SEROPREVALENCE AND MOLECULAR IDENTIFICATION OF MEASLES VIRUS IN CHILDREN (0-8 M0NTHS) PRESENTING WITH MEASLES-LIKE SYMPTOMS IN SOME HOSPITALS IN KADUNA STATE, NIGERIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE DEGREE IN MICROBIOLOGY

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FEBRUARY, 2016

DECLARATION

I hereby declare that the work in this thesis entitled "**SEROPREVALENCE AND MOLECULAR IDENTIFICATION OF MEASLES VIRUS IN CHILDREN (0-8 MONTHS) PRESENTING WITH MEASLES-LIKE SYMPTOMS IN SOME HOSPITALS IN KADUNA STATE, NIGERIA"** has been performed by me in the Department of Microbiology under the supervision of Dr. E.E Ella and Prof. J.B Ameh. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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…… ……... ……..….

Name of Student Signature Date

CERTIFICATION

This thesis entitled "**SEROPREVALENCE AND MOLECULAR IDENTIFICATION OF MEASLES VIRUS IN CHILDREN (0-8 MONTHS) PRESENTING WITH MEASLES-LIKE SYMPTOMS IN SOME HOSPITALS IN KADUNA STATE, NIGERIA"** by Olaitan, Ayorinde Elizabeth meets the regulations governing the award of the degree of Master of Science in the Department of Microbiology, Faculty of Science, Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, without whom I am nothing and to my beloved parents, Barr. & Dcns. Kehinde Olaitan for their unending love, care and support.

ACKNOWLEDGEMENTS

Special thanks to God Almighty for His faithfulness over my life and for making this project a huge success, against all odds.

I am highly indebted to my supervisor, Dr. E.E Ella for his guidance and tireless supervision, as well as constant support all through the course of this work. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. You have been a tremendous mentor for me. My appreciation also goes to Prof. J.B Ameh for giving me the necessary co-operation and encouragement needed in completing this project. I wish to appreciate the Head of Department of Microbiology, Prof. S.A Ado, the Postgraduate coordinator, Dr D.A Machido and the entire staff of Microbiology department for their tremendous support and contribution towards the success of this work. God bless you all.

My profound gratitude goes to my family. Words alone cannot express how grateful I am to my parents, Barr. & Dcns Kehinde Olaitan and my parents-in-law, Engr & Mrs G.T Olatoye for all of the sacrifices that they've made on my behalf. Your prayer for me was what sustained me thus far. I am thankful to all my siblings for their love and support and for being a part of my dream. May God bless you all and satisfy you with long life.

My deepest appreciation goes to the man God has given me as husband- a brother, a friend, a father, a soulmate and the father of my kids, Mr Olatoye Oluwafemi George. I may not have completed this study without your constant prayers, love, support and words of encouragement. Thank you for standing strong by me and for being a part of my life. I will always love you, Sweerie.

Furthermore, I would like to acknowledge with much appreciation the crucial roles of Dr Razak Aminu, Mr. Salau Ndochi and Mr Jonathan of Hajia Gambo Sawaba General Hospital, as well as Mr John Magani, Paediatric Laboratory Scientist of Yusuf Dantsoho Memorial Hospital and the entire staff of Gwamna-Awan Hospital, Kaduna. I will also like to thank Dr Y.Y of Vertenary Biotechnology Lab, ABU and the laboratory staff of Microbiology Department. Their roles in this study cannot be over-emphasized.

Let me use this opportunity to express my gratitude to everyone who supported me throughout the course of this study. I am grateful to my friends and colleagues- Chioma, Taiwo, Ben, Edoma, Fatima, Aisha, Amaka, Chinelo, Matthew, to mention but a few, for their inspiring guidance, invaluably constructive criticism and friendly advice during the project work. I appreciate the families of Dr & Mrs Adedoyin, Mr & Mrs Olanrewaju Dauda and Mr & Mrs Emmanuel Mhyas for providing me with a home away from home. God bless you all. I love you all.

ABSTRACT

This study determined the seroprevalence and molecular identification of Measles virus in children (0-8 months) presenting with measles-like symptoms in some hospitals in Kaduna state. A total of 273 blood samples were collected and analyzed for measles virus IgM using ELISA. An overall prevalence of 21.2% (58/273) was obtained. A prevalence of 6.5% (13/200) was obtained in children 0-8 months and 61.6% in children 9-23 months. The seroprevalence of measles virus increased with age, with a higher prevalence (61.6%) recorded in children >8 months. Higher prevalence was found in female children (27.5%) than male children (16.3%) and this difference was statistically significant, showing an association between Measles virus infection and sex ($\chi^2 = 5.006$, df = 1, P = 0.025, OR = 1.942). There was no statistically significant association with the level of parent's education ($\chi^2 = 1.480$, df = 3, P = 0.687), parent's occupation ($\chi^2 = 3.146$, df = 2, P = 0.207) and the number of children in the family ($\chi^2 = 0.891$, df = 3, P = 0.828). With respect to children's vaccination status and breastfeeding, there was significant association (P = 0.031, P = 0.00) respectively. The marital type of family, place of residence and household size showed no significant association with the prevalence of measles virus. However, significant association was observed in relation to mothers' measles history ($\chi^2 = 7.989$, df = 1, P = 0.005, OR = 2.535) and mothers' vaccination status ($\chi^2 = 3.873$, df = 1, P = 0.049, O.R = 1.791). There was significant association between MV infection and all the symptoms presented, except for vomiting, which showed no significant association ($\chi^2 = 3.748$, df = 1, P = 0.053). There was no significant association (P > 0.05) between MV infection and malaria, typhoid and pneumonia which are common childhood-illnesses with measles-like symptoms. This study recognized age, sex, vaccination, breastfeeding, mother's vaccination status and mother's measles history as important demographic and risk factors of measles virus infection in children.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AUG	Adenine Uracil Guanine
Bp	Basepair
C	Capsid protein
CD4	Cluster of Differentiation 4
CD46	Cluster of Differentiation 46
cDNA	Complementary DNA
CNS	Central Nervous System
CPE	Cytopathic Effects
CSF	Cerebrospinal Fluid
CUU	Cytosine Uracil Uracil
DC	Dendritic cells
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
EDTA	Ethylene Diamine Tetraacetic Acid
ER	Endoplasmic Reticulum
F	Fusion protein
GAH	Gwamna-Awan Hospital
H	Hemagglutinin protein
HGSGH	Hajia Gambo Sawaba General Hospital
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IFN	Interferon
IgA	Immunoglobulin A
IgG	Immunoglobulin G

IgM	Immunoglobulin M
IL	Interleukin
KMV	Killed-virus Measles Vaccine
L	Large protein
M	Matrix protein
MMR	Measles Mumps Rubella
mRNA	Messenger RNA
MV	Measles Virus
MVF1	Measles Virus Forward primer 1
MVF2	Measles Virus Forward primer 2
MVR1	Measles Virus Reverse primer 1
N	Nucleocapsid
OR	Odds Ratio
ORF	Open Reading Frames
P	Phosphoprotein
PCR	Polymerase Chain Reaction
PIE	Postinfectious encephalomyelitis
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
SLAM	Signalling Lymphocyte Activation Molecule
SSPE	Subacute Sclerosing Panencephalitis
STAT	Signal Transducers and Activators of Transcription
UNICEF	United Nations Children's Fund
V	Vcore protein
VZV	Varicella Zoster Virus

WHO	World Health Organisation
YDMH	Yusuf Dantsoho Memorial Hospital

CHAPTER ONE

1.0 INTRODUCTION

Measles, also known as rubeola, is an infection of the respiratory system caused by Measles virus. Measles virus belongs to the order *Mononegavirales,* the genus *Morbillivirus* in the family *Paramyxoviridae* and is most closely related to Rinderpest virus (RPV), which is a pathogen of cattle (Furuse *et al.*, 2010). Measles virus is thought to have evolved in an environment where cattle and humans lived in close proximity (Furuse *et al.*, 2010). Measles viruses are spherical, enveloped, single-stranded, negative-sense RNA viruses (Perry *et al.*, 2004). Presently, there are 24 genotypes and only one serotype of the measles virus which affect humans. The World Health Organization (WHO, 2005) has recognized eight clades (clades are used to indicate the relationship between the various genotypes of the virus) of measles virus (designated A–H) that encompass 24 genotypes (Rota *et al*., 2009). In the central and western parts of sub-saharan Africa, mainly clade B viruses have been identified (Kouomou *et al*., 2002). The most common genotype is B3, with its two clusters B3.1 and B3.2. The first B3 sequences in Africa were reported from Nigeria (Hanses *et al*., 1999).

Measles is transmitted primarily from person-to-person by large respiratory droplets, but can also be spread by aerosolized droplets (Perry, 2004). The virus is spread primarily via coughing and sneezing, close personal contact or direct contact with nasal or throat secretions from infected persons. It can be transmitted by an infected person from four days prior to the onset of the rash to four days after the rash erupts. The virus

is extremely contagious; on the average, 90% of those exposed to infected person(s) can be infected unless they had been vaccinated, or have had measles before.

Development of immunity to this disease is either congenital, by vaccination or infection by wild type. Patients who survive a case of measles retain immunity to it for life (Nanan *et al*., 2000).

Measles is most infectious during the prodrome phase. The prodromal period begins with fever, malaise, cough, coryza and conjunctivitis. Koplik spots appear on the buccal mucosa one to two days before rash onset and may be observed for an additional one to two days after rash onset. The rash is an erythematous maculopapular eruption that usually appears 14 days after exposure, often causing itching. After three to four days, the rash fades. Other constitutional signs and symptoms such as anorexia, diarrhoea and generalized lymphadenopathy may also be present. In severe cases, desquamation may occur. In developed countries, the most commonly cited complications associated with measles infection are otitis media, pneumonia, post-infection encephalitis, subacute sclerosing panencephalitis (SSPE) and corneal ulceration (leading to corneal scarring). The risks of serious complications and death are increased in young children and adults but may be more severe in adults (Sension *et al*., 1988).

Measles occurs worldwide and it is still a significant cause of childhood morbidity and mortality despite the existence of effective vaccine. It is one of the typical viral diseases of childhood. However, unlike other common viral diseases i.e. varicella-zoster virus, rubella, mumps, and parvovirus infections, measles often leads to severe complications that may be fatal. In the third world, there may be up to 900,000 measles related deaths per year. Therefore, there is a lot of pressure on health in different countries in controlling the disease through vaccination.

Measles is endemic in Nigeria. It is a highly infectious immunizable disease with potential for eradication but is still responsible for high mortality among children, particularly in developing nations. Measles virus infects approximately 30 million people annually with a mortality of 197,000, mainly in developing countries with inadequate vaccination and medical care, such as Nigeria (Adeboye *et al.*, 2011). Prior to wide spread vaccination, a staggering number of measles-related deaths (up to 95%) occured in developing countries (Adetunji *et al*., 2007).

After an effective measles vaccine was introduced in 1963, the incidence of measles decreased significantly (WHO, 2006). Significant progress has been made during the last decade, with the elimination of measles from the Americas and the dramatic reduction in measles mortality worldwide (Rota *et al*., 2009). Vaccination coverage for measles in Nigeria according to WHO/UNICEF is currently put at 62%. The National Program on Immunization (NPI) in Nigeria stipulates that children be vaccinated against measles by a single dose of injection at nine months. This is because children below this age are believed to possess passively acquired maternal antibodies which protect them against the virus. However, in developing countries where measles is highly endemic, WHO recommends two doses of the vaccine to be given at six and nine months of age. The vaccine should be given irrespective of the HIV status of the child (Helfand *et al*., 2008).

However, measles continues to be endemic in many developing countries and some industrialized countries, with its greatest incidence in children below two years of age in the developing countries (Papania and Orenstein, 2004; Adetunji *et al*., 2007).

1.1 Statement of the Research Problem

Despite the development of an efficient combined vaccine (measles–mumps–rubella), measles remains the leading cause of vaccine-preventable childhood mortality in developing countries and a cause of continuous outbreaks in industrialized countries (WHO, 2009).

Currently, Nigeria is one of the 47 countries in the world where the burden of measles is highest and where it is still responsible for the highest number of vaccine preventable deaths. The current national measles vaccination coverage is put at 62%, with a very wide variation in the country that has once achieved coverage of 80% with routine immunization (Adeboye *et al*., 2011).

In many hospitals today, it is common to see children below nine months with measles-like symptoms, sometimes requiring hospitalisation. Some go mild and unnoticed, while others are wrongly diagnosed as having other childhood-related illnesses. These children are never diagnosed as having measles because it is assumed that maternal antibodies against the virus persist in these children for one year after birth (Perry, 2004). However, measles has been reported in children below nine months in some countries of the world, including Nigeria.

Based on a survey of disease prevalence among children of ages zero to five years in Akwa Ibom state, South-South Nigeria, the prevalence rate of measles was estimated to be 7%, including infants below one year (Chukwuemeka and Hycienth 2013). A study carried out in Maiduguri, Borno State by Ahmadu *et al*. (2013), reported the prevalence of measles in children below nine months of age to be 6%. A prevalence of 8% was equally reported in Bida, Niger state (Adeboye *et al*., 2011).

Low and waning maternal antibody protection, which results in an increased period of susceptibility to natural infection before vaccination eligibility, typically at nine months of age, has been documented in various developing countries (Oyedele *et al*., 2005; Ntshoe *et al*., 2013).

1.2 Justification of the study

Measles continues to be a major childhood disease in terms of global morbidity and mortality. It remains the fifth leading cause of death among young children globally, despite the availability of a safe vaccine. Globally, an estimated 139,300 people died from measles in 2010 – mostly children under the age of five (WHO, 2011). Unvaccinated young children are at highest risk of measles, its complications and death. Children with immunodeficiency due to HIV or AIDS, leukemia, alkylating agent, or corticosteroid therapy, children with immune disorder and children who lose passive maternal antibodies before the age of immunization, could therefore be at risk of the disease (Sension *et al*., 1988).

There is a paucity of information on the prevalence of measles in children (0 to 8months) in Nigeria. The studies carried out in some parts of Nigeria are retrospective and hospital-based (Bassey *et al*., 2013; Ahmadu *et al*., 2013), with very little work done on the molecular identification of the measles virus. There is therefore the need to carry out a survey of children (0-8 months) with measles-like symptoms in order to know if the symptoms are associated with measles or not. Furthermore, the study will contribute towards the identification of the measles virus in Kaduna state.

1.3 Aim

The aim of this study is to determine the seroprevalence and molecular identification of measles virus in children (0 to 8 months) presenting with measles-like symptoms visiting some hospitals in Kaduna State, Nigeria.

1.4 Objectives

1. To determine the presence of measles antibody (IgM) in children (0 to 8 months) using the Enzyme Linked Immunosorbent Assay (ELISA) technique.
2. To confirm the presence of the measles virus using reverse transcription-polymerase chain reaction (RT-PCR).
3. To determine the risk factors associated with measles in children using structured questionnaires.
4. To determine the relationship between measles and other childhood illnesses with measles-like symptoms using structured questionnaires.
5. To review the recommended age of measles vaccination, based on the ELISA results obtained to know if the vaccine has to be administered earlier than nine months in this part of the world.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Background

Measles is most closely related to Rinderpest virus (RPV), which is a pathogen of cattle (Furuse *et al.*, 2010). Measles virus is thought to have evolved in an environment where cattle and humans lived in close proximity (Furuse *et al*., 2010).

The Antonine Plague, also known as the Plague of Galen which occurred between 165–180 AD, described the disease as probably smallpox or measles. The disease killed as many as one-third of the population in some areas, and decimated the Roman army (Norrie, 2007). Estimates of the timing of evolution of measles seem to suggest this plague was something other than measles. The first scientific description of measles and its distinction from smallpox and chickenpox is credited to the Persian physician, Rhazes (860–932), who published *The Book of Smallpox and Measles* (Harminder *et al.,* 2008). Given what is now known about the evolution of measles, this account is remarkably timely.

Measles is an endemic disease, meaning it has been continually present in a community, and many people develop resistance. In populations not exposed to measles, exposure to a new disease can be devastating. In 1529, a measles outbreak in Cuba killed two-thirds of the natives who had previously survived smallpox. Two years later, measles was responsible for the deaths of half the population of Honduras, and had ravaged Mexico, Central America, and the Inca civilization (Byrne, 2008).

In roughly the last 150 years, measles has been estimated to have killed about 200 million people worldwide (Torrey and Yolken, 2005). During the 1850's, measles killed a fifth of Hawaii's people. In 1875, measles killed over 40,000 Fijians, approximately one-third of the population. In the 19th century, the disease decimated the Andamanese population. In 1954, the virus causing the disease was isolated from an 11-year old boy from the United States, David Edmonston, and adapted and propagated on chick embryo tissue culture (Griffin, 2007). To date, 21 strains of the measles virus have been identified (Rima *et al*., 1995). While at Merck, Maurice Hilleman developed the first successful vaccine (Offit, 2007). Licensed vaccines to prevent the disease became available in 1963.

2.2 Virology of the Measles virus

Measles virus is the prototypic member of the *Morbillivirus* genus of the family *Paramyxoviridae*. The viral genomic RNA is single-stranded, non-segmented, and of negative polarity and encodes six major structural proteins. The two viral transmembrane glycoproteins, the hemagglutinin and fusion proteins, are both required for virus-host cell membrane fusion, while attachment to host cells is mediated by the hemagglutinin. The human CD46 molecule has been identified as a cellular receptor for measles virus. Antibodies raised against either viral glycoprotein neutralize measles virus in vitro and protect against infection. Although measles virus remains a single serotype (monotypic), nucleotide sequence analyses have identified distinct lineages among recent wild type isolates. These genetic changes were manifested by detectable antigenic variation between vaccine and wild type viruses and at some point may influence strategies for control, elimination, and eventual eradication of measles virus (Bellini and Rota, 1994).

2.2.1 Structure of the Measles virus

Measles virus, a member of the genus *Morbillivirus* in the family *Paramyxoviridae*, is an enveloped virus with a non-segmented, negative-strand RNA genome (Griffin, 2001). Measles virus has the structure of the family *Paramyxoviridae*, consisting of spherical, enveloped particles with a central helical nucleocapsid. The diameter of the pleomorphic particles varies between 120 and 250 nm. The nucleocapsid contains a monopartite, single-stranded, negative-sense RNA genome (molecular weight 7×10^6). It is surrounded by the nucleocapsid protein N and associated with the enzymatically active phosphoprotein P and the large protein L, both of which are involved in viral transcription and replication. The P gene also gives rise to nonstructural proteins C and V. The bilayered lipid envelope is partly of cellular origin with the matrix protein M inside and bears a fringe of spike-like projections containing the hemagglutination (H) and the hemolytic and cell fusion (F) activities. H virion infectivity is lost readily when the envelope is disrupted spontaneously and when the virus is treated with lipid solvents.

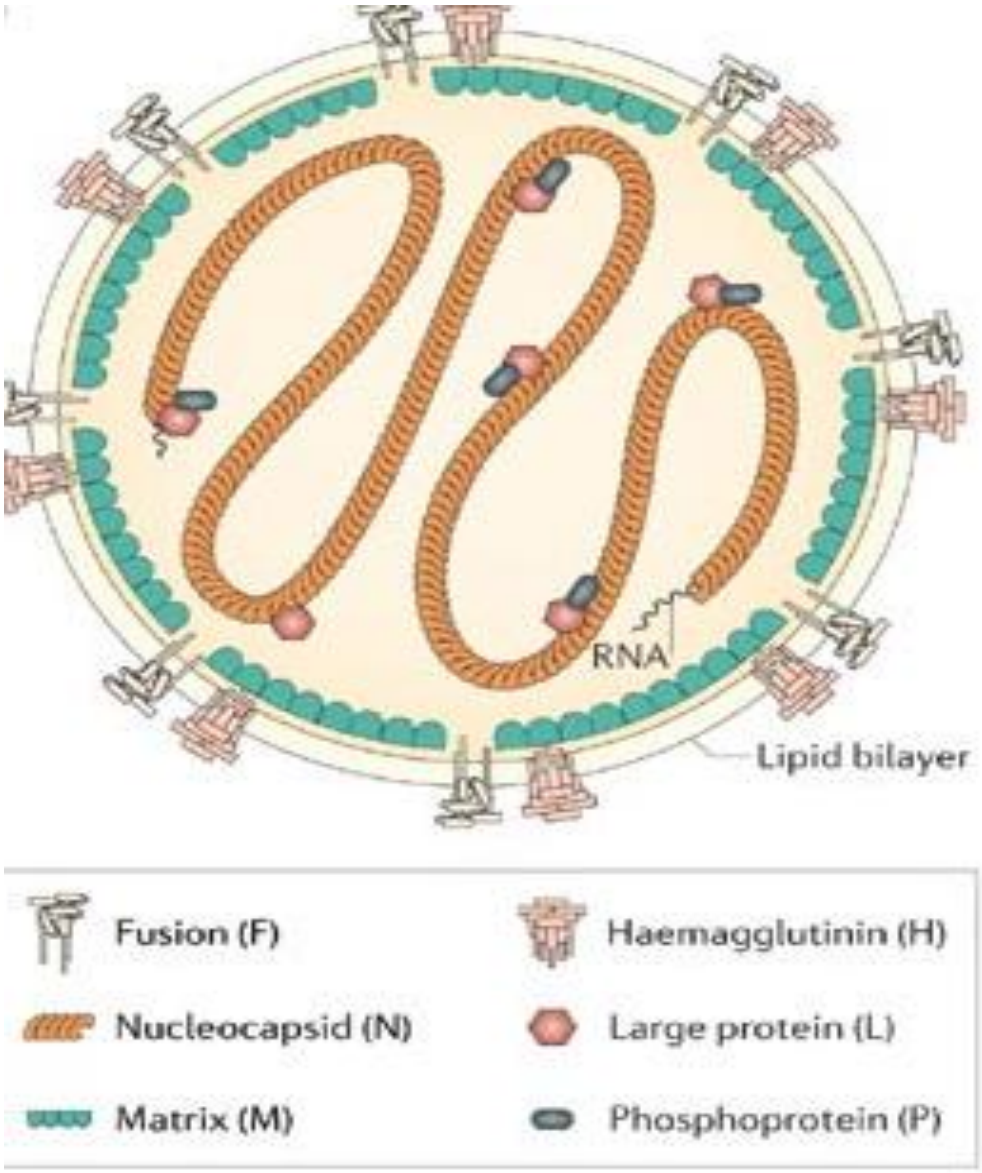


Fig. 2.1: Structure of the Measles virus

Source: (Moss and Griffin, 2006)

2.2.2 Genomic Organisation

The genetic material of paramyxoviruses consists of RNA of negative polarity (Lamb and Kolakofsky, 2001). This RNA is covered by the nucleocapsid protein N, the phosphoprotein P, which serves as an assembly as well as a polymerase cofactor, and the RNA polymerase L. The single-stranded genome contains 15,894-nucleotide genome. The nucleocapsid comprises 2,649 N protein, approximately 300 P protein, and about 20 to 50 L proteins, enclosed within a membrane envelope containing H and F glycoproteins (Rima, 2000). The 15,894-nucleotide MV genome is organized into six contiguous, non-overlapping transcription units that are separated by short untranslated regions and code for the six structural viral proteins in the order 3′-N-P-M-F-H-L-5′ (negative strand) (Griffin, 2001). In addition, the P gene encodes the two non-structural proteins C and V from overlapping open reading frames (ORFs), accessed by different mechanisms. Ribosomal choice during translational initiation results in translation of the C protein from an overlapping ORF downstream of the P AUG codon (Bellini *et al*., 1985). The V protein shares the N-terminal 231 amino acids with the P protein. However, the 68 C-terminal amino acids are translated from a different reading frame accessed by the co-transcriptional insertion of a non-templated G residue, a process referred to as mRNA editing (Cattaneo *et al*., 1989).

Nearly 11% of the 16-kb MV genome is composed of noncoding RNA. The six transcription units are preceded by a 3′ leader and are followed by a 5′ trailer. All cis-acting regulatory elements such as promoters and encapsidation signals are contained within the first 107 and the last 109 nucleotides of the genome (Sidhu *et al*., 1995). Each gene contains 5′ and 3′ noncoding regions with conserved start and end signals for transcription and polyadenylation (Kolakofsky *et al*., 2004).



3′ 5′

Fig. 2.2: Organization of the measles genome

Source: (Moss and Griffin, 2006)

2.2.3 Viral Proteins

The genome contains six genes that encode the nucleocapsid (N), phospho- (P), matrix (M), fusion (F), haemagglutinin (H) and large (L) proteins, respectively. The genomic RNA is encapsidated with the N protein and, together with RNA-dependent RNA polymerase composed of the L and P proteins, forms a ribonucleoprotein complex. The M protein that lines the inner surface of the envelope plays a role in virus budding (Cathomen *et al.*, 1998) and transcription regulation (Suryanarayana *et al.*, 1994). The P gene encodes additional proteins, the V and C proteins, by a process of RNA editing and by an alternative translational initiation in a different reading frame, respectively. The functions of the V and C proteins are not understood completely, but some of their functions are concerned with their activities as interferon (IFN) antagonists (Palosaari *et al.*, 2003). Measles virus has two envelope glycoproteins, the H and F proteins, which are responsible for receptor binding and membrane fusion, respectively. Measles virus enters a cell by pH-independent membrane fusion at the cell surface. Binding of the H protein to a cellular receptor is believed to induce the conformational change of the H protein, as well as that of the adjacent F protein. The hydrophobic fusion peptide inside the F protein is then exposed and inserted into the plasma membrane of the target cell. Further structural change of the F protein probably promotes the fusion of the viral envelope with the host-cell membrane. Upon infection of susceptible cells, measles virus causes cell–cell fusion, producing multinucleated giant cells, the typical cytopathic effect of measles virus infection.

2.2.3.1 Functions of the Measles virus proteins

The most abundant structural polypeptide, the nucleoprotein (N; 525 amino acids), is essential for packaging the genome into a ribonucleoprotein complex (RNP) that serves

as a template for transcription, replication and is packaged into progeny virions (Griffin *et al*, 2007). The amino-terminal region of the N protein (N_{CORE}; amino acids 1–400) forms the core of the helical nucleocapsid, whereas the carboxyl-terminal region ($N_{TAIL;}$ amino acids 401–525) constitutes a disordered domain located outside of the core (Longhi *et al*., 2003). This region is hypervariable among wild-type viruses, and the nucleotide sequence of this region is used for genotype assignation (Rota *et al*., 2009). The N_{TAIL} region interacts with the P protein and the M protein. Two leucine residues at amino acid positions 523 and 524 are required for the interaction with the M protein (Iwasaki *et al*., 2009). The N protein also interacts with multiple cellular proteins. The major inducible 70-kDa heat shock protein, hsp72, promotes MV RNA synthesis and virus propagation by interacting with the N_{TAIL} region. An asparagine residue at amino acid position 522 (Asn522) in the N_{TAIL} region is important for the interaction with hsp72 (Carsillo *et al*., 2006).

The phosphoprotein (P; 507 amino acids) is a subunit of the viral RNA polymerase and acts as a chaperone that interacts with and regulates the cellular localization of N protein and probably assists in nucleocapsid assembly (Sedlmeier *et al*., 1998). The P protein forms oligomers and binds the N protein and the polymerase protein. A domain in the amino-terminus of P is required for interactions with soluble N protein, whereas the unique carboxyl-terminus contains the self-assembly domain and binding sites for the nucleocapsid and the polymerase (Curran *et al*., 1995). The P, V (299 amino acids), and C (186 amino acids) proteins all play roles in antagonizing the host interferon (IFN) responses. The V protein blocks the Jak/STAT signaling pathway and counteracts cellular IFN signaling. It also blocks the MDA5-mediated IFN induction pathway. Direct interference with IFN signaling by the P and C proteins has also been reported (Fontana *et al*., 2008). The C protein controls the levels of viral RNA synthesis to

circumvent IFN induction. The V protein binds RNA and can also regulate viral RNA synthesis (Parks *et al*., 2006).

The matrix protein (M; 335 amino acids) lines the inner surface of the viral envelope and participates in virion maturation. It plays a crucial role in virus assembly by interacting with the cytoplasmic tails of the H and F proteins (Cathomen *et al*., 1998). It binds the nucleocapsid and negatively regulates transcription, presumably by sequestering nucleocapsids to the plasma membrane (Hirano *et al*., 1993).

The fusion protein (F) is translated as a precursor of 550 amino acids that is cleaved by cellular furin-like proteases into disulfide-linked F1 and F2 proteins. It is a cell surface-expressed, type I glycoprotein that mediates fusion with the host cell at neutral pH. Three N-linked glycosylation sites are located in the F2 protein at amino acids 29, 61, and 67. Amino acids 113–145, located at the new amino-terminus of F1, form the hydrophobic fusion peptide. This domain inserts into the membrane of the host cell during the fusion process (Samuel and Shai, 2001). A cytoplasmic tail of 33 amino acids at the carboxyl terminus of F1 interacts with the M protein and contains sorting signals for intracellular localization.

The hemagglutinin (H; 617 amino acids) protein is the second glycosylated surface-expressed protein. This type II glycoprotein mediates attachment to specific protein receptors on the host cell and is a required co-factor for fusion (Griffin, 2007). There are four N-linked glycosylation sites between amino acids 168 and 200, and some wild-type isolates are additionally glycosylated at amino acid 416. A cytoplasmic tail of 34 amino acids at the amino-terminus interacts with the M protein and contains sorting signals for intracellular localization (Moll *et al*., 2001). Two receptors have been identified for MV. HSLAM (human signaling lymphocyte activation molecule, CD150) is a common

receptor for all MV strains, whereas CD46 (membrane cofactor protein, MCP) functions as a receptor for vaccines and some laboratory-adapted strains, but not for wild-type strains (Dorig *et al*., 1993). HSLAM is only expressed on a subset of immune cells. All vaccine strains have acquired the ability to use CD46 as an alternative receptor during passage in various SLAM-negative cultured cells (Yanagi *et al*., 2009). The ubiquitous expression of CD46 explains the ability of vaccine strains to infect various human and monkey cell lines. Recent studies have revealed that another, presently unidentified receptor exists for wild-type MV on epithelial cells that form tight junctions (polarized epithelial cells) (Tahara *et al*., 2008). This yet uncharacterized receptor is referred to as "EpiR". The H protein is the major target for neutralizing antibodies induced by vaccination (De Swart *et al*., 2005).

The Large gene encodes the multifunctional catalytic subunit of the RNA dependent RNA polymerase (L; 2183 amino acids), which is responsible for both transcription and replication. It is thought to carry out most (if not all) enzymatic activities required for transcription and replication, including nucleotide polymerization, mRNA capping, and polyadenylation (Ogino *et al*., 2005). On the basis of amino acid sequence conservation among non-segmented negative strand viruses, six domains of higher conservation have been characterized in the L protein. Within these domains, several functional motifs have been identified. The conserved domains may represent concatenated functional domains that are connected by less-conserved hinge regions. L forms oligomers and binds its co-factor, the P protein. Binding sites for these interactions have been identified. Together with the genome, the N, P, and L proteins constitute the transcriptionally active RNP (Cevik *et al*., 2004).

2.2.4 Measles virus receptors

Measles virus (MV), a member of the genus *Morbillivirus* in the family *Paramyxoviridae*, is an enveloped virus with a non-segmented, negative-strand RNA genome. It has two envelope glycoproteins, the haemagglutinin (H) and fusion proteins, which are responsible for attachment and membrane fusion, respectively. Human signalling lymphocyte activation molecule (SLAM; also called CD150), a membrane glycoprotein of the immunoglobulin superfamily, acts as a cellular receptor for MV.

SLAM is expressed on immature thymocytes, activated lymphocytes, macrophages and dendritic cells and regulates production of interleukin (IL)-4 and IL-13 by $CD4^+$ T cells, as well as production of IL-12, tumour necrosis factor alpha and nitric oxide by macrophages. The distribution of SLAM is in accord with the lymphotropism and immunosuppressive nature of MV. *Canine distemper virus* and *Rinderpest virus*, other members of the genus *Morbillivirus*, also use canine and bovine SLAM as receptors, respectively. Laboratory-adapted MV strains may use the ubiquitously expressed CD46, a complement-regulatory molecule, as an alternative receptor through amino acid substitutions in the H protein.

Furthermore, MV can infect $SLAM^-$ cells, albeit inefficiently, via the SLAM and CD46-independent pathway, which may account for MV infection of epithelial, endothelial and neuronal cells *in vivo*. MV infection, however, is not determined entirely by the H protein-receptor interaction. Other MV proteins can also contribute to its efficient growth by facilitating virus replication at post-entry steps. Identification of SLAM as the principal receptor for MV has provided an important clue for better understanding of MV tropism and pathogenesis (Yanagi *et al*., 2006).

2.2.5 Life cycle of the Measles virus

As for all members in the *Paramyxoviridae* family, MV replication occurs entirely in the cytoplasm of the host cell. Upon receptor recognition and attachment, the envelope is fused to the cell membrane and the nucleocapsid is released to the cytoplasm and replication can begin. The genomic RNA of negative-sense RNA viruses has two functions: to serve as a template for primary transcription of mRNAs and as a template for synthesis of the antigenome (+) strand. For both purposes it uses its own viral RNA-dependent RNA polymerase. The mRNA synthesis is initiated at the N gene start signal and the genes are each separated by a trinucleotide intercistronic junction, CUU (Rima *et al*., 1986). The polymerase ends transcription at the stop signal and restarts after the junction at the start signal of the following gene, thereby synthesizing the capped and polyadenylated monocistronic mRNAs, which subsequently are translated. The P gene is an exception since it not only produces the full-length copy P mRNA**,** but also the C and V mRNAs, by alternative translational initiation at an overlapping reading frame and by a process of RNA editing, respectively (Cattaneo *et al*., 1989). The efficiency of transcription is weaker towards the 5´end as the polymerase occasionally detaches from the template at the junctions, resulting insubstantially less L mRNA as compared to N mRNA (Cattaneo *et al*., 1987). Protein synthesis begins early on after virus infection and is apparently required for genome replication.

For replication of the (-) genome, the viral polymerase must ignore the stop signals at the gene boundaries to produce a full-length complementary copy called antigenome. The only function of this (+) intermediate strand is to serve as a template to new genomic RNA. There have been no structural differences noted in the viral polymerase working as a transcriptase to generate mRNAs compared to functioning as a replicase to

generate nascent genomic RNA. This nascent RNA is immediately encapsidated by N protein. Transition from mRNA transcription to RNA replication seems to be dependent on the availability of unassembled N protein, suggesting a straight forward self-regulatory mechanism, but the details for these events, however, still remain unclear (Horikami and Moyer, 1995).

The final assembly of the virus particle takes place at the plasma membrane. The glycoproteins H and F are glycosylated in the endoplasmic reticulum (ER). They are then assisted by several folding enzymes and molecular chaperones of the host cell to undergo conformational maturation (Plemper *et al*., 2001) before transported out of the ER to the Golgi apparatus, where F protein is cleaved and H protein undergoes oligosaccharide modification (Watanabe *et al*., 1995). From the Golgi apparatus, the glycoproteins are transported to the plasma membrane where they are anchored by hydrophobic amino acid residues, thereby leaving the cytoplasmic tails to attract M protein. In infected cells, M protein forms a continuous layer on the inner surface of the plasma membrane and interacts with the cytoplasmic domains of the glycoproteins as well as with the nucleocapsids to initiate virus assembly and the budding process (Cathomen *et al*., 1998). M proteins are believed to play a major role in this, yet not fully understood, assembly process by directing the newly synthesized nucleocapsids to the plasma membrane. The current model suggests that mature nucleocapsids accumulate in inclusion bodies and later in infection, all viral components: the nucleocapsids, the M protein and the glycoproteins, gather at the plasma membrane where budding occurs.

Recently, it was suggested that the nucleocapsids are co-transported to the plasma membrane as a complex with M protein, sufficient accumulation of M protein being a

prerequisite (Runkler *et al*., 2007). Cytoskeleton structures are likely to be involved in the transportation of the different viral components and the new virus particles obtain the lipid envelope when they are released from the host cell by budding.

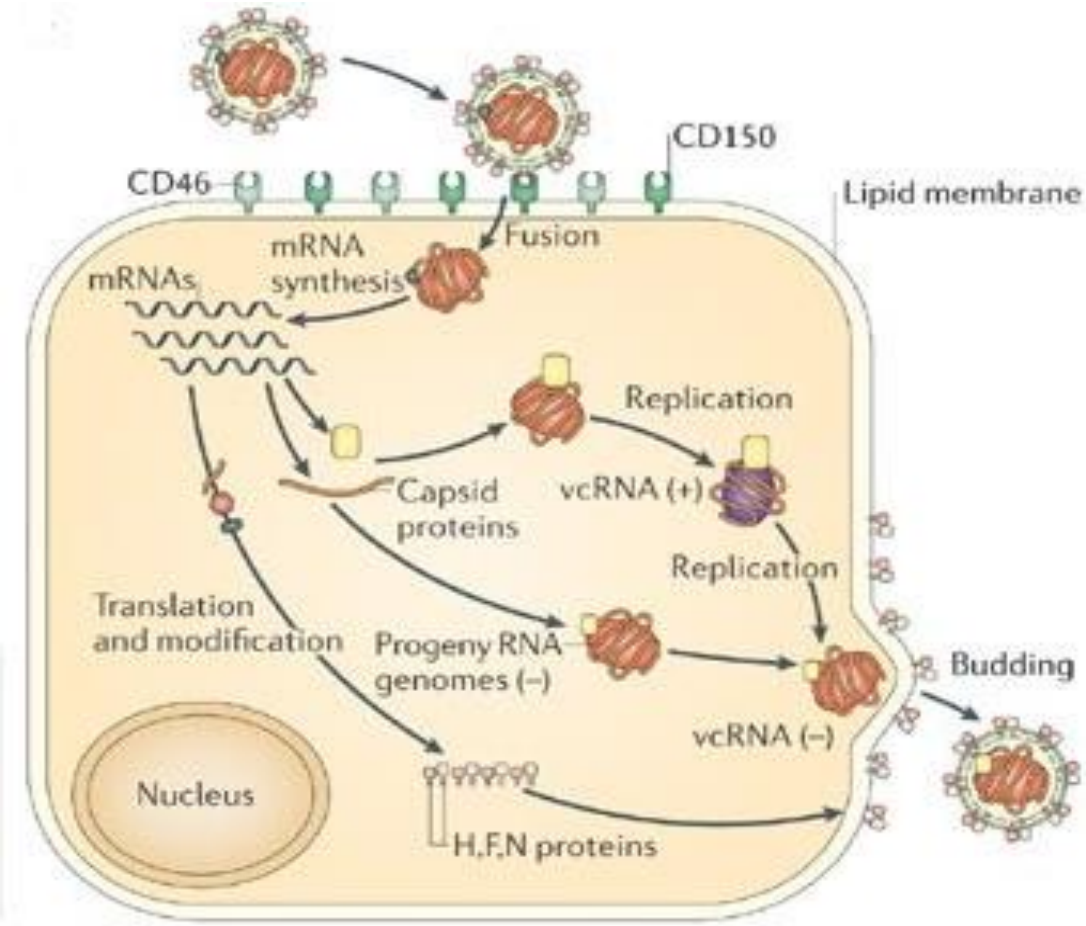


Fig. 2.3: Life cycle of Measles virus

Source: (Moss and Griffin, 2006)

2.2.6 Viral Replication

Measles virus multiplies like the other members of the family *Paramyxoviridae*. Attachment of particles to the cell surface is followed by fusion of the virus envelope and the cytoplasmic membranes and penetration of the nucleocapsid structures into the cytoplasm. The negative-sense RNA is transcribed by the nucleocapsid-associated enzymatically active P and L proteins. The order of genes in terms of their products is N, P, M, F, H and L. The virion RNA serves not only as a template for production of mRNA, but also for replication of intact RNA via a positive-stranded intermediate. After accumulation of genomic RNA and the different structural proteins in the cell cytoplasm, maturation takes place by budding of the virus from the cell. The cell membrane is modified by attachment of N-linked carbohydrate chains of cellular origin before virus transmembranous proteins appear at the cell surface.

The release of viral particles from single cells varies from a few hours, if the cells succumb rapidly to cytopathology, to an unlimited time in chronic, steady-state infections. Development of chronic infection and diseases in the central nervous system (CNS), such as in subacute sclerosing panencephalitis may be caused by a variety of mutations. These result in a lack of viral budding, reduced expression of the viral envelope proteins, and spread of ribonucleoprotein (RNP) through the CNS in spite of massive immune response (Enders, 1996).

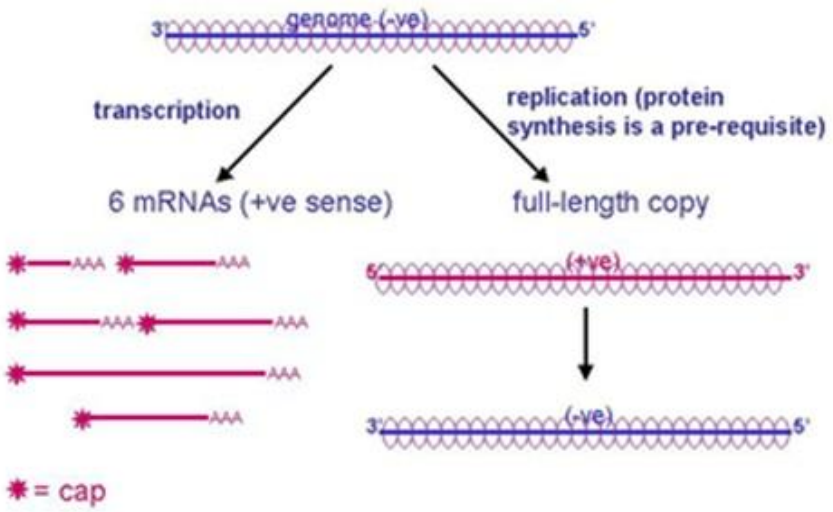


Fig. 2.4: Transcription, translation and replication of Measles virus

Source: (Murray *et al*., 2013)

2.2.7 Classification and antigenic type

Measles virus is a member of the genus *Morbillivirus*. It differs from other paramyxoviruses in lacking neuraminidase and in having hemagglutination activity restricted to monkey and some human red blood cells. Measles virus and the other *Morbilliviruses* occur only as one cross-reactive antigenic type. The natural disease is limited to humans and monkeys.

Although measles virus is serologically monotypic, virus diversity within the complete hemagglutinin (H) gene and the C-terminal part of the nucleoprotein (N) gene has allowed the classification of MV into eight clades, designated A to H (clades are used to indicate the relationship between the various genotypes of the virus), containing 24 genotypes (Rota *et al*., 2009). In the central and western parts of sub-Saharan Africa, mainly clade B viruses have been identified (Kouomou *et al*., 2002). The most common genotype is B3, with its two clusters B3.1 and B3.2. The first B3 sequences in Africa were reported from Nigeria (Hanses *et al*., 1999).

2.3 Epidemiology

Measles is one of the infectious childhood diseases. Infants who become susceptible to measles when passively acquired maternal antibodies disappear are the main source of new susceptible (Clements *et al*., 1995).

In the pre-vaccine era, measles occurred throughout the world, in all races and all climates, with humans as the only host. The maximum incidence was seen in children aged 5 - 9 years. By the age of 20, approximately 99% of subjects have been exposed to the virus. The main factors accounting for the epidemiological pattern are universal

susceptibility to infection in the absence of antibody, extreme contagiousness, population density, and standard of living (Miller *et al*., 1993).

With the introduction of vaccine, measles infection has shifted to the teenagers in countries with an efficient programme. In contrast, in third world countries, measles infection has its greatest incidence in children under two years of age. Here, the disease is a serious problem with a high mortality (10%), with malnutrition being an important factor. In general, measles mortality is highest in children below two years and in adults (Peltola *et al*., 1994).

Following implementation of routine childhood vaccination at age 12 to 15 months, the age at which measles incidence reaches its peak during epidemics in the United States shifted to six months of age. This is approximately the time at which transplacentally acquired maternal antibodies are no longer present if the mother has vaccine-induced immunity (Papania *et al*., 1999).

Sporadic cases occur throughout the year, with peak incidence in the late winter and early summer months. Epidemics occur every two to four years in urban areas with a non-immunized population and every four to eight years in rural areas, when the number of susceptible persons reaches about 40% of the population. The epidemics last three to four months, until the number of susceptible persons falls below 20%. Local outbreaks occur in crowded institutional settings, even when less than 2% of the population is susceptible (Helfand *et al*., 2008).

The source of infection is the virus-containing respiratory tract secretions, either airborne or transmitted by fomites. The contagious period lasts about six days,

beginning with the prodromal symptoms and persisting until about two days after rash develops, at which time antibodies first appear (Perry, 2004).

In developed societies, measles infects children within the ages of four and seven years. In developing societies, measles occurs before age four. By age seven to twelve years, in all but the most isolated areas, nearly all children have had measles and possess specific antibodies. In countries such as the United States, in which vaccine is used extensively, the incidence of reported cases of measles and its complications have dropped more than 95%. As a result of this decreased transmission, a transitory shift to older teenagers has occurred (Peltola *et al*., 1994).

In the year 2000, the WHO estimated that there were approximately 45 million cases of measles worldwide with 800,000 deaths from it. Mortality in developed countries is approximately one out of every one thousand. In sub-Saharan Africa, mortality is 10%. In cases with complications, the rate may rise to 20–30%.

In the year 2003, the World Health Assembly endorsed a global goal to reduce measles mortality by 50% by 2005, compared with the mortality in 1999 (WHO, 2003). Through measles control strategies that included increasing routine immunization coverage and mass vaccination campaigns, the goal was achieved and a new goal was established to achieve 90% reduction by 2010, compared with the mortality in 2000 (Bilous *et al*., 2006).

The WHO-recommended strategies for measles control in Africa, established in 2001, include the following components: (i) increasing routine vaccination coverage with the first dose of measles-containing vaccine (MCV1) for all children, (ii) providing a second dose of MCV to be given through supplemental immunization activities (SIAs),

(iii) improving measles case management, and (iv) establishing case-based surveillance with laboratory confirmation for all suspected measles cases (WHO, 2010). During 2001–2008, routine measles vaccination coverage in Africa increased from 54% to 73% and approximately 400 million children were vaccinated during SIAs, resulting in a decrease in estimated measles mortality from 395,000 deaths in 2000 to 28,000 in 2008, a 92% reduction (CDC, 2006). In the year 2010, approximately 380 deaths occurred every day from measles.

2.3.1 Measles in Nigeria

Measles is a highly infectious immunizable disease with potential for eradication, but is still responsible for high mortality among children, particularly in developing nations like Nigeria. Currently, Nigeria is one of the 47 countries in the world where the burden of measles is high and it is still responsible for the highest number of vaccine preventable death. The current national measles vaccination coverage is 62% with a very wide variation in the country that has once achieved coverage of 80% with routine immunization. The disposition of parents or guardians of the Nigerian children is however key to improved vaccination coverage, and by extension to significant reduction in the morbidity and mortality associated with measles (Adeboye *et al*., 2011).

An increase in measles resurgence was reported in Nigeria from 383 cases in 2006 to 2,542 in 2007 and 9,510 in 2008 (WHO, 2009). About 19,184 measles cases were suspected in 2011, out of which 17,248 were confirmed to be measles, with an annual incidence rate of 18.2 per 100,000 children (WHO, 2011). Several deaths have been reported in 2013 in Kebbi and Kaduna States, northern Nigeria. These show that measles burden is still heavy and severe in Nigeria, even as measles case fatality rate in 2008 was 1.2% (Weldegebriel *et al.*, 2011). Other states that were involved include

Katsina, Yobe, Nassarawa, Bauchi, Taraba, Benue, Zamfara, with varying number of deaths. This outbreak has been linked to drop in immunizations due to vaccine shortages in regional health clinics and widespread suspicion of the vaccine. Many parents have declined to vaccinate their children against measles as they believe the vaccine is harmful. Suboptimal routine coverage and a wide interval between the follow-up campaigns likely led to an accumulation of children susceptible to measles (Weldegebriel *et al*., 2011).

2.4 Pathogenesis

Measles virus enters the host through the oropharynx and possibly through the conjunctiva. Multiplication of the virus is localized at the respiratory tract and the regional lymph nodes; it is followed by primary viremia with virus spread to the rest of the reticuloendothelial system, where extensive replication takes place. A secondary viremia, which occurs 5 to 7 days later, disseminates virus to the mucosa of the respiratory, gastrointestinal, and urinary tracts, to the skin, and to the central nervous system. In these organs, the virus replicates in epithelial cells, endothelial cells, and in monocytes and macrophages. With development of serum antibodies, free virus is quickly cleared from the blood and body fluids, but virus persists for various periods in lymphoid, lung, bladder tissue, and in polymorphonuclear leucocytes (Anderson and Heilman, 1995).

The main pathologic change attributable to viral replication in the main target organs is an inflammatory response. Virus-infected cells contain virus antigens and inclusions in the cytoplasm and nuclei. Infected cells may fuse to form giant cells. The temporary loss of delayed-type hypersensitivity during acute measles may be due to virus multiplication in T and B lymphocytes.

The maculopapular rash is a consequence of the interaction between virus-infected endothelial cells and immune T cells. The simultaneous onset of rash and appearance of serum antibodies suggests an antibody-dependent cellular cytotoxic cause of the exanthem. In cases of dysfunction of T cells, no rash is seen and relentless progression of the infection may lead to giant-cell pneumonia with fatal outcome. Abnormal encephalograms are common during measles, suggesting frequent viral invasion of the brain (Markowitz *et al*., 1990).

In subacute sclerosing panencephalitis patients, mainly noninfectious viral ribonucleoprotein (RNP) inclusion bodies occur in different cell types in the gray and white matter with a strong inflammatory response and some demyelination (Godec *et al*., 1990). RNA can be detected in brain biopsies.

2.5 Clinical Manifestations of Measles virus

The initial symptoms of measles appear about 10 days after a person becomes infected and generally last for up to 14 days. Measles typically begins with a mild to moderate fever, often accompanied by a persistent cough, runny nose (coryza), inflammed eyes (conjunctivitis) and sore throat. Two or three days after symptoms begin, tiny white spots (Koplik's spots) may appear inside the mouth. Koplik spots, is the pathognomonic sign of measles and appears one to two days prior to the onset of the rash. However, these spots are not often seen, even in real cases of measles because they are transient and may disappear within a day (Bernstein *et al*., 1998). This relatively mild illness may last three to four days.

The skin rash typical of measles develops after about four or five days of illness. The rash is a generalized, erythematous maculopapular rash that starts on the back of the

ears, and after a few hours, spreads to the head and neck before spreading to cover most of the body, often causing itching. When the rash appears, a person's fever may spike to more than 104 or 105^0F (40 or 40.6^0C). Over the next three to four days, the fever subsides and the rash fades (Norrie, 2007).

Other constitutional signs and symptoms, such as anorexia, diarrhoea and generalized lymphadenopathy may also be present.

2.5.1 Classic Measles

Classic measles infection can be subdivided into the following clinical stages: incubation, prodrome, exanthem, and recovery (Perry *et al*., 2004).

2.5.1.1 Incubation period

The incubation period begins after measles virus entry via the respiratory mucosa or conjunctivae. The virus replicates locally, spreads to regional lymphatic tissues, and is then thought to disseminate to other reticuloendothelial sites via the bloodstream. The incubation period of measles is usually 10 days with a range generally of eight to ten days. Infected individuals are characteristically asymptomatic during the incubation period, although some have been reported to experience transient respiratory symptoms, fever, or morbilliform rash (Cherry, 2009). The dissemination of measles virus due to viremia, with associated infection of endothelial, epithelial, monocyte, and macrophage cells, may explain the variety of clinical manifestations and complications that can occur with measles infection. A second viremia occurs several days after the first, coinciding with the appearance of symptoms signaling the beginning of the prodromal phase (Bernstein *et al*., 1998).

2.5.1.2 Prodrome

The prodrome phase is defined by the appearance of symptoms which typically include fever, malaise, and anorexia, followed by conjunctivitis, coryza, and cough. The severity of conjunctivitis is variable and may also be accompanied by lacrimation or photophobia (Cherry, 2009). The respiratory symptoms are due to mucosal inflammation from viral infection of epithelial cells. Fever is typically present; the pattern may be variable. Various fever patterns have been described; fever as high as 40ºC can occur. The prodrome usually lasts for two to three days but may persist for as long as eight days (Griffin *et al*, 1996).

Patients may develop an enanthem known as Koplik's spots; these are one to three milimeters whitish, grayish, or bluish elevations with an erythematous base, typically seen on the buccal mucosa opposite the molar teeth, though they can spread to cover the buccal and labial mucosa as well as the hard and soft palate. The Koplik's spots have been described as "grains of salt on a red background" (Bernstein *et al*, 1998). The spots subsequently may coalesce and generally last 12 to 72 hours. It is important to search carefully for Koplik's spots in patients with suspected measles, since they are considered pathognomonic for measles infection and occur approximately 48 hours before the characteristic exanthem. However, this enanthem does not appear in all patients with measles (Perry *et al*., 2004). Uncommonly, patients with severe measles develop generalized lymphadenopathy and splenomegaly.

2.5.1.3 Exanthem

The exanthem of measles is a maculopapular, blanching rash beginning on the face and spreading cephalocaudally and centrifugally to involve the neck, upper trunk, lower

trunk, and extremities. The lesions may become confluent, especially in areas such as the face, where the rash develops first. The rash may also have some petechiae; in severe cases it may appear hemorrhagic (Abramson *et al*., 1995). In general, the extent and degree of confluence of the rash correlates with the severity of the illness in children. The palms and soles are rarely involved. The cranial to caudal progression of the rash is characteristic of measles but is not pathognomonic (Cherry, 2009).

Other characteristic findings during the exanthematous phase include lymphadenopathy, high fever (peaking two to three days after appearance of rash), pronounced respiratory signs including pharyngitis, and nonpurulent conjunctivitis. Koplik's spots often begin to slough when the exanthem appears. Clinical improvement typically ensues within 48 hours of the appearance of the rash. After three to four days, the rash darkens to a brownish color and begins to fade, followed by fine desquamation. The rash usually lasts six to seven days.

2.5.1.4 Recovery and immunity

Cough may persist for one to two weeks after measles infection. The occurrence of fever beyond the third to fourth day of rash suggests a measles-associated complication.

Immunity after measles infection is thought to be lifelong, although there are rare reports of measles re-infection. A measles surveillance program conducted in mid 1960s, for example, identified measles in a 16-year old female with a prior history of measles at age eight. A rise in anti-measles IgG but not IgM was noted, suggesting an anamnestic response (Schaffner *et al*., 1986).

Measles infection can cause transient immunosuppression due to suppression of T-cell responses. Anergy may be present before the appearance of the exanthem and for

several weeks after measles infection. This is exemplified by reports of tuberculosis reactivation in the setting of recent measles infection (Gershon, 1995).

2.5.2 Modified Measles

Modified measles is seen in patients who, though were unimmunized, received immune globulin after being exposed to a patient with measles. It is also seen occasionally in young infants who have limited immunity from their mothers. The immune globulin prolongs the time from exposure to onset of symptoms (incubation period). The incubation period may be as long as 21 days (Lievano *et al*., 2004). When the symptoms do occur, they are similar but much milder than those seen with classic measles and tend to last a shorter period of time. The prodrome is shortened or absent, the fever is reduced, koplik's spots when present are few and rapidly disappear, the rash is short-lived and marked attenuated and complications are extremely rare. Patients with modified measles rarely transmit the infection to others (Lievano *et al*., 2004).

2.5.3 Atypical Measles

Atypical measles occurs in individuals who were vaccinated with the original killed-virus measles vaccine (KMV) between 1963 and 1967 and who have incomplete immunity. The KMV unfortunately sensitizes the patient to the measles virus but does not offer any protection. After exposure to the measles virus, a mild or subclinical prodrome of fever, headache, abdominal pain and myalgia precede a rash that begins on the hands and feet and spread centripetally. The eruption is accentuated in the skin folds and may be macular, vesicular, petechial, or urticarial.

Despite its severity, atypical measles is generally self-limiting, although pulmonary abnormalities may persist. People having this type of measles do not appear to transmit the virus to others (Norby *et al*., 1990).

2.5.4 Congenital Measles

Measles during pregnancies has not been associated with congenital abnormalities in the fetus, but it is associated with a marked increase in premature delivery. In this type of measles, the rash is present at birth or appears during the first ten days of life varying from a mild illness to a fatal disease. Mortality rate is higher in premature than in infants who fail to develop the rash. The high prevalence of passively acquired maternal antibody to the virus result in protection of the newborn (Gershon, 1995).

2.6 Complications of Measles virus

Complications with measles are relatively common and are usually more severe in adults who catch the virus (Sension *et al*., 1988). Measles virus infects multiple organ systems and targets epithelial, reticuloendothelial and white blood cells including monocytes, macrophages, and T lymphocytes. Pathological studies of children dying during acute measles have found multinucleated giant cells typical of measles virus infection throughout the respiratory and gastrointestinal tracts and in most lymphoid tissues. Measles virus infection leads to a decline in CD4 lymphocytes, starting before the onset of rash and lasting for up to one month, and resulting in suppression of delayed-type hypersensitivity as measured by anergy to skin test antigens, including tuberculosis antigen (Moss *et al*., 2002).

Complications from measles have been reported in every organ system. Many of these complications are caused by disruption of epithelial surfaces and immunosuppression

(Atabani *et al*., 2001). Rates of complications from measles vary by age and underlying conditions.

2.6.1 Respiratory Complications

2.6.1.1 Otitis media

Otitis media is the most common complication of measles reported in the United States and occurs in 14% of children under-five years old. Presumably, inflammation of the epithelial surface of the eustachian tube causes obstruction and secondary bacterial infection. Lower rates of otitis media are noted with increasing age, most likely a function of the increasing diameter of the eustachian tube and the decreasing risk of obstruction.

2.6.1.2 Laryngotracheobronchitis

Laryngotracheobronchitis or "measles croup" was noted in 9%–32% of US children hospitalized with measles. The majority of affected children were below two years old. In one-third to one-half of such cases, culture of samples from the trachea yields positive results for bacterial pathogens, with a purulent exudate and evidence of secondary bacterial tracheitis, pneumonia, or both. The most commonly cultured organism is *Staphylococcus aureus,* although *Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Escherichia coli,* and *Enterobacter* species have also been identified (Ross *et al*., 1992).

2.6.1.3 Pneumonia

Measles infects the respiratory tracts of nearly all affected persons. Pneumonia is the most common severe complication of measles and accounts for most measles-associated deaths (Hussey and Clements, 1996).

Pneumonia may be caused by measles virus alone, secondary viral infection with adenovirus or HSV, or secondary bacterial infection. Measles is one cause of Hecht's giant cell pneumonia, which usually occurs in immunocompromised persons but can occur in otherwise normal adults and children (Kides *et al*., 1999). Studies that included culture of blood, lung punctures, or tracheal aspirations revealed bacteria as the cause of 25%–35% of measles-associated pneumonia. *S. pneumoniae, S. aureus,* and *H. influenzae* were the most commonly isolated organisms. Other bacteria (e.g. *Pseudomonas* species, *Klebsiella pneumoniae,* and *E. coli)* are less common causes of severe pneumonia associated with measles (Mason *et al*., 1993).

Pneumomediastinum and mediastinal emphysema have been reported as complications of measles in several countries (Sharma, 1993). Some children have the clinical pattern of bronchiolitis. Because viral cultures are not always done, the possibility of co-infection with other respiratory viruses cannot be ruled out.

Among immunocompromised persons, diffuse progressive pneumonitis caused by the measles virus is the most common cause of death (Carmena, 1996). These patients may first have typical measles with pneumonia, or they may have a nonspecific illness without rash followed by pneumonitis without a rash. In general, signs of pneumonitis develop in the two weeks after the first onset of symptoms (Quiambao *et al*., 1998).

2.6.2 Gastrointestinal Complications

Measles probably infects the intestinal tracts of most persons with measles. Several cases of appendicitis have developed before and during measles rash, and characteristic giant cells typical for measles have been found in appendix tissue (Pancharoen *et al*., 2001).

2.6.2.1 Diarrhoea

Measles-associated diarrhoea typically begins just before rash onset, suggesting that measles virus is responsible for most of the diarrhea episodes, but that secondary bacterial or viral infections may contribute to the severity and duration of illness.

High rates of gastrointestinal complications that occur after measles in developing countries include mouth sores, decreased food intake, protracted diarrhoea, weight loss, and precipitation of severe protein calorie malnutrition. In young adults, measles is associated with hepatitis, hypocalcemia, and elevation of creatinine phosphokinase levels (Morcos and McHugh, 1997).

2.6.3 Neurological Complications

2.6.3.1 Febrile seizures

Febrile seizures occur in 0.1%–2.3% of children with measles in the United States and are usually benign and not associated with residual damage. Most children with uncomplicated measles have changes visible on electroencephalography, but these changes are most likely due to fever and other metabolic changes (Cherry, 1998).

2.6.3.2 Postinfectious encephalomyelitis (PIE)

PIE occurs in 13 per 1000 infected persons, usually 3 to 10 days after onset of rash. Higher rates of PIE due to measles occur in adolescents and adults than in school-aged children. PIE usually begins with the abrupt onset of new fever, seizures, altered mental status, and multifocal neurological signs. PIE appears to be caused by an abnormal immune response that affects myelin basic protein. As many as 25% of people with PIE due to measles die, and approximately 33% of survivors have lifelong neurological sequelae, including severe retardation, motor impairment, blindness, and sometimes hemiparesis (Cherry, 1998).

2.6.3.3 Subacute sclerosing panencephalitis (SSPE)

SSPE is caused by persistence of measles virus in central nervous system tissue for several years, followed by a slowly progressive infection and demyelination affecting multiple areas of the brain (Cherry, 1998). The initial SSPE symptoms, usually, decreased school performance and behavioral disorders, are often misdiagnosed as psychiatric problems. Subsequently, myoclonic seizures develop, and a characteristic burst-suppression pattern may be seen on electroencephalography. Measles antibody is present in the cerebrospinal fluid. The disease slowly progresses until affected persons are in a vegetative state. Wild-type measles viruses, but not measles vaccine viruses, have been found in brain tissue (Perry and Halsey, 2004).

SSPE occurs on average in one per 8.5 million persons who develop measles in the United States but the rate appears to be higher in some other countries. Half the SSPE patients have contracted measles before the age of two years. The course of SSPE is highly variable but usually starts with generalized intellectual deterioration or

psychological disturbance. It may be several months before other neurological signs appear such as convulsions, aphasia, myoclonic jerks. In 75% of cases, the retina, a chorioretinitis develops leading to blindness. The progression of the disease is very variable, the illness lasts from one to three years and inevitably leads to death.

2.6.3.4 Measles encephalitis in immunocompromised patients

A progressive central nervous system measles virus infection, termed "measles inclusion body encephalitis," occurs in immunocompromised persons with disorders such as human immunodeficiency virus (HIV) infection or leukemia. Onset is usually five weeks to six months after acute measles. The illness begins with mental-status changes and seizures in the absence of fever; less than 80% of deaths occur within weeks (Mustapha *et al*., 1993).

2.6.4 Ocular Complications

Conjunctivitis occurs in most persons with measles and inflammation of the cornea (keratitis) is common. In well-nourished persons, these lesions usually heal without residual damage. However, secondary bacterial (e.g., *Pseudomonas* or *Staphylococcus)* or viral infections (e.g., HSV or adenovirus) can lead to permanent scarring and blindness. Vitamin A deficiency predisposes to more severe keratitis, corneal scarring, and blindness. Measles associated with vitamin A deficiency is one of the most common causes of acquired blindness in children in developing countries. Blindness can also result from cortical damage from measles encephalitis (Wairagkar, 1999).

2.7 Measles in pregnancy

Measles in pregnancy result in a high rate of spontaneous abortion and premature delivery and low-birth-weight infants. There is some evidence that measles may be transmitted transplacentally as infants delivered during the mother's incubation often develops a rash simultaneously with the mother. While some infants with perinatally acquired measles have mild illnesses, others develop severe disease with pneumonia.

2.8 Factors affecting Measles morbidity and mortality rates

2.8.1 Sex

Historically, males have had higher case-fatality rates than did females. An analysis of vital statistics data from several countries (primarily in the Americas and Europe) for the years 1950–1989 suggests that women and girls may have slightly higher mortality rates after measles than do men and boys, but recent surveillance data from the United States and United Kingdom show equal rates of complications for men and women. Pregnant women have an increased risk of complications, including death, following measles (Garenne, 1994).

2.8.2 Age

Complication rates, including mortality, from measles are highest in children below five years and adults. Most infants are protected during the first months of life via maternally derived antibodies. However, when immunity is lacking, measles can be severe. Adults more commonly have encephalitis, hepatitis, hypocalcemia, or pancreatitis after measles. The increased severity of measles in adults most likely reflects the decline in cell-mediated immunity that begins in adulthood. Studies showed

that young infants and adults have more severe and a longer duration of lymphopenia after measles than do children (Okada *et al*., 2000).

2.8.3 Crowding

Several studies from West Africa and Europe shows that children who develop measles after within-household exposure have higher case-fatality rates than do children who are exposed to measles outside the household. This phenomenon is most likely secondary to a higher inoculum from more intensive and prolonged exposure compared with more casual exposures outside the home (Butler *et al*., 1994).

2.8.4 Immunosuppression

Children with defects in macrophage function only (e.g., chronic granulomatous disease) do not have increased rates of complications from measles (Pickering, 2000). Suppression of lymphocyte function, resulting from congenital defects in T lymphocyte function, bone marrow transplantation, chemotherapy for cancer, or immunosuppressive doses of steroids, is associated with increased severity of measles (Cherry, 1998). In some immunosuppressed patients with measles, multiple organ systems are affected. Measles has developed after bone marrow transplantation even when both donor and recipient have histories of measles vaccination (Nakano *et al*., 1996). Patients with B cell immune deficiency syndromes without T cell abnormalities do not appear to have increased rates of complications associated with measles.

Children born to HIV-infected women become susceptible to measles at an earlier age than do children born to HIV-negative women because the former transmit reduced amounts of antibodies to their infants. HIV-infected infants not taking highly active

antiretroviral therapy (HAART) have decreased responses to measles vaccination and a faster decline in vaccine-induced immunity (Moss *et al*., 2002).

2.8.5 Malnutrition

Malnourished children have impairments in multiple aspects of the immune system, prolonged excretion of measles virus, and higher measles case-fatality rates. Measles contributes to the development of malnutrition because of protein-losing enteropathy, increased metabolic demands, and decreased food intake. Children who have measles early in life have significantly lower mean weights for age than do children of the same age who do not develop measles (Vargas *et al*., 2000).

2.8.6 Vitamin A deficiency

Children with clinical or subclinical vitamin A deficiency in many developing countries have increased case-fatality rates. Measles and other illnesses are associated with reductions in serum retinol concentrations and may induce overt vitamin A deficiency. Hospitalized U.S measles patients frequently have deficiencies in vitamin A; these children are more likely to have pneumonia or diarrhea after measles (Perry and Halsey, 2004).

2.9 Immune response

Little natural resistance to measles virus infection exists. Nonspecific substances, such as interferon, appear to contribute to early limitation of virus spread. Interferon may be detected until virus-specific antibodies appear. The cell-mediated immune response is associated with recovery from primary infection and also with resistance to re-infection at the portal of entry. The humoral immune response helps to eliminate extracellular

virus during primary infection and to prevent systemic spread at re-infection (Norby *et al*., 1990).

In primary acute infection, T-cell and B-cell responses can be detected to most of the six measles virus proteins. Both IgG and IgM antibodies are initially produced, however IgM antibodies peak at 7 to 10 days after rash onset and fall rapidly, rarely being detectable more than 4 weeks after rash onset. The presence of IgM is generally accepted as evidence of primary measles infection (by disease or vaccine). IgG becomes detectable in the serum soon after rash onset, peaks within 4 weeks and subsequently declines, but persists for life (Stokes *et al.*,1961). The IgG antibodies to the H protein appear to be most important in determining immunity. Immunity after natural infection is usually life-long. Serum and secretory IgA are also produced but are usually transient.

Cell-mediated immunity plays an important role in recovery from, and possibly, prevention of measles and it has been postulated that sufficient stimulation of cell-mediated immunity may be a prerequisite for the development of lifelong protection. However, tests for cell-mediated immunity are less readily available than those for humoral immunity. Acute measles infection is associated with a wide range of immunological abnormalities, including depressed general cellular reactivity (manifest, for example, in a depressed delayed hypersensitivity reaction to the tuberculin test) and cytokine production abnormalities.

The humoral immune response occurs in the three immunoglobulin classes- IgG, IgM and IgA. Lifelong persistence of serum antibodies may be due to persistence of viral antigen. Maternal IgG antibodies completely protect the infant for six months; between six and twelve months of age, subclinical infection or modified disease may occur (Helfand *et al*., 2008).

In patients with subacute sclerosing panencephalitis, strikingly high titers of measles oligoclonal antibody (IgG) are present in serum and cerebrospinal fluid. Antibodies are directed against the viral protein.

However, infection is also associated with persistence of viral RNA and several weeks of immune suppression, including loss of delayed type hypersensitivity responses and increased susceptibility to secondary infections. The initial T-cell response includes CD8+ and T-helper 1 CD4+ T cells important for control of infectious virus. As viral RNA persists, there is a shift to a T-helper 2 CD4+ T-cell response that likely promotes B-cell maturation and durable antibody responses but may suppress macrophage activation and T-helper 1 responses to new infections. Suppression of mitogen-induced lymphocyte proliferation can be induced by lymphocyte infection with MV or by lymphocyte exposure to a complex of the hemagglutinin and fusion surface glycoproteins without infection. Dendritic cells (DCs) are susceptible to infection and can transmit infection to lymphocytes. MV-infected DCs are unable to stimulate a mixed lymphocyte reaction and can induce lymphocyte unresponsiveness through expression of MV glycoproteins. Thus, multiple factors may contribute both to measles-induced immune suppression and to the establishment of durable protective immunity (Griffin, 2010).

2.10 Mode of Transmission

The causative agent, measles virus (MV), is generally transmitted by aerosolized secretions deposited on upper-respiratory-tract mucosal surfaces. Measles virus spreads by airborne droplets or by direct contact with nasal or throat secretions of infected people. Measles spreads through the air by breathing, coughing or sneezing. Measles is highly contagious and can be spread to others from four days before to four days after

the onset of the rash. Exposure leads to local respiratory tract replication; infection of regional lymphoid tissues then occurs, followed by viremia and systemic dissemination as revealed by the characteristic skin rash. Most children recover uneventfully from the illness, but serious complications can occur, including pneumonia and involvement of the central nervous system. Measles virus is a highly contagious agent which causes a major health problem in developing countries. Measles is so contagious that if one person has it, 90% of the people close to that person who are not immune will also become infected with the measles virus (Perry and Halsey, 2004).

The virus lives in the mucus in the nose and throat of the infected person. When that person sneezes or coughs, droplets spray into the air. The droplets can get into other people's noses or throats when they breathe or put their fingers in their mouth or nose after touching an infected surface. The virus can live on infected surfaces for up to two hours and spreads so easily that people who are not immune will probably get it when they come in close contact with someone who is infected (Cherry, 2009).

2.11 Prognosis

The majority of patients survive measles, though in some cases, complications may occur, which may include bronchitis, and in about 1 in 100,000 cases, panencephalitis, which is usually fatal. The patient may spread the disease to an immunocompromised patient, for whom the risk of death is much higher, due to complications such as giant cell pneumonia. Acute measles encephalitis is another serious risk of measles virus infection. It typically occurs two days to one week after the breakout of the measles exanthem and begins with very high fever, severe headache, convulsions and altered mentation. A patient may become comatose, and death or brain injury may occur (Berggren *et al*., 2005).

2.12 Diagnosis

The symptoms of acute measles are so distinctive that laboratory diagnosis is seldom required. Clinical diagnosis of measles requires a history of fever of at least three days, with at least one of the three C's (cough, coryza, conjunctivitis). Observation of Koplik's spots is also diagnostic of measles (Baxby, 1997).

However, as the vaccination program progresses, atypical forms of measles have emerged and laboratory diagnosis may be required. Laboratory confirmation of measles infection requires isolation of measles virus, detection by Polymerase Chain Reaction (PCR) or detection of Measles IgM by serological testing (Helfand *et al*., 1997).

2.12.1 Microscopy

The production of multinucleate giant cells with inclusion bodies is pathognomonic for measles. During the prodrome phase, such cells are detectable in the nasopharyngeal secretions. This is more rapid and practical than virus isolation.

2.12.2 Virus isolation

Measles virus can be isolated from a variety of sources, e.g. throat or conjunctival washings, sputum, urinary sediment cells and lymphocytes. Primary human kidney (HEK) cells are the best, although primary monkey kidney can be used as well. Continuous cell lines such as vero cells can also be used although they are not as efficient as primary cell lines. A CPE develops between two to fifteen days, and consist of either a broad syncytium or a stellate form with inclusion bodies visible. The presence of measles can be confirmed by haemadsorption. In acute measles, the isolation rate is difficult and the success rate is low. Isolation is most likely to be successful from material taken in the prodrome phase but not in the later stages after the

rash has developed. Therefore isolation should only be attempted in complicated cases such as suspected SSPE where the lymphocytes may carry the virus, and in immunocompromised individuals developing pneumonia (WHO, 2000).

2.12.3 Measles culture and immunofluorescence

Measles virus may be identified by cell culture from blood, conjunctival swab, throat swab, nasopharyngeal swab or aspirate if taken within five days of the onset of the rash, and from urine for up to ten days after the onset of rash. Throat swabs and nasal swabs are suitable for culture. Antigen testing by immunofluorescence may also be performed on respiratory or urine samples collected during the prodrome or within five days after rash onset. Nasopharyngeal aspirates or nasopharyngeal swabs are the preferred samples for antigen detection by immunofluorescence (WHO, 2007).

2.12.4 Serology

Diagnosis of measles infection can be made if the antibody titres rise by four fold between the acute and the convalescent phase or if measles-specific IgM is found. The methods that can be used include hemagglutination inhibition test, complement fixation test, neutralization test and Enzyme Linked Immunosorbent Assay (ELISA) test (Helfand *et al*., 1997).

2.12.4.1 Measles IgM

This is an antibody test that measures the presence of a specific antibody class (IgM) that is only present during acute illness. Measles-specific IgM is a sensitive and specific marker of recent measles infection, but can also be detected for one to two months following immunization. An IgM response will be present in approximately 75% of

measles cases three days after rash onset, rising to approach 100% after seven days (WHO, 2000).

2.12.4.2 Measles IgG

Both acute and convalescent sera are necessary to determine whether there has been a four-fold or greater rise in total measles antibody. A measles IgG antibody test should preferably be performed together with the IgM assay to aid interpretation (WHO, 2000).

2.12.5 Polymerase Chain Reaction (PCR)

This test is most useful in the first few days of illness when serology may be negative. The test is also useful as a test for measles infection in severely immunocompromised patients where antibody response is unreliable. Respiratory specimens and early catch urine (collected up to three weeks after onset of the rash) or sera collected early in the illness can be used. Nasopharyngeal aspirates or nasopharyngeal swabs are the preferred specimens for measles PCR. Throat swabs or nasal washes are also suitable for PCR (Helfand *et al*., 1997).

2.13 Treatment

There is no specific treatment for measles. Most patients with uncomplicated measles will recover with rest and supportive treatment. It is, however, important to seek medical advice if the patient becomes more unwell, as they may be developing complications (Norby and Oxman, 1990).

Some patients will develop pneumonia as sequelae to the measles. Other complications include ear infections, bronchitis, and encephalitis. Acute measles encephalitis has a mortality rate of 15%. While there is no specific treatment for measles encephalitis,

antibiotics are required for bacterial pneumonia, sinusitis, and bronchitis that can follow measles.

2.13.1 Ribavirin

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

2.13.2 Vitamin A

The use of vitamin A in treatment has been investigated. Vitamin A supplements have been shown to help prevent serious complications arising from measles infection. Supplements are generally recommended for children living in countries with high prevalence of vitamin A deficiency (common in the developing world). Treatment with vitamin A may be offered to people with measles. A systematic review of trials into its use found no significant reduction in overall mortality, but it did reduce mortality in children aged below two years (D'Souza and D'Souza, 2002).

Vitamin A has profound and diverse effects on the immune system and the integrity of epithelial barriers, and its beneficial effect on measles is almost certainly mediated in part by effects on the immune system. However, the immunologic basis for the reduction in measles mortality following vitamin A administration is not understood (Moss *et al*., 2004).

2.13.3 Immune Globulin (Human)

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

The Advisory Committee on Immunization Practices (ACIP) recommends immune globulin for susceptible household contacts of measles patients, particularly those for whom the risk for complications is increased (i.e. infants aged less than or equal to 12 months, pregnant women, or immunocompromised persons). Immune Globulin (Human), BayGam is licensed for use as prophylaxis in susceptible persons exposed to measles (Sawyer, 2000).

All other treatments address symptoms. Ibuprofen or acetaminophen (paracetamol) helps to reduce fever and pain and if required, a fast-acting bronchodilator for cough. As for aspirin, some research has suggested a correlation between children who take aspirin and the development of Reye's syndrome- a rare but potentially fatal disease (Starko *et al*., 1980). Some researches have shown aspirin may not be the only medication associated with Reye's syndrome, even antiemetics have been implicated. Nevertheless, most health authorities still caution against the use of aspirin for any fevers in children under sixteen years (Macdonald, 2002).

2.14 Prevention of Measles

Measles is a vaccine preventable disease. The best way to prevent measles is through immunization.

In developed countries such as the United States, a combined vaccine against measles, mumps and rubella (MMR) is used to protect children against measles. The first dose of the vaccine is usually administered to children between twelve to fifteen months of age. Children are given a second dose or the booster dose before they start school, usually between the ages of four and six years. The second dose of the vaccine is recommended to protect the 5% who did not develop immunity in the first dose, and to give booster effect to those who developed an immune response. Widespread use of the measles vaccine has led to a greater than 99% reduction in measles cases in the United States, compared to the pre-vaccine era (Rutter, 2005).

In developing countries where measles is highly endemic, WHO recommends two doses of vaccine be given at six and nine months of age, irrespective of the HIV status of the child (Helfand *et al*., 2008).

A special immunization (immune globulin) may be necessary for certain high-risk people after they are exposed to measles. These include children younger than one year, children with weakened immune systems and pregnant women.

2.15 MMR Vaccine

Two measles vaccines were first licensed in 1963. One was a live attenuated vaccine and the other was an inactivated (killed) virus vaccine, which was withdrawn in 1967 due to lack of effectiveness and an increased risk for atypical measles. The other vaccine was withdrawn in 1975 because of a relatively high frequency of fever and rash reactions.

An improved measles vaccine was developed in 1968. Vaccines for the mumps and rubella became available in 1967 and 1969 respectively. The three vaccines (for

measles, mumps and rubella) were combined in 1971 to become the measles-mumps-rubella (MMR) vaccine. It was first developed by Maurice Hilleman. It is a mixture of live attenuated viruses of the three diseases, administered via injection. In 1973, Merck's further attenuated combination of measles-mumps-rubella vaccine was licensed and, as of July 2012, it is the only measles-containing vaccine available in the U.S (Edmonston-Enders strain, formerly called "Moraten") (CDC, 2012).

Vaccination with MMR vaccine provides significant improvement in the efficiency of paediatric immunization through the administration of three vaccines in a single injection, reducing costs while increasing immunization coverage against the three diseases. MMR vaccine is usually administered at 12-15 months and 45 years of age in the U.S (Jefferson *et al*., 2003).

Measles vaccine produces an inapparent or mild, non-communicable infection. Measles antibodies develop in approximately 95% of children vaccinated at 12 months of age and 98% of children vaccinated at 15 months of age. Seroconversion rates are similar for single-antigen measles vaccine, MMR and MMRV. Approximately 2%–5% of children who receive only one dose of MMR vaccine fail to respond to it (i.e., primary vaccine failure). The MMR vaccine is generally administered to children around the age of one year, with a second dose before starting school, between ages four and six years in the United States. The second dose is a dose to produce immunity in the small number of persons (2–5%) who fail to develop measles immunity after the first dose. In the United States, the vaccine was licensed in 1971 and the second dose was introduced in 1989. It is widely used around the world. Since introduction of its earliest versions in the 1970s, over 500 million doses have been used in over 60 countries. It is usually

considered a childhood vaccination. However, it is also recommended for use in some cases of adults with HIV (Helfand *et al*., 2008).

MMR vaccine failure may occur because of passive antibody in the vaccine recipient, damaged vaccine, incorrect records, or possibly other reasons. Most persons who fail to respond to the first dose will respond to a second dose. Studies indicate that more than 99% of persons who receive two doses of measles vaccine (with the first dose administered no earlier than the first birthday) develop serologic evidence of measles immunity (CDC, 2010).

2.15.1 Inactivated Vaccine

This vaccine was intended for use in young children less than one year of age who are most prone to severe complications. It was thought to be advisable to avoid the use of a live vaccine. It was found that at least three doses were needed to elicit a protective antibody response but the antibody levels soon waned. This leaves the vaccines open to attack by the natural virus. In some cases, the nature of the partial immunity led to serious hypersensitivity reactions to infection (atypical measles). The exact mechanism is still uncertain but it was thought that the vaccine lacked an important antigen of the virus and thus immunity was not complete. In view of the above and the fact that antibody levels decline rapidly after administration of the killed vaccine, live vaccination is now generally recommended and individuals previously immunized with the killed vaccine should be re-immunized with the live vaccine. The killed vaccine has now been withdrawn.

2.15.2 Live vaccine

Live vaccines are now usually used. The seroconversion rate is 95% and the immunity lasts for at least 10 years or more, possibly lifelong. The virulence of the attenuated strain now in use is so low that encephalitis has only been noted in one in one million recipients. SSPE has been reported in children given the live vaccine. However, the rate is lower than that following natural infection. Therefore the vaccine is safe for use in very young children. The live vaccine is now incorporated as part of the MMR vaccine. As vaccine-induced measles antibody develops more rapidly than following natural infection, MMR vaccine can be used to protect susceptible contacts during a measles outbreak. To be effective, the vaccine must be administered within three days of exposure. If there is doubt about a child's immunity, vaccine should be given since there are no ill effects from immunizing individuals who are already immune. Immunoglobulin should be given to those for whom the vaccine is contraindicated.

2.15.3 Adverse reactions following Measles vaccination

Adverse reactions following measles vaccine (except allergic reactions) represent replication of measles vaccine virus with subsequent mild illness. These events occur 5 to 12 days post-vaccination and only in persons who are susceptible to infection. There is no evidence of increased risk of adverse reactions following MMR vaccination in persons who are already immune to the diseases.

Fever is the most common adverse reaction following MMR vaccination. Although measles, mumps, and rubella vaccines may cause fever after vaccination, the measles component of MMR vaccine is most often associated with this adverse reaction. After MMR vaccination, 5% to 15% of susceptible persons develop a temperature of 103°F

(39.4°C) or higher, usually occurring 7 to 12 days after vaccination and generally lasting 1 or 2 days. Most persons with fever are otherwise asymptomatic.

Measles- and rubella-containing vaccines, including MMR, may cause a transient rash. Rashes, usually appearing 7 to 10 days after MMR or measles vaccination, have been reported in approximately 5% of vaccines.

Rarely, MMR vaccine may cause thrombocytopenia within two months after vaccination. Estimates of the frequency of clinically apparent thrombocytopenia from Europe are one case per 30,000–40,000 vaccinated susceptible persons, with a temporal clustering of cases occurring two to three weeks after vaccination. The clinical course of these cases was usually transient and benign, although hemorrhage occurred rarely. The risk for thrombocytopenia during rubella or measles infection is much greater than the risk after vaccination. Based on case reports, the risk for MMR-associated thrombocytopenia may be higher for persons who have previously had immune thrombocytopenic purpura, particularly for those who had thrombocytopenic purpura after an earlier dose of MMR vaccine.

Transient lymphadenopathy sometimes occurs following receipt of MMR or other rubella-containing vaccine, and parotitis has been reported rarely following receipt of MMR.

Arthralgias and other joint symptoms are reported in up to 25% of susceptible adult women given MMR vaccine. This adverse reaction is associated with the rubella component. Allergic reactions following the administration of MMR or any of its component vaccines are rare. Most of these reactions are minor and consist of a wheal and flare or urticaria at the injection site. Immediate, anaphylactic reactions to MMR or

its component vaccines are extremely rare. Allergic reactions including rash, pruritus, and purpura have been temporally associated with mumps vaccination, but these are uncommon and usually mild and of brief duration (CDC, 2012).

2.15.4 Measles vaccine and Autism

To date there is no convincing evidence that any vaccine causes autism or autism spectrum disorder. Concern has been raised about a possible relation between MMR vaccine and autism by some parents of children with autism. Symptoms of autism are often noticed by parents during the second year of life, and may follow administration of MMR by weeks or months. Two independent non-governmental groups, the Institute of Medicine (IOM) and the American Academy of Pediatrics (AAP), have reviewed the evidence regarding a potential link between autism and MMR vaccine. Both groups independently concluded that available evidence does not support an association, and that the United States should continue its current MMR vaccination policy. Additional research on the cause of autism is needed (CDC, 2012).

2.16 Control

Quarantine is futile, because by the time the rash signals the disease, shedding has been in progress for two or three days. Passive prophylaxis with measles immunoglobulin is recommended for exposed, susceptible individuals, especially those at high risk (e.g., patients with cancer, immunosuppressed and immunodeficient patients, infants younger than one year of age and pregnant women). To completely prevent measles infection, viremia must be prevented by an appropriate dose of immunoglobulin given within three days of exposure. Administration of immunoglobulin between days five and nine after exposure cannot prevent the secondary viremia, but will modify the disease and

allow immunity to develop. Disease also can be modified within three days of exposure by reducing the dose of immunoglobulin. Immunoglobulin may protect recipients for about four weeks.

Active immunization with the combined measles-mumps-rubella live-virus vaccine is recommended for all healthy 12 to 18-month-old children. Vaccine-induced antibody develops in about 94% of the seronegative recipients and usually persists in declining titres for more than 18 years. Natural exposure to virus may cause an antibody booster response. Re-vaccination is recommended in some countries at the age of six and in others at the age of twelve years to reach primary vaccine failures (6-7%) and to boost low levels of antibody. Vaccination is also emphasized in the USA for adolescents entering college. Furthermore, live-virus vaccine should be given to anyone who does not have a history of measles or has not received live virus vaccine after the age of 15 months (Enders, 1996).

2.17 Post-exposure prophylaxis

Administration of MMR vaccine within 72 hours of exposure may be effective as post-exposure prophylaxis for previously unimmunized or incompletely immunized persons.

2.17.1 Immunocompetent patients and staff

- Non-immune, immunocompetent, non-pregnant adults should be offered MMR irrespective of time since exposure
- Non-immune immunocompetent children less than one year should be offered MMR vaccine irrespective of time since exposure. The second dose should be given one month after the first.

2.17.2 Immunocompromised patients

- Measles immune status of immunocompromised patients should be determined prior to exposure.
- Non-immune immunocompromised patients less than twelve months of age should be considered for use of immunoglobulin if within six days of exposure.
- Patients with severe defects of cell-mediated immunity, less than twelve months of age should be considered for use of immunoglobulin if within six days of exposure, irrespective of whether IgG can be detected.

2.17.3 Pregnant patients/staff

- The measles immune status of pregnant patients should ideally be determined prior to admission to hospital
- Non-immune pregnant patients or staff should be considered for use of immunoglobulin if within six days of exposure.
- Pregnant patients or pregnant staff who have had at least one previous dose of MMR vaccine do not require immunoglobulin

2.17.4 Infants

- In exposed infants between six and twelve months of age, if immunocompromised, use of immunoglobulin should be discussed with the clinical team caring for that patient if within six days of exposure. If immunocompetent, MMR vaccine should be offered if within 72 hours of exposure. Alternatively, if within six days of exposure, use of immunoglobulin should be discussed with the clinical team caring for that patient.

- In exposed infants less than six months of age, if the mother is non-immune and exposure occurred less than six days earlier, use of immunoglobulin should be discussed with the clinical team caring for that patient
- Premature infants less than 28 weeks gestation should be considered non-immune irrespective of maternal immune status.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Kaduna State. Kaduna State occupies part of the North West region of Nigeria, covering a land area of about 48,473.2km^2. It shares boundaries with Kano, Katsina, Bauchi, Niger, Nassarawa, Plateau and Zamfara States, as well as the Federal Capital Territory, Abuja. The global location of the state is between longitude $06^0$00 and $09^0$00 east of the Greenwich Meridian and also between latitude $09^0$00 and $11^0$30 north of the equator. The state has three senatorial zones and 23 Local Government Areas, with a population of 6,066,562 and annual growth rate of 3.3% (NBS, 2006).

The study was conducted in three major hospitals in Kaduna state which included; Hajia Gambo Sawaba General Hospital, Kofar-Gayan, Zaria located in Zaria LGA, Yusuf Dantsoho Memorial Hospital and Gwamna-Awan Hospital, both located in Kaduna metropolis. These hospitals were selected using simple random sampling method.

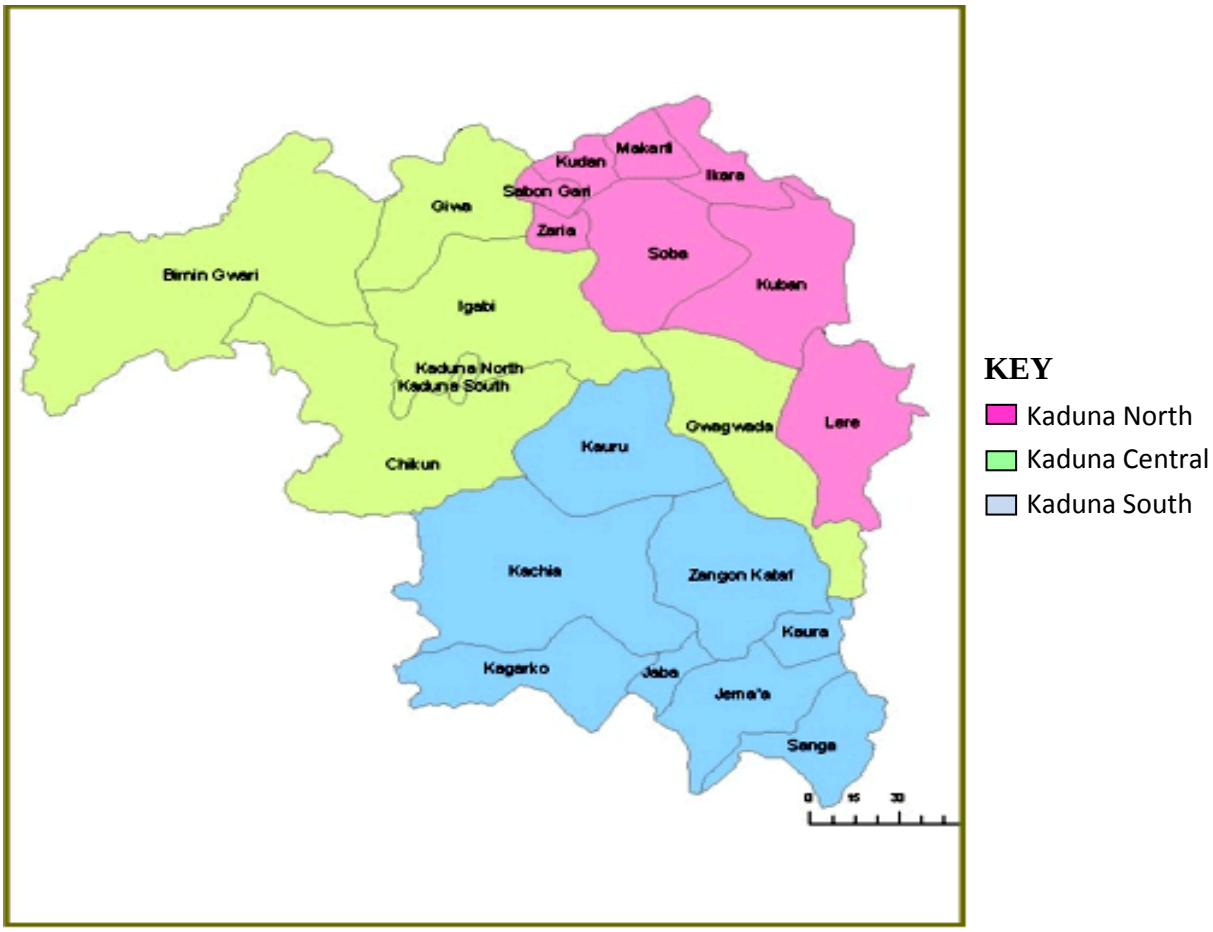


Figure 3.1: Map of Kaduna state showing the three senatorial zones

Source: (http://www.ramalanyero.com/img/kaduna-state/kaduna-general-map.jpg)

3.2 Study Population

The study population included children (0-8 months) presenting with measles-like symptoms, attending the hospitals selected for the study. These symptoms include fever, cough, coryza, conjunctivitis, diarrhoea, vomiting, rash and koplik's spots.

3.3 Control Population

Children (9-23 months) presenting with measles-like symptoms, attending the hospitals were used as control population because these children are expected to be negative for measles virus due to the vaccine-induced antibody in them.

3.4 Ethical Approval

Ethical approval was obtained from the ethical committee of Kaduna State Ministry of Health. The purpose and procedure of the study was explained to the parents or caregivers and their consent was obtained for collection of samples.

3.5 Inclusion and Exclusion Criteria

The inclusion criteria for this study were children (0-23 months) whose parents or caregivers gave their consent.

The exclusion criteria were children (0-23 months) whose parents or caregivers failed to give their consent, as well as children over 23 months of age.

3.6 Sample Size

The sample size was determined using the following equation;

$n = \frac{Z^2pq}{d^2}$ (Naing *et al*., 2006)

Where; n = sample size

Z = standard normal distribution at 95% confidence interval = 1.96

p = prevalence or seroprevalence = 8% (Adeboye *et al*., 2011)

q= 1- p

d = absolute desired precision = 0.05

Therefore; $n = \frac{(1.96)^2 \times 0.08 \times (1-0.08)}{(0.05)^2} = 113.10$

= 113 samples

However, in order to have true representation of the study population, a total of 273 samples were collected for the study. Out of the 273 samples collected, 200 were from children of ages 0-8months (study population) and 73 were from children of ages 9-23 months (control population). Samples were randomly collected from the various hospitals.

3.7 Study design

The study design was a hospital-based cross-sectional and experimental study. The essence of the research was explained to the target population and their verbal consent was obtained, after which structured questionnaire was administered and blood samples

collected. A total of two hundred and seventy three (273) blood samples were collected, of which 112 samples were collected from Yusuf Dantsoho Memorial Hospital, 82 samples from Hajia Gambo Sawaba General Hospital and 79 samples from Gwamna-Awan Hospital, Kaduna. A total of 191 samples were obtained from Kaduna metropolis while 82 samples were obtained from Zaria. This is because Kaduna metropolis is more populated than Zaria.

3.8 Data Collection

Structured questionnaires were administered to the parents of the children who consented to the study. Demographic information on the target population (vaccination status, vitamin A deficiency, overcrowding, etc.) associated with the prevalence of measles virus were collected.

3.9 Sample Collection and Processing

All materials (sterile containers, syringes and needles, tourniquet, cotton swab) required for the collection of venous blood were assembled and labeling of the sterile anticoagulant containers with the patient's identification number was done. About two millilitre (2ml) of blood sample was collected from each patient aseptically by venipuncture and dispensed into sterile labeled anticoagulant container, containing EDTA. The blood samples were transported in an ice box to the laboratory. The blood samples were allowed to settle and the plasma was separated using clean Pasteur pipettes into sterile plain sample containers. The samples were stored at refrigeration temperature (4^0C) until required for analysis.

3.10 Screening for Measles IgM using ELISA

Plasma samples were analysed for the presence of measles virus using Enzyme-Linked Immunosorbent Assay (ELISA) test procedure using the Measles IgM Assay kit (Diagnostic Automation, U.S.A).

3.10.1 Principle of the ELISA test:

Enzyme-Linked Immunosorbent Assay (ELISA) relies on the ability of biological materials (e.g., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum/plasma, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing with wash buffer solution. This is followed by the addition of goat anti-human IgM globulin conjugated with horseradish peroxidase which will bind to the antibody-antigen complexes. The excess conjugate is removed by washing with the wash buffer, followed by the addition of Chromogen/Substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum/plasma, a blue color develops. When the enzymatic reaction is stopped with 1M H_2SO_4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum/plasma, can be read on a suitable spectrophotometer or ELISA microwell plate reader.

3.10.2 Procedure for the ELISA test:

All samples and reagents were removed from the refrigerator and allowed to come to room temperature (25^0C). The coated strips were placed in a holder and labeled (one blank well, one negative control, two calibrators, one positive control and 91 wells for

sample specimens). About 3µl of the test samples, negative control, positive control and calibrators was added to 240µl of the serum diluent and mixed well to make 1 in 80 dilutions. Hundred microlitre (100µl) each of the diluted samples was dispensed into appropriate wells, ensuring that there were no air bubbles. Air bubbles present in the liquid were removed by tapping the holder. Hundred microlitre (100µl) of the serum diluent was added into the reagent blank well. The wells were incubated at room temperature (21 to 25^{o}C) for 30 minutes. After incubation, liquid from all wells was removed by washing three times with 300µl of the wash buffer. Hundred microlitre (100µl) of enzyme conjugate was added into each well and incubated at room temperature for 30 minutes. Excess enzyme conjugate was removed by washing three times with the wash buffer. Hundred microlitre (100µl) of chromogen/substrate solution (TMB) was dispensed into each well and incubated at room temperature for 15 minutes. The reaction was stopped by the addition of 100µl of stop solution (1M H_2SO_4) and the plate was tapped gently to mix contents of the wells. The reading was done using ELISA microplate reader at 450nm.

3.10.3 Interpretation of Results

Results were interpreted according to the manufacturer's recommendation. The Mean Calibrator O.D (Optical Density) value was calculated from the two Calibrator determinations. The Cut-off Calibrator Value for each assay was determined by multiplying the Correction Factor (provided by the manufacturer) by the Mean Calibrator O.D. determined. The ISR (Immune Status Ratio) value for each plasma sample was calculated by dividing the plasma O.D value by the Cut-off Calibrator Value obtained.

Cut-off Calibrator Value = Correction Factor x Mean Calibrator O.D

ISR Value = O.D obtained for patient's plasma

Cut-off Calibrator Value

ISR Value $\leq$ 0.90 was taken as negative which means that no significant level of detectable antibody to measles was present in the sample. ISR Value within 0.91-1.09 was taken as equivocal and the sample was retested. ISR Value $\geq$ 1.10 was taken as positive, indicating significant level of detectable IgM antibody to measles in the sample, which was indicative of current or recent infection.

3.11 Detection of Measles RNA using Semi-nested PCR

3.11.1 RNA Extraction using Q1Aamp Viral RNA Mini Kit (QIAGEN)

Two hundred microlitre (200µl) of each positive blood sample was transferred into an eppendorf tube. Five hundred microlitre (500µl) of lysis buffer AVL was dispensed into each eppendorf tube and incubated at room temperature for 10minutes. The samples were mixed by pulse-vortexing for one minute. The sample mixtures were centrifuged at 12000rpm for five minutes. The supernatants were transferred into spin columns (in a 2ml collection tube) and centrifuged at 12000rpm for five minutes. The spin columns were transferred into a clean 2ml collection tube and the filtrate was discarded. Five hundred microlitre (500µl) of wash buffer AW1 was added to each spin column and centrifuged at 12000rpm for two minutes. The filtrate was discarded. Five hundred microlitre (500µl) of wash buffer AW2 was added to each spin column and centrifuged at 12000rpm for two minutes. The filtrate was discarded. The empty spin columns were centrifuged at 10000rpm for two minutes. The spin columns were transferred into new eppendorf tubes and 60µl of elution buffer AVE was added into each spin column and allowed to stand at room temperature (25^0C) for 2minutes. Centrifugation was done at 10000rpm for two minutes to elute the RNA from the spin columns into the eppendorf

tubes. A double elution was done by adding 100µl of elution buffer AVE into each spin column and centrifuged after two minutes at 10000rpm for two minutes. RNA samples were stored at -20^{0}C until required for use.

3.11.2 Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

Measles virus (MV) RNA was reverse-transcribed at 42°C for one hour, using avian myeloblastosis virus reverse transcriptase (Promega) and specific primers MVR1 (nt 1629–1609, 5′ AACAATGATGGAGGGTAGGCG 3′) and MVF1 (nt 985–1008, 5′ TACCCTCTGCTCTGGAGCTATGCC 3′) in a one-step RT-PCR process. Following reverse transcription, a 229 bp fragment of the MV Edmonston strain (vaccine strain) N gene was targeted with first-round primers, MVF1 and MVR1, using DreamTaq Green PCR master mix kit. The reaction was performed in 50µl PCR mixture volume that contained 25µl of master mix (containing DreamTaq^{TM} DNA polymerase, $MgCl_2$ and dNTPs), 1µl each of forward and reverse primers, 1µl RT enzyme, 15µl of nuclease free water and 7µl of RNA template.

The PCR cycling program proceeded, consisting of initial denaturation at 94^{0}C for four minutes, followed by 40 cycles (first round) of denaturaion at 94°C for 60secs, annealing at 58°C for 60secs and extension at 72°C for 60secs, with final extension at 72°C for 10minutes in a DNA thermocycler (Bio-Rad Gene AMP PCR SYSTEM 9710).

3.11.3 Amplification of Measles virus DNA

A second-round semi-nested PCR was carried out to amplify a 229 bp fragment, using second-round primers MVF2 (nt 1101–1121, 5′ GATGGTAAGGAGGTCAGCTGG 3′) and MVR1 (nt 1629–1609, 5′ AACAATGATGGAGGGTAGGCG 3′). Semi-nested

PCR was performed in 50µl PCR mixture volume that contained 25µl of master mix (containing DreamTaq^{TM} DNA polymerase, $MgCl_2$ and dNTPs), 1µl each of forward and reverse primers, 16µl of nuclease free water and 7µl of DNA template.

The PCR cycling program consisted of denaturation for four mins at 94^0C, followed by 25 cycles (second round) of denaturaion at 94°C for 60secs, annealing at 58°C for 60secs and extension at 72°C for 60secs, with final extension at 72°C for 10minutes in a DNA thermocycler (Bio-Rad Gene AMP PCR SYSTEM 9710).

3.11.4 Detection and Identification of the Amplified PCR Products

Agarose gel of 1% concentration was prepared by weighing one gram of agarose in a conical flask and adding 100ml of 1x TAE buffer (Tris Acetate EDTA). The agarose was solubilized by heating in a microwave oven for two minutes. The solubilized agarose was allowed to cool to 40˚C and 5µl of ethidium bromide was added. The gel was poured into the gel tray with the combs in place and was left to solidify for about 50minutes. Electrophoresis tank was filled with the running buffer (Tris Acetate EDTA) and the gel slab was totally submerged in the buffer at a level not more than two milimetre above the gel. The combs were gently removed to give well-formed wells. About 20µl of each amplified PCR product was loaded into respective wells and 100volts was applied for 30minutes using Bio-Rad power pack (powerpac^{TM}HC). The power supply was turned off after 30minutes. The gel was removed and examined using Bio-Rad Gel Doc Molecular Imager System (Gel Doc^{TM}XRT). The basepair of the amplicon was determined using the standard (100bp DNA ladder).

3.12 Data analysis

Data generated from questionnaire and results obtained from laboratory analysis were entered into Microsoft excel. The data obtained were analyzed with SPSS (statistical package for social sciences) version 20.0 software program. Pearson's Chi-square (X^2) was used to test the significance of variables at 95% confidence interval and P-value <0.05 was considered significant. Odds ratio (OR) was used to measure association for 2 by 2 contingency tables and OR > 1 was taken for positive association. Side-by-side bar charts were used to visually display the results of cross-classification data of disease prevalence.

CHAPTER FOUR

4.0 RESULTS

A total of 273 blood samples were collected from children aged 0-23months presenting with measles-like symptoms in three major hospitals in Kaduna state: Gwamna-Awan Hospital, Kaduna, Yusuf Dantsoho Memorial Hospital and Hajia Gambo Sawaba General Hospital, Zaria. Of the 273 samples screened, 58 (21.2%) were positive for Measles virus IgM, with YDMH having the highest prevalence of 25.0%. There was no significant association between MV infection and the hospital location (P = 0.086) (Table 4.1).

From the 273 samples collected, 200 samples were from children aged 0-8months (study population) and 73 samples were from children aged 9-23months (control population). The distribution in relation to age showed that age group 9-12 months had the highest prevalence (64.3%) and age group 0-2 months had the lowest prevalence (1.4%). This difference was highly significant (P = 0.000) (Table 4.2). The overall prevalence was lower in children 0-8 months (6.5%) and higher in children >8 months (61.6%), showing a significant association between MV infection and age of children (P = 0.00) (Table 4.3).

The prevalence was significantly higher in female children (27.5%) than the male children (16.3%) showing an association between measles virus infection and the gender of the children (P = 0.025) (Table 4.4).

The parent's socio-demographic data were assessed in relation to MV infection. Though there was no significant association with the level of parent's education and MV

infection (P = 0.687), children of parents with primary education had the highest prevalence (24.3%) and the least prevalence was recorded in children of parents with tertiary education (16.3%) (Table 4.5). With respect to occupation, there was no significant association (P = 0.207), though children whose parents were civil servants had the highest prevalence (28.1%), followed by children whose parents were housewives (24.3%) and the lowest prevalence was reported in children whose parents were self-employed (16.7%) (Table 4.5). Similarly, there was no significant relationship between the number of children in the family and MV infection (P = 0.828) (Table 4.5).

In relation to the marital type of family, there was no significant association (P = 0.716). Children from polygamous family had a higher prevalence (22.4%), while children from monogamous family had a prevalence of 20.6% (Table 4.5). The result was analysed according to the place of residence and it was observed that those living in self-contained apartments had a higher prevalence of 21.4% while a lower prevalence was observed in those living in shared apartments (21.2%). Hence, there was no significant association between MV infection and place of residence (P = 0.966) (Table 4.5). Measles virus infection was not significantly associated with number of persons living in a house (P = 0.931) but those living in houses with more than five persons had a higher prevalence (21.4%) while lower prevalence was observed in those living in houses with less than five persons (20.9%) (Table 4.5).

Table 4.6 presents the association between measles virus infection and the children's vaccination status; there was a significant association (P = 0.031), with vaccinated children having a lower prevalence of 10.0%, as against unvaccinated children with prevalence of 23.8%. Also, there was significant association between MV infection and breast feeding babies (P = 0.000) (Table 4.6).

There was significant association between MV infection in children and the mothers' measles history (P = 0.005). Children of mothers with past measles history had a lower prevalence (12.7%) than children of mothers with no measles history (27.0%) (Table 4.6). Likewise, MV infection was significantly associated with the mother's vaccine status (P = 0.049). Children of vaccinated mothers had a lower prevalence (17.1%) than children of unvaccinated mothers (27.0%) (Table 4.6).

Table 4.7 shows the association between MV infection and symptoms presented. Fever was a common symptom to all the children. Out of 273 children with fever, 58 (21.2%) were positive for Measles virus IgM. There was significant association between MV infection and all the symptoms presented, except for vomiting, which showed no significant association (χ^2= 3.748, df = 1, P = 0.053).

There was no significant association (P > 0.05) between MV infection and malaria, typhoid and pneumonia which are common childhood-illnesses with measles-like symptoms (Table 4.8).

Table 4.1: Prevalence of Measles Virus Infection in Children according to Hospitals

Hospital	Number Examined	Number Positive	Prevalence (%)	P-Value
GAH	79	10	12.7	0.086
YDMH	112	28	25.0	
HGSGH	82	20	24.4	
Total	**273**	**58**	**21.2**	

$\chi^2 = 4.910$, df =2, P = 0.086

KEY:

GAH- Gwamna-Awan Hospital

YDMH- Yusuf Dantsoho Memorial Hospital

HGSGH- Hajia Gambo Sawaba General Hospital

P - Level of significance

Table 4.2: Seroprevalence of Measles Virus Infection in Children according to Age

Age group (months)	Number examined	Number positive	Prevalence (%)	P-value
0 – 2	72	1	1.4	0.00**
3 - 5	46	2	4.3	
6 - 8	82	10	12.2	
9 – 12	28	18	64.3	
>12	45	27	60.0	
Total	**273**	**58**	**21.2**	

χ^2= 100.225, df = 4, P = 0.000

KEY:

P - Level of significance

** = significant association exists at $p < 0.01$

Table 4.3: Seroprevalence of Measles Virus Infection in Children in relation to

Their Age Category

Age group (months)	Number examined	Number positive	Prevalence (%)	P-value
0 – 8	200	13	6.5	0.00**
9 – 23	73	45	61.6	
Total	**273**	**58**	**21.2**	

χ^2= 97.195, df = 1, P = 0.00, OR = 23.118

KEY:

P - Level of significance

** = significant association exists at $p < 0.01$

Table 4.4: Seroprevalence of Measles Virus Infection in Children according to Sex

Sex	Number examined	Number positive	Prevalence (%)	P-value
Female	120	33	27.5	0.025
Male	153	25	16.3	
Total	**273**	**58**	**21.2**	

χ^2= 5.006, df = 1, P = 0.025, OR = 1.942

KEY:

P - Level of significance

Table 4.5: Seroprevalence of Measles Virus Infection in Children in relation to Some Socio-demographic Factors

Factor	Number Examined	Number Positive	Prevalence (%)	P-Value
Parent's Educational status				
Primary	107	26	24.3	0.687
Secondary	88	17	19.3	
Tertiary	43	7	16.3	
Others	35	8	22.9	
Parent's Occupation				
Housewife	115	28	24.3	0.207
Self-employed	126	21	16.7	
Civil servants	32	9	28.1	
No. of children				
1 – 3	129	25	19.4	0.828
4 – 6	105	25	23.8	
7 – 9	32	7	21.9	
>9	7	1	12.1	
Family type				
Polygamy	98	22	22.4	0.716
Monogamy	175	36	20.6	
Type of residence				
Self-contained	117	25	21.4	0.966
Shared apartment	156	33	21.2	
Household number				
>5	187	40	21.4	0.931
2 – 5	86	18	20.9	

$\chi^{2=}$ 1.480, 3.146, 0.891, 0.132, 0.002, 0.007 respectively; **P**- level of significance.

Table 4.6: Seroprevalence of Measles Virus Infection in Children in relation to Possible Risk Factors

Factors	Number examined	Number positive	Prevalence (%)	P-value	Odd Ratio	Confidence Interval
Vaccination status						
No	223	53	23.8	0.031*	2.81	1.059-7.432
Yes	50	5	10.0			
Breastfeeding						
No	28	17	60.7	0.00**	7.69	3.36-17.62
Yes	245	41	16.7			
Mother's Measles history						
No	163	44	27.0	0.005*	2.535	1.312-4.900
Yes	110	14	12.7			
Mother's Vaccination status						
No	115	31	27.0	0.049*	1.791	0.998-3.211
Yes	158	27	17.1			

Vaccination status – χ^2 = 4.828, df = 1, P = 0.031, OR = 2.806

Breastfeeding – χ^2 = 29.048, df = 1, P = 0.000, OR = 7.69

Mother's Measles history – χ^2= 7.989, df = 1, P = 0.005, OR = 2.535

Mother's Vaccination status – χ^2= 3.873, df = 1, P = 0.049, OR = 1.791

KEY

P - Level of significance

* = significant association exists at P < 0.05

** = significant association exists at P < 0.01

Table 4.7: Seroprevalence of Measles Virus Infection in Children in relation to Disease Symptoms

Symptoms	Response	Number Examined	Number Positive	Prevalence (%)	P-Value	Odds Ratio	Confidence interval (95%)
Fever	Yes	273	58	21.2	0.00*	Undef	Undef
	No	0	0	0.0			
Cough	Yes	127	34	26.8	0.037*	1.858	1.032-3.346
	No	146	24	16.4			
Coryza	Yes	7	24	32.0	0.008*	2.270	1.234-4.177
	No	198	34	17.2			
Diarrhoea	Yes	182	46	25.3	0.021*	2.227	1.113-4.454
	No	91	12	13.2			
Vomiting	Yes	153	39	25.5	0.053	1.819	0.988-3.348
	No	120	19	15.8			
Rash	Yes	66	31	47.0	0.00*	5.905	3.14-11.09
	No	207	27	13.0			
Conjunctiva	Yes	14	7	50.0	0.007*	4.078	1.37-12.15
	No	259	51	19.7			
Koplik's spots	Yes	4	3	75.0	0.008*	11.67	1.19-114.41
	No	269	55	20.4			

Fever $\chi^2 = 0.000$, **Cough** $\chi^2 = 4.334$, **Coryza** $\chi^2 = 7.148$, **Diarrhoea** $\chi^2 = 5.298$, **Vomiting** $\chi^2 = 3.748$, **Rash** $\chi^2 = 34.426$, **Conjunctiva** $\chi^2 = 7.292$, **Koplik's spots** $\chi^2 = 7.011$

KEY

P – Level of significance, * = significant association exists at $P < 0.05$

Table 4.8: Seroprevalence of Measles Virus Infection in Children in relation to Common Childhood Diseases with Measles-like Symptoms

Disease	Response	Number Examined	Number Positive	Prevalence (%)	P-Value	Odds Ratio	Confidence interval (95%)
Malaria	Yes	45	9	20.0	0.823	0.913	0.412-2.024
	No	228	49				
Typhoid	Yes	14	0	0.0	0.056	Undef.	Undef.
	No	259	58	22.4			
Pneumonia	Yes	4	0	0.0	0.295	Undef.	Undef.
	No	269	58	21.6			

Malaria – $\chi^2 = 0.050$, df = 1, P = 0.823, OR = 0.913

Typhoid - $\chi^2 = 3.981$, df = 1, P = 0.056

Pneumonia - $\chi^2 = 1.095$, df = 1, P = 0.295

KEY

P - Level of significance

4.1 Molecular Identification of Seropositive Samples

The molecular analysis to confirm the presence of Measles virus infection was carried out using specific primers MVF1 and MVR1 for the RT-PCR and specific primers MVF2 and MVR1 for the semi-nested PCR, which yielded products of 200bp. All the fifty-eight seropositive samples from ELISA were used for the RT-PCR and the products from the RT-PCR were used for semi-nested PCR.

Plate I shows some of the products of the RT-PCR. Lanes 1 to 18 were loaded with the RT-PCR products of the positive ELISA samples while lane M19 represented the negative control. The cDNA products obtained confirms the presence of measles virus in the samples.

Plate II shows the amplification products following semi-nested PCR. Lane M1 represents the DNA size marker (100bp ladder) which served as our positive control, lane M2 represents the negative control, while lanes 1 to 13 were loaded with the products from the semi-nested PCR. An amplicon size of 200bp was obtained in lanes 3, 7 and 11.

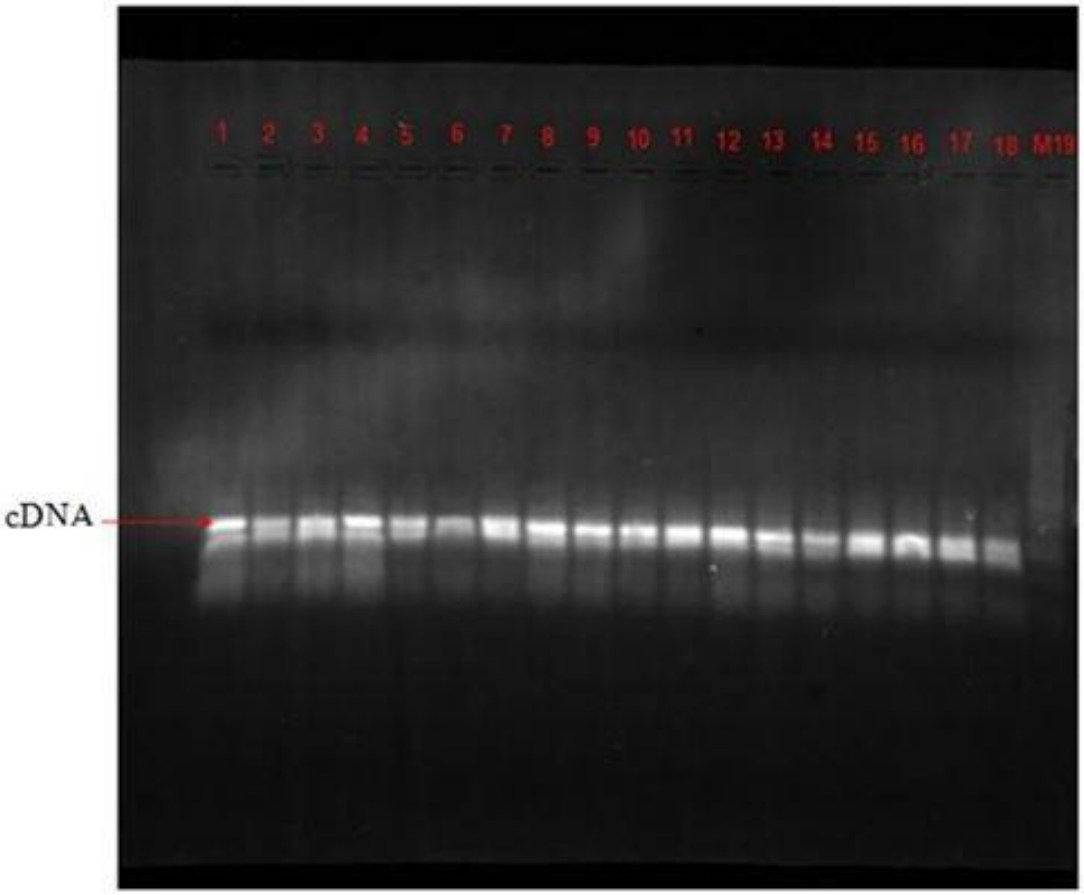


Plate I: Agarose gel electrophoresis of the RT-PCR products using MVF1 and MVR1 primers.

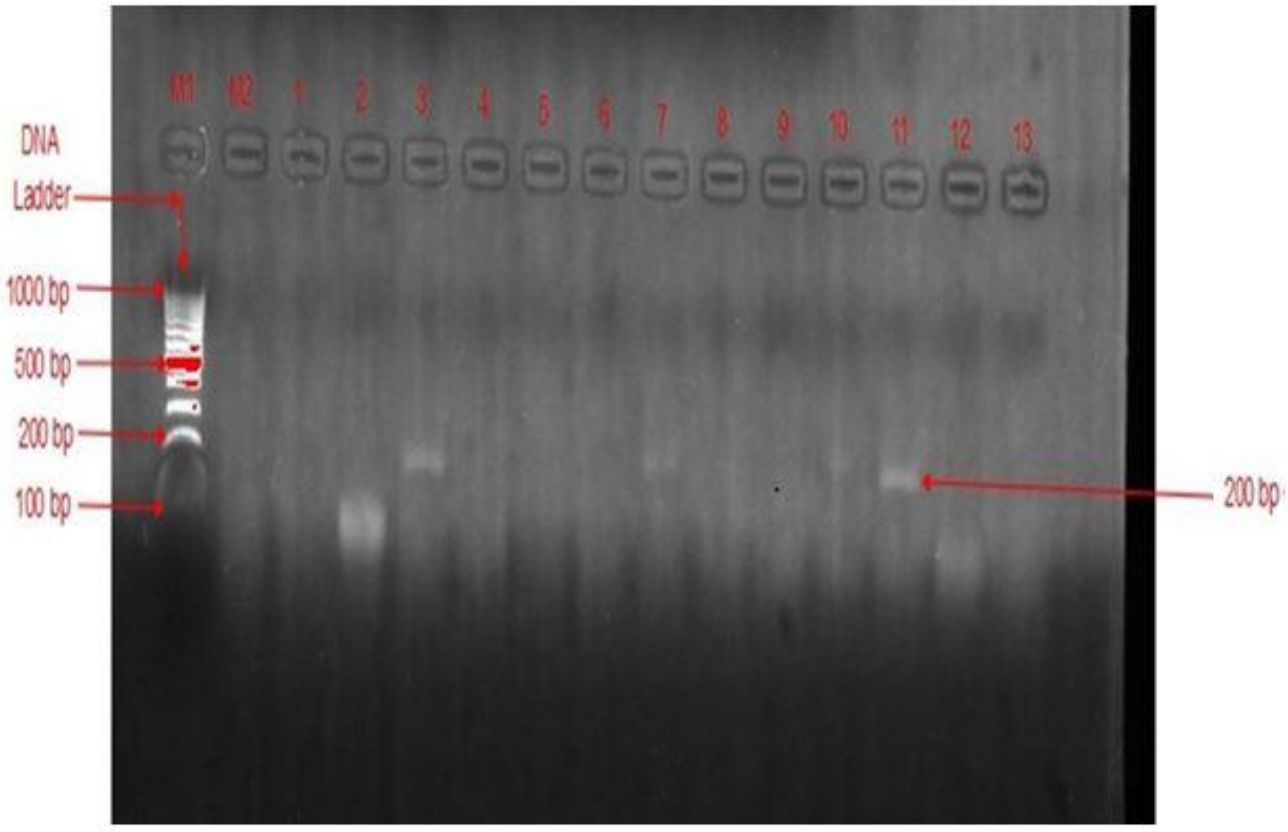


Plate II: Agarose gel electrophoresis of the semi-nested PCR products using MVF2 and MVR1 primers.

CHAPTER FIVE

5.0 DISCUSSION

In this study, the prevalence of measles virus in children (0-8 months) was 6.5%. This is in agreement with the prevalence of 7.0% reported in Akwa Ibom state (Chukwuemeka and Hycienth, 2013) and 6% prevalence reported in Maiduguri, Borno state (Ahmadu *et al*., 2013). A prevalence of 61.6% was obtained in children of ages 9-23 months. The overall prevalence obtained in children (0-23 months) from this study was 21.2%. This is lower than the prevalence of 30.2% reported in Akwa Ibom (Bassey *et al*., 2010), 32% reported in Ilorin, North Central Nigeria (Babanniyi *et al*., 2005), much lower than 55% recorded in a study conducted in Lagos (Adetunji *et al*., 2007) but higher than 15.6% reported in another study in Southwestern Nigeria (Omilabu *et al*., 1999). Elsewhere in Africa, 73.7% has been reported in Ghana (Ishiwada *et al*., 2001), 86.1% in Alexandria, Egypt (Tayil *et al*., 1998) and 69.61% reported in Bolivia (Aumatell *et al*., 2013). The reason for the observed decline in the prevalence may be attributed to the proper measles virus vaccine immunization coverage in Nigeria. Accelerated measles control activities, including improved provision of a second dose of measles vaccine at supplementary vaccination activities in certain countries of the world and case-based surveillance with laboratory confirmation, may have reduced measles-associated morbidity and mortality (Manirakiza *et al*., 2011).

Several studies in different regions of the world have identified socio-demographic variables like age, gender, socio-economic status and environmental conditions as risk factors for acquisition of measles virus infection (Desai *et al*., 2003; Aumatell *et al*., 2013). For example, age has consistently shown to be a major predictor of measles virus IgM.

Seroprevalence of measles virus had no significant association with the hospital locations. The lowest prevalence was observed in children attending Gwamna-Awan Hospital, while the highest prevalence was in children attending Yusuf Dantsoho Hospital located in Kaduna metropolis. The prevalence of 21.2% obtained in this study (Table 4.1), makes this study area fall in the category of measles endemic states.

This study showed that seropositivity increased with age in children (0-8 months), with the highest prevalence in age group 6-8months (Table 4.2). This result is in agreement with previous reports (Desai *et al*., 2003; Adeboye *et al*., 2011). This early presentation of measles has been attributed to waning maternal antibodies, especially in the setting where immunity is from vaccination, not natural infection. This result is in agreement with previous studies which showed that 58% of infants lose these protective antibodies by four months and 97% between six and nine months (Oyedele *et al*., 2005; Ntshoe *et al*., 2013).

The age-specific prevalence indicates that children 9-12 months in Kaduna state are more susceptible to measles virus infection. This may be due to failure to receive vaccine against measles. This study also revealed that in children (9-23 months), positive cases of measles decreased as the age of children increased (Table 4.2). This trend may be attributed to physiological status of the individuals and variation in prevailing environmental factors (Akramuzzaman *et al*., 2002). In addition, immunity acquired by older children over the years as a result of sub-clinical infections might provide the required protection against measles (Patel *et al*., 2008). The result from this study is similar to previous work (Desai *et al*., 2003).

It was also deduced from this study that children 9-23 months were 23.1 times more likely to be infected with measles virus than children within 0-8 months (Table 4.3).

In this study, the female population was seen to be more susceptible to measles infection than their male counterparts (Table 4.4). This result is in agreement with previous studies in Nigeria (Bassey *et al*., 2010; Aumatell *et al*., 2013), which documented that measles antibody is marginally higher in females than males. This finding showed that female children had two times higher odds of being infected with measles virus than their male counterparts (Fauveau *et al*., 1991; Tharmaphornpilas *et al*., 2009; Grais *et al*., 2007).

The test of association between parents' level of education with seroprevalence of measles showed no significant association (Table 4.5). Children of parents with primary education had the highest prevalence, followed by children of parents with other forms of informal education and then children of parents with secondary education. The least prevalence was observed in children of parents with tertiary education. The high prevalence in children of parents with primary and other forms of education may be due to the parents' ignorance on the need for measles vaccination, myths and other misconceptions associated with measles vaccines. Studies had shown that educated women are more concerned with the immunization of their children and proper immunization schedules than uneducated women (Muhammed and Danish, 2014). This finding is consistent with similar findings in Malawi (Fowlkes *et al*, 2011) and Bolivia (Aumatell *et al*., 2013), where seroprevalence was higher in children of mothers with less than secondary education level, but lower in children of highly educated parents.

Occupation of parents of the children were assessed and there was no significant association between parent's occupation and infection (Table 4.5). However, children of parents who are civil servants had the highest prevalence, followed by children of parents who are housewives. Children of parents who are self-employed had the least

prevalence. These differences could be as a result of the time spent on child care, since civil servants spend more time at work than others and may possibly miss the routine immunization of their children. This study agrees with a study carried out in United States, where children of working class parents were more susceptible to infection (Holmes *et al*., 1996).

There was no significant association between the number of children in the family and seroprevalence of measles infection. Children of parents that had between 4-6 children had the highest prevalence while children of parents that had more than nine children had the least prevalence (Table 4.5). Herd immunity may be responsible for this low prevalence in families with more than nine children. This conforms to a previous study which found that protective effect in large families was stronger from older siblings and from brothers, compared with sisters (Bloomfield *et al*., 2006). The reason for this is yet to be understood.

Family marital status did not affect the prevalence of measles virus in this study as there was no significant difference in the prevalence of MV infection in children from polygamous and monogamous homes. However, a higher seropositivity was observed in children from polygamous homes than children from monogamous homes (Table 4.5). This may be as a result of the large family size. This is in agreement with previous finding in Bolivia where family structure had no significant association with measles prevalence (Aumatell *et al*., 2013).

This study determined the effect of type of residence as well as number of people living in the house on seropositivity of the virus. A higher positivity was observed in children who lived in self-contained apartments compared to those who lived in shared apartments (Table 4.5). This could be attributed to their poor living conditions such as

poor housing and sanitary conditions as well as prolonged exposure within the home, compared with more casual exposures outside the home. This study agrees with a study carried out in Stockholm (Burstrom *et al*., 1999) which showed that children who develop measles after within-household exposure have higher case-fatality rates than do children who are exposed to measles outside the household. The number of individuals in the household was not significantly associated with the prevalence of measles virus. Children from households with more than five persons had a higher prevalence and they are more likely to be infected than children from households with 2-5 persons. Overcrowding leads to a higher and faster spread of infection through the respiratory route. Since Measles virus is transmitted primarily from person-to-person by large respiratory droplets, this finding suggests that overcrowding, which results in poor ventilation and person-to-person contact could be a possible risk factor for contacting measles virus infection. This study is in agreement with previous study carried out in United States (Butler *et al*., 1994) where there was no significant relationship between crowding and measles infection, but in contrast to a study carried out in Bangladesh (Koenig *et al*., 2001).

The vaccination status of children was assessed in relation to the prevalence of Measles virus infection and vaccination was significantly associated with the prevalence of measles infection (Table 4.6). Out of 223 unvaccinated children, 53 (23.8%) were IgM positive. Two hundred (200) of these unvaccinated children had not attained the eligible age of vaccination (nine months). The prevalence of measles infection in the vaccinated population was 10%, this may be suggestive of vaccine failure. This study conforms to the acceptable rate for measles vaccination failure stipulated in the range of two to ten percent (Wilkins and Wehle, 1978). Storage, transportation and maintenance of cold chain system problems can easily affect the potency of vaccines in developing nations

(Adu *et al*., 1996; Omilabu *et al*., 1999). Diversification in measles strains has also been reported to account for the early presentation of measles and occurrence of measles in vaccinated children (Kouomou *et al*., 2002). This study complements previous studies in Nigeria (Bassey *et al*., 2010).

In this study, it was observed that breast feeding had a high significant association with the prevalence of measles infection. The prevalence was higher in children who were not breast feeding, compared to breast feeding children and non-breast feeding children were at 7.69 higher risks of being infected with measles virus than breast feeding children (Table 4.6). The low prevalence among breastfeeding children may be as a result of the high level of antibodies and antimicrobials, as well as some probiotics contained in breastmilk which confer immunity on these children. Breastmilk has also been shown in previous studies to be a good source of vitamin A in children and this helps to protect children against measles. This study is in agreement with previous studies by Adu and Adeniji (1995); Katona and Katona-Apte (2008) which showed that breastfeeding children are more resistant to measles infection.

This study established a relationship between mother's measles history and vaccination status on the prevalence of measles in the children (Table 4.6). Children of mothers with no measles history had a higher prevalence compared to children of mothers with measles history. This low prevalence observed in children of mothers with measles history can be explained by the fact that these mothers had naturally acquired immunity against measles, which provided passive protection to their infants. Children of mothers with no measles history were at 2.5 times higher risk of being infected than children of mothers with measles history. This agrees with previous studies by Brugha *et al*. (1996) and Nicoara *et al*. (1999). Similarly, there was a significant association between

mother's vaccination status and the prevalence of measles in children. Children of unvaccinated mothers had a higher prevalence and they were at 1.8 times higher odds of being infected than children of vaccinated mothers. This could also be as a result of vaccine-induced maternal immunity conferred on the children (Brugha *et al*., 1996; Nicoara *et al*., 1999).

This study determined the relationship between measles virus infection and symptoms presented in children. The result established that all symptoms (fever, cough, coryza, diarrhoea, rash, conjunctiva and koplik's spots) had significant association with measles virus (Table 4.7). This means that a child with measles virus must present with some or all of these symptoms. This agrees with WHO clinical case definition- any person presenting with a history of fever (39 to 41°C), lasting three days or more and generalized maculopapular rash with one of the following, coryza, cough or conjunctivitis (Wharton *et al*., 1990; WHO, 1999). It was observed from this study that fever was a common symptom in all these children, although this symptom may have been caused by other fever-rash childhood illnesses such as rubella, parvovirus or dengue fever. However, vomiting was not statistically associated with the prevalence of measles virus infection.

There was no significant association between measles virus infection and other common childhood illnesses with measles-like symptoms (Table 4.8). Although these illnesses present similar symptoms as measles, they may not necessarily be present in a child with measles infection. Previous studies have also identified pneumonia as a complication of measles virus (Orenstein *et al*., 2004; Yasunaga *et al*., 2010) which occurs in 6% of cases <5 years, often leading to death. The pneumonia cases in this study may have been caused by other bacterial or viral infections, but not measles. This

establishes the fact that we did not come across any case of complication in the course of this study.

In this study, out of the 273 children that presented with measles-like symptoms, only 58 (21.2%) were positive for measles IgM. This study conforms to the fact that measles is endemic in Nigeria (Adetunji *et al*., 2007). Children (0-8 months) accounted for a prevalence of 6.5% in Kaduna state, which establishes the fact that it is possible for children below nine months to come down with acute measles infection. This has been attributed to the early loss of passively acquired maternal antibodies in these children, which varies in individuals. Hence, the need to review the vaccination age in Nigeria and bring it backward to six (6) months, as recommended by WHO (Helfand *et al*., 2008). This study conforms to previous studies by Adeboye *et al*. (2011), Ahmadu *et al.* (2013), Chukwuemeka and Hycienth (2013) who reported the prevalence of measles virus in children (0-8 months). Measles virus is an infection of the respiratory tract and as such, patients should present symptoms such as cough, coryza, conjunctiva, diarrhea, vomiting and koplik's spots in few cases, as well as a generalized erythematous maculopapular rash. However, infants who have low levels of passively acquired maternal antibodies often have subclinical infections or minimal symptoms that may not be diagnosed as measles, some of these symptoms go mild and unnoticed. Seroprevalence in this study has been associated with age, gender, breastfeeding, vaccination, mother's measles history and mother's vaccination status. However, when one considers the low prevalence of measles virus in this study, it may be possible that the timing of this study corresponds to a period of endemicity and low transmission in our environment, since in many regions of the world, transmission follows a seasonal pattern with outbreaks occurring every two to three years in developing countries (Murray and Rasmussen, 2000).

This is the first study to molecularly identify the presence of measles virus in Kaduna state, to the best of our knowledge. Polymerase chain reaction (PCR) has been used over the years to confirm the presence of the virus by detecting specific viral genes. Because serologic methods cannot distinguish between a vaccine-induced antibody response and antibodies derived from natural disease, molecular identification of viral isolates provides the only method to differentiate between natural infection and vaccine-induced adverse effects. Edmonston strain is the vaccine strain produced in the U.S and distributed worldwide. It belongs to genotype A, which is a genotype that is not associated with documented endemic transmission in any part of the world (Rota *et al*., 1994). Studies have recognized genotype B3 as the endemic genotype circulating in most African countries, including Nigeria (Hanses *et al*., 1999; Kouomou *et al*., 2002).

In the investigation, primers for the Edmonston strain were used to determine whether the infection identified by the IgM ELISA was due to the Edmonston strain. The initial RT-PCR products (cDNA) obtained confirms the presence of measles virus while the amplification of the 200bp in lanes 3, 7 and 11 indicates a possible seroconversion of the vaccine-type, which suggests that the infection may have been caused by the vaccine type, since it has been documented that the Edmonston vaccine-type could cause adverse effects characteristic of the wild type (CDC, 2012). It could also imply that the positive cases were caused by a wild-type strain circulating in Nigeria that shares some possible base sequences with the vaccine type. This result agrees with previous works carried out in Croatia (Kaic *et al*., 2010) and Canada (Murti *et al*., 2013) on the seroconversion of measles vaccine strain. This study also conforms to the fact that measles vaccine causes adverse effects, similar to the symptoms of wild-type measles infection (CDC, 2012). This could be because the vaccine is a live vaccine attenuated form of the wild-type virus.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. The findings in this study confirmed the presence of Measles virus in children (0-8 months) in Kaduna state, with seroprevalence comparable to the rates obtained in other parts of the country. The prevalence of 6.5% obtained in children (0-8 months) and 21.2% in children (0-23 months) is an indication that measles is endemic in Kaduna state and still poses a public health problem, despite the availability of a safe and effective vaccine.

2. The results obtained from this study confirmed that both serological and molecular assays have high sensitivity and specificity and are suitable for routine use.

3. This study recognized age, sex, vaccination, breastfeeding, mother's vaccination status and mother's measles history as important demographic information.

4. This study did not establish any relationship between measles virus infection and other childhood illnesses with measles-like symptoms.

5. It is therefore important for health providers and policy makers to recognize the health implication of this virus and review the vaccination age of infants and also, intensify vaccination campaign programs.

6.2 Recommendations

1. The battle against measles should be well mounted. Short-term control measures aimed at eliminating the infection should be vigorously implemented. The provision of effective and potent vaccines, accessible to all children should be ensured. Maintenance of cold chain for vaccines should be a priority as diurnal temperatures vary, and so does the time taken to get to some vaccination locations which reduces vaccine potency as a result of thawing.
2. Public enlightenment and awareness programs for parents, which emphasize the importance of vaccination should be created. Parents should be counseled to always present their children for routine immunization.

3. Changes in timing of the NPI immunization schedule for measles is therefore suggested, as recommended by WHO, such that infants in developing countries can be vaccinated against measles at six and nine months. Early immunization however carries the risk of failed seroconversion. This risk may be worth taking considering the fact that 97% of infants may have lost the passively acquired protection between six to nine months.

4. Exclusive breastfeeding for six months is recommended for infants (WHO, 2001). Diets rich in vitamin A is recommended for children above breastfeeding age, as this will boost immunity to measles virus infection and other related diseases.

5. There is the need to carry out further studies on measles IgG in infants to determine maternal antibody levels and the actual age at which these maternally derived antibodies wane off.

6. ELISA and PCR assay techniques should be employed for routine use due to the high sensitivity and specificity of the techniques.

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APPENDIX I

MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 248084
(062) 248252

Independence Way,
P.M.B. 2014,
Kaduna.
Kaduna State, Nigeria

MOH/ADM/744/VOL.I/ **31st May, 2013**

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PERMISSION TO CONDUCT RESEARCH

I have been directed to convey the Ministry's approval to OLAITAN, AYORINDE ELIZABETH a student from department of microbiology, faculty of Science Ahmadu Bello University Teaching Hospital Zaria, Kaduna State Nigeria. To conduct a research on the Topic: **"SEROPREVALENCE AND MOLECULAR CHARACTERIZATION OF ACUTE MEASLES IN CHILDREN (0-8 MONTHS) PRESENTING WITH LOW-GRADE FEVER ATTENDING SELECTED HOSPITALS IN KADUNA STATE METROPOLIS"** for the award of M.SC.

It is expected that necessary assistance be accorded to him/her in the process of the research, please.

However, it is mandatory for the researcher to submit a copy of his/her research finding(s) to the office of the Honourable Commissioner, Ministry of Health, as soon as it is concluded.

Accept the assurances of the Honourable Commissioner's highest regards.

F. A. KURAH (MRS)
SECRETARY, ETHICAL COMMITTEE

APPENDIX II

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF POSTGRADUATE STUDIES

AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

This Informed Consent Form is basically for Children (0-8 months) presenting with low-grade fever attending hospitals in Zaria/Kaduna selected for this study. We are inviting you to participate in this research work titled "Seroprevalence and molecular characterization of measles virus in children (0-8 months) presenting with measles-like symptoms in hospitals in parts of Kaduna State, Nigeria''. Measles is an infection of the respiratory tract caused by Measles virus. The virus is spread primarily via coughing, sneezing, close personal contact or direct contact with infected nasal or throat secretions. It is assumed that maternal antibodies against the virus persist in children between (0-8 months). Thus, vaccines against the virus are administered to children at nine months. This research will involve the collection of blood samples from children (0-8 months). The results obtained thereby may contribute to the knowledge of the prevalence of measles in children below nine months. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this clinic/hospital.

CERTIFICATE OF CONSENT

I …… of ……. do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that blood samples will be taken for the test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant ……..

Signature/right thumb print of participant ……. Date ……

APPENDIX III

QUESTIONNAIRE

SECTION A: BIODATA

Serial number: .....

Age: .....

Sex: .....

Type of family: Monogamy ( ) Polygamy ( )

Occupation of parent: Housewife ( ) Self-employed ( ) Civil servant ( )

Level of Parent's Education: Primary ( ) Secondary ( ) Tertiary ( ) Others ( )

SECTION B: EPIDEMIOLOGICAL RISK FACTORS

1. How many children do you have? 1-3 { } 4-6 { } 7-9 { } >9 { }
2. Which type of house do you live in? Self-contained { } Shared apartment { }
3. How many people live in your household? 2-5 { } >5 { }
4. Does your child still breastfeed? Yes { } No { }
5. If no, does your child feed on Vitamin A (e.g fruits, vegatables, palm oil, etc)? Yes { } No { }
6. Has your child taken measles vaccine? Yes { } No { }
7. Have you had measles in the past? Yes { } No { }
8. If no, have you taken measles vaccine in the past? Yes { } No { }
9. Nature of illness (e.g malaria, typhoid, pnuemonia, etc): .....
10. Major symptoms of illness: Fever ( ) Cough ( ) Coryza ( ) Diarrhoea ( ) Vomiting ( ) Rash ( ) Conjuctiva ( ) Koplik's spots ( )