EVALUATION OF MEASLES IMMUNITY AMONG CHILDREN AGED 9 TO 59 MONTHS AT SELECTED HEALTH FACILITIES IN KWALE, NAROK AND LAMU COUNTIES OF KENYA

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Evaluation of Measles Immunity among Children Aged 9 to 59 Months at Selected Health Facilities in Kwale, Narok and Lamu Counties of Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any University

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DEDICATION

I dedicate this work to my beloved wife, son and daughter for their unconditional love, encouragement and support. Achieving great success in life isn't always easy, but it is possible.

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I wish to give my sincere gratitude to all individuals who in one way or the other contributed to its success.

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OPERATIONAL DEFINITIONS

Antibody	A blood protein produced by the body in response to and usually counteracts the establishment of a disease causing agent.
Convulsion	A sudden, sometimes violent, irregular movement of the body caused by involuntary contraction of muscles, associated especially with epilepsy or toxins.
Disease	A disorder of structure or function in a human especially one that produces specific characteristics or that affects a specific part of the body.
Encephalitis	Inflammation of the brain tissue.
Haemolysis	The destruction of red blood cells.
Herd immunity	The overall resistance to a disease causing agent in a particular community.
Immune globulin gamma	An antibody of the gamma community mainly involved in providing long term immunity to a particular disease.
Immune	Resistant to a particular infection owing to the presence of specific antibodies or sensitized white blood cells.
Immunity	State of protection from the invasion of the body by disease causing particles.
Infection	The process of affecting a person through introduction of a disease-causing organism on or in to the person's body.

Laryngotracheobronchitis	Inflammation of the mucous membrane in the larynx and tracheal tubes, typically with spasm of bronchial muscle and coughing.
Measles	An infectious viral disease causing fever and a red rash, typically occurring in childhood.
Neutralising antibody	A blood protein that renders infectious agents harmless by blocking them.
Outbreak	A sudden occurrence of a disease beyond the expectation of the community and/or state.
Pathogenesis	The manner of development of a disease.
Pneumonia	A lung infection in which the air sacs fill with pus or watery fluid.
Prodrome	**A**n early characteristic of disease indicating the onset of illness.
Seizures	A sudden attack of illness, usually manifesting as convulsion.
Seroconvert	Undergo a change from a non-immune person to an immune person through the development of specific neutralizing antibodies.
Sero-surveillance	Using blood samples to investigate for the presence of specific indicators of resistance from a particular group of people**.**

Subacute Sclerosing Panencephalitis (SSPE) A chronic, progressive disease involving damage to the sheaths of nerve cells in the brain and spinal cord, whose symptoms may include

	numbness, impairment of speech and muscular coordination, blurred vision, and severe fatigue.
Supplemental Immunization Activities	Additional processes aimed at strengthening immunity in an otherwise already immunized population, this may be in the form of adding vitamin A and/or extra immunization.
Susceptibility	The state or fact of being likely to be influenced or harmed by a particular disease.
Transmission	The action or process of causing a disease to pass on from one place or person to another.
Vaccine potency tests	Laboratory tests employed to determine the strength and effectiveness of vaccines.
Vaccine	An antigenic preparation used to stimulate the production of antibodies and provide immunity against a disease.
Viraemia	The presence of viruses in the blood.
Virus	A sub microscopic infective particle, typically consisting of nucleic acid coated in protein, which is able to multiply only within the cells of a host organism.
Waning immunity	Diminishing protective resistance against disease causing agents.

LIST OF ABBREVIATIONS

$CCID_{50}$	Cell Culture Infectious Dose fifty
CDC	Centers for Disease Control and Prevention (USA)
DBS	Dried blood spots
DMEM	Dulbeccos Modified Eagles Medium
DVI	Department of Vaccines and Immunisation, Kenya
ELISA	Enzyme Linked Immunosorbent Assay
EMEM	Eagles Minimum Essential Medium
EPI	Expanded Programme on Immunization
ESEN	European Sero-Epidemiology Network
FP	Fusion protein
GAVI	Global Alliance for Vaccines and Immunization
GIVS	Global Immunization Vision and Strategy
GVAP	Global Vaccine Action Plan
HA	Haemagglutinin
IgG	Immunoglobulin Gamma
IgM	Immunoglobulin Mew
KEPI	Kenya Expanded Programme on Immunization
McV	Measles-containing vaccine
McV 1	Routine measles containing vaccine 1^{st} dose
McV 2	Routine measles containing vaccine 2^{nd} dose
MDG4	United Nations Millennium Development Goal 4

MMR	Measles, Mumps and Rubella vaccine
NA	Neuraminidase
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
SAGE	Strategic Advisory Group of Experts on Immunisation
SIA	Supplemental Immunization Activities
SSPE	Subacute sclerosing panencephalitis
$TCID_{50}$	Tissue Culture Infectious Dose fifty
VPD	Vaccine-preventable diseases
WHO	World Health Organization

ABSTRACT

Measles burden has been on the decline globally since 1980 due to universal use of measles vaccine; with outbreaks reported in Africa, parts of Europe and Asia in 2008. Population immunity assessment is key to determining progress towards elimination of measles as advised by World Health Organisation (WHO). Kenya relies on incidence reports, since there is scanty information on prevailing measles immunity and risk awareness status. The aim of this study was to evaluate measles immunity among children aged 9 to 59 months at selected health facilities in Kwale, Narok and Lamu Counties of Kenya. This was a hospital-based cross-sectional descriptive study in which 453 children were studied. A structured questionnaire was used, blood was collected and dried blood spots (DBS) prepared. Detection of measles IgG antibodies was done by ELISA. Plaque reduction neutralization test (PRNT) was used to confirm serology equivocal results. Results were correlated with actual vaccination coverage, demographic and vaccination history data. The number and percentages of positive and negative sera were found, Chi-square used to compare proportions and a 95% confidence interval used to describe limits of percentages. Individuals found to have Measles IgG titres comparable to 200mIU/ ml, were considered protected. The study recruited 233 (51.4%) male and 220 (48.6%) female children. Most of the children 408/453 {(90.1%) 95%CI; 81.8% to 98.4%)} were vaccinated, while only 11/453 {(2.4%) 95% CI; 2.2% to 2.6%)} were not vaccinated. Among the study group, 346/453(76.4%) children had vaccination cards, 107(23.6%) did not ($P < 0.001$). Overall, 396/453 {(87.4%) 95% CI; 84.4% to 90.5%)} children had protective antibody titres, while 57/453 {(12.6%) 95% CI; 9.5% to 15.7%)} did not ($P = 0.000$). The proportion of vaccinated children with protective antibody titre was 362/408 {(88.7%) 95% CI; 85.8% to 91.6%}. The general population immunity against measles disease in the children under study was low. At 87.4%, this indicates that the herd immunity in the study group falls below the WHO recommended minimum levels of 93-95%. A country wide assessment of measles population immunity and measles vaccine awareness is required in Kenya. This shall help in identifying the most vulnerable regions and step up strategies to build up herd immunity in these zones, a good step to measles elimination in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Infectious diseases continue to be a major public health concern, especially in developing countries where more than one million people die each year out of these infections (Barskey *et al.*, 2010). Measles is an acute, highly contagious viral disease that is estimated to cause over 114,900 annual deaths globally as by 2014 (Holzmann *et al.*, 2016). Measles is highly communicable, with greater than 90% secondary attack rates among non-immune persons (Naim, 2015; WHO, 2014). Approximately 10 days after exposure, measles in children manifest with an acute picture that initially resembles Rhinovirus common cold (Naim, 2015). The child develops running nose, becomes feverish, Koplik spots are seen in the mouth, conjunctivitis with red eyes sets in, followed by coryza and later child begins experiencing dry cough (Naim, 2015; Siberry *et al.*, 2015; Holzmann *et al.*, 2016). Soon after the acute phase, the child may develop watery diarrhoea, corneal ulcerations and scarring, mouth ulcerations, bronchitis with productive cough, and difficulties in breathing due to viral or bacterial pneumonia. Occasionally, the child may become comatose due to encephalitis and rarely death may occur due to the complications (CDC, 2015; Naim, 2015; WHO, 2009b).

In the 10th century, a Persian physician, Rhazes (Abu Bakr Muhammad Ibn Zakariyya al-Razi), described measles as more dreadful than small pox, another viral disease (William, 1987; Holmlund, 2012). Measles continued to be a universal childhood disease up to the late 1950s prior to the discovery of measles vaccines (Cutts, 1993).

Measles virus remains an important cause of vaccine-preventable disease, disability and death worldwide, more so in resource-poor nations. Among the significant risk factors associated with high transmission of measles in children under 5 years include; malnourishment., immunosuppression, mass movements, civil war or other conflicts, and, more importantly ignorance among the adult population either through lack of information or intentionally as in the case of intentionally unvaccinated groups

(Antona *et al.*, 2013; Barskey *et al.*, 2010). All these factors in one way or the other impedes access to vaccination. Prevention of measles infections by vaccination remains the most significant approach in controlling the high rates of morbidity and mortality among this children (Durrheim *et al.*, 2014; WHO, 2014; Trentini *et al.*, 2017).

Vaccines are an essential component of preventive medicine. They protect those vaccinated from developing potentially serious diseases and the community by limiting the spread of infectious agents. Measles vaccination is a worldwide practice aimed at intercepting the spread of measles, an attempt to eliminate it globally. The world health organization through the expanded program on immunization identified measles as one of the principal diseases against which it is directed (Guerra *et al.*, 2017; WHO, 2009b, 2014). Measles vaccination began with the use of inactivated vaccines in the mid-1950s (CDC, 2015). Whereas inactivated measles vaccine offered substantial protection, it soon emerged that the protection didn't last (Rauh *et al*., 1965; McLean *et al*., 1970). This led to the introduction of attenuated measles vaccines, which have now taken precedent.

Live attenuated measles virus vaccine is stable and can prevent measles effectively (CDC, 2015; WHO, 2009b). However, a valuable vaccine must create high immune response with minimum harm. Therefore, immunisation programmes are supposed to deliver quality vaccines in a safe manner, with age-appropriate coverage rates of above 90%, in an environment that ensures high-quality programme monitoring. The efficacy of a vaccination program can be documented through; immunization records for immunization uptake data, serological surveillance data, vaccine efficacy and effectiveness tests and incidence through integrated disease surveillance programmes (Robert, Gacic-Dobo, *et al.*, 2014; WHO, 2009b, 2014). The more robust such combination of measurements are used, the higher the chances of successfully identifying pockets of the under vaccinated, the better the prospects of realizing measles elimination goal (WHO, 2013c, 2013a).

Europe sought measles elimination by 2015, a total of 37,000 cases were registered in 2014, an increase of 29,900 cases compared to the 7,073 cases that occurred in 2007 revealing a glaring scare (WHO, 2016).

Earlier on, France did very well in controlling measles transmission after introduction of universal vaccination for children in 1983 through 2007. In fact it registered only 40 and 44 cases in 2006 and 2007 respectively, a figure just below the WHO recommended measles elimination threshold of 0.1 cases per 100,000 inhabitants. However, with a target of eliminating the disease before 2015, an upsurge between 2008 through 2011left the country with more than 20,000 measles cases with at least 10 deaths- majority of which were children (Antona *et al.*, 2013).

In March 2014, the World Health Organisation verified measles elimination in the republic of Korea, however, by May the same year, the country recorded 220 measles confirmed cases among which 10 were identified to have been imported (Yang *et al.*, 2015). In the same year, Kenya recorded 356 confirmed cases of measles, and this reduced to 110 confirmed cases in 2015 with an incidence of 2.4 cases per million (Masresh *et al.*, 2017).

In Kenya measles vaccination was introduced through Kenya expanded programme on immunization (KEPI) in the early 1980s. In 2013, a second routine dose at age 18 months was introduced after the nation successfully reached a vaccination status of above 80% as recommended by WHO (DVI, 2013). Currently, routine measles vaccine first dose is given at 9 months of age or first contact after 9 months and second dose is given at 18 months of age or first contact after 18 months. In between, supplemental immunisation activities (SIA) with vitamin A may be given. In the special case of Human immunodeficiency virus infected children, vaccination is done at 6 months and then repeated at 9 and 18 months for routine dose (Chandwani *et al.*, 2011; W. Moss, 2015).

Kenya continues to experience periodic resurgence mainly linked to suboptimal immunization coverage~~s~~ leading to accumulation of susceptible children (Ministry of Health, 2013). Chief among challenges associated with suboptimal coverage~~s~~ as reported by the Health Ministry were inaccessibility of immunisation services due to

distant health facilities and poor geographical infrastructure particularly amongst nomadic communities, poor health seeking behaviour of caregivers due to socio-cultural issues and underdeveloped road network (DVI, 2013).

Within Kenya, Narok County was among the hardest hit regions, according to data from the department of vaccine and immunisation, with 440 cases in September 2012. In the same period Kwale county had 28 cases whereas Lamu County recorded only one case. Among them 197 (42.0%) were children below 5 years. Records show that in 2012, vaccination coverage was at 78.4%, 89.7% and 93.1% for Narok, Kwale and Lamu consecutively. The project intends to conduct a sero-survey to determine measles immune globulin among a sub-population of children aged 9 to 59 months attending selected health facilities in Kwale, Lamu and Narok Counties.

In previous outbreaks casualties included even those who had history of vaccination. One would wonder whether there is disconnect between vaccine coverage and actual protection. Could the serological survey on circulating antibodies provide clue on the effectiveness of the vaccination programmes?

1.2 Statement of the Problem

Despite previous claims of supplemental vaccine uptake of more than 90% (Measles & Rubella Initiative, 2012; Ministry of Health, 2013), episodes of measles cases are still reported in most parts of the country. Up to 45% of the affected population are children below the age of 5 years (CDC, 2012b). The degree of protection against measles infection in the community depends on the populations' herd immunity. Owing to its high reproductive number (Guerra *et al.*, 2017), to achieve herd immunity for measles, 95% of the population needs to be immune to the disease (CDC, 2012b; Guerra *et al.*, 2017; Metcalf *et al*., 2015).

In Kenya, sero-surveillance of neutralising antibodies against measles is not conducted; instead, incidence reports and vaccination estimates are largely relied upon (GoK:DVI, 2014; Masresha *et al.,* 2015; Manakongtreecheep and Davis, 2017). No work has been done to characterise community susceptibility with in the different social-cultural and geographical distinct regions. Therefore, it is difficult to determine

whether adequate levels of measles immunity exist among children below 5 years in the country.

1.3 Justification

By conducting serological surveys on the circulating immunity against measles, the levels of protection against the disease can be determined. This will reflect the population's degree of protection to measles. Through estimation of the proportion of the population who are susceptible to measles, the potential for future outbreaks can successfully be predicted.

By determining the prevailing seroprotectivity rates for the three counties with varying measles transmission rates, the effectiveness of immunization programmes shall be evaluated and gaps identified with possible recommendations to coordinate vaccine policy to ensure that adequate levels of immunity exist.

Among the four indicators of protection against measles and by extension a pointer of measles elimination, is adequate serological laboratory evidence of measles immunity (CDC, 2013), no such survey has been conducted in Kenya and hence no such data exist.

1.4 Research Questions

1. What are the demographic characteristics and measles vaccination history of the study participants?
2. What is the measles IgG ELISA seropositivity rate by age group in selected high, medium and low transmission areas?
3. What is the sero protectivity of antibody titres that are borderline in IgG ELISA assays?

1.5 Objectives

1.5.1 General Objective

To evaluate measles immunity among children aged 9 to 59 months at selected health facilities in Kwale, Narok and Lamu Counties of Kenya.

1.5.2 Specific Objectives

1. To establish the demographic characteristics and vaccination history of the study participants.
2. To determine Measles sero-positivity rate by age group in selected high, medium, and low transmission areas.
3. To determine the seroprotectivity of borderline antibody titres.

CHAPTER TWO

LITERATURE REVIEW

2.1 Characterisation of the Measles virus

Measles virus, also known as Rubeola, is a negative sense, single-stranded unsegmented RNA virus that infects only humans. It belongs to the genus *Morbillivirus* in the family *paramyxoviridae,* housed in the comparatively large order of *Mononegavirales* according to the International Committee on Taxonomy of Viruses (ICTV)(CDC, 2015; Naim, 2015). Measles virus is closely related to the Rinderpest and Canine distemper viruses (CDC, 2015; Naim, 2015). The virus is famed for having retained its monotypic antigenic characteristics for long, a rare feature not exhibited by many viruses, more so RNA based. Despite documented changes in the H protein, changes in vaccine efficacy are yet to be observed (Rota *et al.,* 2009; Beaty and Lee, 2016).

2.1.1 Biology of the Measles Virus

Measles viruses have single-stranded ribonucleic acid (RNA) genome, weighing approximately 6.5×10^6 Daltons, contained in a helical nucleocapsid enclosed within a pleomorphic envelope. The virus measures between100–200 nm in diameter and has its surface largely covered by the Haemagglutinin (H) and the Fusion (F) glycoproteins. These molecules are associated with the virus infectivity and are among the eight (8) proteins encoded by the Measles virus genome (Fontana *et al.,* 2017; Kutty *et al*., 2013).

The composition of the nucleocapsid include the RNA associated with a structural maintenance nucleoprotein (NP), an RNA synthesis facilitator phosphoprotein (P), and the considerable large (L) protein functioning as the RNA polymerase (Beaty & Lee, 2016; CDC, 2015).

Lining along the inside of the virion envelope is the matrix (M) protein which has a close association with the nucleocapsid. The envelope contains two significant glycoproteins, a haemagglutinin (H) protein, and a fusion (F) protein. The H protein is

involved in adsorbing the virus on to blood cells thereby enhancing viral attachment processes (WHO, 2009b). The F protein promotes fusion of the viral envelope and host plasma cell membranes and viral penetration in the course of a replication cycle, it is also the chief agent in haemolytic activities associated with the virion (Naim, 2015).

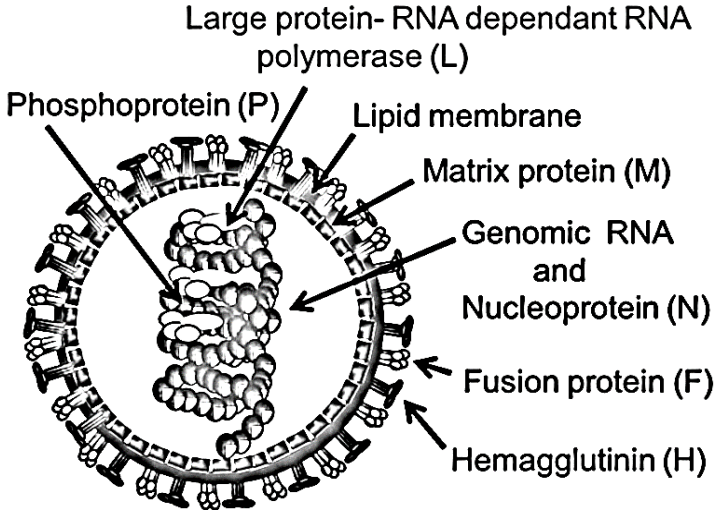

Figure2.1: A section view of the Measles virus showing its major components. *Source;* https://microbeonline.com/

It has been established that the long lasting immunity that usually follows a measles infection come courtesy of the neutralizing antibodies against the H protein (Lech *et al.*, 2017).Up on attachment, proteolytic activities are required to splice and thereby activate the F protein to express its membrane-fusing activity. Cleavage of the F protein produces F1 and F2 glycopeptides that are held together by a disulphide bond (Wen Xu *et al*., 2016).

Genetic characterisation of the nucleotides sequences found in the Haemagglutinin and nucleoprotein genes has been used to group the wild-type virus (Bankamp *et al.*, 2011; Rota *et al.*, 2011). By 1998, 8 distinct groups had been identified (Bellini & Rota, 1998). Among these genetic groups was; The Edmonston strain, isolated in 1954, a prototype group 1 isolate, and a reference virus from which all vaccine viruses are derived *(Bankamp et al., 2011; Penedos et al.,* 2015; WHO, 2009b).This includes Edmonston-Zagreb, AIKC and Schwarz strains. Among the groups are also the

temporally and geographically independent wild-type isolates like Shanghai -191 and Changchun – 47 from China, Leningrad – 16 from Russia, and CAM – 70 from Japan (WHO, 2009b).

Currently 24 genotypes of Measles virus contained within 8 groups are in existence; A, B1-3, C1-2, D1-11, E, F, G1-3, and H1-2 (Bankamp *et al*., 2014; Beaty & Lee, 2016; Penedos *et al.*, 2015). It should be noted however that all these genotypes are found within a single serotype. Despite existence of all these genotypes, only a fraction of this pool is currently circulating (He *et al*., 2012; Hickman *et al*., 2011; Zhang *et al.*, 2007).Generally, there is minimal variation between strains as shown by several nucleotide sequence analysis of selected genes but their effects are negligible (Beaty & Lee, 2016; Fulton *et al.*, 2015).

2.1.2 Transmissibility of Measles virus

Measles, largely spread through respiratory droplets, is the most contagious viral disease on earth (Cutts *et al*., 2013; Durrheim *et al.*, 2014).Having a basic reproduction number (R_0) of between 12 to 18, it is more infectious than Ebola (R_0 = 1.5-2.5), and influenza (R_0 = 1.4- 4) (Stanley *et al,*. 2013; Roberts, 2015; Holzmann *et al,*. 2016). Among susceptible persons, Measles has greater than 90% secondary attack rates and may be transmitted from 4 days before to 4 days after rash onset (CDC, 2012; Monfort *et al*., 2010; Kamau *et al*., 2007). Transmission is primarily through person to person contact, but aerosolized transmission through airborne nuclei has been documented, especially, in closed areas where suspended droplets may remain up to 2 hours (Paul A. Rota *et al.*, 2011; Yanagi *et al.*, 2009). Interruption of transmission has been a world wide effort given the debilitating nature of the disease. Infections associated with this virus has been linked to higher loss of disability adjusted life years worldwide (Mitiku *et al.*, 2011; CDC, 2011).

Transmission interception is a key factor in the process of measles elimination, maintenance of endemic free zone for at least 1 year, and subsequent eradication. Measles is a highly infectious vaccine preventable disease that has continuously afflicted man globally. Well timed, programmed, adequate vaccination done using a vaccine strain that has been maintained in the correct cold chain system can effectively

intercept measles transmission (Uzicanin and Zimmerman, 2011; Mercader *et al.*, 2012; Papania *et al.*, 2014).With such high $R_{0,}$ an estimated population immunity of 95% and above is necessary to achieve herd immunity, overall protection due to total immunized, in a given geographical group (Durrheim *et al.*, 2014).

Measles virus is susceptible to inactivation through heating at 56^0c for 30 minutes, it is not known to resist sunlight, and is rapidly inactivated by acidic pH (Weiss *et al.*, 2013). Measles virus envelop is rapidly digested by ether or trypsin suspension. Although its survival time on objects surfaces or air is usually less than 2 hours, in a closed environment, it has been proved that respiratory droplets can remain infectious for up to 2 hours (CDC, 2015). The virus is highly susceptible to disinfectants and, can be inactivated by 70% alcohol, phenolic solutions, povidone iodine, 1% sodium hypochlorite (domestic bleach), hydrogen peroxide and per acetic acid. Moreover, the virus is readily destroyed by aldehydes such as glutaraldehyde and formaldehyde (CDC, 2015; Plattet *et al.*, 2016; Weiss *et al.*, 2013).

2.1.3 The Measles Burden

Earlier, before licensing of the initial live attenuated vaccine for measles in 1963, more than 90% of individuals used to be infected by the age of ten years with a greater majority showing classical symptoms of the disease (Robert, Gacic-dobo, *et al.*, 2014; WHO, 2009b). Since then there has been massive achievements on reducing measles reported cases globally.

According to the World Health Organization, in 1998, reported measles incidents per a million of total population was 16 cases in the Americas, 82 cases in Europe, 111cases in the Eastern Mediterranean region, 42 cases in South East Asia, 50 cases in the Western Pacific region, and 617 cases in Africa. In 2006, a total of 187 confirmed cases were documented in the whole of Western Hemisphere and this was mainly in the United States, Mexico, and Venezuela (R T Perry *et al.*, 2013; WHO, 2013c), a tremendous improvement in the region.

In 2001, the world lost an estimated total of more than 22 million disability-adjusted life years (DALY) as a result of measles with Africa being the most affected region

(WHO, 2009b).In developing countries, up to 30 million children used to be affected annually and caused almost 1 million deaths in a year. An estimated 15,000 – 60,000 cases of blindness occurred annually as a consequence of Measles virus infection.

Within the period 2000 and 2008, WHO reported cases of measles worldwide had declined from 852,937 cases in 2000 to 278,358 cases in 2008, a 67% reduction (WHO, 2012c). In this period, the global measles mortality dropped by 78%. However, because in majority of countries, especially those with the most serious burden of disease believed to be lacking reliable surveillance data, it is expected that global measles incidences were under reported in many countries (WHO, 2009b).

From early 2008 through 2011, France had been experiencing uncharacteristic upsurge of measles cases with peaks in April 2010 and March 2011. Throughout this period, some 22,178 incidences were documented 447 among them were imported, a majority of which (230 cases) came from Europe. Of this, 8,847 had biological confirmation, and 2,620 cases epidemiologically linked to at least a biologically confirmed one (Antona *et al*., 2013). Over the same period of time, outbreaks were experienced among the 46 WHO member states of the African Region (WHO, 2013c).

The CDC reports estimated that, measles caused 197,000 deaths worldwide in the year 2007 making it to be among the principal causes of deaths in children globally (CDC, 2011; WHO, 2013c). Majority, almost 85%, of the deaths were reported in Southeast Asia and Africa. Between 2000-2007, there was a drop in deaths from measles by 74% (from750,000 in 2000 to 197,000 in 2007), thanks to the increasing cooperation between the several global organizations.

The period 2000–2014 has experienced a comparatively steady reduction in the numbers of annually reported measles incidences worldwide. We have seen a decrease in reported cases from 853 479 to 267 482, an approximately 69% decline. Concurrently, measles incidence has dropped by 73%, from around 146 to a mere 40 cases per million population (CDC, 2012; CDC, 2015). Come the year 2013, Member States that were reporting less than 5 cases in a million were 113 (65%) out of the total 175 members who submitted reports. However, in 2014 there was a decrease in member states reporting less than 5 cases per million. Only 98 out of 169 members

(58%) reported fewer cases in 2014, indicating a global upsurge of measles cases. Within the same period (2000–2014), the WHO Americas (AMR) Region managed to maintain measles incidence at below 5 cases per million (WHO, 2015).

According to WHO statistics, measles incidence decreased in 4 out of the 6 WHO regions in the period 2013/2014. In the WHO Africa Region (AFR), there was decline in reported cases from 171,178 cases in 2013 to 73 914 cases in 2014, a 57% decrease. This marked decrease was largely associated with a convincing decrease in cases in Nigeria and the Democratic Republic of Congo (DRC). Nigeria had 52,852 cases in 2013 which reduced to 6,855 in 2014, while in DRC reported cases declined from 88, 381 in 2013 to a mere 33,711 cases in 2014. However, in 2014, comparatively major outbreaks were reported in Ethiopia with 12, 739 cases, Angola with 11,699 cases, and Somalia with 10,278 cases.

Concurrently, in the other 3 regions; The Eastern Mediterranean Region (EMR), The South-East Asia Region (SEAR), and The European Region (EUR) also reported a decrease in measles cases in the year 2014. However, massive outbreaks were experienced in India which had 24, 977 cases, and The Russian Federation which had 4,711 cases.

There was however a general upsurge in the number of reported cases in 2014 from the Western Pacific Region (WPR) and Americas (AMR) region. This increase in cases was largely attributed to outbreaks in Brazil which had 727 reported cases and the United States which had 667 reported cases. Western Pacific Region reported massive outbreaks in the Philippines which had 58, 848 cases, Viet Nam which had 15, 033 cases, and China which had 52,628) reported cases (Holzmann *et al.*, 2016).

Despite confirmation of measles elimination and subsequent interception of its endemic transmission in the United States of America (USA) in 2000, the status was hardly sustained beyond 2011. In approximately 88% of the cases reported in this period, the virus was suspected to have come from a country bordering USA, with two thirds of persons developing the disease being unvaccinated. Moreover, in 2013, these cases increased three times to a whopping 175 cases, majority of which occurred in unvaccinated children (Papania *et al.*, 2014; ECDPC, 2016; Holzmann *et al.*, 2016).

Most of the non-vaccination of children was resulting from parental refusal of immunization (Lieu *et al.*, 2015).

In the first half of 2014, CDC received 288 confirmed measles cases, a startling statistic compared to a mere 220 cases, the highest ever recorded, annual report in the period 2000 through 2011 (WHO, 2014; Gastanaduy *et al*., 2014).

Of the 288 cases, 280 (97%) were attributed to importations and majority (about 200 cases) occurred in unvaccinated individuals while 58 cases was from persons with unknown vaccination status (Gastanaduy *et al*., 2014).

2.2 Measles as a disease

Measles is a systemic infection (Naim, 2015). Its pathogenesis is chiefly a function of the two surface proteins; F protein and H protein. These two proteins associates with several molecules in the normal viral replication cycle thereby inducing a series of other chain of events of varying implications to the host (Naim, 2015; Plattet *et al.*, 2016). Damage to the host may come as a result of the viral replication process, its outcome or host immune response. Disease production is therefore associated with several characteristics that promote viral multiplication and cell change (Plattet *et al.*, 2016).

Replication of Measles virus begins with the haemaglutinin (H) attachment on the virion envelope to sialic acid residue on the cell surface glycoproteins. The H reception on to the cell surface is dependent on two important cell surface molecules, CD46 and CD150. The CD46 is a surface membrane cofactor protein (MCP) available on surfaces of majority of body cells and is involved in protection of the cell from complement mediated hydrolysis by regulating complement activation (Prescott *et al*., 2005). The CD150 is a signaling lymphocyte activation molecule (SLAM). It is present on surfaces of all or at least majority of activated T and B cells, and is involved in regulating the responses of T-Helper 2 (TH2) and T-Helper 1 (TH1). This regulation can be destabilized when the CD150 molecule is targeted by the measles virus (CDC, 2012; Naim, 2015).

Measles virus Adsorption on to cell surface is followed by fusion (F) protein proteolytic cleavage which activates it thus promoting fusion between virion envelope and the plasma membrane of the host cell. Expression of virus specific F proteins on cell surfaces promotes multinucleated giant cells (syncytia) formation through induction of cell-cell fusion. (CDC, 2015).

The conjunctiva of the eye and the respiratory epithelium of the nasopharynx are usually the primary foci of infection. In the body, the virus is distributed through the lymphatic system and spread via cell-associated viremia majorly involving lymphocytes and monocytes as are the most targeted cells. About three to four days after the primary involvement of the epithelium, the initial cell related (primary) viraemia takes place. This is followed by a further invasion of the reticuloendothelial system with subsequent appearance of Koplik spots - tiny white spots with reddened background randomly distributed from the inside of the mouth (CDC, 2012). Secondary viraemia occurs five to seven days after initial infection emanating from further viral replication within distal and regional reticuloendothelial locations. Disseminated spread of the virus leads to involvement of major parts of the body including the blood and lymphatic system, the respiratory system, urinary tract, gross conjuctiva, and the central nervous system (CDC, 2015). This leads to the development of distinguishing measles prodromal features of cough, conjunctivitis, coryza and photo phobia with fever and rash.

The rash usually begins as pale, eruptive papules and macules on the face that follows the hairline, behind the ears, and along the neck. In about two days, lesions condense to form plaques that spread cephalocaudally to the body. Eventually, lesions spread to the extremities including the palms of the patient. Targeting of small blood vessels by immune active Thymus derived (T) cells is thought to account for generation of the maculopapular rash (Naim, 2015). Beginning from the prodromal period until three to four days after rash onset there is continuous shedding of the Measles virus from the respiratory system (CDC, 2015; Naim, 2015).

Despite the fact that robust cell-mediated immunity accounts for a great majority of the characteristic pathological signs, it is still paramount in the overall control of

measles disease. Measlesvirus is an excellent inducer of interferon-α and -β, these are potent molecules involved in the activation of natural killer (NK) cells. Activated NK cells kill viral associated or rather viral affected cells non-specifically thereby reducing the population of such cells (CDC, 2015).

Eventually, this infection leads to a pronounced generalized suppression of immunity marked with decreased interleukin-12 (IL-12) productions and reduced delayed-type hypersensitivity (Takeuchi *et al.,* 2002; Naim, 2015). Immunosuppression increase chances to secondary opportunistic infections, particularly bronchopneumonia, which is arguably the chief cause of measles-related deaths in children below 5 years in the developing world (CDC, 2015; Griffin, 2016).

Moreover, when individuals have defective or weak cell mediated immunity, measlesvirus infection may often cause progressive and frequently fatal giant cell pneumonia (Naim, 2015).

2.3 Clinical Implication

Measles is usually a mild childhood disease, however, it can result in to residual impairment (Prescott *et al*., 2005). More often, complications are attributable to the immunosuppressive nature of the disease which clears the way for other infectious agents to simultaneously establish themselves (Murray *et al*., 2004).

Fatal Measles cases are usually encountered in greater frequencies in malnourished, at least with vitamin A deficiency, mostly children below 5 years from overcrowded dwellings. Occasionally, fatality may be experienced in persons with deranged immunity such as due to advanced cancers, HIV infections or other immune targeting agents. In general, persons younger than 5 years or elderly are more prone to complications of measles (CDC, 2015).The incidence is particularly increased among people with deficient immunities, poor nutrition, inadequate vaccination and inadequate vitamin A. Therefore, immunocompromised non-measles protected children are even at a higher risk of developing severe measles infections and superinfections (Naim, 2015).

During measles infection, the virus targets mostly thymus derived (T) cells thereby destabilizing cellular systems leading to suppressed effects of the hosts immune responses. As a consequence, the host may be affected through reactivation of viral latent infections or bacterial superinfections (CDC, 2015; Naim, 2015).

The most common measles complication is pneumonia originating from primary or secondary viral or the more often bacterial infections (CDC, 2015). Hepatomegaly, splenomegaly, lymphadenopathy, para or hyperesthesia with pleural effusions may also be encountered. Other notable complications in measles infected persons include; sinusitis, encephalomyelitis and laryngotracheobronchitis (Bellini *et al.*, 2005; CDC, 2012; Naim, 2015). Disseminated intravascular coagulation (DIC), thrombocytopaenia with frequent haemorrhages, inflammations of the pancreas, appendix or pericardium, are the more fatal although rarely encountered (Cutts *et al.*, 2013; Siberry *et al*., 2015). Moreover, pregnant mothers may have premature labour that results to preterm births or spontaneous abortions (CDC, 2015).

Subacute sclerosing panencephalitis (SSPE), an even rarer complication with incubation period of approximately 10.8 years, is a degenerative CNS disease that can result from a persistent measles infection(Bellini *et al*., 2005; Hinman *et al*., 2004). This disease is characterized by the emergency of behavioural and intellectual deterioration with or without seizures several years after measles infection.

Generally, in about 1 in 1000 of reported cases of measles, encephalitis ensues with a small percentage often progressing to permanent brain damage. In the United States of America (USA) deaths are reported in approximately 0.1 to 0.2% of cases and, with ranges between 3 - 20% in other regions (CDC, 2012). Complications are seen more often in young children under the age of five years. Pneumonia is among the chief causes of complications and deaths, and can be directly attributed to measles pathogenicity or accompanied bacterial (CDC, 2015; Naim, 2015).

Acute encephalitis, which presents with drowsiness, headache, fever, stiff neck, ending up with convulsions and coma, generally occurs 6 days after rash onset (CDC, 2015). Seizures associated with meningeal damage, accompanied with or without fever, are

reported in 6 to 7 out of 1000 cases. Cases of deaths are more frequent among children below 5 years (CDC, 2012a, 2013).

Subacute sclerosing panencephalitis, a more severe degenerative form of encephalitis, occurs in about 0.0001% of measles cases (CDC, 2015; Kutty et al., 2013), but can be generally higher in measles cases among children below 5 years. (Campbell *et al.*, 2007; Holzmann *et al.*, 2016).

2.4 Measles control programmes and elimination efforts

Whereas complete eradication still remains a global headache (Papania *et al.*, 2014; Wang *et al*., 2014), major efforts are directed to the lesser target of bringing down the burden of measles transmission (Ferrari *et al.*, 2013; WHO, 2013c). The success of measles control and ultimate eradication is largely dependent on a well organised surveillance system, programmed and well-coordinated pre exposure prophylactic means, and adequate post exposure measures (CDC, 2015; WHO, 2014). These, coupled with frequent awareness programmes aimed at early case reporting, improved nutrition, understanding of vaccination strategies and accepting vaccines, could go a long way in arresting indigenous transmission. Such practices are being embraced by several member countries of the World Health Organisation (WHO, 2014; Masresh *et al.*, 2017).

Towards the end of the year 2012, the World Health Assembly (WHA), an arm of the World Health Organisation (WHO), established as a target measles elimination by 2020 in at least 5 of its regions. The Strategic Advisory Group of Experts on Immunization (SAGE) reviewed the guidance on monitoring of the progress towards measles elimination as a control measure. Owing to earlier successes in reducing measles-related deaths demonstrated by the elimination of endemic measles in the region of America in 2002 (Cutts *et al*., 2013), the remaining 5 WHO regions adopted measles elimination target by 2010. This was revised to 2015 (WHO, 2014) and later 2020 (Strebel *et al.*, 2011; Durrheim*et al.*, 2014; Holzmann *et al.*, 2016).

Elimination success in the American Region came through full implementation of a vaccination strategy that included high, two routine measles vaccine doses coupled

with supplementary immunization activities (SIA) that were religiously adhered to (Holzmann *et al.,* 2016). This was supplemented with high, coordinated monitoring of immunization coverages and robust disease surveillance systems (Cutts *et al*., 2013; Strebel *et al*., 2011).

2.4.1 Vaccination programmes

Earlier on after it was discovered that the best control of measles was through vaccination, in mid 1970s, programmes were set and specific age for vaccination was introduced by WHO (CDC, 2013, 2015; Cutts, 1993). Considerations were made to balance immunological response and the danger of being infected at a given age (CDC, 2013). It was realized that, behavioural and demographic factors affected developing countries to an extent that transmission of measles was so high immediately after losing maternal antibodies at 6 to 8 months of age. Thus, by the time children will lose maternal protection, many will already have been infected. Measles vaccination using Schwarz vaccine was then recommended from 6 months of age (Cutts, 1993). However, after studying data from Kenya (1977), Latin America (1982), and Haiti (1985), it was seen that maximum benefit would have been achieved at ages 8 to 10 months hence WHO recommended, through a policy, a single dose at 9 months in 1986 (Cutts, 1993; C. L. Martins *et al.*, 2008).

Increasing the age had been anticipated to decrease transmission in children below 9 months as well, however, cities in Africa continued reporting high mortality and morbidity rates despite some reporting up to 60% vaccination coverage (Cutts, 1993). This prompted the search for a vaccine that could be used at a lower age yet offer considerable protection (C. L. Martins *et al*., 2008). Studies from The Gambia and Guinea Bissau revealed that the measles vaccine Edmonston Zagreb used between ages 4 to 6 months could at least be comparable to the Schwarz vaccine when used at 9 months of age (C. Martins *et al.*, 2013; C. L. Martins *et al*., 2008). Based on this, in 1989 the use of high titre Edmonston Zagreb vaccine at the age of 6 months was recommended (Cutts, 1993).

However, this recommendation was not put in to use as high titre usage of the vaccine proved to be extremely expensive, and offered suboptimal immunogenicity than earlier

seen (Ferrari *et al.,* 2013; Holzmann *et al.,* 2016). Other data have proved that vaccination at an early age, 6 months, is associated to poor maturation of the humoral immune system especially against the measles vaccine (CDC, 2013).

Furthermore, high dose titre showed a high potential to reduce survival rate of infants recipients, hence, developing countries upheld vaccination at 9 months. However, special consideration was put for HIV infected children and in cases of mass vaccination during outbreaks or SIA which normally starts at 6 months (Cutts *et al.*, 2013; Holzmann *et al.,* 2016). Nonetheless, children vaccinated at 6 months are supposed to be revaccinated at 9 months and get a third opportunity when others are receiving their second dose (Siberry *et al.*, 2015; Fowlkes *et al.*, 2016).

In developed countries, routine first dose is administered at the age of 12 to 15 months followed by a second routine dose at between 4 to 6 years (CDC, 2013).

2.4.2 Surveillance

The hallmark for restricting spread of measles is swift identification, documenting, and tracing of measles cases. Early and rapid case identification complemented with prompt response that includes vaccination and seclusion of vulnerable contacts with or without convincing indication of protection is of paramount importance (Cutts *et al.*, 2013; Williams *et al*., 2016).

Disease surveillance information is useful in describing the current measles epidemiological characteristics. It goes a long way in evaluating current national or global preclusion guidelines and accomplishment of target goals including maintenance of disease-free states. Data from surveillance work directs on which supplementary procedures to be followed in the reduction of the chances of measles outbreaks.

Different countries or entire geographical regions have come up with operational procedures on the conduction of local disease surveillance, be it a routine surveillance procedure or an outbreak investigation. The most preferred course of action is; a case is reported, an active investigation to identify the original source is initiated, screen

for more contact cases, and avert further spread through vaccination (Bose *et al*., 2014; Wenbo Xu *et al.*, 2017).

Case reporting to a central referral organisation is essential. Nasal secretion and blood samples can be collected from cases and their contacts and forwarded to a reference laboratory for verification and, where possible molecular characterisation (Gidding *et al.*, 2016).

During the period between 2001 and 2011, the US recorded no endemic transmission except for importation associated outbreaks. Furthermore, most of those affected were unvaccinated (65%), or their vaccination status was unknown (20%) (Papania *et al.*, 2014). The WHO region of the America in general maintained less than 5 confirmed cases per million between 2001 and 2011 (Papania *et al.*, 2014; WHO, 2013c).In 2011 the West Pacific Region (WPR) confirmed cases were 12 per a million population, Eastern Mediterranean Region (EMR) had 35 923 cases, and South East Asia Region (SEAR) had 65 161 while European Region (EUR) and the Region of Africa (AFR) had 37 073 and 194 364 confirmed cases respectively (Perry *et al.*, 2013; WHO, 2013c; Perry, Gacic-dobo, *et al.*, 2014; Masresha *et al.*,2015)

Current Measles surveillance statistics indicate that by 2016, WHO Africa region reported 27.9 confirmed measles incidence per a million population, a significant decrease compared to the 76.3 reported in 2013 (Masresh *et al.*, 2017).Despite this improvement however, the number of member states with incidences of less than 1 reduced from 16 in 2015 to 15 in 2016 (Masresh *et al.*, 2017; Who/Unicef, 2015). In Kenya, the figure of reported confirmed measles cases were 110 by 2015, and the reported estimated incidence was 2.4 confirmed cases per a million population (Masresh *et al*., 2017; Who/Unicef, 2015).

On the other hand, surveillance of vaccine protectivity and immunisation coverages plays a vital role in identifying pockets of under vaccinated population. Moreover, it gives assurance on how far a country is in achieving the heard immunity threshold of more than 95% seroprotectivity as required to shut off indigenous measles transmission (Holzmann *et al.,* 2016; Moss and Strebel, 2018). This also exposes pockets of under immunization which usually form the weak points from where

measles transmission may be blown out of proportion in the event of an outbreak (Papania *et al.*, 2014; Liu *et al.*, 2015).

By 2010, at least 94.8% of children joining kindergarten in the united states had evidence of 2 doses of routine measles vaccine while state coverage estimates for the first dose ranged from 85.1% to 97.8% (Papania *et al.*, 2014). Seroprevalence survey for measles IgG done on the US population between 1999 and 2004 revealed evidence of seroprotectivity in 95.9% of the population (Papania *et al*., 2014). In contrast, in the African region, McV one had reached a mere 74% by 2015 (Masresh *et al*., 2017). Moreover, only 7 (15%) of African countries had maintained the McV 1 coverage at more or equal to 95%, a decrease from 8(17%) attained in 2013. Twenty three African countries (49%) were offering McV 2 with a vaccination coverage of 18% in 2015 (Masresh *et al.*, 2017). In the same year, in Kenya, McV 1 coverage was reported at 75% while McV 2 was estimated at 28% (Masresh *et al*., 2017; Who/Unicef, 2015).

2.4.3 Pre-Exposure Prophylaxis

Vaccination is the sole prophylactic treatment accorded to the population as pre exposure protective measure to prevent future infections or disease states. For protection against measles disease, at least two routine doses of measles vaccine are advised for children. In the developing countries, the first dose is usually at 9 months with the second dose following at between 15 months and 18 months of age (Hall & Jolley, 2011; R T Perry et al., 2013; Subaiya *et al*., 2015). In the developed countries, the first dose is provided at between 12 to 15 months of age and the second routine dose given at between four to six years of age (Kutty *et al*., 2013).

In most states, live attenuated measles virus vaccine is available as a monovalent or polyvalent vaccine in combination with mumps and rubella (MMR) or with additional varicella as MMRV. Some developed countries like the United States don't use the monovalent measles vaccine (Kutty *et al.*, 2013). Protection in vulnerable children for example; malnourished, or those with chronic immunosuppressive conditions like cancers and HIV infections is very important. Such cases, vaccination is an indication rather than contraindication, and, it is given at 6 months of age and again at the normal immunisation time as per schedule (Chandwani *et al*., 2011; Embree *et al.*, 1992).

Vaccination is also recommended for prevention of measles among adults at high risk of infection. Two doses are recommended for adult health professionals born after 1957 and international travellers (CDC, 2013).

2.4.4 Post Exposure Prophylaxis

Measles being a viral disease has no known treatment. Live attenuated measles vaccine offers lifelong immunity and may protect against disease when given within 3 days of exposure (CDC, 2015). If given within 6 days post exposure, Immune globulin (IG) can prevent or at least modify disease and offer brief shield. Immunoglobulin is given intramuscularly at a dose of 0.25 µL/kg body weight, with a maximum of 15 µL. In the case of immunocompromised persons, the suggested dose is 0.5µL/kg of body weight (up to 15 µL) intramuscularly (Arciuolo *et al.*, 2017). For the case of intravenous use, the suggested dose of immuneglobulin is normally 400mg/kg.

In the case of vulnerable household contacts especially when such contacts are less than one year old, when the risk of complications is increased, Immune globulin has been insisted. Moreover, in the case of children from 6 months to 11 months old, a measles containing vaccine (McV) is usually given in place of IG as long as it can be done within 72 hours post exposure (Orenstein *et al.*, 2004; Perry *et al.*, 2013). If IG is used, the attenuated vaccine must be given to 1 year or older children to give an allowance of at least 5 months for the clearance of passive measles antibodies. Immune globulin therapy has never been indicated for the control of measles outbreaks (Barskey *et al*., 2010; CDC, 2015).

Generally, all household contacts should receive post exposure prophylaxis without necessarily waiting for presumptive evidence of immunity. This need be extended to other high priority groups such as close contacts other than family, health care settings, and other care centres to minimise the transmission potential. In all circumstances, persons who may not show any convincing indication of having measles immunity ought to be included for post exposure prophylaxis (PEP) or be taken out of the outbreak setting (WHO, 2009b, 2014).

2.5 The Measles Vaccine

Measles vaccines are prepared from killed or live wild type measles virus that has undergone continuous propagation in non-familiar conditions rendering them avirulent without necessarily losing their immunogenicity. Measles virus was originally isolated in mid 1954 by John Enders (CDC, 2015; Strebel *et al.*, 2011). Tissue culture virus isolates from throat swabs and blood samples taken from a student (Edmonston D.) who was suffering from measles infection in 1963 was used to make the first measles vaccine. Following this breakthrough, the United States licensed the use of inactivated and live attenuated vaccines (Edmonston B strain) in the same year (CDC, 2015; McLean *et al.*, 1970). Owing to its inability to protect against measles disease development, the killed vaccine was later removed from circulation in 1967 (McLean *et al.*, 1970; Schmidt, 1965).

The original Edmonston B vaccine was later to be removed in 1975 due to questionable safety threshold as recipients invariably developed fever and rash, hence had to be used in conjunction with immune globulin(C. Martins *et al*., 2013; WHO, 2009b).

The reactogenic nature of the Edmonston B vaccine led to search for new vaccine strains in mid 60's. This was achieved through extended passaging of the original strain in cultures using human diploid cells, sheep kidney, chick embryos, chick fibroblasts and sometimes dog kidney (Bankamp *et al.*, 2011). New vaccines such as Schwarz, Rubeovax, Edmonston Zagreb, Moraten, and AIK-C were generated this way.

Elsewhere, 4 genotypically separate wild type isolates (Leningrad 16, CAM-70, Changchun-47, and Shanghai-19) were distinctly developed in much similar way to generate other more safer vaccines (Bankamp *et al.*, 2011; CDC, 2012a; Duraisamy *et al.*, 2012). In 1965 Schwarz vaccine strain, a further attenuated vaccine, was introduced but was not used for long. In 1968 Edmonston- Enders vaccine strain which is also a further attenuated strain was licensed (CDC, 2012a). These new vaccine strains caused less reactions than the former Edmonston B vaccine and needed no simultaneous administration of immune globulin (Druelle *et al.*, 2008; Bankamp *et al.*, 2011).

2.5.1The vaccine strains

The Edmonston strain of measles virus is the major source of live, attenuated measles vaccines. The virus strain, isolated from a sick child, was originally isolated in 1954 in a primary cell culture using Human and monkey kidney tissues by two scientists, John Enders and Peebles (CDC, 2012a; Uzicanin & Zimmerman, 2011; WHO, 2009b). Most strains including; Moraten, Schwarz, Edmonston–Zagreb and AIK-C were derived from the original Edmonston isolates. These vaccine strains have been in use since early 1960s to date with minor modifications being introduced where need arose but maintaining their original antigenicity. Nucleotide sequence comparisons on selected genes of the existing Edmonston derived strains has revealed negligible variations (Penedos *et al*., 2015; Tahara *et al*., 2013; WHO, 2009b).

Whereas Edmonston derived strains have shown little genetic variations, Non-Edmonston derived strains, including TD-97 and Leningrad-16 strains from Russia, CAM-70 strain from Japan, and Shanghai-191 strain from China, demonstrate high nucleotide disparities (Uzicanin & Zimmerman, 2011). Despite documented sequence divergence, internationally available measles vaccines have been proved to equally protect against the current available wild genotypes. Attenuation derived mutated H gene has reduced affinity to interact with inhibitory complement receptor (CD46), an important step for attachment. Furthermore, attenuation enhances interferon activation of the vaccine virus compared to the wild type, thereby increasing chances of virus destruction in the body. This makes it extremely difficult for Measles vaccine strains to attack new victims, in fact such transmission has never been reported yet (WHO, 2009a; Holzmann *et al.,* 2016; Tahara *et al.*, 2016).

2.5.2 Evolution of measles vaccines

Most current measles vaccines evolve from the initial Edmonston strain, a handful are from other wild Measles virus strains. Initially, both inactivated and live measles vaccines were produced, these were later in 1963 authorised for use in the United States of America (USA) (CDC, 2012a). In 1967, inactivated vaccines were withdrawn because they couldn’t impart significant immunity and patients developed atypical measles after encounter with the wild Measles virus (CDC, 2015; McLean *et al.*, 1970;

WHO, 2009b).Although the Edmonston B vaccine (Rubeovax) produced exceptional seroconversion, it generated fever in almost 50% of the vaccine recipients. The vaccine was later withdrawn from use in 1975 because of excessive fever and rash in recipients (CDC, 2015).

The Schwarz vaccine strain, first produced in 1965 through further passaging of the Edmonston strain in chick embryo, is currently produced and used in Brazil and parts of Europe (C. Martins *et al*., 2013). Moraten vaccine strain, later changed to Edmonston –Enders, was developed by supplementary passaging of the Edmonston-B virus strain in chick fibroblasts in 1968.

Separately, another isolate developed in 1957 in St Petersburg in the federal government of Russia, was passaged to develop the Leningrad-4 vaccine strain. Like the previous vaccines, this also proved to have been insufficiently attenuated and was passaged further in China to produce the current Chinese vaccine strain Changchun-47 (Stanley Plotkin, Walter Orenstein, 2013). Another vaccine strain, Shanghai-191, was arrived at through a series of passages in avian and human fibroblast cell lines of an isolate obtained in Shanghai in 1960 (WHO, 2009b; Uzicanin and Zimmerman, 2011).

In Japan, a wild isolate, Tanabe, was serially passaged in chorioallantoic membrane and chick embryo fibroblasts to develop CAM-70. This vaccine strain is mainly in use in Indonesia and Japan (Bankamp *et al.*, 2011).

More improved vaccine strains currently in use include: Schwarz F88, derivative of Schwarz strain in Japan, Leningrad-16, derivative of Leningrad-4 in Russia and a Tanabe strain derivative (TD97) also from japan (Bankamp *et al*., 2011; WHO, 2009b; Zhang *et al.*, 2007).

2.5.3 Strategies in vaccination

Measles immunization is universally recommended for all children to whom no advice against its use was prescribed. Most reports indicate that the current internationally available live attenuated vaccines are safe. They have been proved to impart a long

lasting protection in healthy vaccinees, and that, can be changed within schedules (Menezes *et al.*, 2014; Durando *et al.*, 2016). As a standard, national immunization programmes aim at reaching all children with at least two doses of measles containing vaccine as a strategy.

In national settings whereby measles transmission is ongoing, due to high chances of mortality resulting from measles disease, the initial routine vaccination (McV 1) is done earlier at the age of 9 months. Here, for optimal protection during this extremely vulnerable period, timely delivery of the McV 1 should be emphasised (CDC, 2011; Cutts, 1993; WHO, 2009b, 2012a). In a population affected by high incidences of Measles and is concurrently affected by high prevalence of Human immunodeficiency Virus (HIV), the first dose is even given at a more earlier age of 6 months for enhanced defence (Chandwani *et al*., 2011; Fowlkes *et al*., 2016; W. Moss, 2015).

Where countries are reaching near elimination status, very low chances of measles transmission amongst children, McV1 administration at 12 to 15 months will serves well (Cutts *et al*., 2013; Menezes *et al*., 2014; Subaiya *et al*., 2015). In each case a booster dose, usually supplementary immunization activities (SIAs) is advised. It is stopped only when population immunity reaches more than 93% for three consecutive years, when routine two doses will be sufficient to maintain measles immunity (CDC, 2013; Perry *et al.*, 2014; Trentini *et al.*, 2017).

The additional use of a routine second dose of vaccine containing measles (McV 2) is aimed at further reducing the accumulation of susceptible children and increasing overall immunity. According to WHO estimates, countries that have attained more than 80% McV1 coverage in 3 consecutive years, can introduce McV2 and maintain regular SIAs as required (CDC, 2015; WHO, 2013a). SIAs will be stopped only when 90% – 95% immunization coverage for both routine doses has been maintained nationally for three consecutive years.

For success, optimum timing for routine McV2 is mandatory. Countries that have achieved more than 80% (WHO estimates) coverage with McV1 for three consecutive years with constant measles threats, the McV2 is administered at between 15 and 18 months of age. The minimum accepted gap between routine McV1 and McV2 should

be one month (CDC, 2013, 2015; WHO, 2009c, 2013a). In low transmission zones, more than 90% McV1 coverage with good school enrolment (more than 90%), routine McV2 can be done at school entry to achieve high coverages. In special conditions, like high HIV prevalent zones, a dose at 6 months is required in addition to the other routine doses (Chandwani *et al*., 2011; W. Moss, 2015; WHO, 2009c).

Previously, at the beginning of the Expanded Programme on Immunization (EPI), a single McV dose was deemed sufficient enough. However, the realization that primary vaccination failure occurs in up to 15% of vaccine recipients at the age 9 months, the approach has proved ineffective in preventing measles outbreaks (Hall & Jolley, 2011; Li *et al.*, 2013; WHO, 2009b).As of 2008, a strategy to deliver two doses of McV was all but one WHO member state. In these, 132 member states used a routine two-dose programme with 49 conducting consistent SIAs. Among them, 44 relied only on provision of the two routine doses. Another 60 member states, Kenya among them, used a routine first dose in addition to regular SIAs (CDC, 2012b; Kamau *et al.*, 2007; WHO, 2009c).

2.5.4 Immune response to measles vaccine

Measles vaccines are known to elicit immune response, humoral and cellular, similar to that following natural infection albeit with a comparable lower antibody titre. At 12 months of age vaccination can lead to antibody production in about 99% of children, this is reduced to approximately 89% of children when done at the age 8 to 9 months. Immunological response are comparatively lowered for children vaccinated at an early age mostly because of existing passive antibody in recipients. Besides induction of IgM, IgG and mucosal IgA, chief humoral immune agents, immunization induce measles specific CD8 and CD4 positive thymus derived (T) cells as well (Naim, 2015; Uzicanin & Zimmerman, 2011).There is no variation in immunogenicity between the single-antigen measles vaccine and the multivalence vaccine (Durando *et al*., 2016; Menezes *et al*., 2014).

Up to 5% of children administered with a single dose of measles vaccine will fail to respond to it in the widely known primary vaccine failure (Menezes *et al*., 2014; WHO, 2014). Measles vaccine failure usually occur due to existence of passive anti measles

antibodies in the vaccine recipient, poor cold chains, expired vaccine, incorrect records, malnutrition especially vitamin A deficiency or severe immunosuppression amongst other reasons (Sugerman *et al.,* 2010; Mercader*et ak.,* 2012; Rosen *et al.,* 2014). Nonetheless, majority of recipients who responds poorly to the first measles vaccine dose responds well to the second dose (Tavajohi *et al.,* 2005; WHO, 2009a; Chandwani *et al.,* 2011).

Studies have shown that serologic evidence of measles protection is seen in more than 99% of two dose vaccine recipients when the first dose was administered at the first birthday or later (Griffin, 2016; Menezes *et al.*, 2014). Despite the fact that vaccine-induced immune antibody titre is lower compared to that following natural disease, serologic and epidemiologic evidence show that immunity achieved is long lasting with a high possibility of being lifelong in most individuals (Roberts, 2015; WHO, 2009a). Although previously vaccinated persons may appear to have lost antibodies, they usually show a quick response after revaccination, a good indication that they are still immune (Rosen *et al*., 2014).

Whereas the general trend is an increase in antibody titre following subsequent vaccination, occasionally, such increased titres may not be sustained. Studies indicate that secondary vaccine failure, waning immunity, may occur after successful vaccination, but this appears to occur rarely and to play only a minor role in measles transmission and outbreaks (Hickman *et al.,* 2011).

Though attenuation disables the virus pathologically, the virus receptors are largely preserved hence vaccine strains retain the ability to infect and successfully replicate in the host without necessarily causing the wild type associated symptoms. Replication exposes the vaccine strain to the immune system sufficiently enough to induce considerable long immunity comparable to the wild strain. Given in the correct dose, and at the optimal age, measles vaccine is highly immunogenic (Chandwani *et al*., 2011; Menezes *et al*., 2014).

Studies have shown that vaccine induced protection can last longer than 20 years (Griffin, 2016). Although, a considerable drop in immune antibody levels is expected, re exposure of patients to measlesvirus usually stimulates a rapid response that

generates sufficient antibody pool by day 10 in a majority of population (Griffin, 2016). Normally, reinfection with wild type virus only boosts the antibody levels but occasionally, symptomatic measles may occur due to secondary vaccine failure (Naim, 2015).

Despite waning in immunity over time, protection from infection is generally pegged on the ability of the body to rapidly re-establish humoral and cellular immune responses.(Naim, 2015; W H O, 2009)

2.5.5 Viability and potency

Of concern in vaccine application is its quality, this necessitate the testing of each individual vaccine batch as a requirement towards insurance of safety and effectiveness before its use (WHO, 2009). Due to this, the potency test is geared towards assessing the ability of a vaccine to protect against subsequent challenges from the active constituent of the pathogen accounting for disease production (Smith, 2010).

It looks at vaccines capacity to withstand environmental changes, like temperature changes, for long hours with minimal loss to its effectiveness. Such measures represent an indispensable tool in measuring the factual relative strength of artificial vaccines (WHO, 2001). Vaccine efficacy testing offers an essential tool for insurance of the reliability of commercial vaccines taking into account that all biological-based manufacturing approaches are fundamentally variable (Smith, 2010). Vaccine potency tests are however cumbersome and relatively expensive hence resource-poor nations may overlook this leaving the manufacturer the task of supplying quality vaccine.

2.6 Population immunity

The long-lasting, arguably lifelong, protection against measles disease following natural measles virus infection owes its existence to excellent memory. Thanks to the continued low-level production of measles virus-specific antibodies as well as the persisting circulation of measles virus-specific CD4+ and CD8+ T lymphocytes (CDC, 2015; Fulton *et al.*, 2015). To eliminate measles, overall population susceptibility must

be kept below 5% and ideally even lower within confined settings such as schools(Antona *et al*., 2013; De Serres *et al*., 2012)

Although, like any RNA virus, measles virus undergoes error-prone replication, it has largely remained monotypic – a factor contributing to the long-lived immunity. Neutralizing antibodies for measles virus mainly targets the Haemagglutinin (H) and Fusion (F) glycoproteins, these are thought to occupy a very little evolutionary space thereby resisting changes in the face of persistent selective pressures (Fulton *et al*., 2015). Despite the existence of 24 distinctive genotypes, the vaccine strain although prepared from genotype A, confers protective immunity against all known wild genotypes (Beaty & Lee, 2016). This implies that the population herd immunity, protection in 95% and above of the population in all age cohorts, is achievable.

Challenges involved in achieving population herd immunity include the existence of intentionally unvaccinated pockets, biologically challenged vaccinees, and under vaccination because of geographical constraints (Antona *et al.*, 2013; Barskey *et al*., 2010; W. Moss, 2015).

2.7 Recommended Herd Immunity for Measles

Owing to its high transmissibility nature (CDC, 2015; Holzmann *et al.*, 2016), measles virus is among the most feared infection causing viral agents that is vaccine preventable (CDC, 2015; Cutts *et al*., 2013; Trentini *et al*., 2017). The fact that it's mostly respiratory based, at least in its early entry stages, it is easily transmitted in aerosolized form making it a very successful agent. This coupled with the highly immunogenic surface receptors which have not been shown to vary, add to its attachment prowess on cell surfaces of the respiratory membranes (Tahara *et al.*, 2016; Gonçalves-Carneiro *et al.*, 2017). This transmissibility nature of measles virus always allow immunity gaps to be exposed in cases of vaccination coverage inadequacy (Holzmann *et al.*, 2016).

The herd immunity, a threshold level of immunity above which the disease causing agent no longer spreads, is a hypothetical level of immunity within the community derived from calculations based on the basic reproduction number of that particular

disease causing agent (Jacobson *et al.,* 2015; Guerra *et al.,* 2017). Owing to its high reproduction number, number of people infected by a single case in partly immune community, which has been variably reported as between 12-18, measles virus is an excellent transmissible agent currently holding the tag of the most infectious agent (Holzmann*et al.,* 2016; Guerra *et al.*, 2017).

In recognisance of the high reproduction number in measles virus infections, a high level of protective immunity is a must within the community. In this regard the recommended herd immunity for measles virus ranges between 93% – 95% as suggested by several researches (Holzmann *et al.,* 2016; Guerra *et al.,* 2017; Manakongtreecheep and Davis, 2017).

2.8 Progress of Measles control in Kenya

In the year 2000, African countries represented by the WHO established a goal to reduce the 506,000 deaths from measles estimated in 1999 to 50% by the end of 2005 (CDC, 2007). These strategies included the strengthening of routine vaccination, supplemental immunization activities (SIAs), monitoring disease trends, and improving measles case management (WHO, 2001).

In Kenya, through the then Kenya Expanded Programme on Immunization (KEPI), the Ministry of Health implementation of these strategies began in 2002. This reduced the number of reported measles cases by about 99%, from 11,304 in 2001 to 20 in 2004 (Kamau *et al*., 2007). KEPI was established 1980, with the goal of immunizing all children in the country against six vaccine-preventable diseases among them measles. Single dose vaccine coverage increased in the early 1990s to an uptake of about 84% but later decreased to 72% in 2002 (CDC, 2007). Currently, the Division of Vaccines and Immunisation (DVI) does what KEPI used to do in the Ministry of Health.

One of the 2002 goals was to achieve and maintain national average measles vaccination coverage of 90%, with every district expected to attain coverage of above 85%. Since then, reported national measles vaccination coverage increased to 77% in 2006 and the proportion of districts with coverage of above 85% increased from 8 districts in 2002 to 27 districts in 2006 (Kamau *et al*., 2007).

In contrast, during the period 2004-2005, more than majority of suspected cases were reported with a blood specimen, and the proportion of districts reporting at least one suspected measles case increased from 69% in 2004 to 99% in 2005 indicating high level of measles incidence reporting (WHO, 2005). This was followed by a more pronounced outbreak of measles from September 2005-May 2007. During this outbreak, a total of 2,544 confirmed measles cases were reported from 71 (91%) of the 78 districts, with peak monthly totals of 375 and 332 confirmed cases reported in the months of April and August 2006, respectively (Kamau *et al*., 2007; Wolfson *et al*., 2007).

Majority of the 2,544 confirmed cases in this period 944 (37.1%) were aged between 9 and 59 months, 491 were persons aged between 5 and 14 years, and 658 were aged above 15 years. Of this, 466 (18%) patients had history of vaccination including 220 (23%) of children aged 5-59 months and 95 of those aged 5-9years. Among those who died, 24 in total, 9 (38%) of them had history of vaccination.

As a counter measure to the outbreak, massive immunization program was launched in April 2006 that saw 670,016 children between the age 9 and 59 months receive measles vaccine, which was 120% of the 558,699 children targeted. By July 2006, 4,590,225 children had received measles vaccine, which was 110% of the 4,180,330 targeted. In 2010, McV1 WHO estimates was recorded at 86% and 2011 raised to 87%, however, number of districts reporting coverage of 80% and more dropped from 66% to 65%. Moreover measles incidences increased from 3 cases per million in 2010 to 59 cases per million in 2011. By April 2011, more than 1,000 cases of measles were reported in Nairobi, North Eastern and Rift Valley districts with 80 cases and 11 deaths confirmed (CDC, 2012b).

As late as October 2012, serious measles outbreaks were reported in Kenya, with 45 out of 47 counties reporting cases. Thirty two (32) children succumbed to complications of new measles outbreak in the country with in a period of nine months (DVI, 2012; Wanjiku & Adetifa, 2018). The first cases were reported in North-Eastern and then in Eastern province but it spread to 217 districts out of a total of 285 districts (Measles outbreak in Kenya, 2012). During this period a total of 3,056 suspected cases

were recorded although only 767 were confirmed. Whereas the public health department estimated 85% coverage, during that vaccination campaign an independent monitoring unit confirmed coverage of 74% (Measles outbreak in Kenya, 2012).

In line with the WHO-African region resolution of 2012, Kenya adopted the goal of measles elimination by 2020 (Ministry of Health, 2013). Owing to this, routine measles vaccine second dose was introduced in 2013. These two routine doses of measles vaccine are coupled with catch-up programmes, supplemental immunisation activities (SIA) in between (Government of Kenya: Ministry of Health (GoK:DVI), 2013). In addition to the first routine monovalent measles dose given at 9 months of age, a second opportunity dose, either a combination of Measles, Mumps and Rubella (MMR) vaccine or a monovalent measles vaccine, is given at 18 months of age. This is in tandem with the WHO policy guidelines (WHO, 2009b, 2012a, 2013b). However, data shows that routine second dose was still at 28% by 2015 (Masresh *et al.*, 2017).

The polyvalent vaccine, MMR, is recommended for the second opportunity because of its advantage of benefiting the community with Mumps and Rubella which have a low prevalence in children less than 1 year. It is also aimed at maximising the rate of sero conversion among the vaccinees which is raised to about 98% – 99% in children vaccinated on or after their 15 month of age (Chandwani *et al.*, 2011; Mercader *et al.*, 2012; Papania *et al.*, 2014; Naim, 2015).

In the event of a measles outbreak, the Ministry of Health recommends that the age of the primary dose of the monovalent measles vaccine be lowered to 6 months. Despite this, children are still required to return for the routine monovalent vaccine dose at 9 months of age. In the same note, children born to HIV positive mothers or HIV positive infants should receive the monovalent measles vaccine at 6 months of age and the routine dose at 9 months (GoK:DVI, 2014). Documented studies have shown beyond doubt that early vaccination is still immunogenic even in HIV-infected subjects with a good seroconversion rate when the first dose is given at 6 months of age (Chandwani *et al*., 2011; Kizito *et al*., 2013; Siberry *et al*., 2015).

Moreover, the Ministry recommends that situations characterised by mass movements of displaced people within or through our national boundaries requires that all children

aged between 6 months and 12 years be vaccinated regardless of previous vaccination status (GoK:DVI, 2014). Although it is the prerogative mandate of the Ministry of Health to determine the target age groups during mass vaccination exercise, this is also highly monitored and supported by WHO (WHO, 2009b; Masresha *et al.,* 2015; Subaiya *et al.*, 2015).

A single dose of 0.5 ml is given as a deep subcutaneous injection, of the monovalent vaccine, over the deltoid muscle of the left upper arm. Under normal circumstances, this standard dosage delivers at least 1000 $TCID_{50}$ of live measles virus (Durando *et al.*, 2016; Naim, 2015).

Despite this great policy, there is no clear formulation on how to deal with the few pockets of groups not willing to be vaccinated. Evidence indicate that such intentionally unvaccinated groups is a worldwide problem affecting even the highest developed nations (Antona *et al.*, 2013; Barskey *et al.*, 2010; Filia *et al*., 2013). In the same context, some communities in Kenya especially those with a pastoral lifestyle like The Maasai occupying most parts of Narok have very poor health seeking behavior. Majority, up to 80%, of their women prefer local herbs and traditional consultants more than hospital services and, seldomly visit hospitals and usually in emergencies (Christian Aid, 2013).

Moreover, challenges arising from within the immunization programme in Kenya has led to poor performance in vaccination coverage and hinders making of refined conclusive surveillance reports (DVI, 2013). Hurdles include difficulties in accessing immunization services due to distant health facilities especially among the nomadic communities along the rift valley region. The general poor health seeking behaviour of caregivers emanating from socio-cultural issues is compounded by the rather poor geographical infrastructure and underdeveloped road network in Kenya (DVI, 2013), and elsewhere (Ibrahim *et al.*, 2010; Metcalf *et al*., 2015). Among the management, low quality support supervision that is not evidence-driven has been mentioned as part of the problem (DVI, 2013). Negative attitude, limited knowledge and capacity of health workers has been attributed to missed opportunities in Kenya (DVI, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was undertaken in three different counties of Kenya; Kwale, Narok, and Lamu Counties. In each County, a hospital was chosen and recruitment of participants was done at the mother-child welfare clinic of that specific hospital. These were chosen according to the 2012 measles outbreak data from the Ministry of Health (MoH). Narok was leading in measles cases, Kwale was moderately affected while Lamu was the least affected in Kenya. Laboratory analysis was conducted at Kenya Medical Research Laboratories (KEMRI) headquarters in the Polio – Measles Viruses department of the Centre for Virus Research Nairobi.

3.1.1 Msambweni District Hospital in Kwale County

The study was conducted partly at Msambweni Hospital in Kwale County. Kwale County is located in the southern part of coastal Kenya. To the East is the Indian Ocean beach and borders Tanzania from the south and southwestern regions. It forms an important portal of entry and exit from Tanzania and the larger southern Africa region. The Digo and Duruma are the dominant inhabitants. Minority groups include mostly the Kamba people and, Kikuyu, Meru, Kissii, Luo, Somali, Makonde among others. Main economic activities include crop, livestock and fish production (Kwale County, 2013).

In the previous outbreaks, as by 25th September 2012, Kwale County had 28 suspected measles cases of which 24 were laboratory confirmed (IgM positive). The county benefited from a measles immunisation programme supplemented with vitamin A given to children aged between 9 and 59 months. This Campaign was conducted between 3rd and 7th November 2012 and covered approximately 73.9% of the target population (DVI, 2013).

Being along the Likoni – Lungalunga highway, the hospital is the most accessible and thus serves a larger population than the rest.

3.1.2 Narok District Hospital in Narok County

A part of the study participants was drawn from Narok County. A region found within the Great Rift Valley and is made up of 3 constituencies; Narok North, Kilgoris and Narok South. Elevated to 1827 metres above the sea level, the county experiences temperatures of up to 28^{o} C and rainfall ranging from 500 to 1800 mm per year. Agriculture is the main economic activity with both crop production and livestock farming. The Maasai people are the predominant inhabitants of Narok occupying Narok North and Narok south. Minority groups in the county include the Kalenjins and Kisii people which occupy mainly Transmara (Christian Aid, 2013). The kikuyu, another minority community occupy mostly the urban zones. The Maasai practice a pastoral lifestyle with a very poor health seeking behavior. As by 2012 up to 80% of women preferred herbs more than hospital visits and used hospitals in mostly emergency cases (Christian Aid, 2013).

During the recent outbreaks (September 2012), Narok county had 441 suspected measles cases of which 45 were laboratory confirmed (IgM positive) and 324 patients were linked to positive cases with only 13 laboratory confirmed negative cases (WHO, 2012b). The county benefited from supplemental measles immunisation programme (mop-up programme) with vitamin A given to children aged between 9 and 59 months. This Campaign was conducted between 17th and 21st September 2012 then repeated between 3rd and 7th November 2012 during the countrywide SIA campaign (DVI, 2012, 2013).

Narok district Hospital serves mostly people from Narok North although it houses the county heads of the different health departments. Patients from Kilgoris and Narok South districts also do attend this hospital in considerable large numbers.

3.1.3 Lamu District Hospital in Lamu County

The last part of the study drew its participants from Lamu County. Lamu County is located in the northern coast of Kenya bordering Indian Ocean to the East, Garissa County to the West and Tana River County to the South. Fish production characterised by large scale and small scale fishing is the main economic activity. Livestock and

crop farming also practiced here (Republic of Kenya, 2015; Veldpaus, 2012). Lamu has four main tribes; Bajuni, Orma, Sanye, and Aweer (Boni). The Bajuni, owing their ancestry to Bantu and Arab are the largest in population and are mainly fishermen and farmers, and recently have ventured in to tourism and related activities. The Sanye and Aweer are Cushitic in origin and are mainly hunters and farmers primarily living along Boni forest. The Orma are mainly pastoralists. Other minority communities include Kikuyu, Kambas, and Luyha (Veldpaus, 2012).

During the 2011/2012 measles outbreaks Lamu County was one of the five counties in Kenya not to record a confirmed measles case, and the only county to record a single suspected case, the lowest in Kenya (WHO, 2012b). Nevertheless, the county benefited from a measles mop-up immunisation programme supplemented with vitamin A given to children aged between 9 and 59 months. This campaign was conducted between 3rd and 7th November 2012 and covered approximately 92.0% of the target population (DVI, 2012, 2013). Lamu District Hospital (King Fahd Hospital) is found in the island and serves the whole island in addition to a considerable proportion of the mainland population, which is mainly served by Mpeketoni Hospital.

Figure 3.1: Map showing geographical location of the study sites in Kenya.
Source; https://www.shutterstock.com/search/kenya+counties+map

3.5 Study Design

This was a cross-sectional hospital-based descriptive study done in the period between July and December 2014.

3.6 Study Population

The study targeted outpatient children of both sexes aged between 9 and 59 months attending the selected health facilities. This was the target group during the previous immunization programme from the department of Vaccines and Immunisation data in the ministry of Health.

3.6.1 The Inclusion Criteria

- All children aged between 9 and 59 months
- Children attending Maternal child health clinic MCH
- Parental/guardian consent to participate in the study

3.6.2 The Exclusion Criteria

- All in-patient children

3.7 Sample Size Determination

Manirakiza and others in 2011, in their work 'Seroprevalence of measles and natural rubella antibodies among children in Bangui, Central African Republic', found out that the prevalence of IgG-specific measles antibodies among children aged between 9 months and 5 years was 57.3% (Manirakiza *et al.*, 2011).

Using Fishers method, thus, the following population size was arrived at:

$$n = \frac{Z^2P(1-P)}{\delta^2}$$

Where Z score is 1.96 at 95% confidence interval (CI), P is past prevalence documented (57.3%), q is (1 - P), and δ is the error at 95% CI which is 0.05.

= [1.96 x 1.96 x 0.573 x (1 – 0.573)]/0.0025

= 375.97

= 376 participants

In the Department of Vaccines and Immunisation (DVI) of the Ministry of Health (MoH) in Kenya, statistics data records of 2012, Kwale, Lamu and Narok Counties had 126,194, 17,129 and 161,921 children less than 5 years of age respectively. Accordingly, participants from the Counties were drawn in the ratio 12.6:1.7:16.2 (Kwale:Lamu:Narok), which approximately gave us 155 participants in Kwale, 22 participants in Lamu and 199 participants in Narok.

3.8 Sampling Method

Proportionate based sampling was conducted to determine the appropriate number of participants in each County. Consecutive sampling technique was then employed to sect participants in each site. Participants were selected on a first come first served basis as long as they met the inclusion criteria. This was done in all study sites until the desired sample size for that site was attained.

Upon satisfying the inclusion criteria, the guardian of the child was taken through an informed consenting form (Appendix II). The Guardian was then taken through a structured demographic and vaccination history questionnaire (Appendix I)

3.9 Blood collection and handling procedures

Whole blood was collected from each child by finger prick (or heel prick for younger children). Each participant's finger or heel was disinfected and then pricked with a sterile, single-use micro lancet. Up to four drops of whole blood was each collected on a separate circle of a four circled standardized filter paper (Whatman S&S No. 903) – commonly known as dried blood spot (DBS) collecting card. The card was labelled

with the child's specimen identification number, age, sex and the collection date. The blood spots were allowed to air dry for 60 minutes. Each dried filter paper was individually placed into a glycine envelop, put in sealable plastic bags to prevent possible cross contamination and to protect from dust and moisture in readiness for transportation (Appendix III).

3.10 Sample Transportation

Well labelled, sample charged, dry DBS cards were packed individually in glycine paper envelops. Sheets of glycine papers were then wrapped in filter papers which in turn were stacked into sealable plastic bag, containing desiccant packets, in such a way as to avoid shuffling of cards and expel moisture (Appendix III). This was meant to prevent dampening the DBS cards and maintain integrity of samples. Labelled bags were then transported in well-sealed boxes to KEMRI-Mbagathi laboratories for analysis.

3.11 Laboratory Procedures

3.11.1 Initial sample preparation

Dried blood was removed from the filter by cutting circles through tracing of pre marked partially cut dotted lines along the circumference of each circle. The cut was placed in a sterile 10 by 75 mm test tube containing 0.5 ml of phosphate buffered saline (PBS). Specimen disks were soaked for 1 hour at ambient temperature. Filters were then removed and any remaining liquid squeezed from them using duckbill forceps against the sides of the test tube. Specimens processed from dried blood were considered to have a practical starting dilution of approximately 1:10.

3.11.2 Measles IgG antibody screening ELISA

Samples were analysed using ELISA kit from Novatec Immuno diagnostica GMBH (NOVALISA ^{TM}) and optical density measured according to manufacturer's instructions (Appendix IV). Optical density results were converted to Nova Tec units as per the manufacturer, multiplied by the dilution factor (10) and results categorised as positive, negative or equivocal accordingly.

According to the manufacturer, results that turned out positive in this kit were comparable to 220 IU/ml in the third international standard. Several studies suggest that a concentration of 200 IU/ml anti-Measles antibodies is sufficient to protect against measles disease, therefore, all positive results in this study were considered protective.

Specimens that were found to be equivocal for measles virus IgG were retested and those that remained equivocal were assessed by a neutralization assay.

3.11.3 Measles IgG neutralization assay

Viral antigen preparation

Vaccine strain of measles virus was sub-cultured to increase virus titre. This was done through culturing of virus suspension in vero cells, once cytopathic effects (CPE) appear, infected cells were frozen then thawed once and contents centrifuged.

Virus-containing supernatant was collected into vials and kept frozen at -70^0c. Virus titre was then determined by measuring the tissue culture infectious dose 50($TCID_{50}$). This was adjusted to obtain a virus suspension of approximately 1000 $TCID_{50}$ per millilitre (Appendix V).

Serum neutralization assay

Done on ELISA equivocal samples, 2 positive and 2 negative sera. Vero cells were grown in 12, 96 well (flat-bottomed) microtitre plates.

Sera heated at 56^0C for 30 minutes to inactivate non-specific inhibitors was double diluted serially from 1:10 to 1:1280. Volumes of 50 µl of each serum dilution was mixed with 50 µl of approximately 100 $TCID_{50}$ viral antigens and incubated at 37^0c for 1 hour.

Subsequently, 50 µl of serum-virus mixture of sample one was put in the first 2 rows and serum-virus mixture of sample two was put in the 3rd and 4th rows as test samples. 50 µl of tissue culture put in the 5th and 6th rows as negative controls, and 50 µl of viral

antigen suspension only put in the 7^{th} and 8^{th} rows as positive controls. This process was repeated for all the remaining samples.

Preparations were then incubated at 37^0c in presence of 5% carbon dioxide (CO_2) until the time when the positive control was showing CPE. Residual infectivity as indicated by the observation of CPE in the test specimen was used to show the presence of non-neutralizing antibody. The highest titre inhibiting CPEs was used as the neutralization titre (Appendix V). A neutralization test (NT) titre of 1:120 and above was considered protective in this study.

3.12 Data Management

Data was collected in structured demographic questionnaire that captured sex, age, county of residence, guardian's relation to child, vaccination status of child, awareness about vaccines etc. (Appendix I). Data was then fed into computer spreadsheets, cleaned and verified for consistency. Information was only accessible to persons involved in the study.

3.13 Data Analysis and Presentation

Demographic questionnaire results were categorised per risk factor attribute with each attribute making up a variable. Results from serological survey was either positive, negative or equivocal per person. Samples that remained equivocal after ELISA retesting were subjected to Plaque reduction neutralization test (PRNT) to ascertain their positivity. Serological results were categorised and compared to international standards and findings interpreted to indicate the level of protection of each patient. Immunological data was linked to the vaccination records and compared to demographic and risk factors using chi-square and student t-test. Proportions were expressed as percentages and their confidence intervals, odds ratios and P-values determined. A p-value of less than 0.05 was considered statistically significant.

3.14 Information Dissemination

Research results on measles immunity status were communicated back to the participants through their respective hospital administrations. Explanations were done by a paediatrician in a confidential manner.

3.15 Quality Control Measures

All processes involved in the study strictly followed the specific laid down standard operating procedure. All reagent preparations and utilization were done in accordance to the manufacturer's instructions.

Positive and Negative controls were run concurrently with test samples in both ELISA and Neutralization tests.

All machine used were first conditioned using specific provided standardized calibrators. Morning and evening temperature and carbon dioxide concentration charts were closely monitored before and during incubations.

3.16 Limitations of the Study

Effects of confounding factors in vaccine administration processes were not assessed. These include Human Immune Deficiency (HIV) status, cancers, malnutrition, cold chain effects, individual personnel factors and the technique of vaccine administration.

3.17 Ethical Considerations

The study commenced after ethical approval was sought and granted by the University of Nairobi/Kenyatta National Hospital ethical review committee research number P400/2013 (Appendix VIII). All participants were informed about the objectives and purposes of the study, procedures and possible sources of discomfort by being taken through the informed consent before giving their consent (Appendix II).

Participants did not meet any expenses of the study and were granted freedom to decline to take part in the study.

To maintain confidentiality samples were assigned laboratory numbers from which vaccination status, residence, and other demographics were detailed.

CHAPTER FOUR

RESULT

4.1 The demographic characteristics and vaccination history of study participants

A total of 453 children were drawn into the study. Out of 453 participants, 210 (45.85%) were from Narok, 185 (41.27%) from Kwale and 58 (12.88%) from Lamu County. Of this, 220(48.68%) were female, while 233 (51.31%) were male. Out of the 220 females, 103 (46.8%) were from Narok, 91(41.4%) from Kwale, while 26(11.8%) were from Lamu. Among the 233 males, 107 were from Narok, 94 from Kwale and 32 were from Lamu.

The demographic characteristics of the study participants was tabulated to give an overview of participant's healthy behaviour within the three Counties (Table 4.1). While there was no significant difference in sex ($P = 0.8$), there was significant variation in age ($P < 0.00$) and guardian accompanying the children ($P < 0.001$).

The information relating to vaccination history and awareness status of guardian is presented in Table 4.2 below. This describes the general participants' attitude towards matters of public healthy importance. The table shows that there is significant difference between participants who are aware about measles and those who are not ($P = 0.03$), as well as those vaccinated ($P < 0.001$) and card presentation ($P < 0.001$).

Table 4.3 is describing the proportions of vaccination accompanied with the 95% confidence intervals for the same across the different age groups.

The proportions of participants that were not vaccinated, those vaccinated once, twice and thrice, and their 95% confidence intervals was presented in the form of bar graph (Figure 4.1) to depict the general status of vaccination in the study group and give an inference on the general population through confidence interval. This showed strong variations in vaccination status among the participants ($P < 0.001$).

Analysis of vaccination status among study participants was done for the various demographic and vaccination history groups to gauge the likelihood of a participant being vaccinated given the underlying characteristics (Appendix VI).

Table 4.1: Demographic characteristics of the study participants.

Category		Kwale		Lamu		Narok		Totals		95% CI limits		P value
		N	%	N	%	N	%	N	%	lower	Upper	
Sex												
	Male	94	50.8	32	55.2	107	51	233	51.4	46.7%	56.1%	0.830
	Female	91	49.2	26	44.8	103	49	220	48.6	44.1%	53.0%	
Age_group in months												
	≤12	27	14.6	3	5.2	14	6.7	44	9.7	8.9%	10.6%	0.000
	12_24	65	35.1	12	20.7	60	28.6	137	30.2	27.5%	33.0%	
	24-36	46	24.9	12	20.7	71	33.8	129	28.5	25.9%	31.1%	
	36-48	26	14.1	15	25.9	48	22.9	89	19.6	17.9%	21.4%	
	48- 60	21	11.4	16	27.6	17	8.1	54	11.9	10.9%	13.0%	
Residency												
	Permanent	175	94.6	47	81	183	87.1	405	89.4	86.6%	92.2%	0.050
	Moved in	10	5.4	11	19	27	12.9	48	10.6	7.8%	13.4%	
Duration of stay in months												
	<1	0	0	1	9.1	0	0	1	2.1	0.8%	3.4%	0.119
	1 - 6	5	50	3	27.3	5	18.5	13	27.1	23.0%	31.2%	
	7 - 12	2	20	0	0	7	25.9	9	18.2	14.7%	21.8%	
	>12	3	30	7	63.6	15	55.6	25	52.1	47.5%	56.7%	
Guardian												
	Parents	174	94.1	36	62.1	189	90	399	88.1	85.1%	91.8%	0.000
	Siblings	4	2.2	2	3.4	18	8.6	24	5.3	3.2%	7.4%	
	Uncle	4	2.2	1	1.7	2	1	7	1.5	0.4%	2.6%	
	others	3	1.6	19	32.8	1	0.5	23	5.1	3.1%	7.1%	

Table 4.2: Participants Vaccination and Awareness status.

Category	Kwale		Lamu		Narok		Totals		95% CI limits		P value
	N	%	N	%	N	%	N	%	lower	upper	
Vaccination card											
Yes	152	82.2	45	77.6	149	71	346	76.4	69.40%	83.40%	0.032
No	33	17.7	13	22.4	61	29.1	107	23.6	21.50%	25.70%	
Awareness about measles disease											
Yes	171	92.4	49	84.5	166	79	386	85.2	81.93%	88.47%	0.001
No	14	7.6	9	15.5	44	21	67	14.8	11.52%	18.07%	
Awareness about measles vaccine											
Yes	173	93.5	56	96.6	184	87.6	413	91.2	82.80%	99.60%	0.036
No	12	6.5	2	3.4	26	12.4	40	8.8	8.00%	9.60%	
Awareness about measles outbreaks											
Yes	11	5.9	30	51.7	12	5.7	53	11.3	8.38%	14.22%	0
No	174	94.1	28	48.3	198	94.3	400	88.7	85.78%	91.62%	
Source of information											
Health worker	105	60.7	46	82.1	129	70.1	280	67.8	61.30%	74.30%	0.008
Others	68	39.3	10	17.9	55	29.9	133	32.2	29.10%	35.30%	
Vaccination status											
Vaccinated	178	96.2	55	94.8	175	83.3	408	90.1	81.80%	98.40%	0
Not	3	1.6	2	3.4	6	2.9	11	2.4	2.20%	2.60%	
Unknown	4	2.2	1	1.7	29	13.8	34	7.5	6.90%	8.10%	
Vaccination times											
Once	167	90.2	36	62.1	151	71.9	354	86.8	78.40%	95.10%	0
Twice	11	6	14	24.1	24	11.4	49	12	10.90%	13.10%	
Thrice	0	0	5	5.2	0	0	5	1.2	1.20%	1.30%	
Latest time vaccinated											
<1 month	12	6.7	25	45.5	2	1.1	39	9.6	6.90%	12.30%	0
Btwn 1&6 months	41	23	4	7.3	22	12.6	67	16.4	13.00%	19.80%	
Btwn 7&12 months	25	14	8	14.5	36	20.6	69	16.9	13.50%	20.40%	
> 12 months	100	56.2	18	32.7	115	65.7	233	57.1	52.50%	61.70%	
History of Measles infection											
Yes	4	2.2	3	5.2	6	2.9	13	2.9	1.35%	4.45%	0
No	178	96.2	55	94.8	153	72.9	386	85.2	81.93%	88.47	
Unknown	3	1.6	0	0	51	24.3	54	11.9	8.92%	14.88	

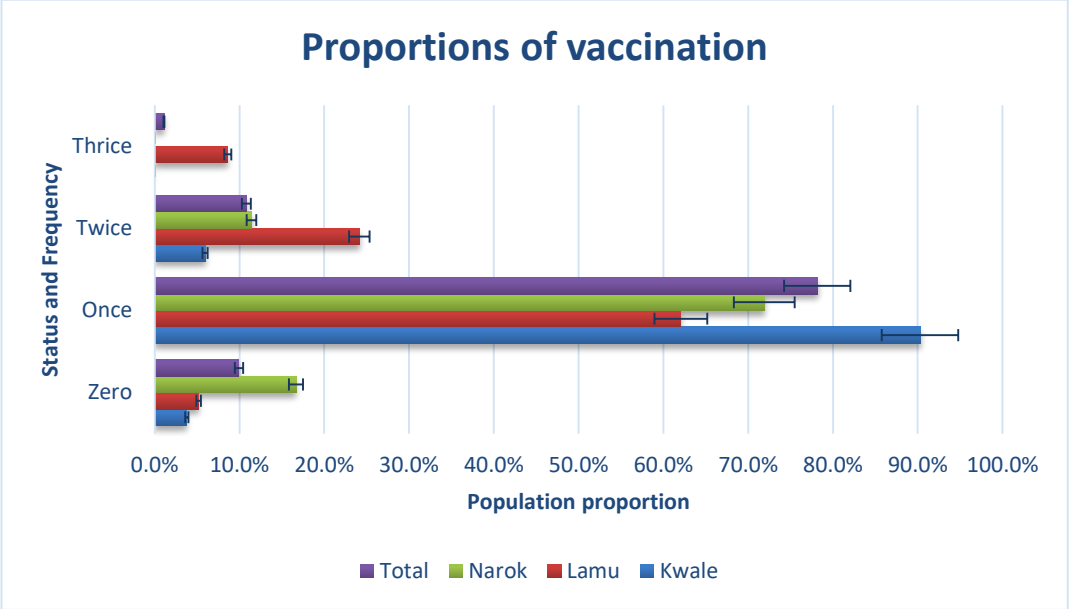

Figure 4.1: Bar chart showing proportion of vaccinated and vaccination times among study participants across the three counties and the overall population.

Table 4.3: Proportions of vaccination across the different age cohorts

Age Cluster (Months)	Number examined (n)	Number Vaccinated	Proportion vaccinated (%)	95% Confidence Interval	P - value
≤12	29	29	100%	88.06% -100%	0.217
12_24	131	130	99.2%	95.74% - 99.98%	0.028
24-36	111	98	88.3%	80.82% - 93.62%	0.001
36-48	90	70	77.8%	67.81% - 85.88%	0.000
48- 60	92	85	92.40%	84.96% - 96.89%	0.252

4.2 Participants' measles IgG seropositivity.

4.2.1 Measles IgG ELISA Results

ELISA results showed that; 376 (83.0%, 95% CI; 75.4% to 90.6%) of children were positive for Measles specific IgG antibodies, 57 (12.6%, 95% CI; 11.5% to 13.7%) were negative, while 20 (4.4%, 95% CI; 4.0% to 4.8%) had equivocal sera. Out of 408 vaccinated children, 346 (84.8%) were seropositive, 46 (11.3%) were negative while 16 (3.9%) had equivocal sera. Out of 11 none vaccinated children, 2 (18.2%) were sero positive, 6 (54.5%) negative, and 3(27.3%) had equivocal sera. Distribution of participants IgG ELISA results for the various demographic categories and vaccination history is presented in table 4.4. Measles IgG sero-status varied significantly ($P<0.001$) between children whose parents had vaccination cards and those lacking cards. Significant variation of sero status ($P<0.001$) was also noted between vaccinated and non-vaccinated children, although there was no significant variation in sero status with in frequency of vaccination ($P=0.813$).

Table 4.4: Participants Measles IgG ELISA results summary.

Category	Examined	Positive	Negative	Equivocal	P Value
	n	n (%)	n (%)	n (%)	
Gender of child					
Female	220	179(81.4)	29(13.2)	12(5.5)	0.520
Male	233	197(84.5)	28(12.0)	8(3.4)	
Age group of child in months					
<12	44	33(75.0)	8(18.2)	3(6.8)	0.851
12_24	137	117(85.4)	15(10.9)	5(3.6)	
24-36	129	109(84.5)	15(11.6)	5(3.9)	
36-48	89	74(83.1)	10(11.2)	5(5.6)	
>48	54	43(79.6)	9(16.7)	2(3.7)	
County of residence					
Kwale	185	158(85.4)	18(9.7)	9(4.9)	0.419
Lamu	58	50(86.2)	7(12.1)	1(1.7)	
Narok	210	168(80.0)	32(15.2)	10(4.8)	
Residential status					
Permanent	404	338(83.7)	49(12.1)	17(4.2)	0.557
Moved in	49	38(77.6)	8(16.3)	3(6.1)	
Vaccination card presentation					
No	107	68(63.6)	31(29.0)	8(7.5)	0.000
Yes	346	308(89.0)	26(7.5)	12(3.5)	
Awareness about vaccines					
No	40	32(80.0)	7(17.5)	1(2.5)	0.534
Yes	413	344(83.3)	50(12.1)	19(4.6)	
Awareness about Measles disease					
No	67	47(70.1)	17(25.4)	3(4.5)	0.003
Yes	386	329(85.2)	40(10.4)	17(4.4)	
Awareness about Measles outbreaks					
No	400	336(84.0)	46(11.5)	18(4.5)	0.161
Yes	53	40(75.5)	11(20.8)	2(3.8)	
Relationship to child					
Parent	399	334(83.7)	49(12.3)	16(4.0)	0.565
Sibling	24	17(70.8)	4(16.7)	3(12.5)	
Uncle/Aunt	7	6(85.7)	1(14.3)	0(0)	
Cousin	23	19(82.6)	3(13.0)	1(4.3)	
History of Measles disease					
Unknown	54	41(75.9)	10(18.5)	3(5.6)	0.111
No	386	324(83.9)	47(12.2)	15(3.9)	
Yes	13	11(84.6)	0(0)	2(15.4)	
Vaccination status					
Unknown	34	28(82.4)	5(14.7)	1(2.9)	0.000
No	11	2(18.2)	6(54.5)	3(27.3)	
Yes	408	346(84.8)	46(11.3)	16(3.9)	
Vaccination times					
Once	354	298(84.2)	41(11.6)	15(4.2)	0.813
Twice	49	43(87.8)	5(10.2)	1(2.0)	
Thrice	5	5(100)	0(0.0)	0(0.0)	

4.3 The seroprotectivity of IgG ELISA borderline samples through Plaque Reduction Neutralization assay

Samples that remained equivocal after ELISA retesting (20 samples), 2 positive and 2 negative (controls) samples were subjected to Plaque reduction neutralization test (PRNT). Out of this, 20 (100%) samples had a PRNT titre above 1:120, while none had titres less than 1:120.

The distribution of seroprotectivity of borderline samples for selected demographic and vaccination history categories of study participants is presented in Table 4.4 below. This table shows the proportions of samples that denotes participants immunity against measles disease that could not be satisfactorily elucidated with the measles IgG ELISA.

Table 4.5: Plaque Reduction Neutralization test results analysis for selected risk categories

Risk Category	No. Examined	Protected	Protectivity Rate
Overall	20	20	100%
Sex			
Female	12	12	100%
Male	8	8	100%
County			
Kwale	9	9	100%
Lamu	1	1	100%
Narok	10	10	100%
Vaccination Card			
Absent	8	8	100%
Present	12	12	100%
Vaccination Status			
Unknown	1	1	100%
Not Vaccinated	3	3	100%
Vaccinated	16	16	100%
Vaccination Times			
Once	15	15	100%
Twice	1	1	100%

4.3.1 Measles Seroprotectivity

This combined the results from measles IgG ELISA and plaque reduction neutralization assay on the borderline samples from ELISA which were used to categorise participants as protected or not protected against measles. All participants whose ELISA tests had 11 Novatec units (comparable to 220 IU/ml 3rd international standard according to the kit used - NovaLisa^{TM}) or above were considered protected. Moreover, all equivocal samples that posted a neutralization titre of 1:120 or higher after PRNT were also considered protected. Overall results showed that; out of 453 participants, 396 (87.4%, 95% CI; 84.3% to 90.5%) of children were protected against Measles while 57 (12.6%, 95% CI; 9.5% to 15.7%) were not protected.

Among the 408 children vaccinated, 362(88.7%) had evidence of protection while 46(11.3%) had no evidence of protection. Among the 11 children with no history of vaccination, 5(45.5%) had evidence of protection while 6 (54.5%) were not protected. Within the 34 children whose history of vaccination was unknown, 29(85.3%) had evidence of protection while 5(14.7%) were not protected.

Kwale had 167 (90.3%, 95% CI; 87.6% to 93.0%) of children protected, by far the greatest protected proportion. Lamu had 51 (87.9%, 95% CI; 84.9% to 90.9%) of protected children, while Narok had 178 (84.8%, 95% CI; 81.5% to 88.1%) of its children protected. Proportion of unprotected children was; 18 (9.7%, 95% CI; 7.0% to 12.4%) in Kwale, 7 (12.1%, 95 CI; 9.1% to 15.1%) in Lamu and, 32 (15.2%, 95% CI; 11.9% to 18.5%) in Narok.

Comparatively, there was no significant difference in Protectivity when Kwale County (90.3%) was compared with Lamu County (87.9%), P = 0.60, nor with Narok County (84.8%), P = 0.10. Moreover, no significant difference in protectivity was seen between Lamu County (87.9%) and Narok County (84.8%), P= 0.55.

The overall distribution of measles seroprotectivity among children aged between 9 and 59 months in Kwale, Lamu and Narok Counties in the study period for the various socio-demographic categories and vaccination history is summarised in table 4.5 below. This summarizes numbers and proportions of children who are protected and

those that are not protected. There was no significant difference in protectivity between males (87.9%) and female (86.9%) children (P = 0.5), but significant difference was seen between vaccinated (88.7%) and unvaccinated children (45.5%), (P < 0.0001).

Analysis of seroprotectivity in the various demographic characteristic group and vaccination history groups was done to explore the possibility of them increasing or decreasing the chances of participant being protected against measles disease (Appendix VI).

Table 4.6: Distribution of participant's seroprotectivity in the study regions.

Attribute	Protected							Not protected						
	Kwale		**Lamu**		**Narok**		**P value**	**Kwale**		**Lamu**		**Narok**		**P value**
	n	**%**	**n**	**%**	**n**	**%**		**n**	**%**	**n**	**%**	**n**	**%**	
Gender														
Female	82	49.1	21	41.2	88	49.4	0.498	9	50	5	71.4	15	46.9	0.557
Male	85	50.9	30	58.8	90	50.6		9	50	2	28.6	17	53.1	
Age group														
<12	23	13.8	1	2	12	6.7	0.000	4	22.2	2	28.6	2	6.2	0.478
12_24	61	36.5	11	21.6	51	28.7		4	22.2	1	14.3	9	28.1	
24-36	40	24	10	19.6	64	36		6	33.3	2	28.6	7	21.9	
36-48	25	15	14	27.5	39	21.9		1	5.6	1	14.3	9	28.1	
>48	18	10.8	15	29.4	12	6.7		3	16.7	1	14.3	5	15.6	
Residency														
Permanent	159	95.2	41	80.4	155	87.1	0.003	16	88.9	6	85.7	28	87.7	0.975
Moved in	8	4.8	10	19.6	23	12.9		2	11.1	1	14.3	4	12.5	
Guardian														
Parent	159	95.2	31	60.8	160	89.9	0.000	15	83.3	5	71.4	29	90.6	0.386
Others	8	4.8	20	39.2	18	10.1		3	16.7	2	28.6	3	9.4	
Presence of vaccination card														
No	21	12.6	8	15.7	47	26.4	0.004	12	66.7	5	71.4	14	43.8	0.185
Yes	146	87.4	43	84.3	131	73.6		6	33.3	2	28.6	18	56.2	
Awareness about vaccines														
No	11	6.6	2	3.9	20	11.2	0.140	1	5.6	0	0	6	18.8	0.225
Yes	156	93.4	49	96.1	158	88.8		17	94.4	7	100	26	81.2	
Awareness about measles disease														
No	11	6.6	8	15.7	31	17.4	0.008	3	16.7	1	14.3	13	40.6	0.130
Yes	156	93.4	43	84.3	147	82.6		15	83.3	6	85.7	19	59.4	
Awareness about measles outbreaks														
No	157	94	27	52.9	170	95.5	**0.000**	17	94.4	1	14.3	28	87.5	0.000
Yes	10	6	24	47.1	8	4.5		1	5.6	6	85.7	4	12.5	
Source of information														
Health worker	96	61.5	40	81.6	111	70.3	0.023	9	52.9	6	85.7	18	69.2	0.269
Others	60	38.5	9	18.4	47	29.7		8	47.1	1	14.3	8	30.8	
Vaccination status														
Unknown	2	1.2	1	2	26	14.6	0.000	2	11.1	0	0	3	9.4	0.461
No	1	0.6	0	0	4	2.2		2	11.1	2	28.6	2	6.2	
Yes	164	98.2	50	98	148	83.1		14	77.8	5	71.4	27	84.4	
Vaccination status														
Vaccinated	164	99.4	50	100	148	97.4	0.200	14	87.5	5	71.4	27	93.1	0.270
Not vaccinated	1	0.6	0	0	4	2.6		2	12.5	2	28.6	2	6.9	
Vaccination times														
Once	154	93.9	31	62	128	86.5	0.000	13	92.9	5	100	23	85.2	0.537
More than once	10	6.1	19	38	20	13.5		1	7.1	0	0	4	10.9	
Latest time of vaccination														
One year or less	71	43.3	34	68	55	37.2	0.001	7	50	3	60	5	18.5	0.048
> a year	93	56.7	16	32	93	62.8		7	50	2	40	22	81.5	
History of measles infection														
Unknown	3	1.8	0	0	41	23	0.000	0	0	0	0	10	31.2	0.009
No	160	95.8	48	94.1	131	73.6		18	100	7	100	22	68.8	
Yes	4	2.4	3	5.9	6	3.4		0	0	0	0	0	0	

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMENDETIONS

5.1 Discussion

This study brings open a rare glimpse, at least for the first time, in to the seroepidemiological profile of Measles-specific IgG antibodies among Kenyan children. This work shows that levels of vaccination varied between the counties of study and were generally low although they affected measles protection positively. The current study also suggest that the demographic characteristic of a population plays a role in community attitudes towards vaccination.

In this study, participants' demographic characteristics and vaccination history varied across the study region. There was no significant difference in gender representation across the three Counties under study P=0.830 with male children being slightly higher (51.4%) than female (48.6%). This difference could probably have arisen by chance considering that it was not significant, or due to more female children being admitted at the time of study thus failing to meet the selection criteria.

Among the participants, majority 89.4% were permanent residents in their Counties and very few 10.6% were either fresh residents or visitors and the difference between this two groups was significant (P=0.05). Respondents who were not permanent residents had lived there for period ranging between few days and slightly over a year. Although an insignificant proportion their inclusion helped broaden the research inference. It is normally that in each region there is a continuous inflow, and consequently outflow, of residents as they undertake their day to day activities. In this study the inflow affected negatively the vaccination rates consequently affecting the general seroprotectivity. This factor needs to be looked at broadly whenever implementing a public health intervention policy as it is natural that new entrants into a community bring with them different characteristic that could influence the overall behavior of this new community.

The current study discovered that not all guardians carried vaccination cards when taking children to child health welfare clinics. Vaccination cards were presented in only 76.4% ($P < 0.000$) of the respondents while in approximately one quarter of the respondents such vital document could not be traced. Although the study didn't go further to establish the reason behind this, probably, frequent movements as experienced by some pastoralist populations may make it difficult to safely maintain documents or rather records. This may also be attributed to low awareness in public health matters especially the significance of vaccination as was evidenced by low awareness on measles disease (14.8%) and measles vaccine (8.8%) in the study population. This is alarming considering that the same card bears all other information regarding the child fully vaccination history in the case of other vaccine preventable diseases like mumps, polio and others. Such a gap in vaccination history, although did not affect this study, might stagnate the process of achieving our set vaccinations targets as in cases where the card is not available care givers depend entirely on guardian recall ability. The alternate vaccination mark on the outer right hand is not reliable either as this mark may not be evident in some vaccinees.

From the current study, most people know something about measles disease (85.2%) and vaccines (91.2%), ironically, only a small population (11.3%) could recall having heard about measles outbreak cases within the country. Moreover, a very small proportion (2.1%) acknowledged their children to have suffered from measles before while 11.9% were not sure. There is very scant work on people's knowledge of measles and its awareness status currently, thus couldn't get a good comparison. However, this goes far in describing the general perception and attitude towards the whole subject of vaccination and immunity generation among the study participants which can be best described as low. From this observation, it may seem that measles outbreaks are very rare, contrary to this, measles cases were reported in 2012 and 2013 by the Government and are contained in the department of vaccines and immunization records of 2013. The same was reported by WHO and other sources (Masresh et al., 2017; WHO, 2017b) yet not much information reached the community.

This study revealed that parents had high chances of carrying vaccination record cards when taking their children to welfare clinics as compared to non-parent guardians

(77.2% vs 70.4%, odds; 0.702, 95% CI; 0.374 – 1.316). The same holds true when it comes to recalling information regarding awareness about vaccines (91.5% vs 88.9%), and more importantly ability to recall their children's vaccination status in absence of vaccination record cards (13.0% vs 6.8%). Probably, due to expected long hours of parents-children contact, there develops a natural bonding that make parents always wanting to know what is happening to their children. Thus, parents seem to be superior in mastering events surrounding their loved children and this knowledge is hardly erased from their minds. This goes a long way to assert the pivotal role parents play in their children wellbeing. Parents are therefore the ideal advisors of health-related issues at home and hence can be relied on to pass public health related issues satisfactorily.

Even though, official records on vaccination coverage for the three counties of study had on average 82.8%, a 4.3% margin lower than the national averages, still this did not differ significantly with the study results (P=0.001). The WHO put the estimates of McV 1 vaccination at 79% (Masresh *et al.*, 2017; Who/Unicef, 2015), and it is thought that the level 87.1% was reached after inclusion of catch up and follow up SIA campaigns (Who/Unicef, 2015). SIAs have been found to be responsible for more than 25% of immunised individuals and as high as 45% in Ethiopia (Trentini *et al.*, 2017), however the study did not find out how SIA data is relayed in Kenya. Concluding on this, one would say that our vaccination programme is still inadequate and coverage is suboptimal as this is still far from the WHO recommended levels of at least 95% national vaccination coverage with the McV1 and McV2 (Bose *et al*., 2014; WHO, 2017c). Low vaccination coverage could have partly been attributed to low vaccine awareness among the population. Research conducted elsewhere have revealed the difficulties in reaching some places, long distances from health centres, few community health workers and vaccine refusal as some of the challenges in attaining sufficient coverages (Cutts *et al.*, 2013; Ferrari*et al.*, 2013; Lieu *et al.*, 2015; Metcalf *et al.*, 2015).

Internationally, a measles IgG titre of 200IU/ml or more is considered protective as suggested elsewhere (Chen *et al.*, 1990; Kizito *et al*., 2013; W. Moss, 2015), although, reinfection may not be completely ruled out in individuals whose immune systems

have had previous priming through the wild or vaccine strains (Chen *et al*., 1990; Fu *et al*., 2010; Manirakiza *et al.*, 2011) attaining of protective titres notwithstanding. However, in this study an ELISA IgG threshold titre of 220IU/ml was used. Through this ELISA analysis, the study established that a significant proportion (83.0%, $P < 0.0001$) of participants had evidence of immune antibodies (seropositive) against measles disease. Earlier ELISA studies on immune antibody prevalence especially in developed countries had reported higher seroprevalence values of up to 99% (Menezes *et al.*, 2014; Papania *et al*., 2014; Wang *et al.*, 2014). However, in a few instances, results showed lower percentages of circulating antibodies (Manirakiza *et al*., 2011). These differences may be attributed to infrastructural setup as experienced between the developing and developed countries. Kenya cannot match the USA in many aspects of development hence hitting 83% here is still a commendable feat.

Despite there being variations in the proportions of seropositive individuals among the three counties of study, there was no significant difference in seropositivity between them. In this study, immune antibodies distribution was high in male 84.5% than female 81.4% participants although this difference was not significant $P = 0.381$.While some studies concur with this (Shamsizadeh *et al*., 2012), in the past, other studies have found women to have significantly higher titres than men (C. Martins *et al.*, 2013; Wang *et al.*, 2014). These results are a probable indicator that immune antibody distribution is not necessarily affected by sex but rather a combination of other factors like vaccination time and health status at the time of vaccination as explored elsewhere (Trentini *et al*., 2017).

A recent study in Gambia found seroconversion rates of up to 91% in children (Wanjiku & Adetifa, 2018). Although slightly lower, the current study found out that vaccination significantly affected seropositivity, a considerable proportion (83.0%) of vaccinated participants were positive for measles IgG antibodies through ELISA as compared to unvaccinated participants (18.2%, $P < 0.0001$). Previous researches across the globe support the significance of vaccination as an immune antibody generation tool (Antona *et al*., 2013; CDC, 2013; Lieu *et al*., 2015; Wanjiku & Adetifa, 2018; WHO, 2017a). High levels of immune antibody circulation in vaccinated individuals is attributed to the fact that measles virus is highly immunogenic (Durando

et al., 2016; Naim, 2015), hence under normal circumstances it leaves an excellent circulating memory. It is therefore natural that a large pool of vaccinated individuals remains positive for these antibodies.

Whereas all measles ELISA IgG positive samples in this study were considered protected, the equivocal samples had to be retested with neutralization technique (PRNT) to confirm their seroprotectivity.

In the current study, all of the equivocal samples had protective titres (≥ 1:120). These results are suggestive that presence of equivocal sera can be regarded as a sign of protection from developing measles disease. Several previous research work concur with this and actually go ahead to suggest the utilisation of equivocal sera as a presumptive evidence of their being active circulating immunity (Bose *et al.*, 2014; CDC, 2013; Gidding *et al*., 2016). This probably could be attributed to the fact that in equivocal reactions there is usually the presence of the specific antibodies in question albeit at a non-convincing concentration. However, in an immune reaction we just need an existing memory and however low the antibody titre, we still have some lowly active memory cells. The other presumptive reason for this may be attributed to the fact that while ELISA searches for the presence of circulating IgG antibodies only but, in totality immunity is a function of other molecules and cells as well which augments the work of antibodies.

The study found a strong association between measles vaccination and consequent protection against measles disease as compared to non-vaccination and subsequent protection (Chi-square; 18.45, $P < 0.0001$). Among the vaccinated (90.1%), protection stood at 88.7% whereas among the non-vaccinated (7.5%) protection was at 45.5%. This suggests that vaccination using the McV is very effective as far as protection against measles is concerned. Such association has been highlighted by world health organization (CDC, 2013; Perry, *et al.*, 2014; WHO, 2014), and mentioned in reports from other studies as well (Penedos *et al.*, 2015; Strebel *et al*., 2011; Uzicanin & Zimmerman, 2011; Wanjiku & Adetifa, 2018). This is attributed to the strong memory imparted by attenuated vaccines amongst which the McV is one of them.

Although there was no significant variation in measles protection between children vaccinated once and children vaccinated more than once, P = 0.615, children vaccinated more than once had higher chances of being protected than those vaccinated once (Odds Ratio; 1.284, Table 6.3 in appendix VI). Previous studies concur with this work on superiority of multiple measles vaccination doses as compared to a single vaccination dose in vaccine effectiveness (Siberry *et al*., 2015; Uzicanin & Zimmerman, 2011). Presumably, getting a second and probably third dose of measles containing vaccine (McV) after the initial one, improves the memory several folds higher. This improves the robustness or rather alertness of the general immune system as regards measles disease protection. The robustness of the immune system can be demonstrated as high titres of circulating measles IgG antibodies in a larger proportion of individuals.

Protection against measles does not favour any gender, males and females were equally protected in this study. Despite there being a slight tilt in the risk of not being protected, the risk being higher in females than males, there was no significant difference in protection between male and female populations (P; 0.709, Odds ratio; 1.112, 95% CI; 0.638 - 1.937). Previous studies found no significant difference in measles protection between male and female populations under normal circumstances (Manirakiza *et al*., 2011; Shamsizadeh *et al*., 2012). However, some studies found out that women were significantly less protected than men although the mechanisms behind these variations was not known (Jaber, 2006; Menezes *et al*., 2014). Probably other factors come to play here like one gender could be favoured by age of vaccination and number of doses received hence the difference.

Information regarding measles disease is among the most useful knowledge required by the population in order to eliminate measles virus. The current study revealed that children whose guardian had some knowledge about the disease were considerably protected when compared to their counterparts whose guardians were not aware about the disease. There was a significant difference in seroprotectivity between children whose guardians were aware of Measles disease and those not aware (Chi-square = 11.694, P = 0.001).

Interestingly, the general knowledge about vaccines seems inferior to knowledge about measles disease as far as protection against measles is concerned. The study revealed that there was no significant difference in protection between children whose guardians were aware about the vaccines and those whose guardians were not aware (Chi-square = 0.964, P = 0.326). However, despite there being no significant difference, the risk of not being protected was still higher among children whose guardians were not aware about the vaccine as compared to children whose guardians were aware (82.5% vs 87.9%; Odds ratio = 1.54; 95% CI, 0.647 – 3.667). Other studies agree with the current work on the importance of vaccine awareness (Brieger *et al*., 2017; Duron *et al.*, 2017). Probably, the awareness about vaccines makes a parent to be more decisive in taking their children for vaccination thereby increasing the chances of such children being protected against measles disease.

The presence or absence of vaccination card has a direct effect in the overall protection of a community. The revelation that children whose guardian had their vaccination cards were more protected than those who didn't is emphasising this. Children whose guardians had no vaccination cards were less likely to be protected with the specific measles IgG antibodies than those with vaccination cards (66.8% vs 90.1%; P= 0.000, Odds ratio = 5.02; 95% CI, 2.816 – 8.949). Incidentally, such comparative studies are extremely scanty.

Although there is scant data on awareness studies, this study found out that the general awareness of vaccines and measles disease play a significant role on decision making when it came to safe guarding children's vaccination cards and availing them when required during vaccinations. The same can be said on the competence of the said guardians when it came to taking their children for vaccination or at least heading calls for vaccinations. The study found out that a significant proportion of guardians who carried vaccination cards were either aware of vaccines (P < 0.001) or the measles disease (P < 0.001) or both. In the same breadth, it is the same cadre of population that showed a significant difference in the actual vaccination process. This research revealed the existence of a significant difference in vaccination between children whose guardians were aware of either vaccines (P = 0.003) or the measles disease (P = 0.011) against those who were not aware. This solidifies the fact that both the

awareness of vaccines and the awareness of measles disease play a significant role on the probability of getting individuals vaccinated when other factors are kept constant, thus affecting the overall population immunity in a given population.

This study brings open, a rare glimpse, at least for the first time in to the seroepidemiological profile of Measles-specific IgG antibodies among a sub population of Kenyan children. The study results suggest that the general population sero - immunity among children between 9 to 59 months as in 2014, was 87.4%, well below the acceptable herd immunity threshold (93-95%) necessary to interrupt measles transmission as suggested by several studies (Penedos et al., 2015; Poethko-Müller & Mankertz, 2012; WHO, 2009a). Comparatively, this is much higher than what another recent (2011) study in Bangui, Central African Republic (CAR) found (57.3 %) on a research conducted in 2008 despite having reached immunisation coverages of more than 90% in 2006 (Manirakiza et al., 2011). Whereas CAR is among the regions poorest countries with Human Development Index (HDI) of 0.352, Kenya is among the medium developing countries, HDI of 0.555 (U.N. D. P, 2012) and this could probably be the attributing factor for the difference in sero-immunity despite having almost the same immunisation coverages albeit at slightly different times.

None of the three counties of study – Lamu, Kwale and Narok- reached the threshold of herd immunity. However, individually, there was some good hope in Kwale and Lamu counties that established protective rates of 90.3% and 87.9% respectively. Although Kwale showed an edge above Lamu and Narok counties in seroprotectivity. Whereas the difference in protection was not statistically significant (P=0.256), the chances of being seronegative were lower in Kwale as compared to Lamu (Odds Ratio; 0.785, 95% CI; 0.311 – 1.986, P=0.60) or Narok (Odds Ratio = 0.600, 95% CI 0.324 – 1.109, P=0.10). Consequently, the risk of being seronegative was lower in Lamu when compared to Narok (Odds Ratio = 0.763, 95% CI; 0.318 – 1.832, P=0.55).

The low seroprotectivity rate in Narok is alarming, considering that this is still considered among the high measles transmission zone. This could have been attributed to, mostly, low levels of vaccinations (83.3%) in the region. This is due to, partly, frequent movements of people, in accessible terrain which could have led to low

awareness on vaccination related issues. Incidentally, in Narok all children (100%) in the study who moved in from elsewhere were vaccinated compared to the permanent residents (80.8%), hence movement into the county had a positive impact rather than a negative one in terms of vaccination and consequently protection. Therefore, it is probably the movements within the county that negatively affects access to vaccination services in Narok County. Regular relocations in search of pastures coupled with difficult terrain could have made it difficult for mobile health teams to access residents during catch-up vaccination exercises or population targeted awareness programmes as suggested elsewhere (Cutts *et al*., 2013; DVI, 2013; Metcalf *et al*., 2015). The distance from health centres could also limit access to immunisation services(Lieu et al., 2015). This is complimented by the fact that this county had the least proportion of vaccinated individuals (83.3%) when compared to the other two.

Among children aged between 9 and 59 months, vaccinated children had more chances of having measles-specific IgG protective antibodies than those that were unvaccinated and this was significantly so at 95% CI (88.5% vs. 45.5%; chi-square = 18.45; P = 0.000). McV is an integral part in protection against measles, this has been proved beyond doubt in several studies (Antona *et al*., 2013; Defay *et al.*, 2013; Uzicanin & Zimmerman, 2011), and hence are next only to Measles natural infection as far as protection against measles disease is concerned. In another study, 84.8% of confirmed measles cases were found not to have been vaccinated (European Centre for Disease Prevention and Control, 2016).

From the research findings, the levels of protection against measles disease from the three study counties is higher (87.4%) when compared with the vaccination coverage data from the government records (82.8%). This is very interesting as such reports are very rare and, in most cases, one expects protection levels to be lower than the vaccination coverage levels. This is probably due to the fact that we always have to give allowance 85% to 90% rates of seroconversion and a 15% rate of primary vaccine failure as suggested in other studies (Cutts *et al*., 2013; Manakongtreecheep & Davis, 2017; Trentini *et al.*, 2017).So, in any vaccination programme we expect a small percentage of failure which is not the case here. The study couldn't pinpoint the exact reason for this unlikely disparity.

However, high protective levels than the recorded vaccination coverages could have arose due to massive SIA activities that somehow failed to be reflected on vaccination reports but whose effects on general measles immunity couldn't go unnoticed. This is probably the case as supported by the fact that while the government of Kenya introduced routine second dose measles (McV2) in 2013, there was still no data for second dose by 2014 and reported a coverage of 28% only by 2015 at a time another research gave a coverage of 55% (Subaiya *et al.*, 2018), thus, there could be challenges in accurately capturing these official records (GoK:KNBS, 2015; Masresha *et al*., 2015; Who/Unicef, 2015). More over SIAs do not segregate previously vaccinated children and hence a child could be vaccinated twice or more times courtesy of SIA leading to superior seroconversion characteristics within the population (Trentini *et al*., 2017; WHO, 2017c). Occurrence of natural immunity from measles infection could also have contributed to a higher immunity level in a population that received low vaccination coverage. Although only 3.6% acknowledged suffering from measles, 10.2% were not sure and going by measles incidence records of 2014 (7.9 per million), natural immunity could not be ruled out either(WHO, 2017b).

As per the relationship between natural infection and vaccination, the study found no significant difference between immunity attained through vaccination with the measles containing vaccine and natural infection with the wild measles virus. However, although there was no significant difference in protection, it was shown that children who suffered from Measles infection had more chances of being seroprotected than those who didn't suffer from the disease (100% vs 89.2%; Odds ratio = 1.9863; 95% CI = 0.1124 to 35.0924; P = 0.6395).Natural immunity has always been proven to be superior to vaccine induced immunity and our case is not an exception (Ella, Olaitan, Ameh & Ella, 2015; WHO, 2014).

Measles containing vaccine imparts an excellent result. This signals the significance of vaccination within in our community. There was no significant difference between the measles IgG seropositivity rate in the region of study when compared against reported vaccination uptake (87.4% vs 90.1%; P = 0.604). This posts a 90% seroconversion rate which, although at the lower side, is still comparable to most effective immunizations done elsewhere (Durando *et al.*, 2016; Menezes *et al*., 2014;

Trentini *et al*., 2017).This characterises excellent vaccine and its handling, coupled with robust host characteristics, and could have positively affected seroconversion rates. However, this still serves as a significant pointer that the higher the vaccination coverage the greater the seroconversion expected and, consequently the more the chances of increasing pools of measles seroprotected children and lowering the residual susceptibility rates in our community. Considering the now greatly reduced global exposure rates to circulating wild measles virus, which would otherwise naturally supplement the vaccination programmes, robust immunisation programme is a must for us to post significant outcomes.

Potential confounding limitations of the study; Subject selection was based on hospital/clinic attendance which could have biased our results, and, levels of protective antibodies may not have been fully and rightly categorised because of the method applied.

5.2 Conclusions

In this study, participants' demographic characteristics and vaccination history varied across the study region. Vaccination awareness was low among the adult study population and large number of children lacked vaccination records.

Through Measles IgG ELISA analysis, the study determined that a significant proportion (83.0%, P <0.0001) of participants had evidence of immune antibodies against measles disease while a small proportion (12.6%) had no immune antibodies against the disease and yet a much smaller group (4.4%) had equivocal results.

Through plaque reduction neutralization assay, the study established that all ELISA equivocal samples from the kit used had evidence of protection against measles disease.

This seroepidemiological study revealed that vaccination coverage is still inadequate (90.1%) and children protective immunity (87.4%) is well below the herd immunity threshold requirement (at least 95%).

5.3 Recommendations

Measures need to be put in place to improve awareness on importance of vaccination and attendance of child health welfare clinics, monitor efficacy of our vaccination programmes and, include automated vaccination records for all children.

The department of vaccine and immunization may consider large-scale serological surveillance to boost the current measles surveillance programme and enhance identification of geographical clusters of under immunization.

In immune protective assays, ELISA-equivocal samples can be regarded as actually protected.

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APPENDICES

Appendix I: Demographic Questionnaire

A questionnaire to collect demographic and related information about the research participants.

Serial Number:_____ Date: _____

Demographic Information

1. Date of birth ……..
2. Residence: Town: …… County: ……..
3. Are you a permanent residence here

yes		No	

4. If no,

   a. How long have you stayed here

Less than a month ago	
Between one and six months	
Between seven months and one year	
More than a year ago	

   b. Where were you staying before.....

Clinical Information

5. Do you know anything about vaccines

yes		No	

6. How did you know about vaccines

Friends	
Health workers	
News	
Books	
Other	

7. Do you know anything about measles

yes		No	

8. Has there been an outbreak that you can rememberof

yes		No	

9. When was that

Less than a month ago	
Between one and six months	
Between seven months and one year	
More than a year ago	

10. How many people were affected if you can remember

Less than 5	
Between 5 and 20	
More than 20	

11. What is your relationship to the child

Father/mother	
Sister/brother	
Uncle/Aunt	
Cousin/Friend	

12. Sex of your child

Male		Female	

13. How old is he/she……months

14. Has the child suffered from a measles infection:

yes		No		I don't know	

15. Measles Vaccination status

Vaccinated		Not vaccinated		I don't know	

16. Number of times

	At --- Months
Once	
Twice	
Thrice	
Many times	

17. When was the last time he/she was vaccinated against measles

Less than a month ago	
Between one and six months	
Between seven months and one year	
More than a year ago	

18. Do you live with other children

yes		No	

19. If yes, how many? _____ how old? _____

20. Have they been vaccinated

yes		No		I don't know	

21. When was that done

Less than a month ago	
Between one and six months	
Between seven months and one year	
More than a year ago	

Thank you for everything!

Appendix II: Informed Consent Form

a. Information sheet

Research Title: Evaluation of Measles Immunity among Children aged 9 to 59 months at selected Health facilities in Kwale, Narok and Lamu Counties of Kenya, 2014

Introduction

This research study is being conducted by Ali Juma Kanga at Jomo Kenyatta University of Agriculture and Technology to determine the proportion of children aged between 9 and 59 months that can resist measles virus infection.

Procedures

You will be requested to give permission for withdrawal of approximately 2 ml of blood from your son/daughter. This sample shall be analysed to determine your child's level of resistance to measles. Research findings shall be made available through reports which shall be free.

You will also be asked to complete a short questionnaire or the questionnaire will be read for you and your response shall be recorded. Questions will include mainly details about your demographic background and your own personal knowledge regarding Measles virus vaccine.

Risks/Discomforts

There are minimal risks for participation in this study. However, you may feel physical discomfort during sample withdrawal.

Benefits

There are no direct benefits to subjects. However, if there are gaps in immunity against measles supplemental immunization activities shall be recommended. Moreover, the information gained from this study is vital for overall elimination of measles in Kenya.

Confidentiality

All information provided will remain confidential and will only be reported as group data with no identifying information. All data, including questionnaires will be kept in a secure location and only those directly involved with the research will have access to them. After the research is completed, excess samples will be discarded and the questionnaires will be destroyed.

Participation

Participation in this research study is voluntary and you have the right to refuse to participate or withdraw.

Questions about the Research

If you have questions regarding this study, you may contact:

Ali J. Kanga

PO Box 90791- 80100

Mombasa.

Tel. Mob: 0721 545650

Email:akanga74@gmail.com

OR,

The Secretary, Kenyatta National Hospital/University of Nairobi- ERC

PO Box 20723 – 00202

Nairobi.

Tel: 726300-9

Email: uonknh_erc@uonbi.ac.ke

b. Certificate of consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily on behalf of the child to participate as a participant in this study.

You are making a decision whether or not to participate. Your signature indicates that you have decided to participate, having understood the information provided above.

Signature……. Date…….

Time……. Relationship to Subject……

I have accurately read or witnessed the reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that consent was given freely.

Signature of Witness……. Signature of Investigator…….....

Appendix III: Dried Blood Spots (DBS) Collection and Handling

Requirements:

- Blood collection card (filter paper; Whatman S&S 309)
- Glycine weighing paper
- Sealable plastic bags
- Humidity cards
- Desiccant packs
- Cotton swabs
- Sterile lancets
- Permanent marker pens
- Personal protective equipment

Safety measures

- Treat all blood samples as though they are infectious
- Wear gloves and apron/lab coat
- Dispose of contaminated sharps and waste appropriately
- Take precaution to avoid needle injury
- Wash hands and dry them aseptically

DBS sample collection

- Clearly label each card with appropriate identification number.
- Prepare participant by explaining the technique.
- Sterilize puncture site with 70% alcohol, let it dry.
- Apply gentle pressure to the finger and allow a large drop of free-flowing blood to collect at the puncture site.
- Working quickly, hold the filter paper by the edges and touch the filter paper gently against the large drop of blood and in one step allow a sufficient quantity of blood to soak through and completely fill or saturate a circle. A completely saturated spot will contain 100 µl of blood.

- Repeat, until you have collected enough blood to fill at least 3 circles on the blood collection card.
- Completly filling the circle is important because the laboratory will need to use a hole puncher to punch a section of the circle of blood for testing
- If collecting spots using a pipette, collect 100 µl of blood and gently apply to filter paper.

DBS sample drying

- Avoid touching or smearing the blood spots
- Allow the specimen to fully air dry horizontally (at least 3hours) at room temperature.
- Keep away from direct sunlight - Care should be taken to avoid exposing DBS to environmental conditions that may compromise the integrity of the specimen. DBS should not be dried near an open window as sunlight, dust and in some cases flying insects may come in contact with the DBS during the drying procedure.
- Do not heat, stack or allow DBS to touch other surfaces during the drying process.

Packaging DBS samples.

- Place weighing or glycine paper between DBS cards before transport to prevent cross-contamination
- Place filter paper between sheets of weighing paper
- Fold weigh ends of weighing paper
- Avoid using bags that are too big as the cards will shuffle inside the bag.
- The bag should be a sealable heavy-duty plastic bag, one that will prevent moisture from entering.
- Insert into sealable plastic bag
- Add desiccant packs

- Add humidity cards and seal bag

- Add humidity cards and seal bag

DBS sample storage.

- Keep packaged DBS (in sealable plastic bags) cool and dry until transported
- Avoid leaving it in a vehicle, as sun and heat will deteriorate DBS
- Avoid placing spots in an malfunctioning refrigerator where water may drip on or soak the spots

Errors invalidating DBS samples

1. Insufficient blood quantity

    - Removing filter paper before blood has completely filled circle or before blood has soaked through to the other side
    - Applying blood to filter paper with a capillary tube
    - Filter paper coming in contact with gloved or ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.

2. Scratched or abraded specimen

    - This may have been caused by applying blood with a capillary tube or other device.

3. Mailing specimen while still wet, DBS must dry a minimum of 4 hours before packaging and shipping.
4. Clotted or layered specimen The volume of specimen will not be uniform between spots resulting in errors during the testing process.

    - Touching the same circle on the filter paper to blood drop several times
    - Filling circle on both sides of filter paper

5. Haemolysed, discoloured, or contaminated specimen

    - Squeezing or “milking” of area surrounding the puncture site
    - Allowing filter paper to come in contact with glove or ungloved hands or substances either before or after blood collection
    - Exposing blood spots to direct heat

6. Specimen exhibiting serum rings (serum becomes separate from cells).

    - Not allowing alcohol to dry at puncture site before making skin puncture
    - Allowing filter paper to come in contact with alcohol, hand lotion, etc.
    - Squeezing area surrounding puncture site excessively
    - Drying specimen improperly
    - Applying blood to filter paper with a capillary tube

Appendix IV: ELISA screening for Measles IgG Antibodies

This was done using Measles IgG ELISA kits from NOVA TECIMMUNODIAGNOSTICA GMBH NovaLisa^{TM}from Germany.

Principle

The qualitative immunoenzymatic determination of IgG-class antibodies against Measles is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with Measles antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured Measles-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Measles specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Materials and Equipment

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

Technique

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.

2. Cover wells with the foil supplied in the kit.

3. Incubate for 1 hour ± 5 min at 37±1°C.

4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

5. Dispense 100µl Measles anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.

6. Incubate for 30 min at room temperature. *Do not expose to direct sunlight.*

7. Repeat step 4.

8. Dispense 100µl TMB Substrate Solution into all wells

9. Incubate for exactly 15 min at room temperature in the dark.

10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Any blue colour developed during the incubation turns into yellow.

Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of

the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.

11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

Interpretation of Results

Results are given by multiplying patient mean absorbance value by ten (10), then dividing this figure with the cut off and expressed as Nova Tec Units (NTUs). Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off. Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative (**GREY ZONE**). Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

Table 6.1: Comparison of kit results with the 3rd International standard.

Result aspect	**NTU**	**IU/ml (3rd International Standard)**
Positive	> 11	> 220
Equivocal	9 - 11	120 - 220
Negative	<9	< 120
Cut off	10	-

Appendix V: Viral Antigen Neutralization Protocol

Viral antigen preparation

Vaccine strain of measles virus (*Edmonstonzagreb* strain) from one of the end user of the cold-chain system, Msambweni Referral Hospital in Kwale County. This was sub-cultured to increase virus titre. Done through culturing of virus suspension in vero cells, once cytopathic effects (CPE) appeared, infected cells were frozen then thawed once and contents centrifuged.

Virus-containing supernatant was then collected into vials and kept frozen at -70^0c. Virus titres were then determined by measuring the tissue culture infectious dose 50($TCID_{50}$). This was then adjusted to obtain a virus suspension of approximately 1000 $TCID_{50}$ per milliliter.

Serum neutralization assay

ELISA borderline samples, four randomly selected positive and negative samples were used for this procedure. To do the test, Vero cells were grown in a 96– flat bottomed well, cell culture microtitre plates.

Sera heated at 56^0C for 30 minutes to inactivate non-specific inhibitors was double diluted serially from 1:10 to 1:1280. Volumes of 50 µl of each serum dilution were mixed with 50 µl of 1000 $TCID_{50}$ viral antigens and incubated at 37^0c for 1 hour.

Subsequently, 50 µl of serum-virus mixture was put in the first 2 rows as test samples, 50 µl of tissue culture put in the 3^{rd} and 4^{th} rows as negative controls, and 50 µl of viral antigen suspension only was put in the 5^{th} and 6^{th} rows as positive controls.

Preparations were then incubated at 37^0c in presence of 5% carbon dioxide (CO_2) until the time when the positive control was showing CPE while the negative controls showing none. Residual infectivity indicated by the observation of CPE in the test specimen would indicate the absence of neutralizing antibody. The antibody titre in the specimen was determined as the highest dilution that showed no CPE, and this was used as the neutralization titre.

Sera samples with titres below 1: 120 were regarded as not protected, while those with 1:120 and above were declared protected. Chen and others in their work 'Measles Antibody: reevaluation of protective titres' in 1990 concluded that a titre of more than 1:120 was required to protect from classical measles disease.

Materials:

• Pipette aid

• Micropipettor: 0-100 µl,

• Sterile micropipette tips: 0-200 µl,

• Bio safety cabinet (laminar flow hood)

• T flask tissue culture bottle

• Incubator: 37^0C, 5% CO_2

• Autoclave

• Inverted microscope

• Sterile dilution tubes: 10 X 100 mm, capped

• Water bath: 37 ^{0}C, 56 ^{0}C

• Light box

• Vortex mixer

Reagents:

• EMEM

• Fetal Bovine Serum (FBS)

• Penicillin/Streptomycin

• L-Glutamine

• Vero Cells

• Sodium Bicarbonate

• HEPES

• Non-essential Amino Acids

Technique

Virus antigen preparation

1. Seed tissue culture wells at a density of cells which will be confluent on the day of virus assay one day earlier.
2. Make serial dilutions of virus suspension in tissue culture maintenance medium. Prepare 10 folds dilution of the given virus starting with 10^{-1} to 10^{-9}
3. Remove tissue culture growth medium from healthy confluent monolayer and replace with appropriate dilution of virus. 0.1 ml in the well of a 96-well plate.

   Set up 4 wells per virus dilution.

4. With sterile technique, inoculate 0.1 ml of each virus dilution into the tissue culture tube beginning at the most dilute (10^{-9}) and working toward the most concentrated level of virus. The same pipette can be used to inoculate several dilutions.
5. Set up 2 control wells which contain diluent alone, i.e.no virus.
6. Incubate at 37^{0} C in 5% Carbondioxide and monitor the development of CPE. Record CPE after the 2^{nd} day up to the 10^{th} day, having observed the cell control wells first.
7. Grade CPE on a 0-4 system;
   a. 0 (no CPE)
   b. 1 (less than 50% of cells showing CPE)
   c. 2 (about 50% of cells showing CPE)

d. 3 (about 75% of cells showing CPE)
e. 4 (the monolayer is totally destroyed or shows 100% CPE).

8. Calculate the $TCID_{50}$ counting all the wells with 1- 4 CPE as being positive.
9. Adjust the titre to $100TCID_{50}$ per 0.1 ml. ($1000TCID_{50}$ per millilitre)

Neutralization Test (Inhibition of CPEs)

1. Determine virus titer as illustrated above
2. Prepare serial serum double dilutions from I:10 to 1:1280
3. Add 50µl volume of the constant virus dilution containing 1000 $TCID_{50}$ per ml to 50µl of each serum dilution in separate labelled tubes.
4. Allow the virus-serum mixture and the virus control of serial 10-fold dilutions to remain at room temperature for 1 hour.
5. Inoculate 50µl of the virus-serum mixture each into 2 culture wells accross.
6. For the control, inoculate 25µl of each virus dilution into a set of cultures, 2 wells per dilution.
7. For negative control put 50µl of diluent across 2 wells
8. Check all wells for CPE under inverted microscope at 3, 5 and 7 days post inoculation.
9. Record the highest serum dilution that prevented CPEs as the neutralization titre.

Appendix VI: Other Tables

Analysis of vaccination in the various demographic characteristic group was done to explore the rates of vaccination in each category including the 95% confidence limits and the chances of an individual not being vaccinated in each demographic category group (Table 6.2).

The analysis of measles seroprotectivity in the various demographic and vaccination history groups was done to explore the possibility of them increasing or decreasing the chances of participant being protected against measles disease in that particular category (Table 6.3).

Table 6.2: Multivariate analysis of the potential of being vaccinated among study participants.

Attribute	Examined	Vaccinated	Prevalence	95% CI limits		Chi square	P value	Odds	95% CI limits	
	n	n	%	lower	upper				lower	upper
Gender of kid										
Female	202	195	96.50%	94.81	98.19	1.077	0.299	0.523	0.151	1.815
Male	217	213	98.20%	96.98	99.42					
County										
Kwale	181	178	98.30%	97.11	99.49	1.174	0.556	2.158	0.351	13.244
Narok	181	175	96.70%	95.05	98.35			1.061	0.208	5.406
Lamu	57	55	96.50%	94.81	98.19			R		
Presence of vaccination card										
No	74	67	90.50%	87.80	93.20	16.42	0.000	0.112	0.032	0.394
Yes	345	341	98.80%	97.80	99.80					
Awareness about vaccines										
No	26	23	88.50%	85.56	91.44	8.615	0.003	0.159	0.04	0.641
Yes	393	385	98.00%	96.71	99.29					
Awareness about measles disease										
No	50	46	92.00%	89.50	94.50	6.416	0.011	0.222	0.063	0.789
yes	369	362	98.10%	96.84	99.36					
Awareness about measles outbreaks										
No	366	356	97.30%	95.81	98.79	0.129	0.719	0.685	0.086	5.459
Yes	53	52	98.10%	96.84	99.36					
Residency										
Permanent	371	360	97.00%	95.43	98.57	1.462	0.227	0.97	0.953	0.988
Moved in	48	48	100.00%	100.00	100.00					
History of Measles infection										
Yes	12	8	66.70%	62.36	71.04	41.57	0.000	0.038	0.009	0.157
No	374	367	98.10%	96.84	99.36					

Table 6.3: Multivariate analysis of seroprotectivity in relation to sociodemographic attributes and vaccination history of the study participants.

Attribute	Examined (n)	Protected (n)	Prevalence (%)	95% CI limits Lower	Upper	Chi-square	P value	Odds Ratio	95% CI limits Lower	upper
Sex										
Female	220	191	86.8	83.7	89.9	0.14	0.709	1.112	0.638	1.937
Male	233	205	88	85	91					
Presence of Vaccination card										
No	107	76	71	66.8	75.2	34.21	0.000	5.02	2.816	8.949
yes	346	320	92.5	90.1	94.9					
Residency										
Permanent	405	355	87.7	84.7	90.7	0.195	0.659	0.825	0.351	1.939
Moved in	48	41	85.4	82.1	88.7					
Awareness about measles										
No	67	50	74.6	70.6	78.6	11.694	0.001	2.941	1.55	5.579
Yes	386	346	89.6	86.8	92.4					
Awareness about vaccines										
No	40	33	82.5	79	86	0.964	0.326	1.54	0.647	3.667
Yes	413	363	87.9	84.9	90.9					
Awareness about measles outbreaks										
No	400	354	88.5	85.6	91.4	3.644	0.056	0.496	0.239	1.031
yes	53	42	79.2	75.5	82.9					
Vaccination status										
Vaccinated	408	362	88.7	85.8	91.6	18.45	0.000	0.106	0.031	0.361
Not vaccinated	11	5	45.5	40.9	50.1					
Vaccination times										
Once	354	313	88.4	85.5	91.3	0.725	0.696	-	-	-
Twice	49	44	89.8	87	92.6					
Thrice	5	5	100	100	100					
Vaccination frequency										
Once	354	313	88.4	85.5	91.3	0.253	0.615	1.284	0.484	3.407
More than once	54	49	90.7	88	93.4					
Guardian										
Parents	399	350	87.7	84.7	90.7	0.278	0.598	0.805	0.359	1.806
Others	54	46	85.2	81.9	88.5					
Source of information										
Health workers	337	298	88.4	85.5	91.4	0.49	0.484	0.773	0.376	1.59
Others	76	65	85.5	82.3	88.8					
History of measles disease										
Unknown	54	44	81.5	77.9	85.1	3.659	0.160	-		
No	386	339	87.8	84.8	90.8					
Yes	13	13	100.0	100	100					

Appendix VII: Swahili version of informed consent form

a. **Kurasa ya Maelezo**

Kichwa cha Utafiti: Kutathmini uwezo wa kujikinga na Ukambi kwa watoto katiya wiki 9 hadi 59 katika vituo vya afya kaunti za Kwale, Narok na Lamu ya Kenya, 2014.

Inkisiri/Utangulizi

Utafiti huu unafanywa\na Bw. Ali Juma Kanga wa chuo kikuu cha kilimo na teknolojia cha Jomo Kenyatta ili kutathmini idadi ya watoto wenye umri katiya wiki 9 hadi 59 ambao wanaweza kuzuia ambukizi la virusi vya ukambi.

Utaratibu

Itakubidi utupe kibali cha kumtowatakriban milimita 2 za damu ya mtoto wako. Sampuli hii itatumiwa kufanyia utafiti wa kiwango cha uwezo wa mtoto wako kujikinga na ukambi. Matokeo ya utafiti yatatolewa Kama ripoti na bila malipo.

Pia utaulizwa kujaza hoja hi fupi au utasomewa hoja ji hiyo na majibu yako yatanakiliwa. Maswali mengi yatahusu mazingira yako na welevu wa kokuhusu chanjo ya virusi vya Ukambi.

Athari/ Kutoridhika

Ipoatharikidogoyakushirikikatikahilizoezi. Hatahivyo,unawezakutoridhikakihali au kuonausumbufuwakatiwakutolewakwasampuli.

Faida

Haitokuwepofaidayamojakwamoja.
Hatahivyo,kamakutokuweponahajayakusaidikajamiikuambatananakingahizi za ukambibasichanjodhidiyaukambitutazipendekeza. Zaidi yahayo,

maelezoniliyoyapatakutokananautafitihuuutatumikakatikakuondshatatizohilisugunchi ni Kenya kabisa.

Usiri

Maelezo yote yatakayopatikanayatabakiakuwasirinazitatolewakamataarifu za kikundibilakuwekawazinimaelezoyananikhususan. Maelezo yote, zikiwemo pia hojajizitahifadhiwasehemumaalumsalamana wale wanaohusikanautafitipekeendiyowatakaozitumiatenakwazoezilengwa.
Baadayakukamilishazoezihili, sampulizote za ziadazitatupiliwambalinahojaji pia kuharibiwa.

Kushiriki

Kushirikinikwakujitoleana una hakiyakukataakushiriki au kujitoakwenyeutafitihuu.

Maswalikuhusuutafitihuu:

Iwaponaswalilolotekuhusiananautafitihuu, unawezakuwasilianao:

Ali J. Kanga

S.L.P. 90791- 80100

Mombasa.

Rununu/ Rukono: 0721 545650

Mtandao: akanga74@gmail.com

AU,

Katibu, Hospitali ya kitaifaya Kenyatta/Chuo kikuu cha Nairobi- ERC

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Nairobi.

Simu: 726300-9

Mtandao: uonknh_erc@uonbi.ac.ke

b. Cheti cha Mashauriano.

Nimeusoma ujumbe huu, au nimesomewa. Pia nilipe wanafasi ya kuuliza maswali na kila swali nililouliza nimejibiwa nikaridhika.

Nimekubali bila kushurutishwa na nimemruhusu mtoto wangu kushiriki katika somohili.

Unakata shauri imakushiriki au kutoshiriki katika zoezi hili. Sahihi yako itamaanisha umekubali kushiriki, baadayakuelewamaelezoyaliyotangulia.

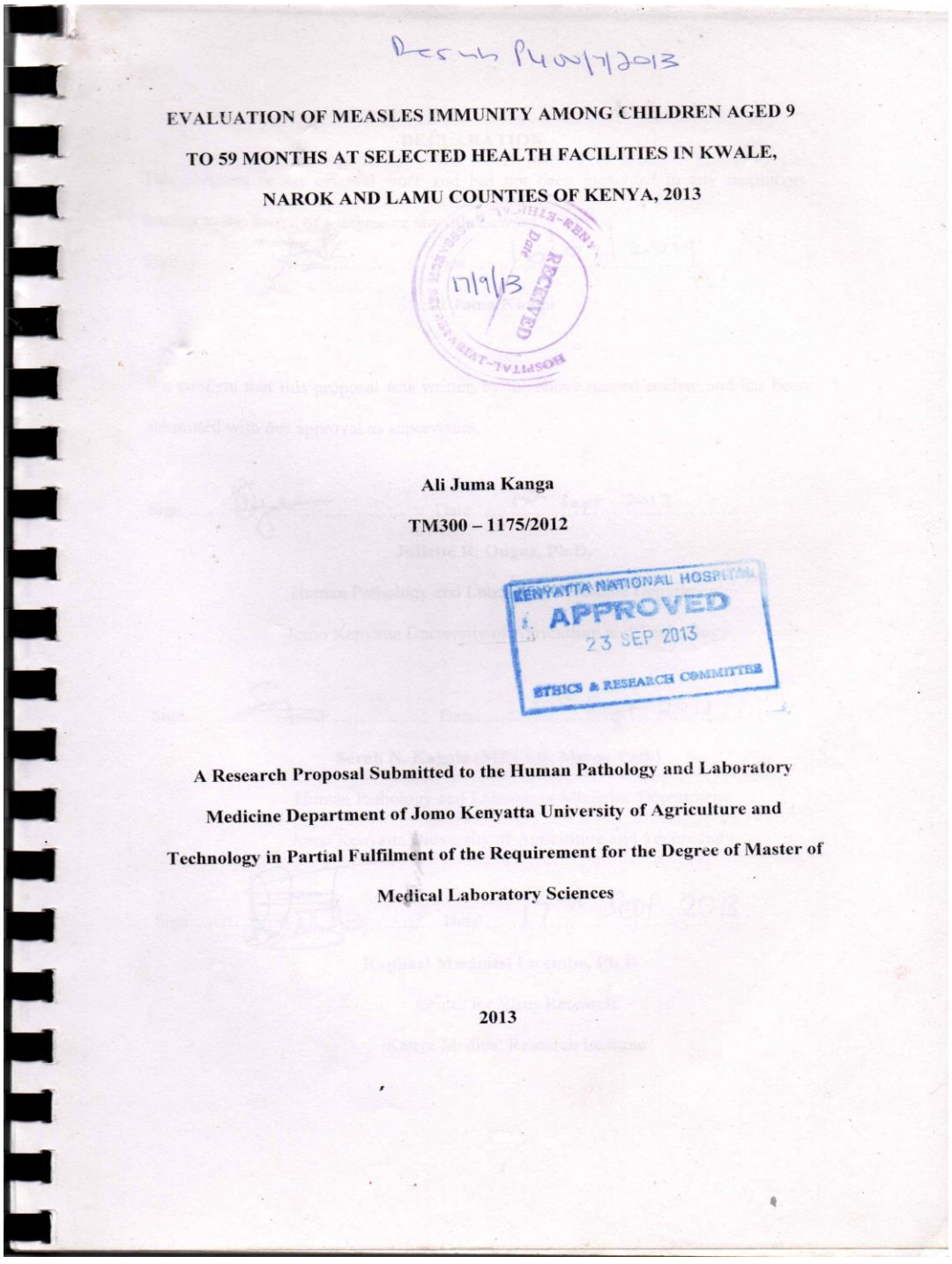
Sahihi……. Tarehe…….

Muda/Wakati……. Uhusianonamuhusika……

Nimesoma kwa ufasaha au nimeshuhudia usomaji wa fomu hii ya kukata-shauri kwamshiriki, na amepata muda mzuri wa kuuliza maswali. Na thibitisha kuwa amekubali kwa uamuzi wake mwenyewe.

Sahihi ya shahidi……. Sahihi ya mchunguzaji…….....

Appendix VIII: Ethics review authorisation



Desk Pkm/7/2013

EVALUATION OF MEASLES IMMUNITY AMONG CHILDREN AGED 9 TO 59 MONTHS AT SELECTED HEALTH FACILITIES IN KWALE, NAROK AND LAMU COUNTIES OF KENYA, 2013

RECEIVED Date 17/9/13

Ali Juma Kanga

TM300 – 1175/2012

KENYATTA NATIONAL HOSPITAL APPROVED 23 SEP 2013 ETHICS & RESEARCH COMMITTEE

A Research Proposal Submitted to the Human Pathology and Laboratory Medicine Department of Jomo Kenyatta University of Agriculture and Technology in Partial Fulfilment of the Requirement for the Degree of Master of Medical Laboratory Sciences

2013