MALARIA IMMUNOSURVEILLANCE AND EVALUATION OF RAPID DIAGNOSIS TEST IN MERU SOUTH SUB COUNTY, THARAKA-NITHI COUNTY, KENYA

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156/CE/14159/2009

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (APPLIED MEDICAL PARASITOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

MARCH 2019
DECLARATION

This thesis is my original work and has not been presented for degree or other awards in any other university.

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To my dear wife Idah Nyaga, daughters Betty, Yvonne and Jane whose moral and material support made it possible to complete this work. May it be a good showcase for them.
ACKNOWLEDGMENTS

Thanks to God for granting me the grace to accomplish this task. My heartfelt gratitude goes to my supervisors; Prof. Michael M. Gicheru and Dr. Jemimah Simbauni for their guidance during the course of this study.

My sincere thanks go to all the Medical Laboratory staff at Chuka Level 5 Hospital for their tireless efforts in coordination and preparation of the laboratory specimens. I am also particularly grateful to Mr. Kithinji Rugendo and Mrs. Aileen Muthoni laboratory technician Chuka level 5 Hospital for their patience and participation in the typesetting of this document.

My appreciation goes to Kenyatta University for offering me a chance to pursue post-graduate studies in the Department of Zoological Sciences. I also acknowledge those in this department whose discussions and suggestions greatly contributed to the successful completion of this study. I acknowledge the School of Pure and Applied Sciences and Graduate School for organizing seminars to guide post-graduate students.

I wish to thank my wife, Mrs. Idah Nyaga who agreed to sacrifice family resources to support my studies this was the source of funding for my research. Finally, I am highly indebted to all my student colleagues whose encouragements and shared ideas were of immense value to the completion of this study.
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ABBREVIATIONS AND ACRONYMS

ACC  Automated Blood Cell Counters
ACT  Artemisinin Combination Therapy
CDC  Centre of Disease Control
CLH  Chuka Level 5 Hospital
CMP  Circumsporozoite Protein
EEF  Exo-Erythrocyte Form
FCM  Flow Cytometry Assay
GNP  Gross National Product
HCPM  Host Cell Plasma Membrane
HR   Histidine Rich Protein
IRS  Indoor Residual Spraying
LAMP Loop Mediated Isothermal Amplification
LDMS Laser Desorption Mass Spectroscope
LLITN Long Lasting Insect Treated Nets
MWJ  Malaria World Journal
MOH  Ministry of Health
PCR  Polymerase Chain Reaction
PEC  Pathogenic Erythrocyte Cycle
PFEMP Plasmodium falciparum Erythrocyte Membrane Protein
PLDH Plasmodium Lactate Dehydrogenase
RBC  Red Blood Cell
RDT  Rapid Diagnosis Test
GOK  Government Of Kenya
UNEP  United Nations Environmental Programme
WBC  White Blood Cell
WHO  World Health Organization
ABSTRACT

Despite intensive worldwide attempt to control malaria, it remains one of the most fatal and widespread protozoan infection of mankind. About 2.4 billion people inhabit malaria prone regions which is about forty percentage of the world population. Prompt accurate, diagnosis and treatment is important to avert suffering of malaria patients. Malaria infection is a serious global challenge in the affected countries. The rapid diagnosis tests of malaria are recent diagnostic techniques whose performance has not been evaluated in many malaria endemic regions like Tharaka-Nithi County. The main purpose of this study was to perform malaria immunosurveillance and evaluate performance of rapid diagnosis test for malaria in Meru South Sub County, Tharaka-Nithi County. The study design was hospital based cross-sectional study in the laboratory at Chuka Level 5 Hospital. Three hundred and eighty four blood specimens were used from febrile patients with clinical manifestation of malaria infection. The blood specimens were used for thin, thick smear for microscopy and rapid diagnosis test. The results were analyzed by t-test to compare the two microscopy and Rapid diagnostic methods. A p-value > 0.05 was obtained meaning there is no difference in performance between the Rapid Diagnostic Test (RDT) and microscopic test. The results indicated that RDT had similar performance with microscopy for both positive and negative cases of malaria infection. Analyses for sensitivity, specificity and predictive values of RDT done stood at 98.9%, 97.1% and 96.9% respectively with reference to microscopic tests. The study further sought to determine the incident rates of malaria infections during wet and dry seasons among infants below five years and expectant females with febrile illnesses. Interestingly, incident rates of malaria were significantly high 47.4% during dry season than wet season 30.9%. The results showed that female children had 42.1% incidence higher than male children 30.8%. Further analysis of incident rate of malaria among children less than 5 years and expectant women gave (p < 0.05). There was significant difference in incident rate of malaria among children and expectant women with febrile illnesses p < 0.05, with children having higher incidence rate than expectant women that is 2 to 5 years at 52.34% followed by children less than one year at 41.41% and lowest in expectant mothers at 6.25%. Intensified protection strategies need to be adopted during the dry months of the year. The predominant Plasmodium species diagnosed from children and febrile patients attending Chuka Level Five Hospital was P. falciparum at 81.37% and P. vivax at 18.63%. P. falciparum is relatively higher than P. vivax which is likely to develop anti-malaria infections. In conclusion RDT is appropriate for the use of malaria diagnosis. The study recommends use of RDTs in mass screening for malaria infection, adopt or intensify protective measures during dry seasons and monitoring infections in children.
CHAPTER ONE: INTRODUCTION

1.1 Background information

*Plasmodium* infections are the major reason for morbidity and mortality in the tropical and sub-tropical countries and about one million people die as a result of this disease (Tagbor *et al.*, 2008). Malaria is the highest protozoan infection within tropical and subtropical areas of the world and results in serious medical challenges. Despite intensive worldwide attempt to control malaria in the tropical, it remains one of the most fatal and widespread protozoan infection of human beings especially in some countries in Africa and especially Kenya (WHO, 2009).

Malaria is a global medical challenge in many countries especially within the tropics where environmental conditions are suitable for vector reproduction and also regions near the tropics. It has been estimated that economic burden as a result of malaria infection is also extremely high in malaria endemic countries and the prolonged effects in these countries is the decline in Gross National Domestic Product (GNDP) by greater than half (Mendis, 2001). The disease was once widespread but was successfully eliminated from many countries within temperate regions in 1890 (WHO, 2013). The cases of malaria once untreated results into approximately greater than 300 million serious sickness and at least one million mortality per year. Ninety percent of mortality is associated with malaria occur in Africa, South of Sahara (WHO, 2008). Most children who survive severe malaria episode may have cerebral hemispheres of brain damaged affecting cognitive abilities in children (Boivin, 2002). The expectant women and children are prone to malaria due to low immunity and is a main cause of mortality, morbidity, low birth weight and maternal anaemia (GOK, 2003). In Africa most expectant females infected by *Plasmodium falciparum* seldom
do not result into clinical signs and symptoms of malaria hence remain unattended resulting in approximately one hundred thousand children fatalities in Africa (Dondrop, 2010).

Several clinical and epidemiological studies show concerted efforts to fight malaria. However the problem still persists in endemic areas. In some countries scientist fear that malaria that was successfully eliminated is re-emerging due to environmental changes in temperature contributed by global warming (Snow, 2005). In Kenya statistics from the Ministry of Health (MOH, 2011) reveal that malaria was the cause of death to 28,660 people year 2010 more than half of them from malaria prone regions of Nyanza and Western Kenya (WHO, 2009). In Meru South Sub County the incidences of confirmed malaria increased from 79,000 cases in February 2011 to 126,781 cases in July 2011 (MOH, 2011). This indicates that malaria is becoming a major health challenge which requires intensified plus systematic study to generate effective solutions and interventions. More than forty percent of global population is likely to be infected by *Plasmodium* parasites in many countries especially within the tropics. Approximately more than half a million people are affected by malaria infection yearly. This causes an estimated one and half million deaths, of which over ninety percent are children in the continent of Africa (Mwangi *et al*., 2005).

The cases of *Plasmodium* infection globally appear to be increasing. Several factors may have contributed like; areas where malaria control programmes have reduced due to donor funding, misuse of antimalaria drugs by self-medication and in a relatively rarely cases of continuous expansion of global tourism and immigration (WHO, 2006). The accurate diagnosis of malaria reduces death and sickness due to malaria infections. It has necessitated raise in demand for effective malaria diagnosis and
coordinated global reporting systems for malaria. Identification and differentiation of malaria based on clinical manifestation or descriptive information about the disease from other infectious parasite diseases may be cumbersome. Hence, in such situations, immediate laboratory diagnosis followed by correct antimalaria drug administration for all positive tests results would prevent mortality.

Malaria diagnosis is mainly done using blood smear which are examined microscopically to observe the presence of *Plasmodium* parasites (Mwangi et al., 2005). The procedure for diagnosis of malaria has not changed significantly since the original discovery of *Plasmodium* parasite (Cox-Singh et al., 2008). Microscopic examination and enumeration of malaria parasites in the thick and thin blood smear films is the standard gold test for malaria infections (WHO, 2007). Microscope slides for thick and thin smear specimens are examined to demonstrate presence of different stages of malaria parasites.

Malaria test requires examination of the *Plasmodium* parasites or antigens or waste products of parasite’s metabolism in the patient blood. The procedure is likely to be simple and easy but may be affected by several factors. One of the factors is external appearance and different developmental stages of the *Plasmodium* parasites. The other factor is the immunity of patients. The final factor is penetration of the parasites in the internal organs where immunity is least active or immune protected sites (Ned et al., 2008). A study conducted by Government of Kenya (2013) revealed that standard malaria tests at the main sub county clinics facilities such as in Meru South Sub County could decrease the administration of antimalaria drugs and treatment of non-malaria fevers. The diagnostic procedure requires an experienced laboratory technician. These technicians are inadequate in many Counties of Kenya. Most
laboratory technologist can enumerate 50-100 *Plasmodium* parasites except experienced microscopist who are able to enumerate up to 5 parasites μl of blood (WHO, 2009). This has led to lowering estimates of malaria infections in situations of low parasitemia transmissions and in non-symptomatic malaria.

The capacity to retain high standards of malaria diagnosis is challenge, especially in rural health care facilities where infection is not common and in situations where malaria management is based on patient’s description of fever or other manifestation of malaria symptoms. Modern laboratory test procedures that show acceptable sensitivity, predictive values and specificity, without high deviations from normal standards are immediately required in many parasitology laboratories. The innovative discoveries of molecular and biochemical techniques such as Rapid Diagnostic Tests (RDTs), Polymerase Chain Reaction (PCR), Loop-mediated Isothermal Amplification (LAMP), microarray, Mass Spectrometry (MS), and Flow Cytometric (FCM) assay techniques, have allowed broad understanding characterization of the malaria parasite and improvement in new methods for malaria diagnosis (Roll Back Malaria, 2010). However, malaria test methods present challenges to laboratory technicians in most countries in Africa because of the limitations of diagnosing malaria using microscopic techniques. An accurate and reliable diagnosis is becoming more and more urgently required, in view of continuous drug resistance of *Plasmodium falciparum* to antimalaria drugs. The rapid test and microscopic examination of thick and thin smear tests are recommended Protocol for malaria diagnosis and the performance of RDTs have not been evaluated in several counties in particular Meru-South Sub County (Roll Back Malaria, 2010).
The utility of malaria Rapid Diagnostic Tests has frequently been used in many regions to ascertain presumed cause of fever possibly due to malaria febrile patients and young children. Rapid Diagnostic Tests (RDTs) for malaria have widely been used for detecting and confirming cases of suspected malaria infection in endemic zones. The RDT may have limitations in low transmission settings due to storage facilities and quality control by different manufacturers. Rapid Diagnostic Tests are relatively cheap since RDT cost Ksh 10 per test while microscopy Ksh 50 per test. The RDT are user friendly, and provide results within 5 to 10 minutes. The RDTs are important in detecting presence or absence of *Plasmodium* and type of species since they have high sensitivity and specificity.

Accurate and reliable malaria diagnosis is important for malaria treatment, prevention and control to avert suffering of the patients especially infants as well as pregnant women. In regions where malaria control and prevention targets have been achieved further measures are undertaken to ensure that *Plasmodium* parasite is eliminated from the population. This phase of malaria elimination is done by continuous surveillance and monitoring using recent diagnostic techniques.

1.2 Statement of the problem

*Plasmodium* infection remains a global challenge despite concerted efforts by scientists and governments in eradication because *Plasmodium* species undergo several developmental stages, each with specific antigens and proteins that have made manufacture of vaccines a real challenge (Kilama and Ntoumi, 2009). In the continent of Africa, *Plasmodium* infection is the major result of death in young children of less than five years and expectant females. In Kenya, malaria is life-threatening and burden in malaria prone areas such as Nyanza and Western Kenya (MOH, 2011).
Incidences of malaria infection in Meru South Sub County have increased rapidly despite various efforts by the government to reduce infection rates. The incident and prevalence rates of malaria in children and pregnant women in Meru South Sub County have not been established.

1.3 Study justification

The febrile patients afflicted by different pathogenic microorganisms like bacteria, viruses or mixed infections manifest clinical signs and symptoms similar to those of malaria. Therefore, accurate and reliable laboratory diagnosis must be carried out to ascertain the cause of sickness. The data and information on febrile patients due to malaria rather than other diseases is insufficient in out-patient clinics and little information on malaria surveillance in Meru South Sub County. The evaluation of performance of RDT in Meru South population has not been done in this region. In addition, malaria species predominant in Meru South Sub County has not been established. Thus, this forms the basis of the current study. Although, WHO released documentary medical guides on handling malaria cases, mainly prevention and treatment the compulsory confirmatory tests are inadequately done due to poor quality standards resulting from poor staffing of medical personnel and resource facilities. In common practice, medical personnel and health practitioners have often administered antimalaria medicine to patients based on malaria fever manifestations. This has led to unacceptable administration of antimalaria medicine, probably causing effects of antimalaria drug resistance that increase the cost of disease management and therapy. Inappropriate antimalaria drug administration causes a lot of suffering to febrile patients, causing rise in sickness and death.
The major purpose of this research study was to conduct malaria immunosurveillance and evaluate performance of rapid diagnostic test for malaria in Meru South Sub County, Tharaka-Nithi County.

Across Sub Saharan Africa, few disease specific studies on patients with febrile sickness have been done. The few studies have mainly focused on bacteria and HIV infections in inpatients in hospital settings (Zerpa et al., 2008). The collected data on the incidence rate of indigenous transmissions is important for accurate and specific intervention on clinical and asymptomatic malaria cases reported or diagnosed.

1.4 Research questions
i. What is performance of rapid diagnosis test for malaria as compared to blood smear diagnosis in Meru South Sub County?
ii. What is the incident rate of malaria in children below 5 years and pregnant women with febrile illnesses during wet and dry seasons in Meru South Sub County?
iii. What is the predominant *Plasmodium* species in Meru South Sub County?

1.5 Null Hypothesis
i. Performance of rapid diagnosis test of malaria is superior than microscopy in malaria diagnosis in Meru South Sub County.
ii. Malaria incident rates is the same in children and pregnant women with febrile illnesses during wet and dry season in Meru South Sub County.
iii. There is no difference in distribution of *Plasmodium* species associated with malaria in Meru South Sub-County.
1.6 General objective

To conduct immunosurveillance and evaluate performance of rapid diagnostic test for malaria diagnosis in Meru South Sub County, Tharaka-Nithi County.

1.6.1 Specific objectives

i. To determine the performance of Rapid Diagnostic Test for malaria parasite diagnosis in Meru South Sub County.

ii. To determine the incident rates of malaria infections during wet and dry seasons among young children of the age less than five years and expectant women with febrile illnesses in Meru South Sub County.

iii. To determine the distribution of *Plasmodium* species associated with malaria in Meru South Sub-County

1.7 Significance of the study

The information and data collected from the research study will have important implications in the application of RDT for diagnosis of malaria and improve on treatment of malaria. It will also provide baseline information for setting up control and prevention strategies of malaria in Meru South Sub County, Tharaka-Nithi County. It will provide more information on use of RDT in health care units, where microscopes and qualified technicians are grossly inadequate.

1.8 Assumptions of the study

It was assumed that the patients or guardians cooperated and no interruptions occurred during the study period. It was also assumed that the patients and
guardians who participated in the study came from all the division of Meru South Sub County.
CHAPTER TWO: LITERATURE REVIEW

2.1 Background to malaria infection in Kenya

Malaria is caused by various species of *Plasmodium* namely; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* (found in primates). In Kenya, there are approximately 6.8 million new clinical cases of malaria resulting in about 3900 fatalities per year and most affected were found inhabiting in Nyanza and Western Kenya regions are exposed to significant danger of *Plasmodium* infection (GOK, 2013).

Malaria is recognized public health case and community problem hindering the national economic progress of Country of Kenya. The Second National Health Sector Strategic Plan (NHSSP II, 2005-2010) expanded to 2012), has given highest priority in mitigation of malaria management and control programmes. The purpose of the 2009-2017 National Malaria Strategy (NMS) was to reduce sickness and death associated with malaria by greater than two thirds of the level recorded in the year 2008. In 2010, clinical signs and symptoms diagnosis for malaria accounted for about thirty per cent of outpatient cases reported at hospitals in Kenya. In Meru-South Sub County, incidences of clinical malaria infection increased to 126,781 cases representing about 48% of all diseases reported at Chuka Level 5 Hospital (CLH) during the month of April (MOH, 2011). The mortality rates are less in the urban centers, although average mortality rates are 25%. The average mortality rate of 25% was recorded in patients referred to the public hospital (WHO, 2013).
2.2 Malaria transmission cycle

A mosquito becomes infected by sucking blood from a person infected with malaria. The infected mosquito bites another person and introduces trophozoites into the bloodstream. Through blood circulation the parasites develop in the liver while others remain dormant for some time. The parasites mature in the liver and infect erythrocytes which lysis to release merozoites plus parasites metabolic waste in the bloodstream. Malaria transmission may occur through infected mother to child and through blood transfusion (Wassmer et al., 2005).

2.3 Signs and symptoms of malaria

Malaria signs and symptoms are mainly due to the parasites and damage of the red blood cells. The immune response to infection also contributes to complicated signs and symptoms of malaria. These include; fever above 38°C, paroxysms, joint pains, headache, sweating at night, jaundice, convulsions, anaemia, vomiting, hepatomegaly,
delirium and psychotic behavior (WHO, 2010). In complicated malaria breathing problems, convulsions and fatigue occur (RBM, 2010).

2.4 Epidemiology of malaria

Study of the factors affecting distribution and intensity of malaria in the world has shown different epidemiological patterns for both cerebral malaria and severe malaria anaemia which is the most frequent forms of malaria (Sackett et al., 1991). Severe malaria anaemia associated with lack of iron is often found in endemic zones with high transmission rates most in young children and expectant women than in older generations (Weber et al., 1997). However, cerebral malaria predominates in zones of moderate transmission, mainly where there is seasonal rainfall, and it is seen most frequently in older children leading to brain damage in delayed cases of treatment (Boivin, 2002). Malaria parasite infection occurs mainly within tropical and subtropical areas of American, most regions of Asia and most parts of Africa. The geographical patterns of distribution of malaria within large zones are complicated and it is in Sub-Saharan Africa where over ninety percent cent of malaria fatalities occurs. The regions heavily infested by mosquitoes and mosquito free zones are often close to each other making vector control and prevention to be a continuous collaborative strategic activity (Snow, 2005). In arid and semi-arid areas within tropics, the phenomenon of malaria infection may be determined and postulated precisely with high degree of accuracy by mapping trends in rainfall patterns.

In comparison of malaria infection in remote and urban centers the danger is lower in large cities than in rural areas associated with limited access to health care facilities and education. Malaria is mostly linked with poverty and certainly may be the cause of poverty, the main drawback to wealth creation (Mendis, 2001). In malaria prone
areas traditional diagnosis involved signs and symptoms of malaria causing prophylaxis to be based on assumption instead of laboratory examination to confirm absence or presence of the disease. More frequent, malaria parasitemia is common among patients in many endemic zones, so that in children and expectant women with febrile illnesses require a laboratory test result to confirm the disease progression or recovery from malaria.

The major positive effects on the programmes in malaria interventions and prevention strategies for more than eleven years and the benefits achieved in decreasing sickness and death are not easy to evaluate or quantify (Tagbor et al., 2008). This is because within the usual tradition health care programmes most febrile patients with fever are treated for malaria. This situation makes it important to carry out intermittent short time individual assessment on situation of malaria (WHO, 2009). Malaria infection is major causes of sickness and death in sub-Saharan Africa. Kenya has taken specific strategies in decreasing sickness and death associated with malaria infection leading country’s main public health concern (WHO, 2010). Increasing reports reveals that the disease infection and trends have in Kenya decreased within a period of ten years from 1999 to 2009. The recently updated malaria epidemiology show reduction in prevalence in epidemic zones but expansion in low transmission zones (WHO, 2009). Approximately 60-70 percent of the Kenyan land has a parasite prevalence about 50% which is inhabited by 78% of the Kenyan population (MOH, 2011). The prevalence of malaria is also changing with age group children less than 5 years affected most followed by those 5 to 15 years of age (Ngasala et al., 2008).

The research and studies on malaria infection in adults particularly in endemic zones is inadequate due to assumption that adults develop some acquired immunity with
prolonged encounter with intense mosquito bites. Malaria prevention and interpretation have focused on young children less than five years plus expectant females, as most of malaria morbidity and mortality are mainly reported in the two age groups (Snow et al., 2005). The research studies in West Africa revealed that clinical malaria epidemics manifested in mature people living in high transmission areas (Scholl et al., 2004).

The danger of *Plasmodium* infection in the local set-up where malaria prevalence is high reduces as the individuals become older, implying that immunity may be a correlated with age (Bell et al., 2005). This acquired immunity subsequently passed on to other generations resulting in a decline in transmission of the disease and the incidence rates in that zone. The process through which acquired immunity develops is still not completely well known requires more research. It may be possible that the more mosquito bites individuals encounter lead to continuous antigen changes in the bloodstream conferring the person some immunity. Further studies have shown that the acquired immunity may be developed after relatively short periods exposure and last for a long time (Mwangi et al., 2005).

The immune process that respond to a malaria infection possibly alter with old age, as implied by the age-dependency, evolution of the parasites and the rate at which transmission occur (Talisuna and Meya, 2007). It is commonly noted that acquired immunity effectively reduces signs and symptoms of malaria attacks and eventually decline in parasitemia but does not stop infection. The effects of this response are that the presence of latent blood-stage parasites in a semi-immune host is not synonymous with the disease. Furthermore, the non-specificity of malaria signs and symptoms in adults, make the individual specific diagnosis of clinical malaria difficult. The
research on malaria in semi-immune adults of Africa are rare and attention to the natural history of malaria infection in adults, also the interaction with the immune system (Reyburn et al., 2007).

2.5 Impact of malaria in Kenya

In Kenya, malaria is the main cause of mortality among all infectious diseases accounting for up to 30% of all the deaths. Approximately more than 4 million cases are reported yearly resulting to a rate of 5.1% in those admitted to health facilities (GOK, 2003). About 8.5 million Kenyans are at danger of infection, mainly in the highlands while estimated 20 million people are exposed to stable transmission including 3.5 million children age below five years (Ratsimbasoa et al., 2008). The overall human suffering caused by malaria infection is great with young with children and expectant females having the greatest burden (GOK, 2003). Malaria account for 19% of all admissions to the health care facilities and each year an estimated 26,000 children die from direct consequences of its infection translating into 72 deaths each day (GOK, 2003). Malaria endemic areas in Kenya are Western, Nyanza, Coast, Eastern and Rift valley regions. Areas that lie at altitude above 1600m like Nairobi, Mount Kenya and the highlands are considered malaria free zones (GOK, 2003).

Unstable malaria includes malaria transmission which differs from year to year. This type of malaria occurs in many areas in Kenya including Garissa, Isiolo, Narok, Kajiado, Turkana and Wajir (GOK, 2003). Areas with seasonal malaria include North Eastern, parts of Eastern (Machakos, Embu and Kitui and Rift valley (Marigat and Ngurumani). This eco-zone has been extended in the recent years by population dynamics and small scale irrigation schemes (GOK, 2003). Since 1988 epidermics have been reported frequently in the highlands. The malaria outbreak of 1994 affected
more than 12 Sub Countys of different climatic conditions ranging from the highlands (Kisii, Nyamira and Kericho) to semi-arid lands (Turkana and Narok). Under certain circumstances, migrating populations such as refugees, settlers in new schemes may import the diseases in areas considered free from malaria (GOK, 2003).

2.6 Malaria surveillance, control and prevention strategies in Kenya

Malaria surveillance recommends that the strategies must be set-up continuously and evaluated periodically. The objective of the surveillance is to detect changes in trends or distribution in malaria and other vector borne diseases in order to initiate investigative or urgent control measures (Ngasala et al., 2008). It also set guidelines, approaches and the standards for effective methods of combating the disease. There is possibility of further considerations to collaborate with other similar disease surveillance system so that both resources are utilized simultaneous at minimum cost. The research reports and success achieved are analyzed and used in other regions with the same environmental and climatic challenges. Increased surveillance programmes are likely to combat the spread of infection and once confirmed the region is declared disease free zone. Thereafter programmes are set in place to ensure that the region has completely eliminated malaria. Such a programme may be very expensive in tropical and subtropical countries because within this region it has optimum conditions for mosquito breeding (WHO, 2013).

Malaria surveillance involves community and activities to be initiated by public health care providers to collect comprehensive data related to the disease in order to reduce progression to unacceptable levels of morbidity and mortality (WHO, 2010). The countries within tropical and subtropical regions have high malaria transmission rates accompanied by weak surveillance system causing great challenges in the
control and prevention of diseases (Teun et al., 2017) Efficient malaria programme is important in identifying population groups, trends and additional intervention measures to regulate the disease severity (Bell, 2005). Generally, such knowledge and resources are channeled to the people who require quick attention and immediate treatment of the disease (Fergusson, 2007). Therefore, improvement in prevention of malaria and reduced misuse of drugs as well as resources (Chotivanich and Nicholas, 2006). The process of setting-up malaria surveillance programmes to collect suitable data on epidemiology of the disease changes worldwide. It is influenced by; (i) sick patients (ii) treatment (iii) type of healthcare provider and reporting system recording systems (Hawkes and Kain, 2007). When correct procedures are done and documented the number of malaria cases are less than 10% worldwide. Malaria cases are few in Europe and America and more in Asia (Wongchotigul et al., 2004). Initial malaria management and treatment were based on clinical manifestations of fever and rarely microscopic test and RDT were done but currently confirmatory test must be done to exclude other diseases since frequent ailments have similar signs and symptoms found in malaria (WHO, 2013).

The guidelines on control and prevention of malaria plus setting up elimination phases of malaria however, implementation and interpretation are affected by several factors (WHO, 2012). Some of the factors are socio-economical, cultural and political goodwill to faithfully adhere to the programmes. During the control and prevention phase measures are undertaken to reduce the incidences of malaria by massive education and provision of resources and knowledge on mosquito vector. The febrile patients are adequately informed to seek treatment for the infectious parasitic diseases. The countries that recorded high malaria transmission rates tend to have
middle income with low expenditure for each patient seeking treatment in public health hospitals. It is generally observed that febrile patients are reluctant to seek medical care attention in public hospitals possibly due to irregular and insufficient drug supplies plus personnel availability. Health programmes in low malaria transmission rate setting are usually stronger than in high transmission rates settings resulting in limited diagnostic test and inappropriate treatment of malaria (WHO, 2009). Malaria cases or out breaks may be concentrated in marginalized people or groups like isolated individuals inhabiting refugee camps and water irrigated projects on agricultural land. Therefore, creative approach must be initiated to such people.

During the elimination phase malaria cases occur occasionally in specific zones and imported malaria involve immigrant workers constitute a high percentage. The purpose of malaria control methods are set to reduce indigenous malaria transmissions followed by foreign or introduced infections due to movement of the people. All new malaria transmissions are significant and require be identified and treated as they cause prolonged infection to other individuals.

In Kenya, malaria affects approximately 20 million Kenyans yearly, the overall effect is to increase mankind constrains and hindrances to high quality life and comfort (Snow et al., 2005). It is approximated that annually 26,000 young children less than five years (about 72 per day die) succumb to death due to direct side effects of malaria infection and pregnant women may develop severe anemia and lead to high likelihood of delivering young children with low birth weight (Kilama and Ntoumi, 2009). All Kenyan people are influenced negatively due to monetary problems resulting from malaria infection. It is approximated that about 170 million working days are wasted
annually due to malaria sickness, which in turn lead to low economic growth, causing increased poverty (MOH, 2009).

The spread of malaria is not even due to climatic and environmental factors that provide favourable conditions for mosquito reproduction. These factors accelerate the transmission patterns as well as increase mosquito population density and increase in frequency of anopheles mosquito when sucking the blood. The government of Kenya is responsible regulating and dealing with malaria menace. This lead to specific policy outcomes and timelines on how to combat the disease. The measures outlined in the National Malaria Strategy document are; management of malaria cases, mosquito vector control, and use of long lasting insects treated nets, indoor spraying and prevention of malaria pregnant women. Periodical treatment of patients with sulfadoxine-pyrimethamine and administration of artemisinin-based combination therapy is recommended (MOH, 2011).

2.7 Malaria diagnosis

2.7.1 Microscopy for malaria diagnosis

Malaria test is routinely done demonstration of malaria parasites in the blood smear microscopically (Warhurst and Williams, 1996). The microscope test for malaria has been used for more than three hundred years since the discovery malaria parasite by Laverran (Kilama and Ntoumi, 2009) and modified staining procedures using Romanosky stains in 1890. After about one hundred years, microscopic determination and identification of Plasmodium parasites in Giemsa stained thick and thin smear films was improved and used. Thick smear films are prepared for screening presence or absence of the parasites while thin smear are specifically prepared for species
identification. The use of microscopic test is the standard gold test for malaria for presence or absence of malaria. The test is done by examination on microscope slide the *Plasmodium* parasites and enumerating the parasitemia. Gently and carefully the patient’s finger is cleaned with 70% ethyl alcohol, permitted to air dry and then third fingertip is pricked with a sharp sterile lancet. Two drops of blood are placed on the microscope slide separately for thick and thin smear preparation. It is followed by adding fixative for thick smear then stained for about ten minutes, washed with distilled buffer solution. The slide is dried then examined microscopically. The thin smear preparation involves touching the drop of blood specimen with slide then move gently upwards at an angle of about 45°C swiftly along the surface. The smear is allowed to air dry then fixed with absolute methanol and washed. It is stained with Giemsa and washed carefully over running water, and then examination is done at high power objective lens to identify the species present. The procedure is simple and cheap but requires experienced microscopist to accurately perform the diagnosis (WHO, 2010).

A research by (Ngasala *et al*., 2008) on effects on training in symptomatic and microscopic detection of malaria in children on anti-malaria drug dispensation and administration in primary medical health care in Tanzania showed reduction in the application of antimalaria drugs. This approach on malaria management has assisted in improvement on treatment of febrile patients with non-malaria fever. The process of staining, fixing, drying of microscope slides and examination of the slides at high power objective lens plus recording results is cumbersome compared with rapid diagnostic tests. Most experienced microscopist can examine accurately up to 5 parasites/µl, of blood the average microscopist examine accurately up to only 50-100
parasites/µl of blood. This lead to possibility of underestimating malaria infection rates, mainly cases with low parasitemia and asymptomatic malaria. The ability to maintain high standards of malaria diagnosis is cumbersome in rural health care facilities particularly in places where disease is not common. The microscopic examination is tedious process used by trained experts therefore unsuitable in some medical health settings especially in poorly equipped healthcare facility.

To diagnose malaria promptly, physicians must obtain a travel history from every febrile patient. Malaria should be included in the differential diagnosis of every febrile patient who has traveled to a malaria prone area. If malaria is suspected, a Giemsa-stained film of the patient's peripheral blood should be examined for parasites as soon as possible. Thick and thin blood smears are prepared correctly because diagnostic accuracy depends on blood smear quality and examination by experienced laboratory personnel. This simple test can quickly detect the presence of malaria parasites and can also be used to determine the species and percentage of red blood cells that are infected, which are important for the appropriate treatment of persons infected with malaria (Farcas et al., 2009). On light microscope examination of the blood film the number, species, and morphological stage of the parasites reported (Figure 2.2).
Figure 2.2: Microscopic view of morphological stage of malaria parasites (Chotivanich et al., 2006). Left: photomicrographs of stage development (ring, trophozoites and schizont) of *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale* from thin smears. Right: photomicrographs of *P. falciparum* from thin blood smear (top), thick blood smear (middle) and brain smear (bottom).
2.7.2 Polymerase Chain Reaction (PCR) technique for malaria diagnosis

Polymerase Chain Reaction-based techniques are a recent development in the molecular diagnosis of Plasmodium parasites, and have proven to be the most specific and sensitive diagnostic methods especially for malaria cases with low parasitemia or mixed infections (Morassin et al., 2002). The PCR method is based on the Plasmodium 18s-rRNA gene and Plasmodium cytochrome oxidase III gene which are detected in the blood sample when there is infection. The PCR method has higher sensitivity and specificity than microscopic method, however it is not commonly used because it is expensive. It is used widely to identify Plasmodium and management of patient with other complications not related to malaria (Hawkes and Kain, 2007). There are different improved types of PCR which are mainly used in research to confirm presence of Plasmodium knowlesi which one of the malaria parasites found in primates (Cox-Singh, 2008).

2.7.3 Loop Mediated Isothermal Amplification (LAMP) technique for malaria diagnosis

Loop Mediated Isothermal Amplification (LAMP) Technique is usually uncomplicated Plasmodium detection which props the coded genes in Re-oxy Ribonucleic Acid (RNA) gene of P. falciparum (Han, 2008). A set of four specially designed primers are used to recognize six distinct regions of the target parasite DNA. The amplification is conducted under isothermal conditions and accumulated approximately 109 copies of the target DNA within a time of less than 30 minutes. In areas of low endemicity, a portable LAMP based Assay (Real-time Fluorescence Loop Mediated Isothermal Amplification) may be used.
2.7.4 Microarrays for malaria diagnosis

The microarray identifies the genome of *Plasmodium* present in the infected erythrocytes. The infected erythrocytes are isolated into microarray chambers and detected by a fluorescence detector applied. It allows very fast profile for many specimen samples for detecting *Plasmodium* parasites in the infected erythrocytes. Microarray test is important and powerful tool for malaria research although it requires specialized equipment for processing and fluorescence scanning (Doolan *et al*., 2004).

2.7.5 Flow Cytometry (FCM) assay for malaria diagnosis

Flow cytometry for malaria diagnosis is based on automated hematology analyzers to detect intra-erythrocytic abnormalities related to malaria infection. This is important in routine laboratory procedures for febrile patients coming from malaria prone zones. The blood cells analyser use Cell-Dyn, Counter-GENS-S and the Sysmex XE-2100 analyser. The Cell Dyn analyzer detects abnormal depolarization of granular monocytes due to *Plasmodium* infection. The Counter analyser detects the malaria factor from lymphocytes size deviations from the normal cells. The Sysmex XE-2100 detects the abnormal patterns in psedoeosinophils and other malfunction hematological features. Further developments in new haematology analyzers with easy, vibrant and cheap automated for malaria identification necessary (Wongchotigul *et al*., 2004).

2.7.6 Automated Blood Cell Counters for malaria diagnosis

An automated blood cell counters (ACC) detects malaria pigment (hemozoin) released from lysed monocytes. The techniques uses depolarized laser light from
DynR-3500 to detect pigment hemozoin from infected monocytes. The high accuracy and reliability of this method promise, suitable technique for detecting malaria parasites in routine malaria-diagnosis in a laboratory (Ratsimbasa et al., 2008).

2.7.7 Mass Spectroscopy for malaria diagnosis
Mass spectroscopy for malaria detection involves ultraviolet Laser Desorption Mass Spectroscopy by direct identifying a specific biomarker (heme) in the blood. The infected erythrocytes release heme-structure specific from lysed red blood cells which is detected by LDMS. Intense ion signal are observed from ferrisoporphyrin IX (heme) released by malaria parasites during their growth in human red blood cells and correlates with the presence of Plasmodium parasites in the blood (Scholl, 2004). The mass spectroscopy diagnosis is usually very fast and results analyzed within less than one minute. Recently electricity has been supplied in many remote rural areas therefore, this method may be suitable for mass malaria screening and surveillance. Several, accurate and reliable malaria diagnosis test procedures have been developed and introduced for malaria research. The Plasmodium parasites have been detected and isolated in tissue autopsy like in spleen, brain and kidneys (Tagbor et al., 2008).

2.7.8 Rapid Diagnostic Test for malaria diagnosis
It is an immune chromatographic assay for initial detection, identification and confirmation of Plasmodium species. The method involves the dynamic flow liquid on the surface of strip paper. Parasite antigen react with antibodies of superficial blood collected in the specimen prepared against a malaria antigen target conjugated to either selenium dye or gold particles in a mobile phase. The Plasmodium Lactate Dehydrogenase (PLDH) enzyme disintegrates almost immediately there is effective
treatment of malaria therefore absent in the patient’s bloodstream but if treatment fails the enzyme persists. (WHO, 2013). The movement of antigen-antibody complex in the mobile phase along a nitrocellose tape allows the labeled antigen to be accurately captured by monoclonal antibodies of immobile phase to form macro-complex molecule. It can detect presence or absence of *Plasmodium* lactase dehydrogenase (PLDH), a 33kD De-oxireductase enzyme. The lack or presence of PLDH after treatment implies that test may be important in determination of effective treatment or inadequate treatment possibly due to drug resistance and other factors or failure. The results are visible in the reader’s window by observing one coloured line for negative test results and two coloured lines for positive test results plus species identified. The RDTs have been improved and modified in various diagnostic test designs. In some RDT kit, they have one well where blood sample is placed followed by the buffer solution which causes the components to be dynamic along the nitrocellose tape.

The blood specimen is usually about one drop obtained from the patient third finger is pricked then placed in the first well and a buffer solution placed in the second well that contains a hemolyzing reagent and a specific antibody that is labeled with a visually detectable marker likes colloidal gold particle (WHO, 2007). In some kits, labeled antibody is incorporated in the reagent and lysing washing buffer introduced. If the target antigen is present in the blood, a labeled antigen/antibody complex is formed and it moves up the nitrocellose tape to be captured by the incorporated antibodies specific against the antigens and against the labeled antibody (as in user guide manual). A rinsing buffer solution is then put to clear remains of the hemoglobin and allow observation of the colored lines developed along the tape the antigen-antibody reaction complex. The pLDH enzyme and aldolase are designed to
detect a parasitemia of greater than 100 to 200 parasites/µL and some of the PfHRP2 protein identifies asexual phase parasites reproduction at very low parasitemia of below 40 parasites/µL. The RDT and microscopic diagnosis have high sensitivity and specificity for malaria test followed by treatment in endemic areas (WHO, 2006). If diagnosis of interested groups of people is found necessary, then, rapid diagnostic test or microscopy may be applied for symptomatic and asymptomatic infections. The microscopic diagnosis is the standard gold test for malaria infection but the most effective and appropriate test for mass diagnosis would be RDT since it is easy to use without skilled personnel. It should be realized that the life of child or expectant woman is threatened from *P. falciparum* malaria infection which is likely to cause death that diagnosis must not be miss out (WHO, 2009).

Figure 2.3: Sample Rapid Diagnostic Test Strips Showing Test Results and demonstrating how RDT works (Farcas *et al.*, 2009)
2.8 Accuracy of malaria diagnosis

Prompt accurate and reliable test for *Plasmodium* malaria parasites in the blood smear is necessary in order to decrease suffering and risk of death. It is not possible to diagnose malaria clinically since the signs and symptoms presentations are similar to other parasitic infections hence confirmatory test must be done (WHO, 2008). The test for malaria may be a challenge in several laboratories due to inadequate quality standards in some health care facilities. The prompt and need for getting fast results from the microscopic slide examination of blood smear from suspected patients with signs and symptoms of malaria make some of more sensitive tests methods impractical for usual laboratory use. High sensitivity and specificity of malaria test is required in all situations and essential for the many susceptible people that risk mortality.

Microscopy blood smear is the presently the gold test for detection and may be incorporated in other related disease prevention and control activities. All malaria cases must be confirmed using microscopic test or RDT before drug administration is done to febrile patients (WHO, 2009). Treatment must always be based on diagnostic test results unless under emergency situations like first aid assistance where patient may not reach to the hospital (WHO, 2010). Parasite examination of *Plasmodium* is presently the recommended global public requirement in treatment of malaria. The laboratory diagnosis should be performed and only positive tests will be required for administration of ant-malaria drugs (WHO, 2012). It is estimated that increasing malaria control strategies will lead to decline in malaria infection to achieve the planned disease elimination strategies (Roll Back Malaria, 2010).
Previously patients were mistakenly treated for malaria by under-dosage or over-treatment leading to serious ant-malaria drug resistance in endemic regions (WHO, 2009). However, all presumed fever must be accurately diagnosed either using microscopy or RDT followed by appropriate interventions but in scarce poor resource settings febrile patients continue to misuse antimalaria drugs. Indeed many public health practitioners depend on manifestation of malaria attack for ant-malaria drug administration treatment of malaria (Reyburn et al., 2007). Thus, most clinicians often rely on clinical signs and symptoms for treatment of malaria, especially where patients are overcrowded in remote health care facilities where diagnostic tests are not available.

2.8.1 Sensitivity of Rapid Diagnostic Test for malaria

Guidelines and protocol on malaria case treatment must be based on appropriate diagnostic test and not clinical signs and symptoms (WHO, 2009). However, in high malaria transmission settings, this protocol may limit the commitment to the guidelines where sources are inadequate or absent. The application immunochromatographic assay using rapid diagnostic test provides important approach for management of malaria in situations where microscopy may not be possible. The limited resources may be complicated by low budgetary allocation due to other emerging tropical diseases requiring immediate attention. Sensitivity of RDTs refers to their ability to demonstrate reliable identification of malaria parasites at moderate parasite densities (200 parasites/μl). The RDTs are not affected by tropical temperatures hence easy to use, store and above all identify the *Plasmodium* species.
Recommended sensitivity for RDTs is 95% at 100 parasites /µl of blood. Sensitivity is mainly associated with the amount of target antigen available, thus change slightly with parasitemia (Zerpa et al., 2008).

Similar diagnosis could attain a high sensitivity in a group of people in which all sick individuals have high parasitemia greater than 1000 parasites/µl and attain low sensitivity and specificity in regions with parasitemia less than 50 parasites/ µl. The sensitivity and specificity of RDTs are stated in the manufacturers user guide although slight variation may occur due to parasite density in the study area. The RDTs must be sensitive and specific enough to accurately and reliably diagnose malaria at moderate parasitemia. The most important approach is the adherence to the manufacture’s guide in performing the test and recording as stated in care Start Malaria Pf, Somerset Journal 08873 USA. This study sought to determine the sensitivity and specificity of RDTs used for malaria diagnosis in Chuka Level Five Hospital, Tharaka-Nithi County.

2.9 Incident rates of malaria infections among children and pregnant women

Incident rate of malaria parasitemia in young children less than five years of age without signs and symptoms of malaria was 42% and 41% in schools, Adansi South and Wa West District in Ghana respectively. The average parasite density in Adansi South and Wa West District showed positive correlation but there were differences observed across individual schools. There was high trend of malaria parasite infection without fever in rural villages (Nsata Subiro, (63%) than in the urban centres (New Edbuiase (21%). While the overall parasite density in the two districts was similar, there was a strong variation of parasitemia across schools. The malaria parasite
infection without fever was higher in the remote villages (Nsata Subiriso, 63%) than in the Sub County capital (New Edubiase, 21%). The incidence rates of malaria were higher in families living in rural areas than in urban areas (Weber, 1997). Microscopic diagnosis of malaria parasites in the research presented may be affected due to lower sensitivity and specificity of RDT than PCR plus information on multiplicity of *P. falciparum* infection is inadequate. The infections of severe malaria in young children affect cognitive and academic performance (Kyabayinze et al., 2008).

A study conducted in Gambia by Weber (1997) also found that the highest incidence of malaria, 26 percent was detected in children 12 to 13 months old and 24 percent was found in young children of age between 24 to 35 months. Malaria was less common among children aged less than one year of age (12 percent) than those aged 48 to 59 months (16 percent). The incidence rate of malaria along Indian Ocean in Kenya was 8 percent and Lake Victoria at 28 percent (WHO, 2009). However, in Meru South Sub County the incident rates of malaria in children and pregnant women with febrile sickness have not yet been established. This was one of the research gaps that this study sought to address. Secondly, the evaluation of performance of RDT and identification of malaria species predominant in Meru South Sub Country has been not established.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

This study was carried out at Chuka Level Five Referral Hospital laboratory, Meru South Sub County in Tharaka-Nithi County. The hospital serves as a referral for patients from the following dispensaries; Mpuoni, Consolata Cottage, Chera, Chogoria, Nkaanwa, Kajuki, Kambandi, Magumoni, Igambang’ombe and private health clinics in the Sub County. The divisions covered were Chuka, Magumoni, Maara, Igambang’sombe and Kajuki which are endemic to malaria (Fig 3.1). Meru South Sub County of Tharaka Nithi County is situated between 37° 19’ and 37° 29’ East and Latitude 00° 08’ and 00° 26’ South. The approximate total area of the Sub County is 102.9 Km².

The altitude ranges from 5200m to 600m above sea level in lower areas. The rainfall occurs in two seasons per year in the months of April to June and in October - December. The rainfall is high (about 2200mm) in Chogoria Forest and (about 500mm) in lower regions of Igambang’ombe sub-division. Temperature in highlands ranges from low 14°C - 17°C while in lower part mainly lowlands ranges from 22°C - 29°C. The population is cosmopolitan and the area is basically urban with most of its land area occupied by residential settlement.
Figure 3.1: Map of the study area  (Extract of Tharaka Nithi County Map, 2014)
3.2 Study population

The study population consisted of febrile sick individuals with clinical manifestations of malaria who were referred for malaria diagnosis in the laboratory by the clinicians and consisted of children of age less than five years and expectant women of different age groups (18-49 years).

3.3 Inclusion and exclusion criteria

The parents or guardians who had lived in Meru South Sub County for more than five years were included in the study. Only the parents or guardians who gave written consent were included in the study (Appendix II). Those who consented were examined by clinical officers and those found to have malaria signs and symptoms included in the study. The children and expectant women who were recommended by clinicians for diagnosis of other infectious diseases apart from malaria were exempted from the research study.

3.4 Study design

The study design was hospital based cross sectional study. The study population was selected through purposive sampling. The sample size was obtained through systematic sampling. The young children of less than five years and expectant women were examined by clinicians and those with febrile illnesses were referred to the laboratory. Purposive sampling technique was used where every three children and one febrile expectant woman who came to the hospital were sampled every day for six months. Data collected in the first three months (January, February, and March) were dry season while the data collected in the months of (April, May and June) were wet season. The blood samples were collected by use of finger pricks by using sterile
needles by laboratory technician for analysis. The samples collected were analyzed to determine the incident rate during the dry season (January - March) and wet season (April - June). The predominant *Plasmodium* species diagnosed from the samples were analysed.

### 3.5 Sample size determination

The sample size was calculated as described by Fisher *et al.*, (2005) and (Wayne, 2010) for calculating sample size where population was less than 10,000.

Thus, \( n = \frac{Z^2 \times P \times q \times D}{d^2} \) where \( n \) is the sample size in population less than 10000, \( Z \) is standard deviation \((Z=1.96)\), \( p \) is the percentage of the target population approximated to have a characteristic infection; (in this case, the general prevalence of malaria was 48% at Chuka Level 5 Hospital (MOH, 2011). \( q=1- p \), hence, 1- 0.48 = 0.52, \( D \) was design effect=1, \( d \) was degree of accuracy at 5% \((0.05)\), Confidence level, 95%

\[
n = \frac{(1.96)^2 \times 0.48 \times 0.52 \times 1}{(0.05)^2} = 384
\]

Therefore three hundred and eighty four blood specimens were prepared for examination and subjected to both microscopic diagnosis and RDT for malaria test. According to Sackett (1991) a minimum sample size of seven hundred and fifty is appropriate for determination of diagnostic test performance. Therefore, eight hundred samples were collected for determination of sensitivity, specificity and predictive values.
3.6 Laboratory procedures

3.6.1 Sampling thick smear

The third finger of the left hand of the patient was held between the thumb and finger at the first phalangeal joint. It was wiped at fingertip with a swap dipped in 70% ethanol, then allowed to air dry. A sterile needle was used to prick the finger and blood was allowed to ooze out. A clean dust free slide collected three drops of blood about 0.3ml. One drop of blood was placed at the centre of the slide and spread uniformly. The microscope slide was marked and allowed to air dry (Talisuna and Meya 2007). Thick smear contained 10 layers of RBC and about 10-12 layers of WBC (Figure 3.2).

![Figure 3.2: Thick blood smear](image)

3.6.2 Thin smear

The second drop of blood was placed at the centre of the slide. The drop of blood was spread at an angle of 30 to 45 degrees from horizontal and pushed the spreader steadily down the surface of the slide to draw the blood behind till the smear was uniform (Figure 3.3). It appeared tongue shaped, and had a single layer of RBC. It was air dried and slide number marked. The thin smear was fixed with absolute methanol for 5 minutes and washed with tap water to remove excess methanol.

![Figure 3.3: Thin blood smear](image)
The thick and thin smear films were stained using freshly prepared 3% Giemsa solution. The stain solution was prepared by measuring 3 ml of Giemsa stock solution to 97 ml of buffered distilled water. The Giemsa stain was poured carefully into the trough until the slides were fully dipped into the solution. The slides were left for 20-35 minutes to stain the parasite. The remaining stain was poured off and the slides rinsed in tap water gently. The slides were removed one by one and the smear was placed downwards on a drying rack to drain out and dry, confirming that thick films did not come in contact with the edge of slide to avoid scratching the smear.

3.6.3 Examination of slides

A drop of oil immersion was added to microscope slide for thick smear preparation and then examined microscopically using X100 objective lens at carefully stained smear free of staining debris with well-populated WBC. The slide was examined for 100 fields by adjusting the specimen along the length of the width of microscope slide. A minimum of 20 fields were examined to confirm the test results. The results for both negative and positive were recorded in a form (WHO, 2011).

Thin film was used for *Plasmodium* species identification. The parasitemia was recorded as number of asexual parasites counted per 200 white blood cells present in the thick smear. The thick smear was considered to be negative, if no parasite had been found in 100 fields at 1,000 × magnification. All the thick and thin blood smears were read immediately by the laboratory technician and secondly read by senior laboratory technician at Chuka Level 5 Hospital as a quality control measure. Blood samples were examined using RDT strips (based on the manufacturer’s instructions) and results were read between 5 and 10 minutes.
3.6.4 Rapid malaria diagnosis test

The RDT contained a stained conjugated antibody (ab) specific for target antigen (Ag) found at the lower end of the nitrocellulose strip or in a well provided in the strip. Antibody specific for the target antigen was bound to the strip in a thin test line and either antibody specific for the labeled antibody or antigen bound at the control line. The drop of blood specimen was placed at the well and mixed with buffer solution as per the manufacturer’s instructions (WHO, 2009). The labeled antibodies move up the tape along the bound antibodies. In case antigen was present some labeled antibodies reacted at the test line and the other labeled antibodies were trapped at the control line to form coloured complexes. The results were read visually by looking and observing coloured line corresponding to *Plasmodium* species and the control line. The results were recorded positive or negative and the species present or absent identified (Figure 3.4). These results were recorded in the check list against microscopic results.

![Negative and positive results for RDT](image)

**Figure 3.4: Negative and positive results for RDT**

3.7 Data management and statistical analysis

The data generated was processed by SPSS (Statistical Packages for Social Sciences) software. Descriptive statistics was analyzed by use of mean, variance, standard deviation and use of percentages while inferential statistics there was the use of t-test, two-way ANOVA and F-test. The t-test and ANOVA were used to compare frequencies of predominant species causing malaria. The t-test was used to compare RDT and microscopy results and F-test was used to analyze the incidence rate of
malaria during wet and dry season in children of less than five years and expectant women with febrile illnesses. The data was presented in tables and bar graphs. Sensitivity, which is the proportion of true positive test results (TP) divided by the total number of infected patients (TIP) multiplied by a hundred. This detects presence of a disease in a given sample population.

\[
\text{Sensitivity} = \frac{TP}{TUP} \times 100 \quad \text{(WHO, 2009)}.
\]

\[
= \frac{380}{384} \times 100
\]

\[
= 98.9\%
\]

Specificity, which is the proportion of true negative test results (TN) was obtained by dividing the total number of uninfected patients. It detects absence of the disease in a sample population.

\[
\text{Specificity} = \frac{TN}{TN+FP} \times 100 \quad \text{(WHO, 2009)}.
\]

\[
= \frac{404}{416} \times 100
\]

\[
= 97.1\%
\]

Predictive value which refers to the percentage of true positive test (TP) which was determined by dividing true positive (TP) by sum of false positive (FP) and true positive result in a sample population.

\[
\text{Positive predictive value} = \frac{TP}{TP+FP} \times 100 \quad \text{(WHO, 2009)}.
\]

\[
= \frac{380}{396} \times 100
\]

\[
= 95.95\%
\]
Negative predictive value, which is the percentage of true negative test result (TN) which was determined by dividing the total true negative result (TN) and false negative result (FN).

Negative predictive value = \( \frac{TN}{TN+FN} \times 100 \) (WHO, 2009). Formula …………… 4

\[ \frac{404}{408} \times 100 = 99.0 \% \]

The performance of RDT was determined by calculating sensitivity positive predictive values, specificity, and negative predictive value. The generated data was programmed and analyzed by using SPSS version 21 and Java Stat –two way contingency table. The sensitivity, specificity, positive and negative predictive values were calculated for microscopic and RDT test. A p-value of less than 0.05 was considered significant in all test comparisons. The collected data were computerized using Excel program, exported and analyzed by SPSS version 21 and Java Stat two-way contingency table analysis. Sensitivity, specificity, and positive and negative predictive values were determined for both tests and compared with one another. Sensitivity was calculated as the proportion of true positive test results obtained among samples containing malaria parasites. Specificity was calculated as the proportion of true negative test results among samples negative in microscopy. Positive and negative predictive values were obtained by the proportion of true-positive results among all positive samples and the proportion of true negative results among all positive and negative samples, respectively. Sensitivity, specificity, positive and negative predictive values were determined for RDT. At 95% confidence interval (95% CI) was given for each parameter.
3.8 Ethical considerations

The quality and integrity of research was upheld. The participants were enlightened on the importance of this research so that they could participate voluntarily. The researcher respected the confidentiality and anonymity of the participant in the research. Throughout the research there was no harm to the participants. The permission to carry out study was granted by Kenyatta University. Authorization to carry out the research was granted by Meru South Sub County Medical Officer of Health.
CHAPTER FOUR: RESULTS

4.1 Socio-demographic characteristics of the participants

In Table 4.1. A total of 384 samples were analyzed from the enrolled participants. These comprised 154 male children and 206 female children and 24 expectant women. Approximately 360 (93.7 %) patients were children aged below 5 years and 24 (6.3 %) patients were expectant women. In this study, 59.9% of patients with malaria were females and 44.1% were males.

Table 4.1 Sample size 1.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Male children &lt; 5 years</th>
<th>Female children &lt; 5 years</th>
<th>Expectant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>384</td>
<td>154</td>
<td>206</td>
<td>24</td>
</tr>
</tbody>
</table>

A sample of 384 was used to compare RTD and Microscopic test for objective 1 and 2.

Table 4.2. Sample size 2.

<table>
<thead>
<tr>
<th>Participants</th>
<th>n cases</th>
<th>Positive cases RDT</th>
<th>Negative cases RDT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasmodium falciparum</td>
<td>Plasmodium vivax</td>
</tr>
<tr>
<td>Male children &lt; 5 years</td>
<td>246</td>
<td>95</td>
<td>22</td>
</tr>
<tr>
<td>Female children&lt; 5 years</td>
<td>336</td>
<td>132</td>
<td>29</td>
</tr>
<tr>
<td>Expectant Women</td>
<td>218</td>
<td>89</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>800</td>
<td>316</td>
<td>68</td>
</tr>
</tbody>
</table>

A sample 800 was used to determine performance of RDT and predominant Plasmodium species for objective 1 and 3.
4.2 Performance of RDT and microscopy for malaria parasite diagnosis

The study evaluated the performance of RDTs in terms of accuracy and reliability compared to microscopy for detecting *Plasmodium falciparum* parasites in the blood. The results show that 274 cases tested positive accounting for 71.35% with microscopic test as compared with 272 positive cases (70.83%) with RDT. Negative cases were 28.65% microscopic test compared to 29.17% for RDT test. The results of tests using RDT and microscopy are shown in Table 4.3

**Table 4.3: Performance of RDT and Microscopy Tests for malaria diagnosis**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Microscopy</th>
<th>Percentage %</th>
<th>RDT</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cases</td>
<td>274</td>
<td>71.35</td>
<td>272</td>
<td>70.83</td>
</tr>
<tr>
<td>Negative cases</td>
<td>110</td>
<td>28.65</td>
<td>112</td>
<td>29.17</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>100</td>
<td>384</td>
<td>100</td>
</tr>
</tbody>
</table>

Further analysis was done by use of paired t- test for microscopic and RDT. From the results, microscopic tests for positive cases showed the positive cases had the highest mean of 46.67 compared to RDT (+ve) which had mean of 45.33. On the other hand, microscopic tests (-ve) had mean of 18.33 compared to RDT (-ve) which had mean of 18.67. The results are shown in table 4.4
Table 4.4: Paired t-Test analysis of Microscopic and RDT tests

<table>
<thead>
<tr>
<th>Tests</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscope (+ve)</td>
<td>274</td>
<td>46.67</td>
<td>4.998</td>
</tr>
<tr>
<td>Microscope (-ve)</td>
<td>110</td>
<td>18.33</td>
<td>3.073</td>
</tr>
<tr>
<td>Pair 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RDT (+ve)</td>
<td>272</td>
<td>45.33</td>
<td>5.432</td>
</tr>
<tr>
<td>RDT (-ve)</td>
<td>112</td>
<td>18.67</td>
<td>3.127</td>
</tr>
</tbody>
</table>

A t-test analysis was carried out to find out whether there was significance differences between the two mean that is RDT(+ve) and RDT (-ve). A p value of 0.953 was obtained which is greater than 0.05 significant level for acceptance of null hypothesis. Therefore, the hypothesized population mean (46.67) in Microscope (+ve) and RDT (+ve) 45.53 is not significantly different from that of RDT (+ve).

Table 4.5: t-Test analysis of Microscopic +ve and RDT +ve tests results

<table>
<thead>
<tr>
<th>Tests</th>
<th>N</th>
<th>Test Value = 46.67</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(P value)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscope (+ve)</td>
<td>274</td>
<td>0.752</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.333</td>
</tr>
<tr>
<td>RDT (+ve)</td>
<td>272</td>
<td>0.953</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.061</td>
</tr>
</tbody>
</table>
Further analysis of negative RDT and negative microscopic were carried out. The t-test result showed that there is no significant difference between the mean of RDT (-ve) and microscopy (-ve) at 0.05 level of significant a P value of 0.617 was obtained. This value is greater than 0.05 therefore we accepted the null hypothesis and conclude that the hypothesized population mean (18.33) of Microscopic (-ve) and the RDT (-ve) mean 18.67 is not significantly different from that of RDT (-ve). The results are shown in Table 4.6.

**Table 4.6: t-Test analysis of Microscopic –ve and RDT –ve tests results**

<table>
<thead>
<tr>
<th>Test</th>
<th>n</th>
<th>t-value</th>
<th>P value</th>
<th>Mean Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope (-ve)</td>
<td>110</td>
<td>0.434</td>
<td>0.682</td>
<td>1.333</td>
<td>-6.57 to 9.23</td>
</tr>
<tr>
<td>RDT (-ve)</td>
<td>112</td>
<td>0.533</td>
<td>0.617</td>
<td>1.667</td>
<td>-6.37 to 9.70</td>
</tr>
</tbody>
</table>

The performance of RDT was determined for sensitivity, specificity and predictive values as shown in table 4.6.

Descriptive analysis was carried out to determine the performance of RDT and Microscope on four parameters; Sensitivity, specificity, positive predictive value and negative predictive value.

In Table 4.7 Malaria Rapid Diagnostic Tests showed 98.9% sensitivity, 97.1% specificity, 96.9% positive predictive value and 99.0% negative predictive value. The
microscopy based test showed 100% sensitivity, 100% specificity, 100% positive predictive value and 100% negative predictive value. (WHO, 2010), recommends that diagnostic test should have performance above 95% in terms of sensitivity, specificity and predictive values. Tests below 90% are considered inferior and unacceptable.

Malaria was noted in 384 (48%) of the 800 samples.

**Table 4.7: Results of RDT and Microscopic**

<table>
<thead>
<tr>
<th>Diagnostic method (n=800)</th>
<th>TP cases</th>
<th>FP cases</th>
<th>TN cases</th>
<th>FN cases</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDT</td>
<td>380</td>
<td>12</td>
<td>404</td>
<td>4</td>
<td>98.9</td>
<td>97.1</td>
<td>96.9</td>
<td>99.0</td>
</tr>
<tr>
<td>Microscopy</td>
<td>384</td>
<td>0</td>
<td>416</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Key: TP: true positive; FP: false positive; TN: true negative; FN: false negative; Sample size for determination of performance of RDT was eight hundred, (n=800).

**4.3 Incidence rate of malaria during wet and dry season in children less than five years and pregnant women with febrile illnesses.**

The study analyzed the incidence rates of malaria during wet and dry season in children and expectant women with febrile illnesses and the cases shown in table 4.8.

The dry season had a total of 182 cases whereas wet season had a total of 119 cases. The incidence rate for dry season was 47.4% and wet season had 30.9%. Table 4.9 shows analyses of incidence rate of malaria during dry and wet season using F- test. A p- value of 0.013 was obtained which is smaller than 0.05 therefore, we rejected the
null hypothesis and conclude that the incidence rate of malaria for dry season differs from that of the wet season.

**Table 4.8: Incident rate of malaria during dry and wet season**

<table>
<thead>
<tr>
<th>Season</th>
<th>n</th>
<th>Incidence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry (Jan, Feb and Mar)</td>
<td>182</td>
<td>47.4</td>
</tr>
<tr>
<td>Wet (Apr, May and Jun)</td>
<td>119</td>
<td>30.9</td>
</tr>
<tr>
<td>Total</td>
<td>301</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 4.9: Analysis of incident rate of malaria during dry and wet Season**

<table>
<thead>
<tr>
<th>F-test analysis</th>
<th>Sum of Squares</th>
<th>d f</th>
<th>Mean Square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>35967.123</td>
<td>1</td>
<td>35967.123</td>
<td>22.763</td>
<td>0.013</td>
</tr>
<tr>
<td>Wet</td>
<td>1580.063</td>
<td>1</td>
<td>1580.063</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the finding on Table 4.9.1, children aged less than 1 year had an incidence rate of 44.2%, children from 2 to 5 years had the highest incidence rate of 55.8% and expectant woman had an incidence rate of 4.8%.

Table 4.9.2 shows analysis of incident rate of malaria in children < 5 years and expectant women with febrile illnesses. P value of 0.002 was obtained which is smaller than 0.05 therefore, we reject the null hypothesis 2 and conclude that the incidence rate of malaria in children and expectant women with febrile illnesses were high in Meru South Sub County.
Table 4.9.1: Incident rate of malaria in children < 5 years and expectant women with febrile illnesses

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Incidence rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1 year</td>
<td>159</td>
<td>44.2</td>
</tr>
<tr>
<td>From 2 to 5 years</td>
<td>201</td>
<td>55.8</td>
</tr>
<tr>
<td>Expectant women</td>
<td>24</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Table 4.9.2: Analysis of incident rate of malaria in children < 5 yrs and expectant women with febrile illnesses

<table>
<thead>
<tr>
<th>F-test analysis</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children &lt; 5 years</td>
<td>39820.907</td>
<td>1</td>
<td>39820.907</td>
<td>5.604</td>
<td>0.002</td>
</tr>
<tr>
<td>Expectant women</td>
<td>14212.053</td>
<td>1</td>
<td>7106.027</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analysis was done further to show monthly distribution of Malaria. The highest incidence rate among children and pregnant women was February and March which coincided with dry season and lowest incidence was in the month of May and June coinciding with dry season (Figure 4.1).

Figure 4.1: Incidence rates of malaria in the period of January- June 2014

Further analyses demonstrated that the highest the incidence was on the female child (42.1%) compared to the male child (30.8 %) and expectant women (4.8%), (Figure 4.1).
Figure 4.2 Mean incident rate of malaria in male, female children and expectant women for period (January-June 2014).

4.4 Predominant *Plasmodium* species in Meru South Sub County

The study sought to determine the predominant *Plasmodium* species diagnosed among Children <5years and expectant women with febrile illnesses attending Chuka Level Five Hospital. The results indicate that the predominant Malaria species is *P. falciparum* (81.37%) followed by *P. vivax* (18.63%). No other *Plasmodium* species was observed in region (Table 4.1).
Table 4.9.3: Predominant *Plasmodium* species diagnosed

<table>
<thead>
<tr>
<th>Participants</th>
<th>n</th>
<th><em>Plasmodium falciparum</em></th>
<th>%</th>
<th><em>Plasmodium vivax</em></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male children &lt; 5 years</td>
<td>154</td>
<td>95</td>
<td>32.75</td>
<td>22</td>
<td>7.58</td>
</tr>
<tr>
<td>Female children &lt; 5 years</td>
<td>206</td>
<td>132</td>
<td>45.52</td>
<td>29</td>
<td>10.00</td>
</tr>
<tr>
<td>Expectant Women</td>
<td>24</td>
<td>9</td>
<td>3.10</td>
<td>3</td>
<td>1.05</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>236</td>
<td>81.37</td>
<td>54</td>
<td>18.63</td>
</tr>
</tbody>
</table>
5.1 Performance of Rapid Diagnostic Test for malaria parasites

This study was aimed at comparing the rapid diagnostic test with that of conventional Giemsa stained thick and thin blood smear microscopy. The results were analyzed and no difference in performance of RDT and microscopic tests was noted. This means RDT is comparable to microscopy in diagnosis for Malaria. Positive cases of *P. falciparum* malaria parasites were slightly higher 384 (100%) in contrast to rapid diagnostic test device 380 (98.9%). A study done in Canada involving 456 cases gave sensitivity 95.5% and 97% specificity with microscopy (Farcas et al., 2009). In a research carried out in Italy to the compare performance of RDT and microscopy involving 571 immigrants obtained a sensitivity of 100% and specificity 98% with microscopy (WHO, 2009). The results of this study are consistent with the findings of (Farcas et al., 2009).

This study was hinged on the fact that malaria was grossly under diagnosed using clinical signs and symptoms and that RDT was recognized to be suitable diagnostic method for malaria test. It was found that RDT can attain a high level of sensitivity like that of microscopy. Therefore, RDTs are sufficient for case management of uncomplicated malaria. This observation is consistent with study by Tidi and Akogun (2005) who established a significant relationship of RDT and Microscopy in terms of performance. Reports from other studies indicated that RDT have shown a similar level of accuracy to microscopy in clinical settings (Scholl et al., 2001).

It is paramount to consider application of RDTs for routine malaria surveillance in malaria endemic area of Meru South Sub County. The utility of RDT may have great
positive effect on treatment and prevention on local malaria transmissions in the community where maintenance and use of microscope may be a challenge. Malaria diagnosis is essential for several purposes; to confirm or rule out malaria infection in symptomatic patients, to guide accurate prescription of treatment and to monitor the incidence of malaria, for targeting prevention activities and evaluating health programmes. The findings of this study show that RDT are appropriate for malaria tests for febrile patients showing signs and symptoms of malaria infection. Consequently null hypothesis is accepted stating that there is no difference in microscopy and RDT in malaria diagnosis in Meru South Sub County.

5.2 Incident rates of malaria infections during dry and wet months

This study determined the incident rates of malaria infections during dry and wet seasons among young children below the age of 5 years and expectant females with febrile illnesses living in Meru South Sub County and attending Chuka Level Five Hospital for treatment. The overall malaria incidence during the dry season was 47.4% as opposed to 30.9% during the wet season. A possible explanation for the high malaria incidence rate in the dry months includes inconsistent use of protective measures by the family (like mosquito treated bed nets, repellants, or antimalaria drug) during the seasons which are latter on abandoned during dry seasons. Further environmental factors like high temperatures and sluggish movement of water in the rivers provide conducive conditions for mosquito breeding. Children under five years of age are at the higher risk of malaria infection during the dry season of the year.

This concurs with the findings of Eskindiran and Bernt (1998) as cited in (Kyabayinze, 2008) who reported high incidence rate of malaria infections during dry months in different localities in Ethiopia. The dry months of the year can thus be said
to predispose children and expectant women to infection with *Plasmodium* causing malaria infection possibly due to inappropriate use vector control and prevention strategies. The incidence rate of malaria in this study was children 42.1% for female children, 30.8% for male children and 4.8% for expectant women. The female children appeared to carry the greatest burden of malaria infection. In Coastal areas near Indian Ocean and Lake Victoria region are high burden and malaria prevalence are 8% and 27% respectively (WHO, 2017). The malaria transmission rate is almost constant throughout the year but slight changes occur during the dry and rainy seasons of the year (MOH, 2011).

Meru South Sub County has a unique climatic conditions ranging from extreme high temperatures in the lower parts to extreme low temperatures near Mount Kenya Forest. The regions with moderate to extremely high temperatures are favourable for mosquito reproduction and possibly a source of malaria infection. These environmental conditions greatly affect the transmission intensity and management of malaria control. The National Government set target of reducing mortality and morbidity by more than 50% per year (WHO, 2009). The individual community based studies are important in evaluating the present malaria burden and appropriate effective measures in the control of malaria in the country. Such studies may help in determining current malaria burden and appropriate measures for individual settings in order to achieve even and meaningful control of the disease throughout the country (Fergusson *et al.*, 2007).

The study was done to determine incident rate of malaria in outpatient attending Chuka Level Five Hospital in Tharaka-Nithi County. It was concluded that the Meru
South Sub County has prevailing environmental conditions that encourage proliferation of mosquito vector causing malaria infection. The incidence rate of malaria infection is most pronounced among children below five years and expectant women (MOH, 2011). Meru South Sub County is located in the belt that receives two rainy seasons in a year and two dry seasons in a year. Therefore, people in Meru South Sub County are more prone to malaria attacks twice per year mainly during two dry seasons. This implies that Meru South Sub County should have special programmes to effectively deal with mosquito vector and urgent treatment of infected people to avoid further local transmissions in the population. In particular, there is the need for a strong collaboration among major stakeholders including the National Government, County Government, Non-Governmental Organizations and the community to devise holistic, effective, and cost-saving methods for prevention, control and treatment of malaria. Null hypothesis that stated incidence rates of malaria in children less than five years and expectant women with febrile illnesses in Meru South Sub County is low is rejected.

5.3 Predominant Plasmodium species in Meru South Sub County

The results show that the most common malaria species detected in this research was *P. falciparum* (81.37%), which is the more virulent than other malaria causing species. *P. falciparum* has a tendency of developing antimalaria drug resistance or tolerance. *Plasmodium vivax* was also detected at 18.63 % in the population. In the Lake Victoria Region *P. falciparum* (81.1%) is the most common species. Along Indian Ocean *P. falciparum* (67%) is most predominant species and Western Kenya *P. falciparum* (34.1%). In South Eastern - Asia (India) predominant *Plasmodium* species was *P. falciparum* (53.0%) and *P. vivax* (47.0%). This finding is consistent
with other reported studies (WHO, 2013). The differences noted on predominant species are due specific drug resistance or tolerance to initial antimalaria drugs like chloroquine. A few reported cases of artemunate drug tolerance was reported in South Eastern Asia (Dondrop et al., 2009). The research finding found out that most malaria cases were caused by *P. falciparum* which triggered great suffering to febrile patients. The findings require further confirmation involving the community on importance of supporting multifaceted approach in implementing integrated malaria control strategies that aim at management of the vector (Snow et al., 2005). The results further emphasize the importance of carrying out many well thought-out control strategies to specifically enlighten community since malaria infection is completely controllable and treatable. It will result to more reduction in sickness and death related infection due to malaria infection. Therefore we accept the alternative hypothesis which states *P. falciparum* is most predominant species in Meru South Sub County.

5.4 Conclusions

Based on the study findings the following conclusions were made:

i. Rapid Diagnostic Test (RDT) is appropriate in large scale screening for malaria parasites and interventions in the management and control of malaria in Meru South Sub County. The RDTs may be used in a mobile clinic and can be performed conveniently at patients comfort. It does not require specialized equipment and personnel.

ii. The incidence of malaria transmission in Meru South Sub County was high in dry seasons 47.4% compared to wet season 30.9%. The female children had higher incident rate (42.1%) than male children (30.8%). Incidence rate in pregnant woman was much lower (4.8%) compared to children.
iii. The predominant species of malaria in Meru South Sub County was *P. falciparum* (81.37 %), which is more prone to developing antimalaria drug resistance or tolerance. *P. vivax* was observed but at 18.63 %.

5.3 Recommendations

i. RDT may be used in malaria surveillance and mass screening of patients during outbreak of epidemics. Malaria immunosurveillance to be conducted in all Counties in Kenya.

ii. There should be intensified malaria control and prevention during dry periods to reduce malaria incidence rates. Intensified protection strategies need to be adopted during the dry months of the year.

iii. Monitoring for drug resistance should be scaled up in Meru South Sub-County.

5.3.1 Suggestion for further studies

Epidemiological studies on risk factors for severe and complicated Malaria should be conducted.
REFERENCES


APPENDIX I: DATA COLLECTION SHEET

Date  ------------------------------------------

Patient Number  ------------------------------------------

Lab Number  ------------------------------------------

IP/OP Number  ------------------------------------------

Age (ys/month)  ------------------------------------------

Sex; Male/Female  ------------------------------------------

Expectant woman  ------------------------------------------

Resident/Village  ------------------------------------------

Investigation Requested  ------------------------------------------

Lab test done by  ------------------------------------------

Designation  ------------------------------------------ Date------------------------------------------

Signature  ------------------------------------------

Stamp
APPENDIX II: AUTHORIZATION LETTER

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: 156/CE/14159/09
DATE: 16th February, 2013

The Permanent Secretary,
Ministry of Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

REF: RESEARCH AUTHORIZATION FOR NJUKI JUSTIN MUGAMBI – REG. NO. 156/CE/14159/09

I write to introduce Mr. Mugambi who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the Department of Zoological Sciences.

Mr. Mugambi intends to conduct research for a proposal entitled, “Malaria Immuno Surveillance and Evaluation of Rapid Diagnosis test in Meru South District, Tharaka-Nithi County, Kenya”.

Any assistance given will be highly appreciated.

Yours faithfully,

[Signature]
MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

18 FEB 2013

DNN/rvm
APPENDIX III: RESEARCH AUTHORIZATION LETTER

We acknowledge receipt of your application for attachment and recommend it.

You are authorized to carry out a research project and use our facility.
APPENDIX IV: INTRODUCTION LETTER

KENYATTA UNIVERSITY
Department of Zoological Sciences
P.O. Box 43844, Nairobi, Kenya
Tel: 810901/811278, Ext. 57305/307
Website: http://www.ku.ac.ke
E-mail: zoologicalku@yahoo.com

4th October, 2011

Medical Officer
Chuka General Hospital
P.O Box
CHUKA

Dear Sir/Madam,

RE: NJUKI JUSTIN MUGAMBI (156/CE/14169/09)

The above is an MSc (Applied Medical Parasitology) student conducting research entitled “Immunosurveillance and microscopic diagnosis of plasmodium species causing malaria” in Meru South District, Tharaka Nithi County.” for his thesis work.

Kindly assist him to get access to hospital facility for the purpose of implementation of this research project. Kenyatta University and in particular the Department of Zoological Sciences appreciate your continued support to our students.

Yours sincerely,

[Signature]

Dr. Gacheru M. M.
Chairman, Dept. of Zoological Sciences
APPENDIX V: CONSENT FORM

RESEARCH TITLE: Malaria Immunosurveillance and Evaluation of Rapid Diagnosis Test in Meru South Sub County, Tharaka-Nithi County, Kenya

Researcher: Njuki Justin Mugambi

I have been given information about research and discussed the research with researcher who is conducting this research as part of masters’ degree supervised by supervisors in the Department Zoological of Sciences Kenyatta University.

I have been advised of the potential risks and burden with this research and had an opportunity to ask any question about the research participation. I understand that my participation in this research is voluntary. I am free to refuse to participate and I am free to withdraw from the research at any time. My refusal to participate or withdrawal of consent will not affect my relationship with the department or hospital.

If there are any enquiries about research, I can contact Njuki Justin Mugambi, Prof, Gicheru Michael or Dr, Simbauni Jemimah. If there is any concern or complain regarding the way research has been done, I can contact the Ethics Officer or Human Research Ethics Committee of Kenya.

By signing below I am indicating my consent to participate in the research. I understand that the data collected from my participation will be used primarily for a thesis and will also be used in summary form for Journal publication.

Signed-----------------------------  Date-----------------------------

Name-----------------------------