THE STATUS OF PROTECTIVE MEASLES ANTIBODIES IN HIV-1 POSITIVE AND NEGATIVE INFANTS AGED 6 AND 9 MONTHS IN URBAN LUSAKA, ZAMBIA

BY

DR MAUREEN SIMWENDA

(MBChB)

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF MEDICINE IN PAEDIATRICS AND CHILD HEALTH

THE UNIVERSITY OF ZAMBIA

LUSAKA

2015

COPYRIGHT DECLARATION

Dr Maureen Simwenda

2015

DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other university.

Signed:_____

Student: Dr Maureen Simwenda, MBChB

Signed: _____

Supervisor: Professor Mary S. Ngoma, MRCP(UK), FRCP(UK), DCH (Glas), Neonates (Jap)

Signed: _____

Co-Supervisor: Dr Evans M. Mpabalwani, Bsc(Hb), MBChB, MSc, MMed

CERTIFICATE OF APPROVAL

This dissertation of Dr Maureen Simwenda has been approved as partial fulfilment of the requirement for the award of the Degree of Master of Medicine in Paediatrics and Child Health by the University of Zambia.

Signed: _____

Head of Department

Paediatrics and Child Health

University Teaching Hospital

Examiners

Name: _____

Signature: _____

Date: _____

Name: _____

Signature: _____

Date: _____

Name: _____

Signature: _____

Date: _____

ABSTRACT

BACKGROUND – Measles remains the leading cause of vaccine preventable disease that contributes to under 5 mortality rate despite the availability of a safe and effective vaccine. During the 2010-2011 measles outbreak, infants aged 0-8 months constituted 39% of the total population of children admitted with measles infection at UTH in Lusaka, Zambia. None of these infants were vaccinated against measles because they were below the age of vaccination of 9 months. The optimal time for measles vaccination in infants has not been revised since the vaccine was introduced in 1966. The age at which passively acquired maternal measles antibodies decay is critical in determining the optimal age for measles vaccination in infants, particularly in HIV exposed infants who may have acquired low maternal antibodies, and even in those who are not HIV exposed.

RESEARCH DESIGN AND METHODS – A cross-sectional study of 126 infants aged six and nine months old along with their respective mothers was carried out and participants were screened for measles antibodies in their oral fluid. The study was carried out at the under 5 clinics at Kanyama and Chilenje health centres in urban Lusaka, Zambia from October 2014 to December 2014. Demographic and clinical data were collected using a structured questionnaire. Measles antibodies were measured in the oral fluids of the mothers and infants using the enzyme-linked immunosorbent assay (ELISA) test.

RESULTS – One hundred and one (81.4%) of the infants included in the study had no protective maternal measles antibodies in their oral fluids. Eighty three percent (83%) of the 6 months and 93% of the 9 months old infants had unprotective measles IgG antibodies detected. Twenty eight (22%) of the infants included in the study were exposed to HIV. Four (14.2%) of the HIV exposed and 8 (8.1%) of the HIV non exposed infants had protective measles antibodies. Only 87 (69%) of the mothers had positive measles IgG antibodies. None of the study variables had significant influence on the status of maternal measles antibodies in the infants - maternal and infant HIV status, infant birth weight, prematurity, maternal measles IgG antibody status as well as maternal and infant nutritional status.

CONCLUSION – The findings in this study suggest that 81.4% of the infants in the studied population are susceptible to measles infection before the age of immunization at 9 months. A lot of other studies done in different regions of the world have demonstrated early loss of maternal measles antibodies below the age of vaccination and have recommended early immunization in infants to reduce susceptibility to measles infection. This study calls for more large scale studies to determine the appropriate age for measles vaccination in the Zambian infant population.

DEDICATION

First and foremost, I dedicate my work to the Lord Jesus Christ. My God whom I love to serve and praise, and in whom all things are possible.

To my son Chrishane, thanks for tolerating mummy's absence from home, and for the lovely and adorable "how was work" question I get from you everyday.

To my husband Sampath, thank you for understanding me the way you do, and for all the unending love and support.

To my angel Luke, you will always live in my heart.

I love you all my boys, to the moon and back.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Professor Mary Shilalukey-Ngoma and Dr Evans Mpabalwani for their guidance and mentorship. I thank Mr. Mulele Kalima for helping me analyse the data, and Dr Suzanna Mwanza for the help and advice she gave me.

I am indebted to Dr Kankasa for the permission to use the Kaposi Sarcoma Laboratory as well as to the staff of the Paediatric Centre of Excellence laboratory for their reliability, professionalism and willing cooperation. In particular would like to thank Mr. John Chisoso and Mr. Rabison Zulu.

I also want to recognize the financial contribution that the University of Zambia/Vanderbilt research scholarship program rendered by providing partial funding to the research. This greatly lightened the financial burden of carrying out this research.

I acknowledge the assistance of my research assistant, Jonathan Gwasupika, as well as the nurses and the community health workers at Kanyama and Chilenje health centers. Lastly, but not the least, I am highly indebted to the mothers and their infants that participated in the study for their willingness to take part in the study.

TABLE OF CONTENTS

Page

Copyright Declaration..…….. i

Declaration …… ii

Certificate of Approval ……. iii

Abstract …… iv

Dedication …… v

Acknowledgements …… vi

Table of Contents …… vii

List of Tables and Figures …… x

Abbreviations and Acronyms …… xi

CHAPTER ONE

1.1INTRODUCTION..…… 1

1.2 LITERATURE REVIEW..……. 3

1.2.1 Measles Epidemiology ……..…… 3

1.2.2 Measles virus and disease ……....…….…….. 4

1.2.3 Vaccine and control of measles ……..... 5

1.3 STATEMENT OF THE PROBLEM …… 7

1.4 STUDY JUSTIFICATION ……... 8

1.5 RESEARCH QUESTION …… 8

CHAPTER TWO

2.1 Main objective …… 9

2.2 Specific objectives …….. 9

CHAPTER THREE

3.1 RESEARCH METHODS ……. 10

3.1.1 Study design …… 10

3.1.2 Study site …… 10

3.1.3 Duration of study ……..……..…… 10

3.1.4 Study population ……..……. 10

3.1.5 Sample size ……..…….. 10

3.1.6 Eligibility …… 11

3.1.6.1 Inclusion criteria ……..……. 11

3.1.6.2 Exclusion criteria ……...…… 11

3.1.7 Description of variables ……. 11

3.1.7.1 Dependent variables …… 11

3.1.7.2 Independent variables …….. 12

3.1.8 Study procedures …… 12

3.1.8.1 Patient screening, study enrolment and collection of demographic

and clinical data ……..…… 12

3.1.8.2 Clinical assessment ……..……. 13

3.1.8.3 Laboratory assessment ……..… 13

3.2 STATISTICAL ANALYSIS ……. 14

3.3 ETHICAL CONSIDERATIONS …… 15

3.3.1 Confidentiality ……..….. 15

CHAPTER FOUR

4.1 RESULTS ……..…… 16

CHAPTER FIVE

5.1 DISCUSSION ……..……. 29

CHAPTER SIX

6.1 CONCLUSION ……. 33

6.2 LIMITATIONS …… 34

6.3 RECOMMENDATIONS ……. 34

REFERENCES ……..…… 35

APPENDICES ……. 40

Appendix A: Consent Form……..... 40

Appendix B: Study questionnaire - infant…….……. 43

Appendix C: Study questionnaire - Mother…..……. 45

Appendix D: Laboratory information form ……... 46

LIST OF TABLES AND FIGURES

Title		Page
Figure 1	Infant Age Distribution	16
Figure 2	Infant Sex Distribution	17
Figure 3	Status of measles antibodies by infant age	18
Table 1	Distribution of measles IgG antibody in mother infant pairs	18
Table 2	Characteristics of infants included in the study	19
Table 3	Characteristics of mothers included in the study	20
Figure 4	Box plot comparing maternal age and infant IgG status	21
Figure 5	Mother vaccine history status by age	22
Table 4	Cross tabulation of history of maternal measles vaccination, infection and Infant IgG status	23
Figure 6	Infant nutritional status	24
Figure 7	Infant HIV status by age	25
Table 5	Bivariate analysis of characteristics with Infant IgG status	26
Table 6	Logistic regression analysis	27

ABBREVIATIONS AND ACRONYMS

AIDS ……	Acquired Immuno-Deficiency Syndrome
ART……..	Antiretroviral Therapy
ELISA ……..	Enzyme Linked Immunosorbent Assay
EPI ……	Expanded Program on Immunisation
HI ……	Haemagglutination Inhibition
HIV ……	Human Immunodeficiency Virus
IgG ……	Immunoglobulin G
IgM ……	Immunoglobulin M
Ltd …….	Limited
MDG ……...	Millennium Development Goals
MMA……	Maternal Measles Antibody
MMR ……	Measles Mumps Rubella
MR ……..	Measles Rubella
PCR..…….	Polymerase Chain Reaction
PRN……	Plaque Reduction Neutralisation
UK……..	United Kingdom
UNICEF……	United Nations International Children Emergency Fund
UTH …….	University Teaching Hospital
WHO ……	World Health Organisation

CHAPTER ONE

1.1 INTRODUCTION

Measles is a major vaccine-preventable disease that contributes to under 5 mortality rate (1). The fourth Millennium Development Goal (MDG 4) aims to reduce the under-five mortality rate by two-thirds between 1990 and 2015. Recognizing the potential of measles vaccination to reduce child mortality, and given that measles vaccination coverage can be considered a marker of access to child health services, routine measles vaccination coverage has been selected as an indicator of progress towards achieving MDG 4 (2). The possibility of measles eradication has been discussed for over 40 years (3). The revised global measles mortality reduction set forth in the WHO-UNICEF global immunization vision and strategy for 2006-2015 is to reduce measles deaths by 90% by 2015 compared to the estimated 757,000 deaths reported in 2000 (3).

In 2011, there were 158 000 measles deaths globally-about 430 deaths every day or 18 deaths every hour. More than 95% of measles deaths occur in low-income countries with weak health systems. Measles vaccination resulted in a 71% drop in measles deaths between 2000 and 2011 worldwide (4). In 2011, about 84% of the world's children received one dose of measles vaccine by their first birthday through routine health services-up from 72% in 2000. Despite impressive progress globally, more than 400 children die every day from this completely preventable infection (5). In 2010, there was a measles outbreak in Zambia and WHO reported more than 33000 measles cases in Zambia. Data from UTH indicated that 1310 children were admitted with measles between May 2010 and February 2011. Young infants aged 0-8 months constituted 39% of the total population and none of these infants were immunized because they were below the

immunization age (6). During the 2010-2011 measles outbreak in Zambia, 17% (5,195/30,397) of those infected were below the immunization age of 9 months (7).

The risk of measles disease in infants and children is much lower in developed countries and measles vaccine is administered at 12-15 months of age when virtually all children have lost maternal antibodies and an optimal immune response is achieved (8). In some of these developed countries, measles has been eliminated and others are close to elimination (9). But developing countries still have high rates of endemic measles and routine immunization is recommended at 9 months of age because of the increased risk of infection early in life (10).

It is well documented that measles infection causes transient but profound immunosuppression resulting in increased susceptibility to secondary bacterial and viral infections as well as severe malnutrition (11). Due to the development of these opportunistic infections and severe malnutrition, measles remains the leading vaccine preventable cause of child death worldwide. This means that HIV exposed and infected children who already have some degree of immunosuppression are at risk of mortality when co-infected with measles. Studies have reported that HIV type 1 infection is a risk factor for increased mortality in hospitalized Zambian children with measles (12).

The main intervention for measles control in Zambia has been routine administration of a single dose of measles vaccine at 9 months and also during national immunization campaigns targeting children between 6 months and 15 years. More recently, a second dose of measles has been introduced at 18 months. But the optimal age for vaccination in infants has not been revised since the introduction of measles vaccine in the 1960s. The age at which passively acquired maternal antibodies decay is critical in determining the optimal age for measles vaccination, particularly in HIV exposed children who may have acquired very low maternal antibodies.

Further, it is well known that measles vaccine acquired immunity in mothers also leads to inadequate passive transfer of antibodies to the newborn babies especially in the face of maternal under nutrition and HIV infection (13).

1.2 LITERATURE REVIEW

1.2.1 Measles Epidemiology

Measles is a highly infectious disease caused by the measles virus. In the pre-vaccination era, >90% of individuals were infected by the age of 10 years, the majority with symptoms. Measles occurs only in humans who are natural hosts, no animal reservoirs are known to exist. Measles virus is transmitted by aerosolized respiratory droplets and by direct contact. The incubation period of measles is approximately 10 days and ranges between 7-15 days from the day of exposure to onset of fever, usually 14 days until rash appears. A person is most contagious 4 days before eruption of the rash and 4 days after appearance of the rash. In temperate climates most cases of measles occur during winter and early spring. The incidence of measles increases during dry seasons in tropical climates (14).

Measles can be prevented readily by vaccination. In 2007, worldwide coverage of the first dose of measles vaccine reached 82%. Between 2000 and 2007, the estimated number of deaths from measles dropped from 750 000 to 197 000 (15). However, measles remains an important cause of death and morbidity in countries with limited health infrastructure. In countries where vaccination has substantially reduced the incidence of measles, failure to maintain high coverage of childhood immunization in all areas has resulted in a resurgence of the disease (16).

1.2.2 Measles: Virus and Disease

Measles virus is the causative agent of measles and was first isolated from the blood of infected persons in the 1950s by John Enders and Thomas Peebles (17). The development of vaccines against measles soon followed. Measles virus is one of the most infectious directly-transmitted pathogens and occurs naturally only in humans. Measles virus (genus *Morbillivirus*, family *Paramyxoviridae*) is an enveloped, single-stranded RNA virus. The genome encodes 8 proteins, including the haemagglutinin (H) and the fusion (F) proteins. The lifelong immunity that follows infection is attributed to neutralizing antibodies against the H protein (18).

Towards the end of the incubation period, patients develop prodromal symptoms of high fever, cough, coryza and conjunctivitis. The typical maculopapular rash appears after another 3-4 days, often accompanied by a fever that peaks at 39-40 °C. At the onset of rash, bluish-white Koplik's spots, which are pathognomonic of measles, are seen in the oral mucosa. Patients normally improve by the third day after rash onset and are fully recovered 7-10 days after onset of disease (19).

The severity of measles varies widely, depending on a number of host and environmental factors. The risk of developing severe or fatal measles increases for those aged <5 years, living in overcrowded conditions, who are malnourished (especially with vitamin A deficiency), and those with immunological disorders, such as advanced HIV infection. In developing countries, case-fatality rates among young children may reach 5-10% (20). In industrialized countries, deaths from measles are rare, although severe forms of the disease and even death may occur in previously healthy individuals.

Measles infection produces an immune paradox. Measles virus infection, while inducing lifelong immunity, also suppresses the immune system leading to an increase in susceptibility to other secondary infections (21, 22). Immune suppression can continue for many weeks to months after the apparent recovery from measles (23). Immunosuppression associated with acute measles infection is the major cause of infant death and therefore of substantial clinical importance. Secondary bacterial, protozoal, or viral infections occur because of immunosuppression caused by measles infection. These infections can result in pneumonia, chronic pulmonary disease, otitis media, laryngotracheobronchitis, adult respiratory distress syndrome, hepatitis and diarrhea (24). These secondary infections account for most of the morbidity and mortality associated with acute measles.

Different immune abnormalities have been associated with measles, including disappearance of delayed-type hypersensitivity reactions, impaired lymphocyte and antigen-presenting cell functions, down-regulation of pro-inflammatory interleukin 12 production and altered interferon alpha/beta signaling pathways. Several measles virus proteins have been suggested to hinder immune functions: hemagglutinin, fusion protein, nucleoprotein and the non-structural V and C proteins (11).

1.2.3 Vaccine and Control of Measles

A number of live, attenuated measles vaccines are available, either as monovalent vaccine or in combination with either rubella vaccine (MR) or mumps and rubella vaccines (MMR). Many of the attenuated strains in use are derived from the Edmonston strain isolated in 1954, named after the student the virus was isolated from, and have been in use since the 1960s (25).

Measles vaccine is safe, efficient and cost effective. The efficacy of measles vaccine was established in a series of double-blind controlled field trials which demonstrated a high degree of protective efficacy afforded by the individual vaccine components (26). The studies also established that seroconversion in response to vaccination against measles paralleled protection from the disease (27).

But despite the availability of a safe, cost effective, and efficient vaccine, measles remains an important cause of vaccine-preventable child mortality worldwide, especially in sub-Saharan Africa (28). Morbidity and mortality due to acute measles infection and complications are higher in developing countries compared to developed countries.

In regions of high HIV-1 prevalence, coinfection with HIV-1 more than doubled the odds of death in hospitalized children with measles and increased mortality among HIV-1 infected children is further evidence that greater efforts are necessary to reduce transmission of the measles virus in such regions (29). Studies have also shown evidence that the duration of hospitalization was longer for HIV-infected children (30). And also that HIV-1 infected children with measles may have a prolonged infectious period that potentially enhances measles virus transmission and hinders measles control (31).

During the 2010 measles outbreak in Zambia, 39% of children admitted to UTH in Zambia were below 9 months, the age of routine immunization (6). A study done in Mali showed that only 30% of infants aged 2 months had protective antibody titers and among 6 months old infants none had protective titers (32). In Gabon, the proportion of seronegative infants had reached 90% by the end of 4 months (33). This constitutes the age group which is more at risk of morbidity and mortality caused by acute measles infection and complications.

WHO strategies to control measles include high vaccination coverage, laboratory-backed surveillance, monitoring and evaluation, outbreak preparedness and response, communication and community engagement, and research and development (34). A second measles routine vaccine dose has been recommended for countries with >80 % coverage of the first dose (35). By 2010, Zambia had achieved 84% coverage of measles vaccination in children aged 12 months (3 consecutive years > 80% coverage) (36).

1.3 STATEMENT OF PROBLEM

Measles elimination is one of the major global health priorities. WHO's vision is to achieve and maintain a world without measles. Its main goals are to reduce global measles mortality by at least 95% by 2015 compared with 2000 estimates and to achieve measles elimination by 2020 in at least 5 WHO regions. Measles vaccination has proved to be an extremely helpful and successful public health intervention which has resulted in the elimination of measles in some regions. But while half of the world is close to eliminating measles, many countries in Sub-Saharan Africa, including Zambia are still struggling to control the disease. In the light of many infants falling ill with measles in the age range below vaccination age, the Expanded Program on Immunization (EPI) may need to introduce an earlier second dose of measles vaccine.

1.4 STUDY JUSTIFICATION

There are few studies that have been done to establish the age at which passively acquired measles antibodies decay in infants, particularly in HIV exposed infants in Zambia. The immunization schedule for measles has not been revised from the time the vaccine was introduced in the 1960s. With the recent frequent cyclical measles outbreaks in the era of HIV/AIDS, there is need to revise the immunization schedule especially to target infants that are exposed to HIV and even those that are not. Therefore, there is need to carry out this study to establish the age at which maternal measles IgG antibodies are lost in infants in order to establish the optimal age for immunization.

1.5 RESEARCH QUESTION

What is the status of passively acquired maternal measles antibodies in HIV-1 positive and negative infants aged 6 and 9 months at Kanyama and Chilenje health centres in urban Lusaka, Zambia?

CHAPTER TWO

2.1 Main objective

The main aim of this study was to establish the status of passively acquired maternal measles antibodies in 6 and 9 months old infants attending the under-5 clinic at Kanyama and Chilenje Health Centres.

2.2 Specific objectives

1. To detect the levels of passively acquired measles antibodies in infants aged 6 and 9 months.

2. To detect the levels of measles IgG antibodies in mothers of infants aged 6 and 9 months.

3. To establish the age by which maternal antibodies decay in both HIV exposed and non-exposed infants.

4. To establish the factors that affect the presence or decay of passively acquired measles antibodies in infants aged 6 and 9 months.

5. To help establish the optimal age for measles immunization for both HIV exposed and non-exposed infants.

CHAPTER THREE

3.1 RESEARCH METHODS

3.1.1 Study design

This was a cross-sectional study of mother-infant pairs of infants aged 6 and 9 months attending the under-5 clinics at Kanyama and Chilenje Health Centres in Lusaka.

3.1.2 Study site

The study was conducted from the under-5 clinics at Kanyama and Chilenje Health Centres in Lusaka, Zambia.

3.1.3 Duration of study

The study was conducted for a period of 3 months from October 2014 to December 2014.

3.1.4 Study population

The study was conducted among infants aged 6 and 9 months (paired with their respective mothers) attending the under-5 clinics at Kanyama and Chilenje Health Centres in Lusaka.

3.1.5 Sample size

Approximately 200 infants aged 6 and 9 months are seen at the under-5 clinics per month. The study duration (data collection) was 3 months, therefore the population size was 600 infants.

Epi info version 3.3.2 software was used to calculate the sample size.

Using a population size of 600 infants, an expected frequency of 50% and a precision of ± 5% which brings the worst acceptable results to 55% and at 80% power with 95% confidence interval, the sample size was found to be 122 infants.

3.1.6 Eligibility

Eligibility to participate in the study was based on the following criteria:

3.1.6.1 Inclusion criteria

The children whose biological mothers consented to take part in this study were those aged 6 and 9 months attending the under-5 clinics at Kanyama and Chilenje Health Centres in Lusaka with their mothers.

3.1.6.1 Exclusion criteria

Infants that were not included in the study were disqualified based on the following exclusion criteria:

-Infants that had history of measles infection.

-Infants that had a rash.

-Infants that had received measles vaccine.

-Infants whose mothers refused to consent for participation in the study.

-Infants with caregivers who were not their biological mothers.

3.1.7 Description of variables

The variables for this study are be as listed below.

3.1.7.1 Dependent variables

The primary outcomes of the study were as follows:

-The status of measles IgG antibodies in oral fluid samples of infants.

3.1.7.2 Independent variables

The independent variables for this study are listed below:

-Status of measles antibodies in oral fluid samples of mothers

-HIV status of both infants and mothers

-Age (Both infant and mother)

-Sex of infant

-Level of education of mothers

-Nutritional status of both mothers and infants

3.1.8 Study procedures

3.1.8.1 Patient screening, study enrolment and collection of socio-demographic data

Mothers attending the under-5 clinics at Kanyama and Chilenje Health Centres were asked to take part in the study. The purpose of the study and the procedures involved were explained in the language that they understood best. Mothers who gave consent to take part in the study were enrolled and a written consent was obtained. Study participants were assured that the information they gave and their results were confidential.

Demographic and clinical information was obtained about the participants using structured questionnaires. Personal information that identified the participant was not included on the questionnaires. The only recorded identification for each participant being the study number.

3.1.8.2 Clinical assessment

The anthropometric measurements including body weight and length of each enrolled infant participant were taken and documented. The mothers' weight and height were also measured in order to determine their nutritional status. The body weights and heights of the infants were assessed using the WHO Child Growth Standards (WHO Child Growth Standards 2006). Nutritional oedema and mid-upper arm circumference of the infants was also assessed and documented.

3.1.8.3 Laboratory assessment

For each mother-infant pair, oral fluid was collected using oral fluid collecting devices – Oracol device (Malvern Medical, United Kingdom, UK). The kit consists of an absorbent foam swab (designed to collect up to 1 ml of saliva), centrifuge tube and cap. The absorbent pad was moved along the gums and left stationary between the lower gums and the buccal membrane for a minimum of 2 minutes or until the pad was saturated with oral fluid. Thereafter, the collection device was placed in a centrifuge tube containing the buffer. The oral fluid was then transported to the Kaposi Sarcoma Laboratory at UTH, Lusaka, Zambia. The infant and mother samples were collected in two separate containers and the samples were analyzed separately. The containers were clearly labeled as mother or infant sample.

The Oral fluid specimens were screened for measles-specific IgG using the MicroImmune ELISA test (Microimmune Ltd, UK). The sensitivity and specificity of the IgG assay are 97.5% (95% CI 96.1–98.3) and 86.7% (95% CI 78.4–91.5), respectively (37, 38).

Participants who had no documented HIV status were tested for HIV using the rapid diagnostic antibody tests. HIV exposed infants who tested positive with the rapid diagnostic antibody test were tested for HIV infection using the PCR test and referred to Antiretroviral (ART) clinic for further follow up.

The results were entered on a standard study laboratory form (Appendix D).

3.2 STATISTICAL ANALYSIS

The data was collected, coded, anonymised and later stored on Epi-Info database. Double data entry, data cleaning and validation was done using Epi-Info, version 3.3.2.

All statistical tests were at 5% significance level. T-tests were used to compare mean values between groups and the Chi-squared test was used for comparison of proportions of categorical variables. Study variables were checked for evidence of collinearity based on a Spearman correlation coefficient >0.8. The relationship between study variables and the status of passively acquired maternal measles antibodies was examined using logistic regression. The selection for entry into the logistic regression model was considered at level $p<0.20$ or known clinical significance. Significant factors at $p<0.20$ which were infant age, gestation age, mother age, and education level were fitted into the model along with infant HIV status and mother IgG status.

The p-value, odds ratio, and 95% confidence interval were reported. Both bivariate and multivariate analysis odds ratios with their corresponding 95% CI were calculated. Statistical analyses were performed using SAS version 9.3., and for some preferred graphical output, IBM SPSS Statistics version 21.0 was preferred.

3.3 ETHICAL CONSIDERATIONS

Ethical approval was sought from Excellence Research Ethics and Science (ERES) Converge. Permission was obtained from Lusaka District Health Office for the participating clinics. Informed consent was obtained from the mothers by way of a signature or thumb print if they were unable to write.

3.3.1 Confidentiality

The information obtained from the participants and results of the tests were not shared with anyone outside the study team. The researcher and supervisors were the only ones with access to the information and laboratory results.

CHAPTER FOUR

4.1 RESULTS

This study examined the status of passively acquired maternal measles antibodies in infants aged 6 and 9 months as well as the status of measles IgG antibodies in their respective mothers. Oral fluid was obtained from both infants and mothers and the test used was the ELISA test.

A total of 126 mother-infants pairs were enrolled in the study. The mother-infant pairs studied were from urban Lusaka, Zambia. It was found that 69/126 (57.3%) were from Kanyama while 57 /126 (42.7%) were from Chilenje. Of the infants enrolled, 57/126 (45.2%) were male infants and 67/126 (54.032%) were female. Fifty one (40.8%) out of the 126 infants were aged 6 months and 74/126 (59.2%) were aged 9 months.

Figure 1: Infant Age Distribution

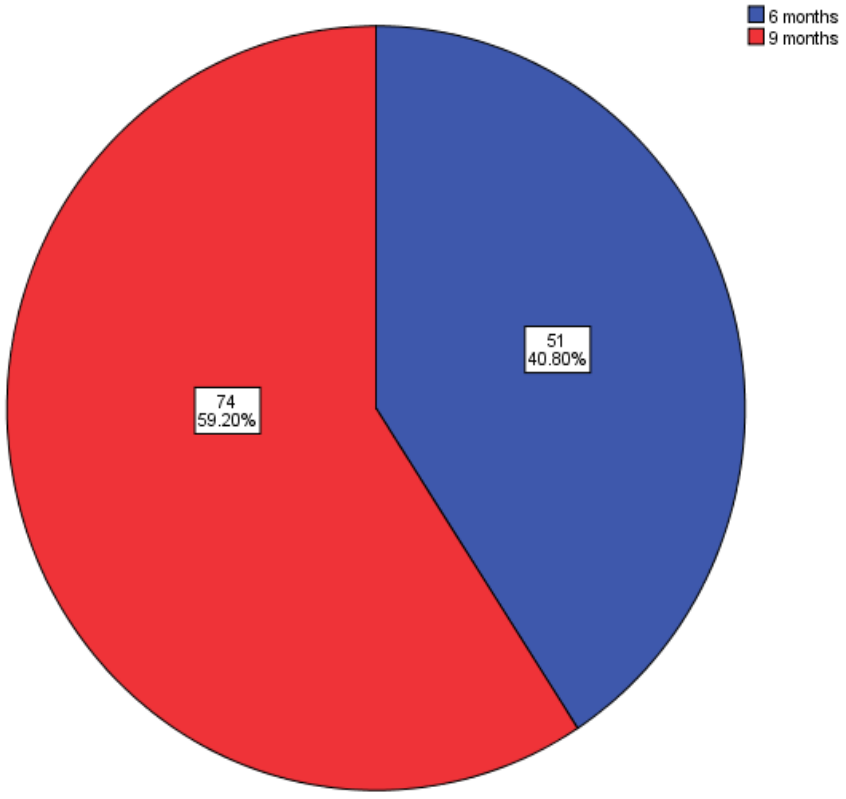
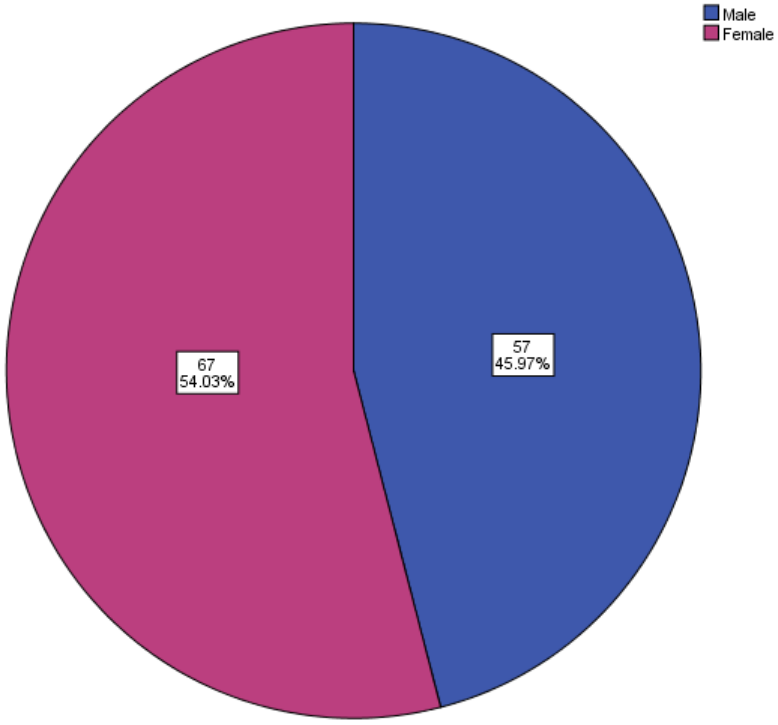


Figure 2: Infant Sex Distribution



Of the 126 infants recruited into the study, 101 (81.4%) tested negative for measles IgG antibodies in oral fluid. Seven (58.3%) out of 12 of those that tested positive were aged 6 months while 5/12 (41.7%) were 9 months old. Ten of the infants that were positive for measles IgG were born to mothers with measles IgG and 2 of these infants were born to mothers that tested negative. This may indicate exposure of these 2 infants to the measles virus. All of the infants included in this study had no symptoms of measles infection at the time the study was done, and none of them had a history of measles infection or contact with anybody who had measles that the mothers were aware of. The proportion of measles positive antibodies in 6 months old infants was higher than the 9 months old infants, 7/42 (16.7%) versus 5/70 (7.1%).

Figure 3: Status of measles antibodies by age

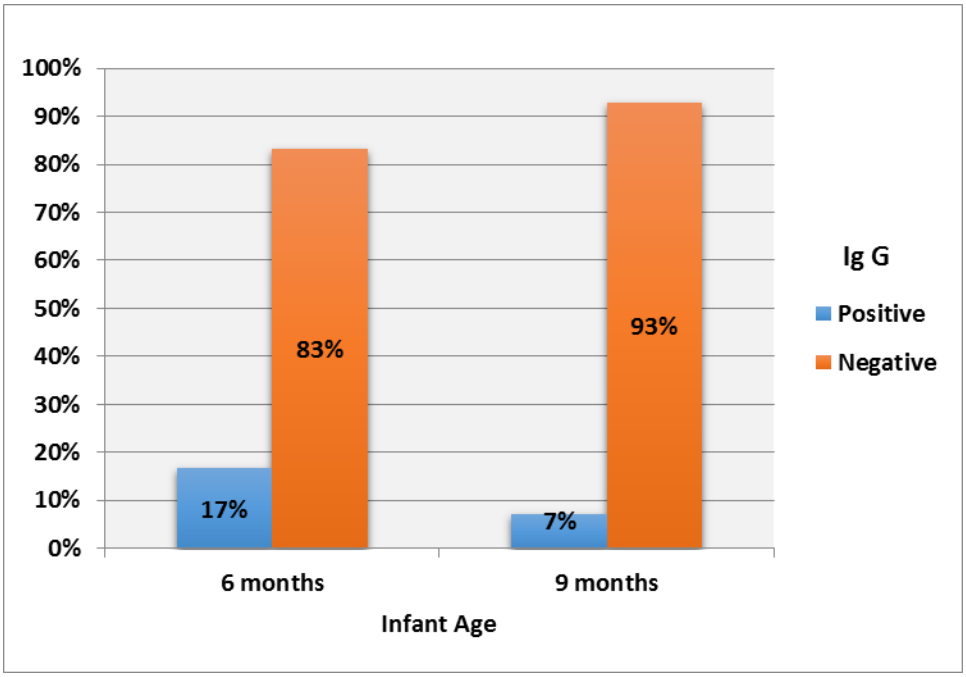


Table 1: Distribution of measles IgG antibody in mother infant pairs

Measles IgG Status	No. (%)
Both mother and infant positive	10 (9)
Both mother and infant negative	28 (26)
Mother positive infant negative	68 (63)
Mother negative infant positive	2 (2)

Table 2: Characteristics of infants included in the study.

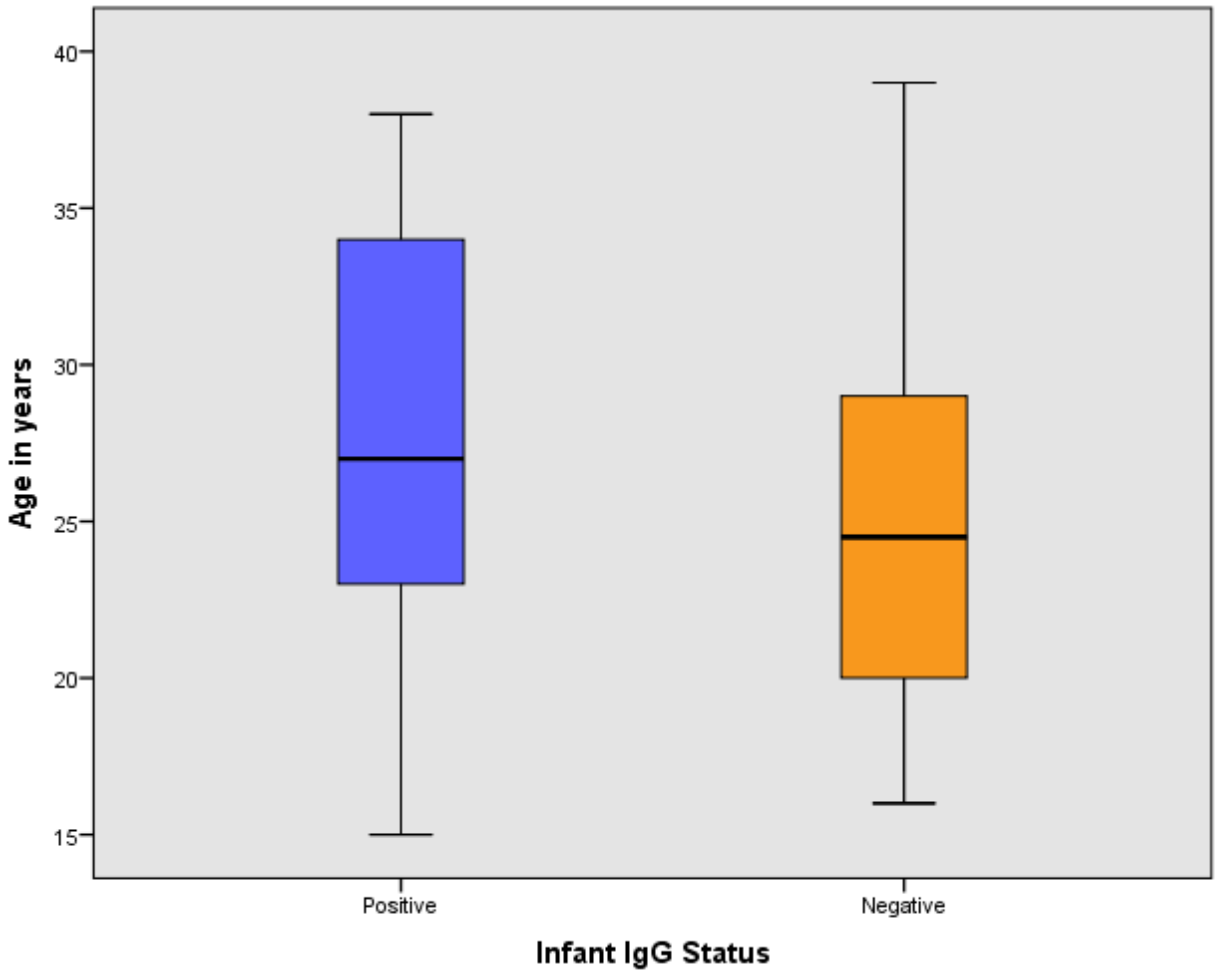
Variable	Number (n=126)	Percent
Sex		
Male	57	45.2
Female	67	53.2
Missing	2	1.6
Age		
6 months	51	40.5
9 months	74	58.7
Missing	1	0.8
Gestation age		
Preterm < 28 weeks	2	1.6
Preterm between 28 and 37 weeks	12	9.5
Term > 37 weeks	112	88.9
Birthweight		
< 2500g	12	9.5
> 2500g	111	88.1
Missing	3	2.4
Infant HIV status		
Exposed	29	23.0
Not exposed	97	77.0
Breastfeeding		
Breastfeeding	119	94.4
Stopped breast feeding	3	2.4
Never breastfed	1	0.8
Missing	3	2.4
Nutritional status		
Normal weight	91	72.2
Underweight	26	20.6
Moderate malnutrition	6	4.8
Severe malnutrition	2	1.6
Missing	1	0.8
Infant IgG		
Positive	12	9.5
Negative	101	80.2
Indeterminate	11	8.7
Missing	2	1.6

Table 3: Characteristics of the mothers included in the study

Variable	Number	Percent
Residential area		
Kanyama	69	54.8
Chilenje	57	45.2
Education level		
None	5	4.0
Primary	51	40.5
Secondary	62	49.2
Tertiary	4	3.2
Missing	4	3.2
Mother's Age		
< 20 years	20	13.9
20 – 29 years	74	58.7
≥30 years	31	24.6
Missing	1	0.8
Maternal Nutritional Status		
Underweight	13	10.3
Health Weight	77	61.1
Overweight	24	19
Obese	11	8.7
Missing	1	0.8
History of Measles vaccine		
Yes	88	69.8
No	9	7.1
Not known	24	19.0
Missing	5	4.0
History of Measles infection		
Yes	27	21.4
No	81	64.3
Not known	11	8.7
Missing	7	5.6
HIV status		
Positive	24	19.0
Negative	95	75.4
Missing	7	5.6

There were 20/126 (15.9%) mothers less than 20 years, the youngest being 15 years old. Seventy four (58.7%) out of 126 of the mothers were aged between 20-29 years, and 31/126 (24.6%) mothers were above 30 years, the oldest was 39 years old. The mean age was 25 and the median was 24 with standard deviation of 5.56. Eighty seven (69%) out of 126 of the mothers had positive measles IgG antibodies in the oral fluid, 32/126 (32.4%) were negative, and 6/126 (4.8%) were indeterminate or borderline. Mothers to infants with positive Measles IgG antibodies were older compared with those with negative antibodies (figure 4).

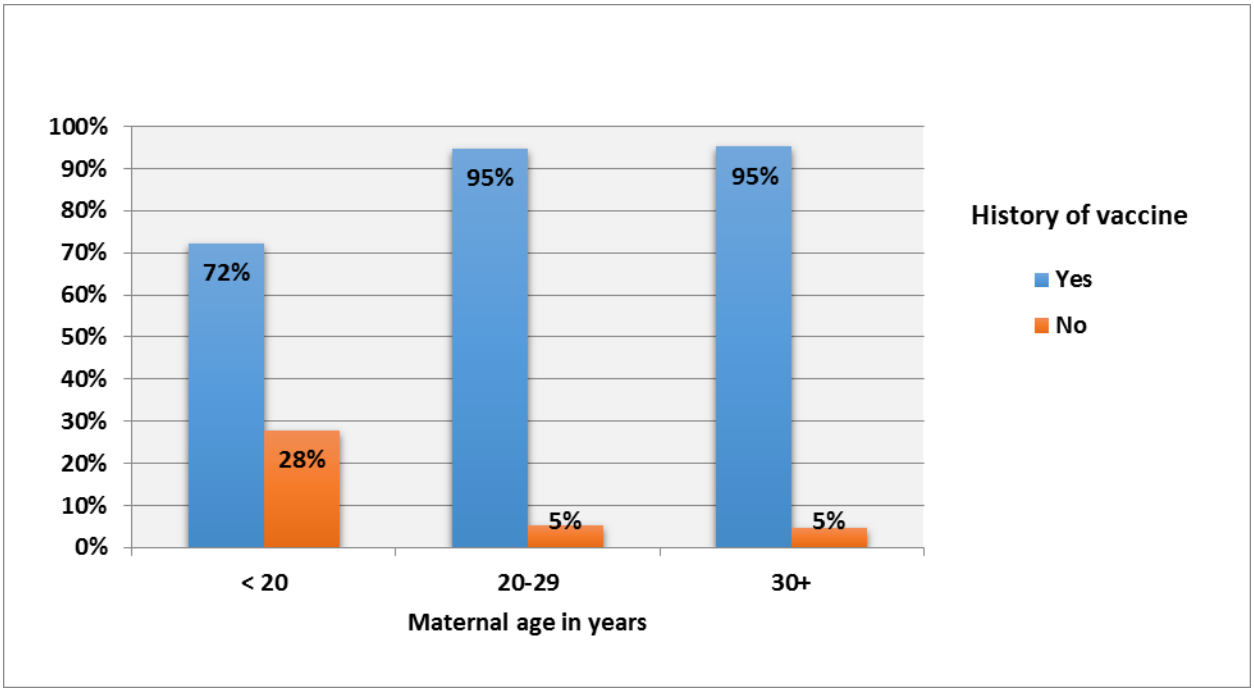
Figure 4: Boxplot comparing maternal age and infant measles IgG status



There were 88/126 (69.8%) mothers with history of measles vaccination in childhood, 9/126 (7.1%) with no history of vaccination, and 24//126 (19%) could not recall having been vaccinated against measles. There were 27/126 (21.4%) mothers with history of measles infection, 81/126 (64.3%) with no history of infection, and 11/126 (8.7%) unknown history of measles infection.

Figure 5 shows history of measles vaccine compared with mother age, mothers < 20 years had the highest proportion of no history of measles vaccination 5/18 (27.8%), and mothers ≥ 30 years had the highest proportion of history of measles vaccine 20/21 (95.2%).

Figure 5: Maternal vaccination history status by age



There were 12 infants with positive IgG status. Three out of the 12 infants were born to mothers with unknown history of measles vaccination and infection. Out of the 9 infants with positive IgG status and known maternal measles vaccination and infection history, 2/9 (22.2%) were born to mothers that had history of both the vaccination and infection. Seven out of nine (77.8%) were born to mothers who had history of vaccination but no infection.

Table 4 shows the cross-tabulation of the maternal history of vaccination, infection, and infant IgG status. The association was not significant, P-value = 0.84.

Table 4: Cross-tabulation of maternal history of measles vaccine, infection, and infant IgG status

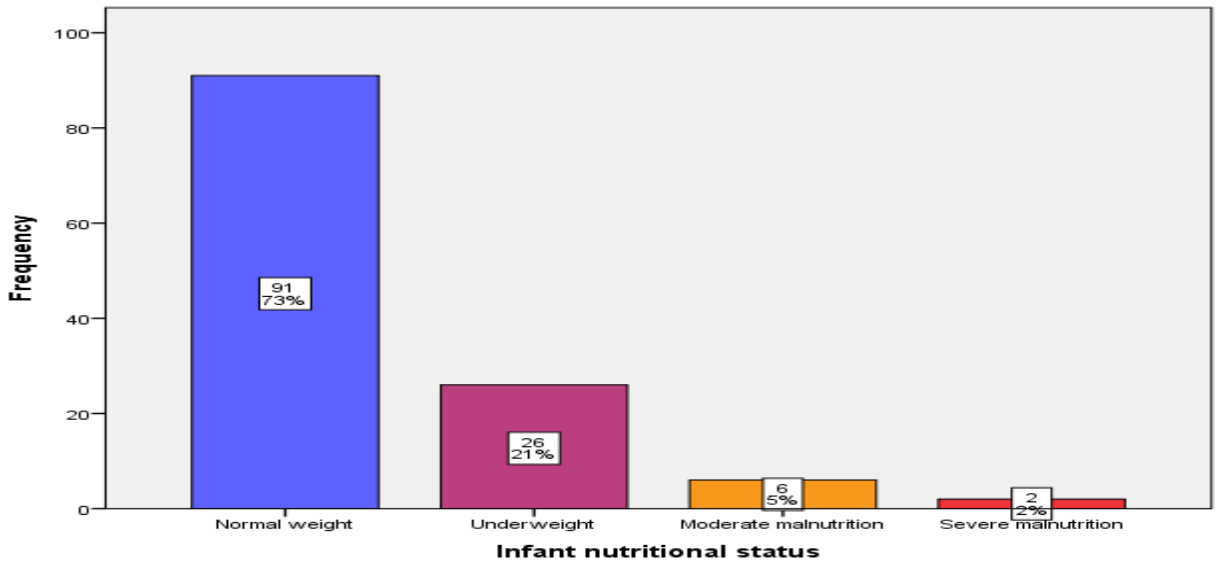
History of Measles Vaccine * History of Measles Infection * Infant IgG Cross tabulation

Infant IgG				History of Measles Infection		Total
				Yes	No	
Positive	History of Measles Vaccine	Yes	Count	2	7	9
			% with Measles Vaccine	22.2%	77.8%	100.0%
	Total		Count	2	7	9
			% with History of Measles Vaccine	22.2%	77.8%	100.0%
Negative	History of Measles Vaccine	Yes	Count	15	47	62
			% with History of Measles Vaccine	24.2%	75.8%	100.0%
		No	Count	1	4	5
			% with History of Measles Vaccine	20.0%	80.0%	100.0%
	Total		Count	16	51	67
			% with History of Measles Vaccine	23.9%	76.1%	100.0%
Total	History of Measles Vaccine	Yes	Count	17	54	71
			% with History of Measles Vaccine	23.9%	76.1%	100.0%
		No	Count	1	4	5
			% with History of Measles Vaccine	20.0%	80.0%	100.0%
	Total		Count	18	58	76
			% with History of Measles Vaccine	23.7%	76.3%	100.0%

Twelve (9.5%) out of 126 of the infants studied were born with low birth weight, 13/126 (10.1%) of the infants were preterm (born at < 37 weeks) while 112/126 (88.9%) were born at term (> 37 weeks). None of the infants born with low birth weight tested positive for measles antibodies, while 3/12 (25%) of those that tested positive were born preterm. Both low birth weight and prematurity had no significant association with presence of measles antibodies in infants (p=0.60 and p=0.14 respectively).

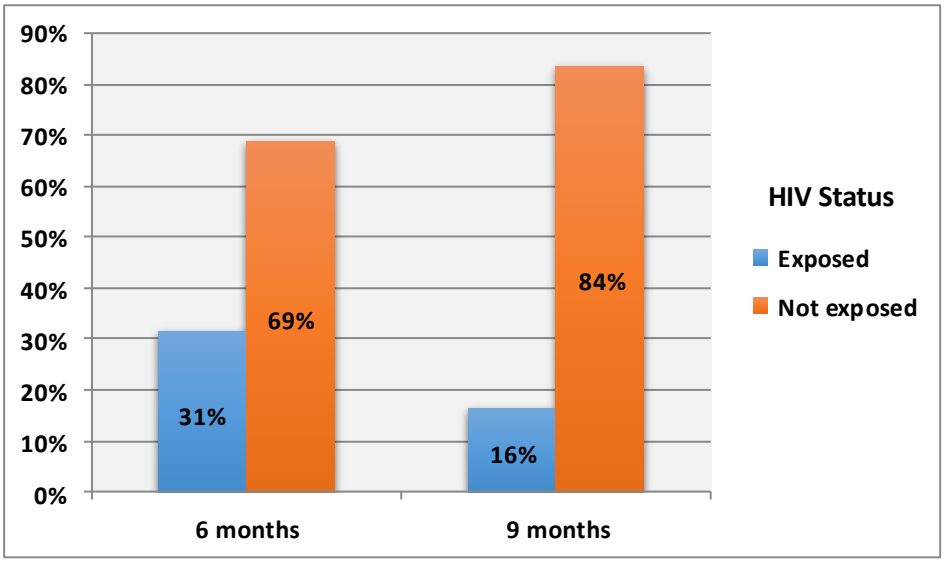
One hundred and nineteen (94.4%) out of 126 infants were exclusively breastfed up to 6 months and were still breastfeeding at the time the study was done. Majority of the infants, 91/126 (72.2%) had weight at median or above median, 26/126 (20.6%) infants were at -1SD, 6/126 (4.8%) infants had moderate malnutrition (-2SD), and 2/126 (1.6%) had severe malnutrition (-3SD). The number of mothers with healthy weight was 77/126 (61.1%), whereas 13/126 (10.3%) were underweight, 24/126 (19%) were overweight, and 11/126 (8.7%) were obese. There was no association between nutritional status of both mother and infant with the presence of measles IgG antibodies (Tables 5 and 6).

Figure 6: Infant Nutritional Status



Of the infants included in the study, 28/126 (22.2%) were HIV exposed. Sixteen out of fifty one (31.4%) of those aged 6 months and 12/74 (16.2%) of the 9 months old were HIV exposed. Four out of twenty eight (14.2%) of the HIV exposed and 8/98 (8.1%) of the HIV non exposed infants had positive measles IgG antibodies. There was no significant association between maternal and infant HIV status with presence of maternal measles antibodies (MMA), p= 0.28 (table 6).

Figure 7: Infant HIV status by age



Of the 12 infants that had positive maternal measles antibodies, stratified by HIV status, there were 2/12 (16.7%) HIV exposed children aged 6 months with positive measles IgG antibodies, and 2/12 (16.7%) HIV exposed children aged 9 months with positive measles IgG antibodies. The proportion of positive measles IgG antibodies in infants not HIV exposed was similar for the infants aged 6 months but different for the 9 months age where 5.1% had positive antibodies.

Tables 5 and 6 show bivariate association analysis for the study variables with infant measles IgG antibodies status. None of the study variables were significantly associated with infant IgG status at 5% significance level. Infant age, infant gestation age at birth, infant birth weight, education level of the mother, and age of mother had P-values less than 0.20. These were considered for multivariate logistic regression together with significant clinical factors such as HIV status and maternal IgG status.

Table 5: Bivariate analysis of mother characteristics with infant IgG status

Variable	IgG Positive		IgG Negative		P-value
	N	%	N	%	
Mother HIV status					
Positive	4	33.30%	20	19.80%	0.28
Negative	8	66.70%	81	80.20%	
Nutritional status					
Underweight	2	16.70%	11	11.00%	0.41
Healthy weight	9	75.00%	58	58.00%	
Overweight	1	8.30%	21	21.00%	
Obese	0	0.00%	10	10.00%	
Mother IgG					
Positive	10	83.30%	68	70.80%	0.50
Negative	2	16.70%	28	29.20%	
Mother age					
< 20 years	1	8.30%	14	14.00%	0.13
20-29 years	5	41.70%	63	63.00%	
≥ 30 years	6	50.00%	23	23.00%	

Table 5: (Continued)

Variable	IgG Positive		IgG Negative		P-value
	n	%	n	%	
Sex					
Male	6	50.00%	47	47.50%	0.87
Female	6	50.00%	52	52.50%	
Infant age					
6 months	7	58.30%	35	35.00%	0.13
9 months	5	41.70%	65	65.00%	
Gestation age					
Preterm ≤ 37 weeks	3	25.00%	10	9.90%	0.14
Term > 37 weeks	9	75.00%	91	90.10%	
Birthweight					
< 2500g	0	0.00%	11	11.10%	0.60
> 2500g	11	100.00%	88	88.90%	
Infant HIV status					
Exposed	4	33.30%	20	19.80%	0.28
Not exposed	8	66.70%	81	80.20%	
Breastfeeding					
Breastfeeding	10	90.90%	96	97.00%	0.35
Other	1	9.10%	3	3.00%	
Infant nutrition					
Normal weight	9	75.00%	73	73.00%	0.99
Other	3	25.00%	27	27.00%	
Residential area					
Kanyama	4	33.30%	52	52.50%	0.29
Chilenje	3	25.00%	11	11.10%	
Other	5	41.70%	36	36.40%	
Education level					
Primary or none	8	72.70%	42	42.90%	0.06
Secondary or better	3	27.30%	56	57.10%	
History of Measles vaccine					
Yes	10	90.90%	70	72.20%	0.38
No	0	0.00%	6	6.20%	
Not known	1	9.10%	21	21.60%	
History of Measles infection					
Yes	2	20.00%	22	22.40%	0.57
No	8	80.00%	67	68.40%	
Not known	0	0.00%	9	9.20%	

Table 6 shows logistic regression analysis predicting positive infant measles IgG antibody status. Adjusting for confounders 6 months old infants had about 4.75 times increased odds on average for positive measles antibodies compared to 9 months old infants [Odds ratio (OR) = 4.75, 95% Confidence interval (CI) = 1.06 - 21.35,]. Adjusting for confounders infants born to mothers aged 20-29 years had about 43% reduced odds for positive antibodies compared to infants born to mothers aged < 20 years, but this was not significant (OR = 0.57, CI = 0.05 - 6.84). Infants born to mothers aged ≥30 years had about 2 times increased odds for positive antibodies compared to infants born to mothers aged < 20 years, but this was not significant (OR=1.84, CI= 0.14 - 24.15). HIV exposed infants had about 10% reduced odds for positive antibodies compared to infants not HIV exposed, however, this was not significant (OR=0.90, CI=0.18 - 4.46).

Table 6: Logistic Regression

Variable	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p-value
Infant age			
9 months	1	1	
6 months	2.60 (0.77 - 8.80)	4.75 (1.06 - 21.35)	0.04
Gestation age			
Term > 37 weeks	1	1	
Preterm ≤ 37 weeks	3.03 (0.70 - 13.07)	4.34 (0.82 - 22.96)	0.08
Education level			
Secondary or better	1	1	
Primary or none	3.56 (0.89 - 14.22)	3.93 (0.86 - 17.88)	0.08
Mother age			
< 20 years	1	1	
20-29 years	1.11 (0.12 - 10.27)	0.57 (0.05 - 6.84)	0.31
≥ 30 years	3.65 (0.40 - 33.59)	1.84 (0.14 - 24.15)	
Infant HIV status			
Not exposed	1	1	
Exposed	2.03 (0.55 - 7.40)	0.90 (0.18 - 4.46)	0.90
Mother IgG status			
Positive	1	1	
Negative	0.49 (0.10 - 2.36)	0.47 (0.07 - 2.96)	0.42

CHAPTER FIVE

5.1 DISCUSSION

Measles remains the leading cause of vaccine preventable disease that contributes to under 5 mortality rate despite the availability of a safe and effective vaccine (1). Vaccination is the most cost effective public health intervention available for preventing morbidity and mortality from measles. Many infants are susceptible to measles infection before the age of vaccination (7).

In the population studied, passively acquired maternal measles antibodies seem to wane earlier in infancy than was shown in previous studies that were the basis of the age of vaccination in Africa (39, 40). Majority of the infants, 81.4%, included in this study did not have protective maternal measles IgG antibodies, meaning that this population of children is at risk of measles infection before the age of vaccination of 9 months. An additional 11% had indeterminate or borderline results which may not be protective levels. Only 8% of the infants included in the study had protective measles antibodies from their mothers.

In this study it was found that 83% of infants aged six months and 93% of those aged nine months had no protective maternal antibodies against measles detectable in their oral fluid. There are a lot of other studies done in various regions of the world that demonstrated early loss of maternal protective measles antibodies and early immunization was recommended.

In South Africa, a longitudinal study done on black infants to measure the loss of maternal measles antibody in order to investigate the feasibility of measles vaccination before the age of 9 months found unprotective measles antibodies in 88% of 6 months old while at 9 months all were susceptible to measles virus infection (41). In Mozambique, it was noted that 82.4% of infants had lost maternal antibodies by 6 months (42).

A study done in Bangladesh to measure MMA decay in rural Bangladesh in 1994 showed that only 12% of infants at age 5 months and 5% at age 8 months had protective maternal measles antibodies (43).

A study in Switzerland demonstrated that IgG antibodies against mumps, rubella and measles were lacking in the majority of infants, the following seroprevalence rates for IgG antibodies were found (measles/mumps/rubella): 0-3 months 97%/62%/91%, >3-6 months 40%/2%/42%, >6-9 months 4%/2%/10%, >9-12 months 2%/0%/12%, >12-16 months 0%/7%/7% (44). Another study in Turkish infants found seropositivity rates at 6 and 9 months to be 61.8% and 3.4% respectively (45). In Argentinian infants, measles antibodies decreased from 85% at 1 month of age to 8% at 8 months and it was found that 80% of infants were susceptible to measles infection for at least 3 months before routine immunization age at 1 year (46).

There are quite a number of factors that have been attributed to influence the decay of MMA in infants. A study done in West Africa (Gambia) to determine the influence of prematurity and low birth weight on transplacental antibody transfer showed that maternal fetal transfer of antibodies is impaired in premature and low birth weight babies (47). Another study done in Sri Lanka also concluded that prematurity and low birth weight may influence the level of maternally acquired immunity in Sri Lankan neonates (48). In India, a study done to look at transplacentally transmitted anti – measles antibodies in term and preterm infants demonstrated a statistically significant decline in transplacentally transmitted anti-measles antibodies in preterm infants over a period of 5 months after birth but no significant correlation in statistical terms was observed between gestational age at birth and antibody status (49).

In this study, prematurity and low birth weight were not significant factors influencing the status of measles antibodies in the studied population. It was also noted that there was no significant

difference in results in infants of different sex (p= 0.87). Gestational age at birth was also not a significant factor (p=0.53). Both the South African and Mozambican studies also showed that prematurity and low birth weight were not associated with the presence of immunoglobulin in oral fluid from infants (41, 42).

Maternal HIV infection was not a significant factor affecting early loss of passively acquired measles antibodies in this study. These findings are in agreement with a study done in a Nigerian tertiary health care facility where it was also noted that maternal HIV infection was not associated with reduced MMA in mother-infant pairs as high protective levels were evident in both mother-infant pairs at birth (50). It was also noted that 94% of mothers exclusively breastfed their infants regardless of the HIV status, and the type of feeding did not have an effect on the presence of antibodies in the infants.

Maternal age and measles vaccination as well as maternal and infant nutritional status in this study were found to have no influence on the status of measles antibodies in the infants. It was noted that older mothers had a higher proportion of infants with positive maternal measles antibodies as compared to younger mothers. Previous studies have shown that mothers believed to have vaccine induced immunity would give birth to offspring with a wider window of susceptibility than mothers with immunity from natural immunity (51, 52). In Antwerp, Belgium, it was found that among infants of naturally infected mothers, only 5% had protective antibodies after 6 months, which is much shorter than believed earlier (53).

This study also found that the status of maternal measles antibody in mothers had little effect on the early decay of passively acquired antibodies as it showed that 69% of mothers had positive antibodies as compared to 8% of infants in the studied population. It is difficult to explain why this is so. There was a small proportion of infants (2) with detectable measles IgG antibodies that

were born to mothers that did not test positive to measles antibodies. The mothers to these infants did not give any history of measles infection in these children. Subclinical infection post exposure to the wild type virus could explain this, however, this does not exclude the possibility of false positive test reactions in these infants. In this group of infants, it would have helped explain these findings if measles IgM antibodies were done as evidence for recent exposure to the wild type virus.

There is some variation in the decay of maternally derived measles antibodies in infants in the studies done in various regions of the world, however all have shown early loss of these antibodies in the different populations studied and have recommended consideration of an earlier dose before the current age of vaccination (9 months in developing countries, 12-15 months in developed countries). Using mathematical models, Mclean and Anderson demonstrated that the rate of loss of maternal derived immunity to measles is broadly similar in developed and developing countries (54). This study has also demonstrated early loss of MMA in the population of infants in urban Lusaka, Zambia.

CHAPTER SIX

6.1 CONCLUSION

A number of studies done in different regions of the world have demonstrated early loss of passively acquired MMA in infants and have recommended early immunization in infants to reduce the susceptibility of measles infection. WHO current recommendation is 2 doses of measles vaccination in countries that have more than 80% of measles vaccine coverage. Zambia has achieved over 80% of measles vaccination, and has introduced a second measles dose to be administered at 18 months, however no studies have been done to justify when the second dosage should be given. This study also has demonstrated early loss of maternal measles IgG antibodies, and therefore an earlier dose of measles vaccination has to be considered in infants aged below 9 months.

The assessment of the status of passively acquired maternal measles antibodies in infants coupled with adjustment of vaccinations schedules and implementation of vaccination strategies could improve protection from measles in infants. This includes undertaking large scale studies to determine the appropriate age for immunization against measles. The optimal age of measles vaccination has to be determined in different regions of the world. It is defined as the age with the highest proportion of infants responding to the vaccine (55) and is dependent on the presence of maternal antibodies against measles virus as well as the maturation of the immune system (55). To achieve elimination of measles, vaccination has to be at the earliest possible time after clearance of maternal antibodies. This will help keep the disease in the susceptible infants as low as possible. Therefore, this study highlights the urgent need to undertake large scale studies to determine the appropriate age for measles immunization in the Zambian infant population.

6.2 LIMITATIONS

Results obtained in this study may not be generalized to the entire Zambian population as the findings are specific to the mother-infant pairs attending Kanyama and Chilenje Health Centres.

Vaccine related and natural immunity to measles in the mothers were not differentiated in the study, this has an implication on the immune status of the mother.

Recall bias was another limitation as the mothers were asked to give information on events that occurred in the past when they were infants (for vaccination).

6.3 RECOMMENDATIONS:

Policy makers have to be aware of the probable early loss of maternal protection against measles infection in the Zambian infant population.

The policy on the first dose of measles vaccination at age nine months needs to be revisited, by considering administering the vaccine earlier at 6 to 7 months and a second booster dose at a later age of 12 months.

The need for further studies to assess the status of measles maternal antibodies in infants below the immunization age at a larger scale should be emphasized and given priority in order to come up with the optimal age for the first dose of measles vaccine.

Policy makers under the Ministry of Community Development, Mother and Child Health will have to conduct further studies to assess the nationwide prevalence of maternal measles IgG antibody in women of childbearing age.

REFERENCES

1.http://www.who.int/immunization_monitoring/diseases/measles/en/index.html.WHO Immunization Surveillance, Assessment and Monitoring 2013 Review

2. www.un.org/millenniumgoals/childhealth.shtm. United Nations Millennium Development Goals, Goal 4

3. Wolfson J.L.et al, Estimating the costs of achieving the WHO–UNICEF Global. Immunization Vision and Strategy, 2006–2015.

4. www.who.int/mediacentre/factsheets/feb 2013/en).

5. http://www.who.int/mediacentre/news/notes/2013/measles_20130117/en/.

6. Data from patients files - University Teaching Hospital, Lusaka Zambia.

7. Mpabalwani E, Matapo B, Katepa-Bwalya M, Mukonka V, Mutambo H, Bubaniyi OA. The 2010-2011 measles outbreak in Zambia: Challenges and lessons learnt. East African Journal of Public Health. Vol 10 No. 1 March 2013 265.

8. World Health Organisation. Immunisation policy, global programme for vaccines and immunization, expanded programme on immunization WHO/EPI/GEN/95.03 Rev1.

9. Moss WJ, Griffin DE: Global measles elimination. Nat Rev Microbiol 2006, 4(12):900-908.

10. Meissner HC, Strebel PM, Orenstein WA. Measles vaccines and the potential for worldwide eradication of measles. Pediatrics 2004;114:1065-9.

11. Kerdiles YM, Sellin CI, Druelle J, Horvat B. Immunosuppression caused by measles virus: role of viral proteins. Rev Med Virol. 2006 Jan-Feb;16(1):49-63.

12. Moss WJ, Fisher C, Scott S et al. HIV type 1 infection is a risk factor for mortality in hospitalized Zambian children with measles, oxford journals, clinical infectious diseases. (2008) 46 (4):523-527.

13. de Moraes-Pinto M, Verhoeff F, Chimsuku L et al. Placental antibody transfer; influence of maternal HIV infection and placental malaria, Arch Dis Child Fetal Neonatal Ed. 1998 Nov; 79(3): F202-F205.

14. Alexander D. Langmuir, M.D. Medical Importance of Measles, *Am J Dis Child.* 1962; 103(3):224-226.

15. http://www.who.int/wer WHO weekly epidemiological record No. 49, 2008, pp. 441–448.

16. http://www.who.int/mediacentre/news/releases/2009/measles_mdg_20091203/en./

17. Enders JF, Peebles TC. Propagation in tissue cultures of cytopathic agents from patients with measles. ProSocExBio Med 1954:86:277-86.

18. Nelson Textbook of Pediatrics; 18th edition, pages 1331 - 1337.

19. Campbell H, Andrews N, Brown KE, Miller E. Review of the effect of measles vaccination on the epidemiology of Subacute Sclerosing Panencephalitis, International Journal of Epidemiology, 2007.

20. Whittle HC, Bradley Moore A, Fleming A, Greenwood BM. Effects of measles on the immune response of Nigerian children. Arch. Dis. Child. 1973;48:753–756.

21. Osler, W. 1904. *The Principles and Pract1ices of Medicine,* 5th ed., p. 88. D. Appleton and Company, New York, N.Y.

22. Kipps A, Vaughan EG. The duration and the possible significance of the depression of tuberculin sensitivity following measles. S. Afr. Med. J. 1966; 40:104–108.

23. Abramson O, Dagan R, Tal A, Sofer S. Severe complications of measles requiring intensive care in infants and young children. Arch. Pediatr. Adolesc. Med. 1995;149:1237–1240.

24. Gavish D, Kleinman Y, Morag A, Chayek-Shaul T. Hepatitis and jaundice associated with measles in young adults. An analysis of 65 cases. Arch. Intern. Med. 1983; 143:674–677.

25. http://www.who.int/biologicals/vaccines/measles/en/. WHO updates March 2013

26. Linneman CC Jr, Dine MS, Roselle GA, Askey PA. Measles Immunity after Vaccination: Results in Children Vaccinated before 10 Months of Age, Pediatrics,69(3): 332-335, March 1982.

27. Hilleman, MR, Buynak EB, Werbel RE, Strokes J Jr, Whitman JE Jr, Leagus MB. Development and Evaluation of the Moraten Measles Virus Vaccine, JAMA2006(3): 587-590, 1968.30.

28. www.who.int/mediacentre/factsheets/feb 2013/en). WHO media facts sheets, February 2013.

29. Moss WJ, Fisher C, Scott S et al. HIV type 1 infection is a risk factor for mortality in hospitalized Zambian children with measles, oxford journals, clinical infectious diseases. (2008) 46 (4):523-527.

30. Moss WJ, Monze M, Ryon JJ, Quin TC, Griffin DE, Cuttis F. Prospective Study of Measles in Hospitalized, Human Immunodeficiency Virus (HIV)—Infected and HIV—Uninfected Children in Zambia, oxford journals, clinical infectious diseases. 2002 Jul 15;35(2):189-96. Epub 2002 Jun 19.

31. Permar SR, Moss WJ, Ryon JJ et al. Prolonged measles virus shedding in HIV infected children, detected by reverse transcriptase-polymerase chain reaction. Journal of infectious diseases. 2001 Feb 15; 183:532-8. Epub 2001 Jan. 18.

32. Tapia MD, Sow SO, Medina-Moreno S et al. A serosurvey to identify the window vulnerability to wild type measles among infants in rural Mali. AM J Trop Hyg; 2005;73(3) 26-31.

33. Gendrel D, Engohan E, Gendrel C et al. Disparition des anticorps anti-rougeoleux d' origine maternel lechezle nourrisong abonais = Disappearing of antimeasles antibodies of maternal origin in the Gabonese infant. SOPEMI; 1987;34:29-32.

34.http://www.who.int/immunization_delivery/adc/measles/measles/en/index3.html.WHO Immunization, Vaccines and Biologicals, Published in Geneva 2013

35.http://www.who.int/immunization/policy/Immunization.pdf. WHO immunization Policy Published in Geneva 2013

36.http://www.undp.org/content/dam/undp/library/MDG/english/MDG/Country/Reports/Zambia/Zambia/MDG/Report/2011.pdf. Millennium Development Goals Progress Report 2011-Zambia.

37. Nokes DJ, Enquselassie F, Nigatu W, Vyse AJ, Cohen BJ, Brown DW, Cutts FT: Has oral fluid the potential to replace serum for the evaluation of population immunity levels? A study of measles, rubella and hepatitis B in rural Ethiopia. Bull World Health Organ 2001, 79(7):588-595.

38. Nigatu W, Nokes DJ, Cohen BJ, Brown DWG, Vyse AJ: Pre-and post-vaccine measles antibody status in infants using serum and oral-fluid testing: An evaluation of routine immunization in Addis Ababa, Ethiopia. Ethip J Health Dev 2003, 17(3):149-155

39. Ministry of Health of Kenya and the World Health Organisation. Measles immunity in the first year after birth and the optimum age for vaccination in Kenyan children. Bull WHO 1977;55:21-31.

40. World Health Organisation. Expanded Programme on Immunisation. Weekly Epidemiol Rec 1981;56: 9-12.

41. Kiepiela P, Coovadia HM, loaning WE, Coward P, Abdool Karim SS. Loss of maternal measles antibody in black South African infants in the first year of life, implication for age of vaccination. SAfr Med J 1991; 1991; 79(3): 145-8.

42. Jagrati VJ, Hansen HC, Mussa T et al. Assessment of measles immunity among infants in Maputo city, Mozambique. BMC Public Health 2008, 8:386 doi:10.1186/1471-2458/8/386.

43. De Francisco A, Hall AJ, Unicomb L, Chakraborty J, Yunus M, Sack RB. Maternal measles antibody decay in rural Bangladeshi infants – implications for vaccination schedules. Vaccine 1998 Apr;16 (6):564-8.

44. Desgrandachamps D, Schaad UB, Glaus J, Tusch G, Heininger U. Seroprevalence of IgGantibodies against measles, mumps and rubella in Swiss children during the first 16 months of Life. Schweiz Med Wochenschir 200 Oct14;130(41):1479-86.

45. Altintus DU, Evliyaoglu N, Kilinc B, Sen'an DI, Guneser S. The modification in measles vaccination age as a consequence of the earlier decline of transplacentally transferred anti-measles antibodies in Turkish infants. Eur J Epidemiol 1996; 12(6): 647-8.

46. Nates SV, Giordano MO, Medeot SI et al. Loss of maternally derived measles immunity in Argentinian infants. Pediatr Infect Dis J. 1998 Aug;17(8):763.

47. Okoko JB, Wesumperuma HL, Hart CA. The influence of prematurity and low birth weight on transplacental antibody transfer in rural West African population. Trop Med Int Health 2001; 6(7):529-34.

48. Wesumperuma HL, Perera AJ, Pharoah POD, Hart CA. The influence of prematurity and low birth weight on transplacental antibody transfer in Sri Lanka. Ann Tro Med Parasitol 1999; 93:169-77.

49. Rau ATK, Dhulia A, Wilsom CG, Chopra GS, SarkarPK. Transplacentally transmitted anti-measles antibodies in term and preterm infants. Indian Pediatrics' 2002; 39:282-288.

50. Baba UA, Mustapha MG, Ashir GM, Rabasa Al, Ibrahim BA, Ibrahim HA. Passive measles immunity in a Nigerian tertiary health care facility: the influence of Human Immunodeficiency Virus infection on measles antibody levels in mother-infant pairs at birth. West Afr J Med 2012 Oct-Dec, 31(4):243-6.

51. Lenon JL, Black FL. Maternally derived immunity in the era of vaccine protected mothers. JPediatr 1986, 108(5 Pt I):671-676.

52. Jenks PJ, Caul EO, Roome AP. Maternally derived measles immunity in children of naturally infected and vaccinated mothers. Epidemiol Infect 1988, 101 (2):473-476.

53. LeuridenE, Hens N, Natse V, Leven M, Aerts M, Van Damme P. Early waning of maternal measles antibodies in the era of measles elimination. BMJ – 28 May 2010.

54. Mclean AR, Anderson RM. Measles in developing countries. Part I. Epidemiological parameters and patterns. Epidemiol Infect 1988 Feb;100(1):111-133.

55. del Buone MB, Vaschetti P, Iannicelli J, Silber R. Age related loss of maternal antibodies against measles in children in -Jun;35(2):102-105.

APPENDICES

APPENDIX A: CONSENT FORM

UNIVERSITY OF ZAMBIA

DIRECTORATE OF RESEARCH AND GRADUATE STUDIES

UNIVERSITY TEACHING HOSPITAL, PAEDIATRICS DEPARTMENT

PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE**: THE STATUS OF PROTECTIVE MEASLES ANTIBODIES IN HIV-1 POSITIVE AND NEGATIVE INFANTS AGED 6 AND 9 MONTHS IN URBAN LUSAKA, ZAMBIA.**

Principal Researcher: **Dr. Maureen Simwenda**

Supervisors: **Prof. M.P.S Ngoma**

Dr. E. Mpabalwani

INTRODUCTION

I am Dr. Maureen Simwenda, a medical doctor working in the Paediatrics department at the University Teaching hospital (UTH). I am pursing a Master's Degree in Paediatrics and Child Health. This study is being conducted as part of the degree. I am responsible for this study.

PURPOSE OF STUDY

This research study will be looking at the protection against measles offered by a mother to her infant aged 6 and 9 months. The study hopes to find out how many infants have this protection before they receive their measles vaccine at 9 months.

You are invited to take part in this study because you have a child who is 6 / 9 months old.

VOLUNTARY PARTICIPATION

Your participation in this study is voluntary. If you decide to not participate in this study, it will not negatively affect your care at this under-5 clinic.

PROCEDURE

Once you agree to take part in this study, you will sign this consent form to show your willingness to take part. Following this, you will be asked some questions from a questionnaire about you and your child. The answers to the questions will be entered on the form. Your name will not be entered on the questionnaire. You will be allocated a study number.

Your child's weight, height and mid-upper arm circumference will be measured. A physical examination of your child will be done, particularly to assess signs of malnutrition like nutritional edema. Your weight and height will also be measured to assess your nutritional status. You will be requested to provide a sample of saliva. Your child will also provide saliva. This will be collected using a special collecting device. If at all you and your child's HIV status is not documented, you will be requested to do an HIV test, after in depth counseling.

RISKS, INCONVENIENCES AND DISCOMFORT

You will need to take time off your usual routine under-5 clinic visit to answer the questions from the researcher, have your measurements taken and saliva samples collected. This will not exceed 30 minutes.

You and your child will experience pain from the finger and or heel prick for the HIV test if we need to do it. There may also be some discomfort when collecting the saliva and scrapings. The procedures will be done by skillful and trained persons so that there is no infection.

BENEFITS OF PARTICIPATION

Participation in the study will give you added exposure to a trained healthworker whom you can consult on other health issues. The findings of the study will help in the prevention of measles in Zambia, which will indirectly benefit your community.

ALTERNATIVE TO PARTICIPATION

If you do not choose to participate in the study, your clinic under-5 visits will not be affected in any way and you will continue to receive the care you always receive.

PAYMENT FOR PARTICIPATION

You will not be paid in any form for your participation in this study.

COSTS TO PARTICIPANT

You will not be required to pay anything in order to take part in this study.

CONFIDENTIALITY

The information you give will not be shared with anyone apart from the researcher, supervisors and University of Zambia examination board. Your name will not be indicated on the forms used to collect information. You will instead be identified by a code. You will not be personally identified in the write up of this research or in future publications.

QUESTIONS ABOUT THE RESEARCH

If you have any questions about the research, please contact the researcher, Dr. Maureen Simwenda on 0977 410 461 or email maursim@hotmail.com. You may also contact the Eres Converge Committee chairperson for any concerns on telephone 0955 155 633 or email eresconverge@yahoo.co.uk.

LEGAL RIGHTS AND SIGNATURES

I _____ consent to take part in this study conducted by Dr. Maureen Simwenda. The nature of the study and what is involved have been clearly explained to me and I understand them. I am not waiving any of my legal rights by signing this form.

My name and signature/thumb print are indicated below as an indication of my consent.

_____	_____	_____
PRINT NAME OF PARTICIPANT	SIGNATURE OF PARTICIPANT	DATE
_____	_____	_____
PRINT NAME OF WITNESS	SIGNATURE OF WITNESS	DATE
_____	_____	_____
PRINT NAME OF RESEARCHER	SIGNATURE OF RESEARCHER	DATE

APPENDIX B: **STUDY QUESTIONNAIRE - INFANT**

STUDY CODE NUMBER: { }

SOCIO-DEMOGRAPHIC INFORMATION

Sex: M { } F { }

Date of Birth (from under-5 card:{ }

Age in months (at last month):
6 months { } 9 months { }

Gestational Age at Birth:
Preterm <28weeks { } Preterm < 37weeks but >28 weeks { } Term >37weeks{ }

Birth Weight:
<2,500g { } >2,500g { }

HIV STATUS (Documented)
Exposed { } Not Exposed { } Infected { }

NUTRITIONAL STATUS / ANTHROPOMETRIC MEASUREMENTS

Breastfeeding
Breastfeeding { } Stopped Breastfeeding { } Never Breastfed { }

Height (in cm, rounded to the nearest 0.1cm): _____

Weight (in kg, rounded to the nearest 100g): _____

Z-score for Weight for Height:_____

Mid Upper Arm Circumference: <11cm { } 11-12cm { } >13cm { }

Presence of Nutritional Edema: Yes { } No { }

APPENDIX C**: STUDY QUESTIONNAIRE - MOTHER**

STUDY CODE NUMBER: { }

SOCIO-DEMOGRAPHIC INFORMATION

Age in years (at last Birthday): _____

Religion:
Christian { } Muslim { } Hindu { } Other { }
Specify if 'Other'……..

Residential area:
Kanyama Area { } Chilenje Area { } Other { }

Highest Level of Education:
None { } Primary { } Secondary { } Tertiary { }

History of Measles Vaccine:
Yes { } No { } Not known { }

History of Measles Infection:
Yes { } No { } Not known { }

If Yes state when ……

HIV Status (Documented):
Positive { } Negative { }

Nutritional Status of Mother:
Weight { } Height { } BMI { }

APPENDIX D: **LABORATORY INFORMATION FORM**

STUDY CODE NUMBER: { }

LABORATORY FINDINGS:

Infant Sample:

IgG Antibodies: Negative { } Positive { }

Mother Sample:

IgG Antibodies: Negative { } Positive { }

Infant HIV Antibody Test

Negative { } Positive { } Not applicable (Documented HIV status) { }

Infant HIV PCR Test

Not Detected { } Detected { }

Mother HIV Antibody Test

Negative { } Positive { } Not applicable (Documented HIV status) { }