MEASLES VACCINE-INDUCED IMMUNITY IN CHILDREN AGE NINE MONTHS AFTER VACCINATION AND MEASLES-VACCINATED CHILDREN AGE FIVE YEARS IN NAIROBI

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JULY, 2014
DECLARATION

This thesis is my original work and has not been presented for the award of a degree or any other award in any other university.

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DEDICATION

This work is dedicated to my wife Katherine K. Kofa Brown, to my late parents Mr. Peter Sieh Brown and Mrs. Mary G. Brown. And my late uncle and his wife Mr. Edwin Brown and Mrs. Toja Brown
ACKNOWLEDGEMENT

I wish to extend my sincere gratitude to Rebuilding Health Sectors (RBHS) – Liberia for the sponsorship and the Mother Patern College of Health Sciences for affording me the opportunity to go through this study. Special thanks to my supervisors Dr. Margaret Muturi, Department of Medical Laboratory Sciences, who has motivated me through every step along the way including Dr. Lucy Kamau, Department of Zoological Sciences and Dr. Caren Emadau, the Pediatrician at the Mama Lucy Kibaki Hospital all of whom were instrumental in guiding and advising me and supported me in the entire process. I am also grateful to the staff of the Mama Lucy Kibaki Hospital most especially to Mr. Peter Magubo of the laboratory, Ms. Veronica Kimani and Ms. Ruth Ingabo both of the Maternal Child Health (MCH) department of the hospital for their support; the family of the African Medical Research Foundation (AMREF) for the cordial reception afforded and permitting me to use their laboratory and the entire family of the Medical Laboratory Department of Kenyatta University.

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Above all, Glory is to God Almighty for the strength and all of his blessings upon me.
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<thead>
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<tbody>
<tr>
<td>AMREF</td>
<td>African Medical and Research Foundation</td>
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<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>DREF</td>
<td>Disaster Relief Emergency Fund</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<tr>
<td>GIVS</td>
<td>Global Initiatives for Vaccination Strategy</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IgG-HRP</td>
<td>Immunoglobulin G Horse-Radish Peroxidase</td>
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<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>mIgG</td>
<td>Maternal Immunoglobulin G</td>
</tr>
<tr>
<td>MLKH</td>
<td>Mama Lucy Kibaki Hospital</td>
</tr>
<tr>
<td>MDG</td>
<td>Millennium Development Goal</td>
</tr>
<tr>
<td>MoPHS</td>
<td>Ministry of Public Health and Sanitation</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>SIAs</td>
<td>Supplementary Immunization Activities</td>
</tr>
<tr>
<td>SLAM</td>
<td>Signaling Lymphocyte Activation Molecule</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nations Children’s Fund</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>WHOAFR</td>
<td>World Health Organization Africa Region</td>
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ABSTRACT

Measles is the leading cause of vaccine-preventable deaths among children under 5 years globally, despite the availability of measles vaccines. Despite national and international efforts in the coverage of measles vaccination in Kenya, the cases of measles continue to rise in recent years. Measles vaccination coverage was about 86% as reported in 2010, in Kenya following the 2009 Supplementary Immunization Activities (SIAs). In Nairobi, as of 2012 the measles vaccination coverage was over 100%. Yet in 2012, the number of measles cases was 2462 up to the 5th of November, with 767 cases reported from September to the 3rd of October 2012, an indication that the disease infects even the measles vaccinated children. The aim of this study was to evaluate the level of measles-specific maternal antibodies (mIgG) prior to vaccination in children, at age nine months; immunity induced by measles vaccine in children after initial vaccination; and the immune status of children aged 5 years who had been vaccinated at age 9 months in Nairobi. The study also investigated factors that lead to measles outbreaks in Nairobi. 2ml of blood was collected from 66 children scheduled for vaccination before and two weeks after vaccination. Another 2ml of blood was obtained from 62 children aged 5 years, who visited the child welfare clinic at the Mama Lucy Kibaki Hospital (MLKH) from August to November 2013. Each mother of the enrolled children was asked to voluntarily fill out a structured questionnaire. The blood samples were analyzed for the levels of measles specific IgM and IgG using ELISA. The results showed that 97.0% of the children had lost maternal IgG to measles before vaccination at the age of 9 months. The results also revealed that 77.5% of the children sampled for post-vaccination analysis had measles-specific IgM levels greater than 15 IU/ml, which is considered positive. 88.7% of the children aged 5 years had IgG levels greater than 200 mIU/ml, which is conventionally considered to be immune. This study concluded that maternal measles antibodies are lost before 9 months in most children in Nairobi, Kenya. It is also concluded that the measles vaccine (Schwarz strain) that is administered at 9 months is immunogenic and that the antibodies (IgG) produced by measles vaccination persist up to 5 years after vaccination. It was also noted that mothers who had secondary level education and above, had a better understanding of measles and the importance of vaccination; therefore they were most likely to have their children vaccinated. The age of the mothers had no significance to the vaccination of the children. It is suggested that initial vaccination be given earlier than age nine months. In addition children could be given a booster vaccination at age 12 months in order to cater for the small fraction (3%) whose maternal IgG were detected at 9 months old. Since the present study was conducted in a relatively small population, a further study is recommended in a larger population.
CHAPTER ONE

INTRODUCTION

1.1 Background information

Measles is the leading cause of vaccine-preventable death among children under 5 years globally. There are vaccines that are available for measles, which was first licensed for use in 1963, and became included in the Extended Programme on Immunization (EPI). In 1980, the Kenya Extended Programme on Immunization (KEPI) now Division of Vaccination and Immunization (DVI) was established, with measles vaccine as one of the first six vaccines in the program (CDC, 2007). Before widespread use of measles vaccination, almost everybody was infected in early childhood globally and acquired lifelong immunity. In the 1980s, measles killed an estimated 2.6 million children globally each year (WHO, 2011).

The widespread adoption of the measles vaccine in national immunization programs since the establishment of the Expanded Programme on Immunization has marked a decrease in the number of reported measles cases. With increasing immunization coverage, the global number of measles deaths mostly in children reduced to about 750,000 in 2000 and to about 197,000 deaths, mostly in children, in the year 2007 (WHO, 2008). Since 2010, there has been an increase in the number and a reemergence of the cases of measles outbreaks worldwide, with approximately 158,000 deaths reported in 2011, mostly in children under the age of five years (WHO, 2013).
Kenya is one of the countries in Africa that has been successfully implementing routine vaccination since the establishment of the EPI in an effort to achieve herd immunity for measles, including other vaccine preventable diseases. However, over the past years, the country has been experiencing increased in the number of measles outbreaks (DREF, 2011). According to the Ministry of Public Health and Sanitation (MoPHS) of Kenya, the year 2012 recorded 2462 confirmed cases of measles by the 5th of November as compared to 2331 cases in 2011 (>5% increase) (WHO, 2012), and over 40 deaths of under-five years despite the series of vaccination campaigns that had been carried out nationwide.

During 2000–2011, the number of measles cases reported worldwide each year decreased by 58%, from 853,480 to 354,922, and measles incidence decreased by 65%, from 146 to 52 cases per million population per year, with declining cases and incidence reported in all WHO regions. However, since reaching a low of 278,417 reported cases worldwide in 2008, annual reported cases have increased each year, from 2010 and large outbreaks of measles have been reported in several countries (CDC, 2013). In 2011 there were 327,305 cases reported with approximately 158,000 deaths from measles mostly children under the age of five years globally (Simons et al., 2012; WHO, 2013).

Despite Measles vaccination coverage in Nairobi being reported to be over 100%, there have been cases of measles. It is important therefore, to find out whether the current measles vaccine is effective and whether the immunity obtained after vaccination beyond
This study determined the levels of measles specific IgM and IgG induced by the measles vaccine administered in Kenya, the levels of maternal antibodies prior to vaccination and the factors associated with measles outbreaks in order to aid in addressing the issues of measles related deaths and outbreaks.

1.2 Statement of the problem

Measles remains the leading cause of vaccine-preventable childhood mortality globally despite the availability of vaccines (Liu et al., 2012). Kenya is one of the countries that have been implementing routine vaccination program; however, in Kenya according to the Global Measles and Rubella management meeting (2012), about 175 out of the 249 districts from all provinces in Kenya reported 4120 measles cases from January to March 2011. And out of the laboratory confirmed cases from the 4120 reported cases, 614 had known vaccination status, of which 223 had previously received at least one dose of measles vaccine. Nairobi has recorded above 100% vaccine coverage (Tabu, 2012) and it is one of the 8 provinces from which these cases were reported. With this level of measles vaccine coverage, it is expected that there should not be measles outbreaks in Nairobi because measles vaccine is attenuated and should induce long lasting immunity to the disease and be able to establish herd immunity, yet there continues to be outbreaks affecting even immunized individuals (Tabu, 2012; Wicker and Poland, 2012). This study aimed at investigating the levels of measles specific maternal IgG antibodies prior to vaccination, and post-vaccination IgM levels at nine months of age. The study also evaluated the persistence of the measles specific IgG antibodies at age five years and the factors associated with measles outbreaks in Kenya.
1.3 Justification

In 2011, many regions of WHO including the African Region of World Health Organization (WHOAFR) set a goal to eradicate measles by 2020 (Strebel et al., 2011; Bellini and Rota, 2011). To achieve this goal, it is important to evaluate the levels of immunity induced by the measles vaccine and find the factors other than immunological that are responsible for outbreaks. This study aimed at evaluating the immunogenicity of the measles vaccine in order to establish the level of immunity induced by the vaccine at age nine months and the persistence of the induced immunity in children of age five years. The age of five years was chosen, because measles is transmissible and 5 years is the average pre-school (kindergarten) going age in Kenya, the time at which children mingle with others from different backgrounds, risking the contraction of measles. Measles vaccination is administered at age 9 months in Kenya and two weeks after immunization the specific IgM induced by administered vaccine peak (Helfand et al., 1999), and therefore is appropriate for detection by commercial antigens.

1.4 Research questions

i. What is the level of immunity induced by the measles vaccine administered to children at nine months in Nairobi?

ii. Does measles maternal antibodies have an effect on measles vaccine-induced immunity?

iii. What is the level of detectable measles vaccine-induced immunity in children at age five years in Nairobi?

iv. What factors contribute to the outbreak of measles in Nairobi, Kenya?
1.5 Null hypotheses

The measles vaccine administered at nine months of age induces low immunity due to interference by maternal antibodies to measles, and the immunity is not detectable at age five years.

1.6 Objectives

1.6.1 General objective

To evaluate the levels of measles specific antibodies pre and post vaccination in children aged nine months and at age five years, attending the Mama Lucy Kabaki Hospital (MLKH).

1.6.2 Specific objectives

i. To measure measles specific maternal IgG in children before vaccination at age 9 months.

ii. To measure measles vaccine induced specific IgM in children of age nine months after initial measles vaccination at Mama Lucy Kibaki Hospital.

iii. To measure measles vaccine induced specific IgG in children of age 5 years attending the MLKH.

iv. To determine factors contributing to measles outbreaks in Nairobi.
CHAPTER TWO

LITERATURE REVIEW

2.1 Measles virus

Measles virus is a member of the family Paramyxoviridae, the genus Morbillivirus and of the order Mononegavirales. It is the causative agent of measles (Griffins and Bellini, 1996). This virus was first isolated from the blood of a measles infected person in the 1950s by John Enders and Thomas Peebles (Enders and Peebles, 1954; Rima and Duprex, 2006). It is an enveloped, non-segmented; negative-sense single stranded RNA and helical symmetrical virus (Griffin, 2001). It is the most closely related member of this family to the rinderpest virus (RPV), which is a pathogen of cattle that has been eradicated (Furuse et al., 2010; Horzinek, 2011; Moss and Griffin 2012).

Measles has been around for centuries and its manifestation was recognized since the 7th century (Sabella, 2010). It is thought to have evolved in an environment where cattle and humans lived in close proximity (Furuse et al., 2010; Moss and Griffin, 2012). It is antigenically stable with few genetic differences between vaccine strains, however, the wild-types are more variable, and several different genotypes of wild measles virus are circulating worldwide (Rota et al., 1992; Bellini and Rota, 1998). Measles virus shares the same name with the disease it causes.
2.2 Epidemiology of measles

Before the introduction of measles vaccines, the virus infected about 90% of children before the age of 15 years with about 20 million cases and 164,000 deaths annually worldwide, with the highest incidences in children less than 5 years (CDC, 2012). After the introduction of measles vaccine, the infection shifted to teenagers in countries with efficient vaccination programs; while in third world countries measles infection has its greatest incidence in children under 2 years of age especially the malnourished and immune-compromised children (WHO, 2013). In 2011, there was 327,305 cases reported with approximately 158,000 deaths from measles mostly children under the age of five years globally (Simons et al., 2012; WHO, 2013).

Measles was almost eradicated in the past decade, if not, in some industrialized countries such as the United States of America where it was declared eliminated in 2000 (CDC, 2012), but has re-emerged in the past few years. Measles remains a common cause of mortality and morbidity causing the deaths of more than 100,000 children each year worldwide (CDC, 2012). In Africa, According to the African Regional Measles Surveillance Feedback Summary (2012), the cases of measles for 2011 was more than 47,703 (WHO, 2012), this figure reflects a small portion of the true number of occurring cases due to the lapses in normal surveillance process.

In May 2005, the 58th World Health Assembly adopted the WHO/UNICEF Global Immunization Vision and Strategy (GIVS), which called for the global measles eradication. This vision required countries to reduce global measles deaths by 90% by
2010. In 2011, the GIVS was adopted by the Africa Region and a target was set to eliminate measles by 2020 (WHO, 2012) as part of the Millennium Development Goal (MDG). The indicator set to measure this target was routine measles vaccination coverage. That is, to reach measles elimination goal, the vaccination strategies, which among others called for national measles vaccine coverage to exceed 90%, must be achieved (WHO, 2012; CDC, 2012). This means that strong immunization programs need to be put in place to have this measles immunization coverage of above the 90% mark in every country.

Kenya is one of the countries that has been having a successful immunization program with the measles coverage rate of 86% achieved nationwide following the 2009 Supplementary Immunization Activities (SIAs) (World Bank, 2012). However, there have been measles outbreaks nationwide since December 2010 with increase in the number of cases annually. The number of confirmed cases of measles for 2012 up to the 5th of November was 2462, with the total of 767 cases reported from September 2012 to the 3rd October 2012 (Maurice, 2012) while in 2011 the number of cases was 2331 (WHO, 2012). To contain these outbreaks, the Government of Kenya through the Ministry of Public Health and Sanitation (MoPHS) has been conducting a series of mop up vaccination campaigns in affected areas, including the nationwide measles campaign conducted in November 2012. These campaigns are aimed at increasing the measles vaccination coverage nationally and addressing the issues of persistent measles outbreak in the country. However, these measles outbreaks have been blamed on either the influx of refugees (migration), or the failure of mothers to take their children for vaccination (un-immunization).
In Kenya, a single dose live attenuated measles virus vaccine is administered at the age of 9 months, for prevention against measles and to provide herd immunity (DREF, 2011). The administration of measles vaccination at 9 months of age in low income countries such as Kenya, is a policy that was decided based on studies of sero-conversion after measles vaccination at different ages between 5 and 12 months, even though the impact on child survival was never tested (Aaby et al., 2012).

Initially, it was thought that maternal antibodies were lost by 12 months of age in the USA (Krugman et al., 1965) and by age 6 months in developing countries (MoH Kenya, 1977), and that these antibodies can persist in some children for several months longer and reduce the effectiveness of immunization (Albrecht et al., 1977), or lead to vaccine failure. It has also been shown that antibody levels are lower after immunization in the presence of maternal antibody than after immunization of children without maternal antibody (Markowitz et al., 1990; Tidjani et al., 1989; Wilkins and Wehrle, 1979). This indicates that even a low level of maternal antibodies can inhibit successful sero-conversion after immunization (MoH Kenya, 1977; Wilkins and Wehrle, 1979).

It is noted that measles vaccine has major non-specific beneficial effects, hence according to Aaby and his colleagues (2012), the earlier the vaccine is given, the earlier the children will benefit by reducing mortality and morbidity due to measles. This is because measles vaccine also reduces mortality from other infections (Shann, 2010; Aaby et al., 2011). The current study highlights the importance of evaluating the level of immunogenicity induced by the measles vaccine administered at the age of nine months; the levels of
measles specific maternal antibodies levels of the children before vaccination at nine months of age; determine the persistence of the measles immunity up to 5 years and the factors that contribute to measles outbreaks, with these arguments at hand.

2.3 Clinical presentation of measles

Measles is a highly contagious viral disease that can be prevented by vaccination. The causative agent is cosmopolitan in distribution (WHO, 2012). Children are the most affected by this disease; hence it is commonly referred to as a childhood disease. It is transmitted through droplets from the nose, mouth or throat of infected individuals. The initial symptoms usually appear 10-12 days after the virus gain entry to the host cells, which include high fever, runny nose, bloodshot eyes, and tiny white spots on the inside of the mouth (Mason, 2011). Days later, rashes are developed starting on the face and upper neck and gradually spreading downwards to the rest of the body.

Measles can also cause serious complications including blindness, encephalitis, severe diarrhea, ear infection, bacterial pneumonia and death; in fact, it is one of the leading causes of childhood deaths worldwide (WHO, 2012). Malnourished children and people with reduced immunity are at high risk of measles (Melissa, 2012). In addition, measles causes immune suppression, resulting in increased susceptibility to opportunistic infections. Indeed, the main causes of measles mortality are secondary infections in the respiratory and digestive tract (de Swart, 2008; de Vries et al., 2012).
2.4 Measles transmission

Measles is transmitted when an infected individual sneezes or coughs; aerosols containing viral particles are released into the air. The released viral particles when inhaled enter through the respiratory route of the new host, and starts replicating within the tracheal and bronchial epithelia cells (WHO, 2013). The virus is subsequently transported from the respiratory tract to the draining lymph nodes, where it amplifies (de Vries, 2012) leading to the production of large multinucleated lymphoid (giant cells) or reticulo-endothelial cells during the prodromal stage (Lemon et al, 2011). These syncytia are seen in sub-mucosal areas of tonsils and pharynx, and are considered to be a major source of virus spread to other organs and tissues through the bloodstream. Measles can also be transmitted through direct contact with the secretions from the infected person's nose and mouth. There is no animal or environmental reservoir for measles virus except human (WHO, 2012). There may be other risk factors for the transmission of measles including people living in crowded areas, immune-potency of the host, the major risk factor for the transmission of measles is the lack of measles immunity, which can be attributed to un-immunization or the waning of immunity (Maldonado et al., 1995; Muscat, 2011).

2.5 Measles pathogenicity

Measles remains a leading cause of childhood deaths. To cause infection, the virus has two types of glycoprotein spikes, designated hemagglutinin (H) and fusion (F) proteins, on the virus envelope. The H protein binds to specific molecules (receptors) on target cells, while the F protein mediates membrane fusion between the virus envelope and the host cell plasma membrane (Griffin, 2007). Signaling lymphocyte activation molecule (SLAM) is also known as CD150. It is a member of the glycoprotein family that is
expressed on cells of the immune system. The family includes activated T and B cells, activated monocytes and mature dendritic cells. The SLAM (CD150) and CD46 are receptors for measles virus found on the host cells (Dorig \textit{et al.}, 1993; Hsu \textit{et al.}, 2001). However, SLAM is a common receptor for all strains of measles virus (Tatsuo \textit{et al.}, 2000), whereas CD46 functions as a receptor for only vaccine strains and some laboratory strains of measles virus (Dorig \textit{et al.}, 1993; Naniche \textit{et al.}, 1993; Manchester \textit{et al.}, 2000; Yanagi \textit{et al.}, 2006). These receptors serve as the point of attachment for measles virus to the host cells.

Measles virus initiates its infectious cycle by attaching the hemagglutinin (H) protein on the virus envelope to a cellular receptor on a target cell. Attachment of the H protein to a receptor triggers membrane fusion between the virus envelope and the plasma membrane of the target cell mediated by the fusion (F) protein (Griffin, 2007; Lamb and Parks, 2007). Infection of memory lymphocytes by measles virus leads to depletion of immunologic memory resulting in immune-suppression. In addition, systemic infection of DCs results in an impairment of antigen presentation functions. Measles virus replicates mainly in lymphoid organs throughout the body and produces syncytia causing damage to the immune system of infected individuals (Griffin, 2007). Massive replication in CD150$^+$ T- and B-lymphocytes then results in the dissemination of the virus throughout the body. In addition, at the peak of virus replication there is a high virus load, which may cause the spillover of viruses to other cell types with low affinity to the virus receptors (Griffin, 2007), leading to disease state.
2.6 Prevention of measles

Measles is a highly contagious disease with high mortality and morbidity rate. Although most patients recover from the illness, serious complications can occur, including pneumonia and invasion of the central nervous system (Moss and Griffin, 2006). Humans are the only natural reservoir of measles virus (Hahm et al., 2003). The causative agent, measles virus, is generally transmitted by aerosolized secretions deposited on upper-respiratory-tract mucosal surfaces. Most children recover uneventfully from the illness, but serious complications can occur (Parks et al., 2001). While there are other likely modes of transmission, airborne spread of measles from a vigorously coughing individual is the most likely mode of transmission (Bloch et al., 1985). It survives at least one hour in the air (Bloch et al., 1985). People with measles are usually infectious for one to two days before the rash appears (Bedford, 2004).

2.6.1 Measles therapeutics

There is no therapeutic treatment for measles, once someone has developed measles. Most people survive the disease measles without taking treatment, however people with measles are advised to rest, drink plenty of fluids, and can take pain killers to help reduce the fever and feel more comfortable. People who are severely ill or who develop pneumonia, middle ear infection, or diarrhea should discuss treatment with their doctor. Although no medicine will make the measles virus go away, there may be a role for other medicines, such as antibiotics, in treating other secondary or opportunistic infections.
2.6.1.1 Prophylaxis

Immune Globulin (Human): BayGam is a solution of immune globulin prepared from plasma pools from human donors is used for intramuscular administration and contains no preservatives. If administered within 6 days of exposure, BayGam can prevent or modify measles in a susceptible person. The usual recommended dose of BayGam is 0.25 ml/kg of body weight, intramuscular (maximum dose -15 ml) (Sawyer, 2000).

2.6.1.2 Ribavirin

Ribavirin is a broad-spectrum antiviral drug with inhibitory activity against many RNA viruses, including measles virus. The monitoring of the CSF ribavirin concentration is necessary because of its toxicity, hence, its concentrations in the CSF after the initial treatment at a dose of 1 mg/kg twice a day should be measured, and the results should be used to adjust the dose and frequency of the next treatment (Hosoya et al., 2004).

2.6.1.3 Vitamin A

Vitamin A is said to have profound and diverse effects on the immune system and the integrity of epithelial barriers, and its beneficial effect on measles is almost certainly mediated in part by effects on the immune system. And its deficiency is a recognized risk factor for severe measles (Moss et al., 2004; D'Souza and D'Souza, 2002).

2.6.1.4 Antiseptics

Measles is said to be sensitive to antiseptics such as povidone-iodine solution and cream, chlorhexidine gluconate, benzalkonium chloride (BAC) and benzethonium chloride (BEC) (Kawana et al., 1997).
2.6.2 Laboratory Diagnosis

Laboratory diagnosis plays an important role in measles control and surveillance as the level of disease control increases. While the recognition of potential measles cases is based on clinical case definition, it is well established that clinical diagnosis is inaccurate during the elimination phase and that laboratory confirmation of suspected cases, complimented by genotyping of circulating measles strains, is critical for effective surveillance (Featherstone et al., 2003).

Culture and Polymerase Chain Reaction (PCR) technique are advance methods used for measles diagnosis particularly in research. Measles virus is excreted from infected cases within the first 57 days after rash onset, and in low concentration enabling the detection of the virus, hence carrying out tests like culture and polymerase chain reaction (PCR) to isolate the virus is not a logic diagnostic tool. However, the detection of measles virus, subsequent genomic analysis, and the availability of an extensive sequence database for wild type measles viruses have enabled molecular epidemiologic studies of measles (Featherstone et al., 2003).

Serum-based IgM Enzyme Immunoassay (EIA) are the current recommended laboratory assays for the confirmation of clinically diagnosed measles. The both indirect and capture EIA formats are reported to have high sensitivity of 83% - 89% higher after the first week of rash onset, and specificity of 95% - 100% with serum specimens collected in the first to fourth week after rash onset. And the EIAs can be done with a single and small serum specimen. This method is relatively rapid and can be used to diagnose acute and active
measles infection from the time of rash onset until 4 weeks after rash onset (Bellini and Helfand, 2003).

The enzyme-linked immunosorbent assay (ELISA)-IgG avidity test is a good tool for evaluating vaccine efficacy of single dose schedules, serologically. This test can discriminate non-responders, primary responders, and those previously immunized, with high avidity indices, it also require small sample and it’s rapid (de Souza et al., 1997).

2.6.3 Vaccination against measles.

The live attenuated measles vaccine was first licensed in 1960s in the USA in the 1960s, while the MMR was licensed in 1970s (Jefferson et al., 2003), since then, the live attenuated Measles virus vaccine has protected hundreds of millions of people. The vaccine is inexpensive and long lasting. However, it has been reported that the presence of maternal antibodies interferes with effectiveness of the vaccine before 9 months of age, and that genetic polymorphisms of the human leukocyte antigen (HLA) system significantly influence the variation in immune responses to the measles vaccine (Ovsyannikova et al., 2004).

Vaccination is a safe, effective and cost-effective means of controlling the morbidity and mortality cause by measles disease. There are two types of measles vaccines, which can be a single antigen dose or in combination with rubella (MR) or rubella and mumps (MMR). These vaccines are initially given to children at age 9 or 12months (UNICEF, 2014). The first measles vaccines were licensed in 1963 in the U.S.A., they were made from measles virus that was isolated by John Enders in 1954 (Jefferson et al., 2003). The
vaccines were killed (inactivated) and live attenuated vaccines of the Edmonston B strain. However, the inactivated vaccine was withdrawn in 1967 because it did not protect against measles virus infection. The original Edmonston B vaccine was also withdrawn in 1975 because of a relatively high frequency of fever and rash in vaccinees. A more potent live attenuated vaccine (Schwarz strain) was introduced in 1965, though it is no longer use in some countries in favor of newer effective vaccines. Other measles vaccine strains include Edmonston-Enders strain, Edmonston-Zagreb, and Moraten strains. The strains, known by different names differ from each other in the number of times the parent strain was “passaged” (EPI Newsl, 1980). There are other strains which are not derived from Edmonston strain, and are licensed for use such as the CAM-70, TD 97, Leningrad-16, and Shanghai 191 (Ji-191) strains (WHO, Biologicals, 2013). The Scharwz measles strain vaccines are the ones currently in use in Kenya.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out at the Mama Lucy Kibaki Hospital (MLKH), which is located in the Umoja II area of Embakasi in Nairobi County (See appendix III).

3.2 Study design

This study was a cross sectional study, which involved sampling blood in children aged nine months for the determination of pre and post-vaccination measles-specific antibodies, and their persistence up to five years of age. Structured questionnaire were administered to the mothers of the children to determine the transmission risk factors of measles. Figure 1 flow chart showing the steps involved in the study.
3.3 Targeted population

The research targeted the population of i) Children nine months of age who visited the MLKH for scheduled vaccination from August to November 2013, ii) Children aged five years who attended the child welfare clinic at the MLKH during the same period (August to November 2013) and iii) Mothers of each enrolled child from the two groups of children of aged 9 months and 5 years.
3.4 Sample size determination

The sample size was calculated using \( N = \frac{Z^2(p)(1-p)}{c^2} \) (Fisher et al., 1998).

Where \( N \) = the sample size

\( Z \) = the Z value of confidence limit at 95% (1.96) was used for this study,

\( P \) = the % estimated prevalence at 5 % (0.05) and

\( C \) = error of the estimate at 5% (0.05).

By calculation; \( N = \frac{1.96^2(0.05)(1-0.05)}{0.05^2} = \frac{3.8416 (0.0475)}{0.0025} = 72.9904 \) (least sample size)

\( N = 80 \) (for each group including mothers).

3.5 Sampling technique

A simple random sampling was done of children attending the Mama Lucy Kibaki Hospital’s Child Welfare Clinic from the Month of August to November 2013. Sampling was based on the average monthly intake of children scheduled for measles vaccination and those attending the clinic. The children were sub-divided into two groups. One group comprised of those children nine months old scheduled for measles vaccination and the other was five year old attending the child welfare clinic. Randomization was carried out using the Random Allocation Software (RAS) version 2.0. Every mother of the qualified and enrolled child was issued with a structured questionnaire to fill, those illiterate were assisted after careful explanation of the questionnaire in Kiswahili by a nurse at the clinic.
3.6 Informed consent

Participation in the study was voluntary. The aim of the study, potential risks and benefits were explained fully to each mother of the qualified and recruited candidate (child). A consent form was signed by each mother upon accepting to take part in the study willingly and each recruited 5 years old child also assented after the mother had consented before enrolment into the study.

3.7 Inclusion criteria

Selection in the study was randomized using the Random Allocation Software version 2.0 based on two categories as: 1) Nine months old children who were scheduled for measles vaccination at the MLKH. 2) And children aged 5 years attending the MLKH child welfare clinic who were vaccinated against measles at 9 months of age (verification by immunization card/record). 3) All mothers who accompanied those children who were qualified and recruited and were mentally sound were also included in the study.

3.8 Exclusion criteria

All children aged five years who were not vaccinated against measles at aged nine months and those who did not consent were excluded from the study.

3.9 Blood sample collection

Two milliliters sample of venous blood from the median cubital veins was aseptically collected from each of the five years old children, and a paired (pre- and post-vaccination) sample was likewise collected from each of the nine months old children. The blood samples were drawn by the researcher, who was assisted by a laboratory
technologist. The collected samples were put into a sterile plain test tube, capped, coded and placed in a test tube rack while waiting for the blood to clot.

3.10 Preparation of sample for storage

The clotted blood samples were centrifuged at 3000G for 5 minutes immediately, and the sera was collected into cryogenic vials and stored at -20°C.

3.11 Transportation of samples

The serum samples were transported in a cool box to African Medical and Research Foundation (AMREF) Public Health Laboratory in Nairobi, and stored at -20°C pending analysis.

3.12 Enzyme-Linked Immunosorbent Assay (ELISA)

Analysis of the samples was carried out using ELISA for Measles-specific IgG and IgM (Serion ELISA Classic Measles Virus IgG/IgM, version 13.11/12-1). The kits were obtained from Institut Virion\Serion GmbH, Germany, and stored at 2-8°C prior to use for the samples analysis.

3.12.1 Preparation of stored samples for analysis

Before use both reagents and sera were brought to room temperature. All the sera samples for IgG analysis were diluted 10µl sample in 1000µl (1:100 v/v) of the ready-to-use dilution buffer reagent of the kit, and for the IgM a rheumatoid factor (Rf)- absorbent reagent was diluted first in the dilution buffer 1 to 4 to obtain a working dilution of the
solution. The sera were then diluted into the diluted Rf-dilution buffer solution 10µl to 1000µl. Rheumatoid factors are auto-antibodies mainly of the IgM class. The Rf-absorbent reagent is used to absorb these auto or irregular antibodies so as to prevent the reaction of these non-specific antibodies with the specific antibodies that were being analyzed for by binding. After the dilutions, the mixtures were vortexed to obtain a homogenous mixture.

3.12.2 Reagents preparation

The wash buffer concentrate was diluted in sterile distilled water before use at 1:30 v/v to obtain the wash buffer working solution. This was made in enough quantity depending on the number of tests to be run at a time. Each of the test kits contained 8 strips of 12 micro-titer wells coated with measles specific antigen and sealed in aluminum pouch. Before a test-run was carried out, the number of strips needed was taken out of the pouch, labeled and placed on the working bench to be brought to room temperature, the rest of the strips were sealed properly in the pouch and returned to the refrigerator for storage.

3.12.3 Test-run procedure

A work sheet of the test-run was prepared which contained the order of the labeled samples corresponding to the layout of the micro-titer well (plate layout) for the results identification. The plates were labeled in order of: Blank in well A1, Negative control in well B1, a pair of positive control (standard serum) in well C1 and D1 and the sera samples were put in duplicates in the rest of the wells as was coded on the work sheet for that run.
One hundred microliter (100 µl) each of the negative control sera, positive controls and samples were dispensed in each corresponding labeled well and the well labeled blank was left empty. The plate was incubated at 37°C for 60 minutes; after the incubation time, the plate was washed 4 times using an automated washer programmed for the dispensation of 300 µl of wash solution per wash. The plate was tapped on an absorbent paper to properly drain the wash solution from the wells after the 4th wash. After the wash, 100 µl of the appropriate conjugate (IgG or IgM) was then added to all the wells except for the blank well and incubated for 30 minutes at 37°C. At the end of the incubation, the plate was washed 4 times and tapped on an absorbent paper after the 4th wash. One hundred (100) µl of the substrate buffer was added to all the wells this time including the blank well and incubated for 30 minutes at 37°C. After the 30 minutes, 100 µl of the stop buffer was added to all the wells to stop the reaction. The plates were read with the ELISA reader at 405 nm against the blank according to the manufacturer’s instructions (that is, the reading of the blank was subtracted from all other readings).

3.13 Tests validation, evaluation and concentration

After each batch of test-run, a validation of the obtained Optical Density (OD) of the ELISA reader was carried out to validate the results obtained in reference to the kit manual.

3.13.1 Validation of the obtained optical density (OD) readings

Validation of the OD was done to insure substrate blanks in all batches of test-run were less than the OD reading of 0.25. The OD reading of the blank was subtracted from every
reading from that batch of test-run to obtain the OD readings of each of the tests against the blank. All readings fell within the given ranges of the kits. The mean of the OD reading of the pair positive standard serum was then obtained from each batch of test-run. The difference (variation) between the mean of the pair standard serum and the reference range of each kit was less than 20%, as Variance exceeding 20% renders a batch of run invalid. This confirmed the validation of the results obtained.

3.13.2 Evaluation of the obtained sample results

The evaluation method used was the determination of continuous antibodies concentration using provided Standard Curves of the kits. A correction factor (F) was calculated after the mean OD value was checked and validated by dividing the reference OD value of the kit by the obtained standard serum mean OD value of the batch for that run. The factor (F) was then multiplied by every sample OD to obtain the factor corrected values. The samples concentration was measured in milli International Unit per milliliter (mIU/ml) or International Unit per milliliter (UI/ml). This was then obtained using the F corrected values against the provided Standard Curve of each test kit.

3.13.3 Samples concentration

The antibodies concentration was measured in mIU/ml for IgG and IU/ml for IgM. The cut-off point of 200 IU/ml was used for IgG and 15 IU/ml for IgM (WHO, 2013).

3.14 Structured questionnaire

Structured questionnaires (Appendix II) were given by the researcher and filled out by every mother of the qualified and enrolled children. The mothers who could not fill the
questionnaire because of some reasons were assisted after careful explanation of the questionnaire in Kiswahili.

3.15 Data analysis
The obtained data were statistically analyzed using SPSS version 12 for windows, and presented using tables, pie charts, graphs and percentages and further subjected to Chi square tests. Ninety-five percent (95%) confidence interval was used to determine the significance.

3.16 Ethical considerations
The proposal to conduct the current study was approved by the Kenyatta University Ethics Review Committee (KU-ERC) (Appendix V). The permission to conduct the study was authorized by the National Commission for Science, Technology and Innovation of Kenya (Appendix VII and VIII). A written consent was voluntarily signed by each of the mothers of the enrolled children, after accepting to take part in the study together with their children, following explanation of the procedures, risks and benefits of the study. In consideration of the community or environment, all used materials and wastes were disposed off according to the bio-safety guidelines of both Mama Lucy Kibaki Hospital and the AMREF (SOP, 2013). To maintain confidentiality, all information gathered from the questionnaires and the laboratory analysis were coded and kept out of reach of unauthorized persons.
CHAPTER FOUR

RESULTS

4.1 Sample population

One hundred and sixty mothers of children attending the Mama Lucy Kibaki Hospital (MLKH) were recruited into the study after consent. In addition, their children also totaling one hundred and sixty were recruited to participate in the study. The initial sample population consisted of eighty mothers and their children aged nine months and another eighty mothers and their five years old children. However, some dropped out of the study at the time of filling the questionnaires, while others dropped out during the blood sampling. After the pullout, a total of 128 children of the mothers responded to the questionnaires. Sixty-six children aged nine months and sixty-two children aged five years were blood sampled. Out of the 66 children age 9 months who were recruited to participate in the study and had their blood sampled for pre-vaccination analysis, only forty returned for the post-vaccination IgM analysis. All the respondents in this study were of Kenyan nationality.

4.4 Levels of serum measles specific antibodies in children

The serological aspect of this study evaluated the levels of measles specific maternal antibodies (IgG) prior to immunization, the levels of IgM antibodies induced by the administered measles vaccination at age nine months and the levels of measles specific IgG antibodies in the 5 years old children.
4.4.1 Levels of pre-vaccination measles specific IgG in children 9 months of age.

The analysis revealed that 3% of the children aged 9 months had maternal IgG levels above 200 mIU/ml, while 97% had IgG levels less than 150 mIU/ml (Table 4.7). The analysis gave the mean IgG level of 58.29 mIU/ml, the standard deviation measured 32.88 mIU/ml and range from 50 mIU/ml to 245 mIU/ml (Table 4.8).

Table 4.1: Pre-vaccination measles specific IgG levels in children aged 9 months

<table>
<thead>
<tr>
<th>Measles Specific IgG (mIU/ml)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (&gt;200) n = 2</td>
<td>3.0</td>
</tr>
<tr>
<td>Negative (&lt;150) n = 64</td>
<td>97.0</td>
</tr>
</tbody>
</table>

Figure 4.1: Levels of measles specific IgG of the children aged 9 months
Table 4.2: Ages of the mothers versus the antibody status of the children prior to vaccination at aged 9 months.

<table>
<thead>
<tr>
<th>Age of the mother</th>
<th>Mean IgG level (mIU/ml) Of the children at 9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 years</td>
<td>61.59 ± 42.35</td>
</tr>
<tr>
<td>25 – 30 years</td>
<td>55.08 ± 16.21</td>
</tr>
<tr>
<td>31 – 40 years</td>
<td>59.71 ± 40.02</td>
</tr>
</tbody>
</table>

Using one-way ANOVA to establish variations in the mean maternal IgG levels of the children and different ages of the mothers showed that there was no significant difference in the levels of IgG of the children aged 9 months and the various ages of their mothers (F = 0.23, P = 0.794).

4.4.2 The Levels of serum measles specific IgM in children aged 9 months

Some of the participants who accepted to take part in the study and were sampled for pre-vaccination analysis were missed out for the post-vaccination sampling. Out of the forty participants who returned, (77.0 %) had the IgM levels >15 IU/ml, (23.0 %) had IgM levels of between 10 – 15 IU/ml (Table 4.9).
Table 4.3: Post-vaccination measles specific IgM in children aged 9 months

<table>
<thead>
<tr>
<th>Post-vaccination IgM (IU/ml)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (&gt;15)</td>
<td>77.0</td>
</tr>
<tr>
<td>n = 31</td>
<td></td>
</tr>
<tr>
<td>Border line (10-15)</td>
<td>23.0</td>
</tr>
<tr>
<td>n = 9</td>
<td></td>
</tr>
<tr>
<td>Negative (&lt;10)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 4.2: Laboratory results for the levels of measles specific IgM of the children aged 9 months
Values less than 10 IU/ml are negative, in the measurement of measles IgM antibodies using ELISA, 10 – 15 are borderline, while values >15 are considered positive. The result obtained averaged 30.10 IU/ml with the range of 12.0 IU/ml to of 75.0 IU/ml. One-way ANOVA analysis of the mean IgM levels of the children and the different age categories of the mothers showed no significant difference (F = 0.19, P = 0.831).

Table 4.4: Measles Specific IgM levels of 9 months old children to mothers of different age categories.

<table>
<thead>
<tr>
<th>Age of the mother</th>
<th>Mean IgM level (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 years</td>
<td>32.1 ± 19.1</td>
</tr>
<tr>
<td>25 – 30 years</td>
<td>31.5 ± 11.7</td>
</tr>
<tr>
<td>31 – 40 years</td>
<td>27.9 ± 18.1</td>
</tr>
<tr>
<td>41 – 50 years</td>
<td>0</td>
</tr>
</tbody>
</table>

4.4.3 Relationship between the levels of pre-vaccination (IgG) and the levels of post-vaccination (IgM) in children Aged 9 months

Levels of IgG and IgM for 9 months old children were subjected to a Pearson moment correlation analysis. The result showed that, there was no significant relationship in the individual levels of IgG to the levels of IgM (r = -0.057, P = 0.727).
4.4.4 The levels of measles specific IgG in children of age 5 years

The levels of measles specific IgG antibodies measured using ELISA, are normally interpreted as negative for result less than 150 mIU/ml, readings between 150-200 mIU/ml are considered borderline, while values from 200mIU/ml and above are considered positive. Majority 55(89%) of the samples analyzed from this study showed IgG levels greater than 200 mIU/ml, (8%) had IgG levels between 150-200 mIU/ml and 2 (3%) had IgG levels less than 150 mIU/ml, indicating the loss of immunity at 5 years (Table 4.11). The average levels of IgG antibodies in this group of children was 587.0 mIU/ml, 329.08 mIU/ml standard deviation with a minimum of 100 mIU/ml and maximum of 1500 mIU/ml (Table 4.12). Using One –way ANOVA, the variations in the mean IgG levels of the children in different ages of mothers revealed no significant
difference (F = 0.81, P = 0.493) (Tables 4.11 and 4.12). Figure 4.14 below shows the variation in the measured pre-vaccination measles specific IgG levels in children age nine months.

Table 4.5: Levels of measles specific IgG in children aged 5 years vaccinated at age 9 months

<table>
<thead>
<tr>
<th>Vaccinated aged 5 years IgG (mIU/ml)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (&gt;200)</td>
<td>88.7</td>
</tr>
<tr>
<td>n = 55</td>
<td></td>
</tr>
<tr>
<td>Border line (150 – 200)</td>
<td>8.1</td>
</tr>
<tr>
<td>n = 5</td>
<td></td>
</tr>
<tr>
<td>Negative (&lt; 150)</td>
<td>3.2</td>
</tr>
<tr>
<td>n = 2</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.4: Variation in levels of measles specific IgG in children aged 5 years
Table 4.6: Mean IgG levels in children of age 5 years old belonging to mothers of different categories of ages

<table>
<thead>
<tr>
<th>Age of the mother</th>
<th>Mean IgG level (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 years</td>
<td>639.3 ± 320.6</td>
</tr>
<tr>
<td>25 – 30 years</td>
<td>555.1 ± 291.3</td>
</tr>
<tr>
<td>31 – 40 years</td>
<td>542 ± 398</td>
</tr>
<tr>
<td>41 – 50 years</td>
<td>995</td>
</tr>
</tbody>
</table>

4.2 Demographic characteristics of the study population

The demographic characteristics assessed included the ages, marital status, religions, education, employment and occupations of the mothers and their spouses.

4.2.1 Ages of the respondents

Forty-six out of 128 mothers (36%) sampled in this study were between the ages of 25-30 years, 32% were less than 25 years, while only one respondent (0.8%) was between the ages of 41-50 years (Table 4.1).

Table 4.7: The Ages and percentage distribution of the respondents

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Number (n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>41</td>
<td>32.0</td>
</tr>
<tr>
<td>25 – 30</td>
<td>46</td>
<td>36.0</td>
</tr>
<tr>
<td>31 – 40</td>
<td>32</td>
<td>25.0</td>
</tr>
<tr>
<td>41 – 50</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Non-committal</td>
<td>8</td>
<td>6.0</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.2.2 Marital status of the respondents

Out of the 128 respondents, 112 (88.0%) were married, 8 (6.0 %) were single, 4 (3.0%) were widowed and 1 (1.0) was divorced (Figure 4.1)

![Marital status of the respondents](image)

**Figure 4.5: Marital status of the respondents**

4.2.3 Religion of the respondents

Most of the respondents 123 (96.0 %) were Christian, 2 (2.0%) were Muslims and 3 (2.0%) had no religious affiliation (Figure 4. 2).
4.2.4 Education level of the respondents and their spouses

Majority of the respondents 50 (39%) had attained secondary education, 49 (38%) had post-secondary education, while 26 (20%) had primary education. Most of the spouses of the respondents 55 (43.0%) had secondary education, while 39 (31%) had post-secondary education. Only 13 (10%) had primary education and 5 (4%) had no formal education (Figure 4.3).
Figure 4.7: Education levels of respondents and their spouses

4.2.5 Employment status of the respondents

Sixty-six of the respondents, representing (52%) were employed either formally or informally and had a means of generating income for themselves, and 60 (47%) were not employed and were found to be either housewives depending on their spouse’s income or students depending on their parent’s income, although the majority 103 (81%) of the spouses of the respondents were employed, 14 (11%) were not employed (Figure 4.4).
A large percentage (55%) of the respondents had formal occupation. Formal occupation by this study refers to those occupations that require education or apprenticeship training. Forty-five percent (45%) had no formal occupation (those occupations that do not necessarily require training) (Figure 4.5).
4.2.7 Categories of the respondents’ occupation

Majority of the respondents 46 (37%) were housewives followed by 40 (31%) in business while 41 (32%) were found to be in other occupations. (Figure 4.6)
4.3 Knowledge on measles disease

Knowledge of measles by the respondents was determined using the structured questionnaire by assessing whether they have heard of measles and by what means, had borne any of their children in a health facility, have had measles, have had any of their children infected by measles, had been vaccinated and had all of their children vaccinated. It is important in assessing the factors that lead to measles outbreak because knowledge about measles enables one to make decisions on the control and prevention of measles outbreaks. In this study, 128 mothers of the children that took part in the study were given questionnaires to ascertain their knowledge on measles. The questionnaires also took into consideration some socio-economic and demographic characteristics that are thought to have an impact on measles outbreaks.

4.3.1 Respondents who had their children borne in a health facility

Majority of the respondents 119 (93.0%) had all their children borne in a health facility, 5 (4.0 %) had some of their children born in a health facility (Table 4.2).

Table 4.8: Respondents who had their children born in a health facility

<table>
<thead>
<tr>
<th>Children born in the health facility</th>
<th>Number (n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>119</td>
<td>93</td>
</tr>
<tr>
<td>Some</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Non-committal</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>128</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
4.3.2 Respondents who had heard of measles

Among the respondents, 115 (90%) had heard of the measles disease and 10 (8%) said they were not aware of the measles disease (Table 4.3).

Table 4.9: Respondents who had heard of measles disease

<table>
<thead>
<tr>
<th>Heard of Measles Disease</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>115</td>
<td>90</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Non-committal</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>128</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

It was noted that the knowledge on measles was significantly associated to the respondent’s education levels ($\chi^2 = 90.779$, $P = 0.0001$); which showed that 96% of the respondents who had post-secondary education had heard of measles disease (Table 4.4).
Table 4.10: Respondents who had heard about measles versus the levels of education

<table>
<thead>
<tr>
<th>Heard of measles</th>
<th>No education n (%)</th>
<th>Primary education n (%)</th>
<th>Secondary education n (%)</th>
<th>Post-secondary education n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>2 (67%)</td>
<td>3 (12%)</td>
<td>6 (12%)</td>
<td>2 (4%)</td>
<td>13</td>
</tr>
<tr>
<td>Yes</td>
<td>1 (33%)</td>
<td>23 (88%)</td>
<td>44 (88%)</td>
<td>47 (96%)</td>
<td>115</td>
</tr>
<tr>
<td>Total</td>
<td>3 (100%)</td>
<td>26 (100%)</td>
<td>50 (100%)</td>
<td>49 (100%)</td>
<td>128</td>
</tr>
</tbody>
</table>

4.3.3 Respondents who had suffered measles

It was also noted that 31 (24%) confirmed that they have had measles but at different times in their lives. Fifty-four respondents (42%) acknowledged that they had not had measles while 43 (34%) could not confirm suffering from measles (Figure 4.7).

Figure 4.11: Respondents who had suffered measles
4.3.4 Respondents who had their children suffered from measles

The majority of the respondent 90% did not have any child suffering from measles prior to the study, while 8% confirmed that at least one of their children had suffered from measles (Figure 4.8).

![Pie chart showing ratios of respondents with children suffering from measles](image)

**Figure 4.12: Respondents whose children had suffered from measles**

It was also observed that 100% of the children who had suffered from measles had been vaccinated at least once.

A cross-tabulation of the parity of mothers versus the number of their children who had suffered measles showed that, there was a significant association in the number of children having measles to the parity of the participating mothers ($\chi^2 = 132.95$, $P = 0.0001$). Mothers with many children had many of their children suffer from measles more than those who had fewer children (Table 4.5).
Table 4.11: Parity of mothers versus the number of children who had suffered measles

<table>
<thead>
<tr>
<th>Had Measles</th>
<th>Number of Children Per Mothers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 child n (%)</td>
<td>2 children n (%)</td>
</tr>
<tr>
<td>No</td>
<td>59 (92%)</td>
<td>40 (95%)</td>
</tr>
<tr>
<td>Yes</td>
<td>5 (8%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (100%)</td>
<td>42 (100%)</td>
</tr>
</tbody>
</table>

4.3.5 Respondents’ number of children

Most of the respondents had 1 child (50%) while 33% had two children, 13% had 3-5 children, 2% had more than 5 children, and 2% of the respondents did not indicate the number of children they had. The result gave an average of 1 child per respondent.

Among the respondents, 98% had heard of measles vaccination, and this information was received either through radio 30%, television 43%, health education 77% and 4% through campaigns and or other means (Table 4.6).
Table 4.12: Means by which respondents got to know about measles vaccination

<table>
<thead>
<tr>
<th>Sources of information</th>
<th>Number of respondents</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>The radio</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>Television</td>
<td>55</td>
<td>43</td>
</tr>
<tr>
<td>Health Education</td>
<td>98</td>
<td>77</td>
</tr>
<tr>
<td>Other sources</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

4.3.6 Vaccination status of the respondents

There were 87 (68.0 %) of the participants who had been vaccinated, 36 (28.0 %) did not know whether they had been vaccinated and 2 (2.0 %) confirmed that they were not vaccinated.
4.3.7 Knowledge on measles outbreak in Kenya

Out of the total number of participants, 96 (75%) have heard of measles outbreaks in Kenya and through radio, television, health education campaigns etc., 30 (23%) said they have not heard of measles outbreak in Kenya.
4.3.8 Frequency of daily meals intake

Majority (79%) of the respondents took meals 3 times per day, while 11% had meals more than three times daily and 9% had meals twice daily, and 8% were non-committal.

4.3.9 Living environment of the respondents

A large percentage (59%) of the respondents lived in a crowded environment. Crowded in this study refers people living in informal settlement where housing units are congested while 39% were found not to live in crowded areas.
5.1 Discussion

5.1.1 Levels of measles specific maternal IgG antibodies in children prior to vaccination

The current study established that majority, (97%) of the 9 months old children had maternal IgG levels of less than 150mIU/ml. These values are below the accepted lower limit of 200mIU/ml, and therefore considered negative. Only 3% of the children were positive for maternal IgG values > 200 mIU/ml. This shows that in almost all (97%) of the children maternal antibodies to the Schwarz strain of measles were lost earlier than 9 months. These findings suggest that there is a window of susceptibility to measles infection contributing to measles outbreaks from the time the maternal antibodies fall below the protective level of > 200mIU/ml to the time of vaccination.

Measles maternal antibodies were initially thought to be lost by 12 months of age in developed countries (Krugman et al., 1965) and by the age of 6 months in developing countries (MoH Kenya, 1977). It is also reported that these antibodies persist in some children for several months longer and reduce the effectiveness of immunization or lead to vaccine failure (Albrecht et al., (1977). It is also observed that measles vaccine induced antibody levels are lower after immunization in the presence of maternal antibody than after immunization of children without maternal antibody (Wilkins and Wehrle, 1979; Tidjani et al., 1989; Markowitz et al., 1990). This indicates that the 3%
who had maternal antibodies levels > 200mIU/ml may not realize the full benefit of measles vaccination at the age of 9 months.

The finding of this study corresponds to a study in Switzerland by Nicoara and his colleagues (1999) in which they found that maternal antibodies reduced by more than 50% in children before the age of nine months and Chowdhury et al., (2008), reported in a study carried out in Dhaka that 75% loss of measles maternal antibodies before the age of nine months.

Maternal antibodies are part of primary immunity acquired by the child from the mother at birth, and these antibodies protect the infants against measles in their early live. Measles vaccine protect infants against measles virus infection. In addition to other benefits such as preventing against other infection that may lead to childhood deaths (Kabir et al., 2003; Aaby et al., 2010; Shann, 2010). Therefore Measles vaccines, if administered immediately after depletion of measles specific antibodies would have positive effect on the reduction of childhood deaths, an important element of millennium development goal (MDG).

5.1.2 Levels of measles specific IgM antibodies after vaccination

Majority, 78% of the post-vaccinated children had IgM levels greater than 15 IU/ml. These are positive results for active measles virus replication. Twenty-three percent (23%) had IgM levels of between 10 – 15 IU/ml, which is considered borderline for effective vaccination (Serion, 2013). This means that all the children aged nine months had IgM antibodies produced in response to the vaccine. These findings agree with a
study in Peru by Bautista-Lopez and his colleagues (2001) in which they obtained high levels of humoral response after vaccination of children with the Schwarz strain. It may be argued that the 23% who had borderline levels, still responded to vaccination however, these values were above negative showing that they responded to vaccination, and that the borderline response could be attributed to either late or early response to the vaccination. In normal circumstances, the rate at which immune response is achieved following the invasion of pathogen differs in individuals, and these individual variations could have led to a fast decline or slow response to the vaccination by some of the children.

The current study has established that the measles vaccines administered at nine months of age is immunogenic and induces immunity, given that 100% of those who were vaccinated responded to the vaccination by producing IgM antibodies.

### 5.1.3 Persistence of measles specific IgG antibodies in children aged five years

The study revealed that majority (89%) of the children aged five years had positive IgG levels greater than 200mIU/ml. These IgG antibodies are presumed to have been obtained from the measles vaccination administered at the age of nine months. Eight percent (8%) of the children indicated borderline values between 150 – 200 mIU/ml and 3% had negative IgG levels less than 150 mIU/ml. This study showed that a large number of the vaccinated children remain immune up for up to five years after vaccination. Similar findings were obtained by Davidkin et al., (2008), in which they reported 95% sero-positivity in individuals twenty years after one dose of measles vaccination. In the
current study, 3% of the children were negative and therefore were at risk of measles attack by the age of five years. This may be the cause of some of the outbreaks that have occurred in Nairobi.

The primary IgM antibodies that are induced after vaccination initiate the production of the adaptive IgG antibodies in what is called “class switch”. The IgM antibodies then decline after the conquering of the invading pathogens, a decline that is concomitant to the increase of the IgG antibodies. The produced IgG antibodies then serve as memory antibodies, and constitute the efficacy of vaccination. A positive IgG value is conventionally considered to be indicating the presence of immunity (Arguelles et al., 2006), that is, IgG levels of >200mIU/ml for measles, while negative values (IgG values < 150) is considered as not immune (Serion, 2013). It is therefore established that measles specific vaccine IgG antibodies persist up to five years.

5.1.4 Demographic and socio-economic characteristics

The demographic and socio-economic factors of the mothers of the children who took part in this study were assessed to establish factors other than biological that lead to the outbreaks of measles.

5.1.5 Effect of the ages of mother on the production of antibodies

Most (32%) of the respondents were aged less than 25 years of age, 36% were between 25 - 30 years, while 25% were between 31 – 40 years. This study showed that the differences in the ages of the mothers did not have significant effect on the levels of the
maternal antibodies of their children (Table 4.8). This current study is in agreement with Pabst (1992) who reported that more than 90% of infants of vaccinated mothers were susceptible to measles by the age of 9 months. Balé et al. (2011), found that children of mothers less than 25 years were at a higher risk of contracting measles than children of older mothers because young mothers transmit lower titers of antibodies to their children. Arguing that a large proportion of these infants become susceptible to measles before vaccination. Most (97%) of the children had lost maternal antibodies before vaccination. This study showed that differences in the ages of the mothers did not have significant correlation with the measured maternal IgG antibodies of the children in the current study.

5.1.6 Social-economic factors of the respondents

Most (88%) of the respondents in this study were married and 96% were Christians. Fifty-one percent of the respondents were employed either formally or informally and had a means of generating income, while 47% were not employed and were found to be either housewives depending on their spouses’ income or students depending on their parents’ income. The majority (81%) of the spouses of the respondents were employed. The results of this study showed that all the children were vaccinated or had come for vaccination. It is therefore observed that marital immunity status in combination with economic empowerment are may be important factors in the ability to have children vaccinated.
It was observed that the welfare of the child including the cost of the process of vaccination, when shared by both parents would be enhanced and may increase the chances of children being vaccinated. Consequently, the combination of single motherhood and economic instability may be some of the challenges affecting the vaccination of children against measles (Hu et al., 2013), who found mothers with a better socio-economic status, such as having occupations and a stable income with improved immunization coverage.

5.1.7 Educational factor to the vaccination of the child

Education levels varied among the mothers in his study, most (39%) had secondary education, while 38% had post-secondary education. This indicates that majority of the respondents were educated. Since all the children in this study were vaccinated the education of the mothers may have contributed to this. This is further seen in the analysis of the level of education to the knowledge of measles as shown in Table 4.4, in which 96% of the mothers who had post-secondary education had knowledge of measles compared to 88% for secondary education, 23% for primary education and 33% for those who were uneducated. The findings are in agreement with a study conducted by Munthali in Malawi (2007), which reported that, the higher the level of education attained by mothers, the higher the likelihood of their children being vaccinated. The demographic health survey of the year 2004 (DHS, 2004) in Malawi showed that 84% of the children whose mothers had secondary education and above were vaccinated. This study further found out that the main source of knowledge about measles was through health education offered at the health facilities and not through the media or any other avenues. The results of the current study suggest that the education of the mothers is an important factor in vaccination against measles virus and subsequently, the prevention of measles outbreaks. Possibly, this may be due to
the fact that an educated mother may be in a better position to weigh the pros and cons of the information she may gather about the disease.

5.1.8 Vaccination status of the mothers versus the maternal antibodies status of the children

The evaluation of vaccination status of the mothers to assess the effect of the vaccination on their maternal antibodies in their children indicated that majority (68%) of the mothers had been vaccinated. 28% did not know whether they had been vaccinated or not. A small percent (2%) were not vaccinated. In this study a large percent of mothers were vaccinated, 97% of the children of these mothers had lost maternal antibodies with maternal IgG levels of less than 150mIU/ml. These findings agree with the results of Waaijenborg et al., (2012) who found that children of vaccinated mothers had low concentration of maternal antibodies. Subsequently, leading to lose of protection at an early age. Measles vaccination produces long lasting antibodies, a mother with these antibodies transfers them to the child at birth as maternal antibodies which protect them against infection during their early live.

5.1.9 Nutritional status

The frequency of meal intake by the mothers and the children to ascertain their nutritional status were assessed in this study. The findings showed that 79% of the respondents had meals thrice daily, 11% had meals more than three times daily while 9% had meals twice daily. The innate immunity is the first line of defense encounter by an invading pathogen and this component of the immunity is strongly influence by the nutritional status of the
Diseases in general encounter the immune system, which is the body’s defense system in a quest to cause infections. Hence, a lack or low nutritional status can lead to immune deficiency and impair host defense against pathogens. The current study established that 90% of the participants were well fed, and had a good nutritional status. These findings agree with Peletier et al., (1993) in which they reported that under-nutrition in children contributes significantly towards the global burden of disease and Caulfield et al., (2004), who reported that 45% of deaths attributed to measles in young children is due to under-nutrition.

5.2 Conclusions

i. Most children (97%) lost maternal immunity (antibodies) to measles before age 9 months, the scheduled time of vaccination putting them at risk of being infected by measles.

ii. The measles vaccine (Schwarz strain) used in Kenya is immunogenic and leads to the production of measles-specific IgM antibodies, even though some children produce low immunogenic responses to this vaccine.

iii. The antibodies (immunity) produced by the vaccine Schwarz strain at 9 months persisted up to 5 years of age in vaccinated children 89% and only 3% of vaccinated children lose immunity by age 5 years.
iv. Education is an important determinant in the ability of mothers to have their children vaccinated.

v. Most of the information mothers got on measles vaccination is at health facilities.

5.2 Recommendations

i. The policy on the one dose measles vaccination at the age of nine months needs to be revisited. Which will mean giving the vaccine earlier than the scheduled time of nine months of age followed by a booster dose at 12 months.

ii. There is need to improve the means through which information on measles vaccine is deliver to the public, so that all mothers get the information.

iii. There is a need for further studies to assess the levels of measles specific maternal antibodies prior to initial measles vaccination at different ages on a larger scale, so as to address the would-be risk of vulnerability to measles before vaccination.

iv. Since the measles vaccine is immunogenic and outbreaks still occur there is a need to do a genetic profile of the circulating strains of measles virus, to determine whether there are re-emerging strains not covered in the vaccine.
REFERENCES


Centers for Disease Control and Prevention (2012). Measles (Rubeola)


Hsu, E., Iorio, C., Sarangi, F., Khine, A., and Richardson, C. (2001). CDw150 (SLAM) is a receptor for a lymphotropic strain of measles virus and may account for the immunosuppressive properties of this virus. Virology 279:9–21.


Serion (2013). ELISA classic Measles Virus IgG/IgM: V 13.11/12-1


APPENDICES

APPENDIX I A

CONSENT FORM

My name is Arthur Brown. I am a MSc. student from Kenyatta University. I am conducting a study on “EVALUATING MEASLES VACCINE-INDUCED IMMUNITY IN CHILDREN AGE NINE MONTHS AFTER VACCINATION AND MEASLES-VACCINATED CHILDREN AGE FIVE YEARS AT MAMA LUCY KIBAKI HOSPITAL”. The information obtained from this study may be used by the Ministry of Medical Services and Ministry of Health to enhance the policy on measles vaccination in order to control and prevent the outbreaks of measles in Nairobi and in Kenya as a whole.

Procedures

Participation in this study will require that I ask you some questions and take some sample of blood from your child in order to test for measles vaccine-induced immunity. I will record the information from you in a questionnaire.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time.

Please remember that participation in the study is voluntary. You may ask questions related to the study at any time.
You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organization now or in the future.

**Discomforts and risks**

Some of the questions you will be asked are on intimate subject and maybe embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview at any time. The interview may add approximately half an hour to the time you wait before you receive your routine services.

We will take small amount of blood (2ml) from the child, which will cause some pain. The child will feel some pains during the insertion of the needle into the vein for the extraction of the blood sample.

**Benefits**

If you participate in this study you will help us to improve on the policy of measles vaccination and reduce the risk of measles outbreaks. You will also benefit from knowing your child’s immune status with respect to measles virus, and if your child is found not to be immune to measles, we will contact you and provide you with the necessary care.

**Confidentiality**

The interviews and examinations will be conducted in a private setting within the hospital. Your name will not be recorded on the questionnaire. The questionnaire that you
will fill in will be kept in a locked cabinet for safe keeping at Kenyatta University and will only be accessible to the investigator and data collected will be password protected. If you have any questions you may contact Dr. Margaret Muturi on 07 227 58523 or the Kenyatta University Ethical Review Committee Secretariat on kuerc@ku.ac.ke.

**Subject’s Statement** The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care I will receive from the clinic today or that I will get from any other clinic at any other time.

Name of participant_________________________________________________________

Sign/Thumb print__________________________ Date: __________

**Investigator’s statement**

I, the undersigned, have explained to the volunteer in a language he/she understands the procedures to be followed in the study and the risks and benefits involved.

Interviewer’s signature: ____________________ Date: __________
APPENDIX I B

ASSENT FORM

My name is Arthur Brown; I am a student of the Kenyatta University. We are carrying out a research to investigate the level of defense that you get after taking measles vaccination. I am asking you to be part of this study. Your parent has been told about this study and she/he knows that you will be asked to take part in the study.

Research Information

The purpose of this research is that measles is a disease which can cause severe sicknesses and even kill, and to prevent this disease, vaccination is given; yet there are some people still get the disease even after they have been vaccinated. So we are carrying out this study to see the level of immunity (defense) that is produced by the vaccination.

Risk

If you agree to be part of this study, we will take small amount of blood from you. You will feel some pains at the area where the blood will be taken.

Benefit

After testing you, the result will be released to your parent through your healthcare provider who will enable you to know the level of your body defense against measles disease.
The report from the study will also help the government to improve on the policy against measles outbreaks in the country.

**Privacy**

The information from this project will be kept private. No names will be included in any reports written about the study.

**Procedure**

Your participation in this study is voluntary; there is no penalty or any bad feelings about you if you choose not to take part. Once you start the project you are always free to stop at any time. Even if your parents gave their permission, you can still decide not to be in the study or to stop at any time, we will respect your decision.

You may ask any questions on what you want to know about the study.

**Subject’s Statement**

I, _________________________________, want to be in this study.

Parent’s / guardian’s Signature: _______________ Date: ______________

Witness Name: _______________ Signature: _______________ Date: ______________

Interviewer’s signature: _______________ Date: _______________
APPENDIX II

QUESTIONNAIRE

I am Arthur, a Masters of Science student in the Department of Medical Laboratory, School of Health Sciences at the Kenyatta University. I am investigating the factors that lead to the outbreaks of measles in Nairobi. I kindly request that you participate in this study by providing the information to the best of your knowledge as outlined in this questionnaire

Serial No: ___________ Date_______________

QUESTIONNAIRE PART I (Socio-economic information)

1. Age in years: i) < 25 (   ) ii) 25 – 30 (  ) iii) 31 – 40 (   ) iv) 41 – 50 (   ) v) >50 (   )

2. Area of Residence: ________________________

3. Religion/Denomination: Christian (   ) Muslim (   ) None (   )
   Others (specify) ____________________________

4. Marital status: Married (   ) Widow (   ) Single (   ) Separate (   )
   Divorce (   )

5. Nationality: ____________________________

6. Educational Level: Primary (   ) Secondary (   ) post-secondary(   )
   None (   )

7. Occupation: ______________________________________________
8. Are you employed? i) yes ( ) ii) No ( )

9. If Yes, as what? ________________________________

10. If No, what is the source of your income? ________________________________

11. Spouse’s Educational Level: Primary ( ) Secondary ( ) Post-secondary( ) None ( )

12. Spouse’s Occupation: ________________________________

13. Is your spouse employed? Yes ( ), No ( )

14. If yes, as what? ________________________________

15. If No, what is the source of income? ________________________________

QUESTIONNAIRE PART II

1. How many children do you have?
   i) 1 ( ) ii) 2 ( ) iii) 3-5 ( ) iv) >5 ( )

2. How many of your children were born in a health facility?
   i) All ( ) ii) Some (specify No.) _________ iii) None ( )

3. Have you at any time heard of measles disease? i) Yes ( ) ii) No ( ) iii) Don’t know ( )

4. Have you ever had measles? i) Yes ( ) ii) No ( ) iii) Don’t know ( )

5. If yes, at what time (age in years) __________________

6. Have any of your children had measles? Yes ( ) No ( )

7. If yes at what time(age) __________

8. Under what condition did they get the measles? i) During an Outbreak ( )
   ii) Outside of measles Outbreak ( ) iii) Don’t Know ( )
9. Do you know a neighbor or anyone whose child has had measles in your community?
   i) Yes ( ) ii) No ( )

10. If yes, was it around the same time your child had the measles? (Not applicable if Q # 6 is No) ________________

11. Have you ever heard about measles vaccine? i) Yes ( ) ii) No ( )

12. If yes, through what means? (Thick as many as possible) i) Radio ( ) ii) Television ( ) iii) Heath education at the health facility ( )
   v) Others (specify) _____________________________

13. Which of the following do you think is the work of a vaccine?
   i) Cures a disease ( ) ii) Makes one sick ( ) iii) Prevents one from getting the disease of that vaccine ( ) iv) Reduces the ability of a woman to conceive ( )
   v) Increases the ability of a woman to conceive ( )

14. Have you been vaccinated against measles? i) Yes ( ) ii) No ( ) iii) iv) Don’t Know ( )

15. If yes, at what age? ________________ If No, why? ________________

16. Are all your children vaccinated against measles? i) Yes ( ) at what age? __________,
   ii) No ( ), why? ____________________________

17. If no, would you love to have those not, to be vaccinated against measles? i) Yes ( )
   ii) No ( )

18. Have you heard about measles outbreak in Kenya? i) Yes ( ) ii) No ( )

19. If yes, what do you think is the cause(s) of the outbreaks?
   i) Migration of people ( ) ii) Cultural/ Religious denial ( )
   iii) Lack of education about vaccination ( ) iv) Other _____________________________
20. How many times does your household take meal in a day? i) 1 ( ) ii) 2 ( ) iii) 3 ( ) iv) >3 ( )

21. How frequent do you take the following food in your household? (Please indicate)

<table>
<thead>
<tr>
<th>Food</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Milk</td>
<td></td>
</tr>
<tr>
<td>2 Eggs</td>
<td></td>
</tr>
<tr>
<td>3 Meat</td>
<td></td>
</tr>
<tr>
<td>4 Maize</td>
<td></td>
</tr>
<tr>
<td>5 Fruit</td>
<td></td>
</tr>
<tr>
<td>6 Beans</td>
<td></td>
</tr>
<tr>
<td>7 Vegetables</td>
<td></td>
</tr>
<tr>
<td>8 Bread</td>
<td></td>
</tr>
<tr>
<td>9 Rice</td>
<td></td>
</tr>
<tr>
<td>10 Potatoes</td>
<td></td>
</tr>
</tbody>
</table>

22. When was the last time you were sick to attend a health center or hospital?

i) <1 month ( ) ii) 1-3 months ( ) iii) 4 months - 1 years ( )

iv) 2-5 years ( ) v) >5 years ( )

23. What was it that you suffered from? i) Malaria ( ) ii) Diarrhea ( ) iii) Flu ( ) iv) Pneumonia ( ) v) Measles ( ) vi) others (specify) ________________________________

24. Have you ever lived among plenty people? i) Yes ( ) ii) NO ( )

25. If yes, where was that? i) A displaced center ( ) ii) A refugees camp ( ) iii) others (specify) ________________________________
APPENDIX III

MAP OF STUDY SITE
APPENDIX IV

PERMISSION FROM MLKH FOR SAMPLE COLLECTION

MLKH/ADM/RES/1/4/ (27)
14th AUGUST 2013

Our Ref:---------------------------
Date---------------------

Chairman, Department of Medical Laboratory
Kenyatta University School of Health Science
Department of Medical Laboratory Science
P. O. BOX 43844
NAIROBI

RE: ARTHUR BROWN- REG NO. PKU/128/1112

Refer to your letter dated 8th August 2013 on the above subject.

This is to inform you that your request has been approved for you to start your study w.e.f. 19th August 2013 to 25th October 2013.

You may continue as scheduled.

Z.A LIUMBazzi
FOR: MEDICAL SUPERINTENDENT
APPENDIX V

ETHICAL APPROVAL

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711578
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844
Nairobi, 00100
Tel: 8710901/12

Ref: KU/R/COMM/51/197

Date: August 7th, 2013

Arthur N. Brown
School of Health Sciences
Kenyatta University
P.O. Box 45844-00100, Nairobi

Dear Mr. Arthur,

APPLICATION NUMBER PKU/128/1112 OF 2013 — EVALUATING MEASLES VACCINE-INDUCED IMMUNITY IN CHILDREN AGE NINE MONTHS AFTER VACCINATION AND MEASLES-VACCINATED CHILDREN AT FIVE YEARS IN NAIROBI — VERSION 2

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic, 'Evaluating Measles Vaccine-Induced Immunity in Children age Nine months after vaccination and measles-vaccinated children age five years in Nairobi' version 2 dated June 28th 2013 received on 9th July, 2013.

2. APPLICANT

Arthur N. Brown
School of Health Sciences
Kenyatta University
P.O. Box 45844-00100, Nairobi

3. SITE

Nairobi

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.5) and the Kenyatta University Ethics Review Committee Guidelines, and is of the view that against the following elements of review,

(i) Scientific design and conduct of study,
(ii) Recruitment of research participant,
(iii) Care and protection of research participants,
(iv) Protection of research participant's confidentiality,
(v) Informed consent process,
(vi) Community considerations.

AND APPROVED that the research may proceed for a period of ONE year from August 7th, 2013.
APPENDIX VI

APPROVAL FROM GRADUATE SCHOOL

KENYATTA UNIVERSITY
GRADUATE SCHOOL

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

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School
TO: Arthur N. Brown
     C/o Medical Laboratory Sciences
     Department

DATE: 29th May, 2013
REF: P150F/21359/10

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 8th May, 2013, approved your Research Proposal for the M.Sc Degree Entitled, “Evaluating Measles Vaccine-Induced Immunity in Two Categories of Vaccinated Children; Nine Months and Five Years Old in Nairobi.”

Thank you.

FOR: JOHN ODONGI
FOR: DEAN, GRADUATE SCHOOL

Cc: Chairman, Department of Medical Laboratory Sciences

Supervisors:

1. Dr. Margaret Muturi
   C/o Department of Medical Laboratory Sciences
   Kenyatta University

2. Dr. Lucy Kamau
   C/o Department of Zoological Sciences
   Kenyatta University
APPENDIX VII

AUTHORITY FROM NATIONAL COUNCIL, SCIENCE AND TECHNOLOGY

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Our Ref:  NCST/RCD/12A/013/142

Arthur N. Brown
Kenyatta University
P.O.Box 43844-00100
Nairobi.

Date: 11th September, 2013

RE: RESEARCH AUTHORIZATION

Following your application dated 22nd August, 2013 for authority to carry out research on “Evaluating measles vaccine – induced immunity in children age nine months after vaccination and measles – vaccinated children age five years in Nairobi,” I am pleased to inform you that you have been authorized to undertake research in Nairobi County for a period ending 31st May, 2014.

You are advised to report to the County Commissioner, the County Director of Education and the County Coordinator of Health, Nairobi County before embarking on the research project.

On completion of the research, you are expected to submit two hard copies and one soft copy in pdf of the research report/thesis to our office.

Said Hussein
FOR: SECRETARY/CEO
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Copy to:

The County Commissioner
The County Director of Education
The County Coordinator of Health
Nairobi County.

"The National Council for Science and Technology is Committed to the Promotion of Science and Technology for National Development"
APPENDIX VIII

CLEARANCE FROM NATIONAL COUNCIL, SCIENCE AND TECHNOLOGY

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.

2. Government Officers will not be interviewed without prior appointment.

3. No questionnaire will be used unless it has been approved.

4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.

5. You are required to submit at least two (2) hard copies and one (1) soft copy of your final report.

6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice.

RESEARCH CLEARANCE PERMIT

Serial No. A 00169

CONDITIONS: see back page.