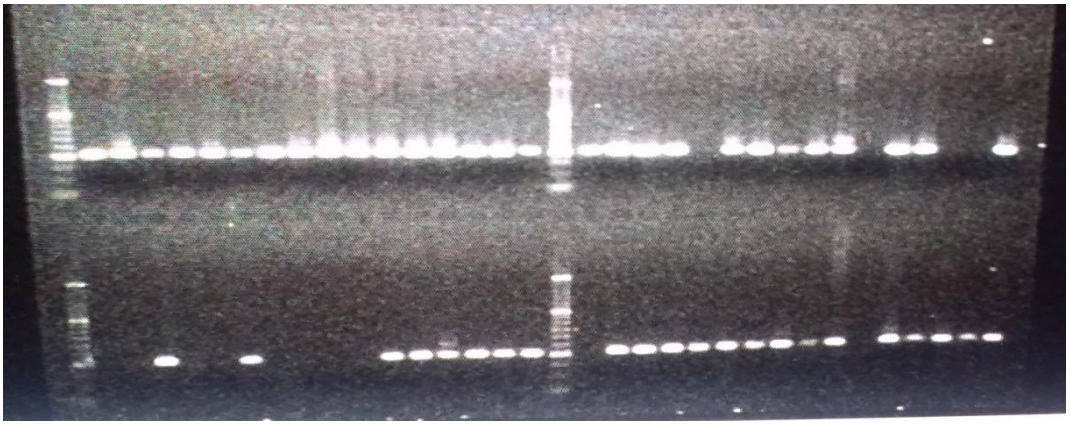
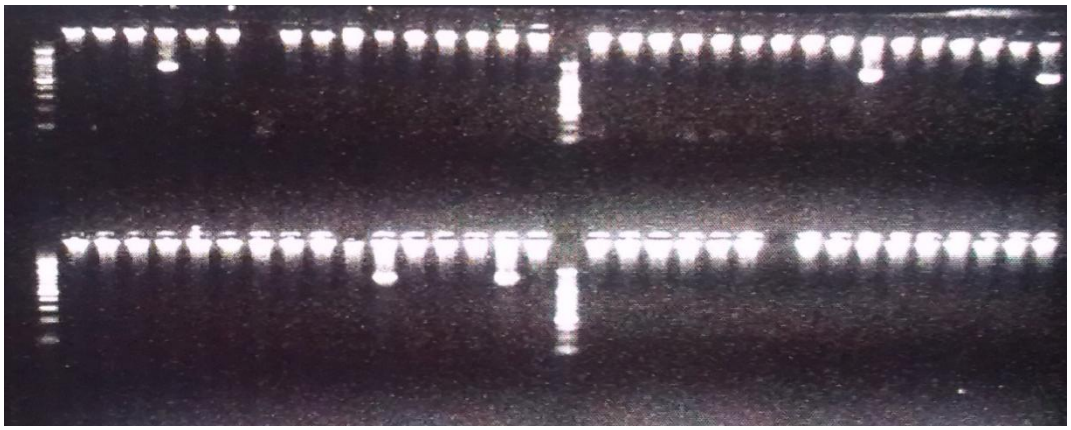


Figure v: Plasmodium falciparum

Both rows were Pf primers, with 100bp ladders on the left and in the centre. Product band size is 205

Figure vi: Plasmodium ovale



Both rows were *Po* primers, with 100bp ladders on the left and in the centre. Product band size is 800bp