SEROPREVALENCE AND GENOTYPIC CHARACTERISATION OF MEASLES VIRUS AMONG CHILDREN 0-10 YEARS IN PARTS OF NORTH-WESTERN NIGERIA

BY

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ZARIA, NIGERIA

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DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCES

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FEBRUARY, 2019

DECLARATION

I hereby declare that the work presented in this thesis entitled "**Seroprevalence and Genotypic Characterization of Measles Virus Among Children 0-10 Years in Parts of North-Western Nigeria**" is original and has been carried out by me in the Laboratory of Department of Microbiology under the close supervision of Prof. O.S. Olonitola, Dr. E. E. Ella and Prof. D.A. Machido.

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 submitted for any degree or qualification.

Aliyu Maryam Yakasai

Name of Student	Signature	Date

CERTIFICATION

This thesis entitled **"Seroprevalence and Genotypic Characterization of Measles Virus Among Children 0-10 Years in Parts of North-Western Nigeria"** by Aliyu Maryam Yakasai meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D.) of Ahmadu Bello University, and is approved for its scientific contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my husband Tijjani Abdul Akaaba and children Amina (Ummi), Farida, Abdullah, Abdul Malik and Saadat (Ihsan).

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ABSTRACT

Measles is a highly infectious disease with potential for eradication but is still responsible for high mortality among children particularly in developing nations like Nigeria. A descriptive cross-sectional study was conducted in some parts of the Northwestern Nigeria with the aim to determine the seroprevalence of measles virus IgM antibody, identify the circulating measles virus genotypes and establish the risk factors associated with the acquisition of the infection. A total of 752 blood and nasal swabs sample and were collected from children (0-10 years) across three states; Kano, Katsina and Zamfara from November 2014 to December 2015. Study subjects were interviewed using self-structured questionnaires. Measles-specific IgM antibodies were screened qualitatively using commercial ELISA IgM kit (Diagnostic Automation and Cortez, Calabasas, CA, USA). Molecular detection and characterization of measles virus were carried out by RT-PCR, Sequencing and phylogenetic analysis. Of the 752 suspected cases of measles screened, three hundred and thirty-four samples (334) 44.4% were measles IgM antibody positive with children from Kano having the highest seroprevalence of 47.8% followed by Zamfara 43.3% and the least was Katsina with a seroprevalence of 41.1%, though the statistical association was not significant (χ^2=2.512. P=0.285). Measles IgM antibody were detected in all age groups with the highest seroprevalence of 53.2% in the age group 1-24 months and the lowest 25.7% in the age group 73-96 months, the difference was statistically significant (χ^2=20.1, p=0.001). Females had higher seroprevalence (46.7%) than their male counterparts (42.1%), though the result was not statistically significant (χ^2=1.619, p=0.203). There was a significant association between measles IgM antibody seroprevalence and parent's occupation

(χ^2=22.58, p=0.001), parent's level of education (χ^2=15.161, p=0.004), previous measles infection history (χ^2=8.678, p=0.013) and involvement with crowd (χ^2=6.298, p=0.012). However there was no statistically significant association with respect to children's vaccination status (χ^2=2.555, P=0.279), number of times of vaccination (χ^2=1.950, p=0.474), travel history (χ^2=3.266, p=0.071), history of contact with infected persons (χ^2=0.030, P=0.862) and vitamin A supplement intake (χ^2=0.153, p=0.696). Measles IgM antibody seroprevalence was found to be associated with all the presenting symptoms rashes, cough, conjunctivitis, runny nose and fever (p value <0.05). This study recognized age, previous exposure to measles, parent's education level, parent's occupation and overcrowding as important demographic and risk factors associated with the acquisition of measles infection.

The study further reaffirms that measles remain endemic in the study area, the presence of measles in this region among unvaccinated children indicate insufficient vaccination. The results of molecular and phylogenetic analysis revealed that N gene sequences of the strains from this study were closely related to each other, and sequence analysis revealed that the viruses belonged to genotype B3, subgroup B3.1; it further confirmed that single endemic genotype B3 viruses are predominantly circulating in Nigeria. Molecular surveillance will help to monitor the progress in measles elimination**.** This study highlights that genetic characterization of measles virus is an essential tool in the laboratory surveillance of measles, especially in countries like Nigeria where measles control is still the major goal.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AUG	Adenine Uracil Guanine
AW1	Washing Buffer 1
AW2	Washing Buffer 2
BLAST	Basic Local Alignment Search Tool
Bp	Basepair
C	Capsid Protein
CD150	Cluster of Differentiation 150
CD46	Cluster of Differentiation 46
CDC	Centre for Disease Control
cDNA	Complementary DNA
CFRS	Case Fatality Ratios
CNS	Central Nervous System
CPE	Cytopathic Effects
CSF	Cerebrospinal Fluid
DCs	Dendritic cells
DIP	Defective Interfering Particles
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
EDTA	Ethylene Diamine Tetraacetic Acid
EGFP	Enhanced Green Florescent Probe
F	Fusion protein

H	Hemagglutinin protein
HIV	Human Immunodeficiency Virus
HAART	Highly Active Antiretroviral Therapy
hSLAM	Human Signaling Lymphocyte Activation Molecule
HSP	Heat Shock Protein
HSV	Herpes Simplex Virus
IFN	Interferon
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
ISO	International Organization of Standards
KMV	Killed-virus Measles Vaccine
L	Large protein
M	Matrix protein
MCP	Membrane Co-factor Protein
MCV	Measles Containing Vaccine
MEANS	Measles Nucleotide Surveillance
MEGA	Molecular Evolutionary Genetics Analysis
MIBE	Measles Inclusion Body Encephalitis
MMR	Measles Mumps Rubella
MMWR	Morbidity and Mortality Weekly Reports
mRNA	Messenger RNA

MSP	Measles Strategic Planning
MV	Measles Virus
MVF1	Measles Virus Forward primer 1
MVF2	Measles Virus Forward primer 2
MVi	Measles Virus Isolate
MVR1	Measles Virus Reverse primer 1
MVs	Measles Virus Sample
N	Nucleoprotein
NCBI	National Center for Biotechnology Information
NCR	Non Coding Region
nt	Nucleotide
OR	Odds Ratio
ORF	Open Reading Frames
P	Phosphoprotein
PAHO	Pan African Health Organization
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Unit
PIE	Post Infectious encephalomyelitis
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
RVP	Rinder Pest Virus
SIAs	Supplemental Immunization Activities

SLAM	Signaling Lymphocytes Activation Molecule
SSPE	Subacute Sclerosing Panencephalitis
TAE	Tris Acetate Edta
STAT	Signal Transducers and Activators of Transcription
UNICEF	United Nations Children's Fund
V	V vcore protein
WHA	World Health Assembly
WHO	World Health Organization
WT	Wild Type

CHAPTER ONE

1.0 INTRODUCTION

Measles virus (MV), a spherical enveloped, non-segmented virus with a single-stranded, negative-sense RNA genome, is a member of the genus Morbillivirus within the family Paramyxoviridae (Maldonado, 2004;Lamb and Park, 2007; Furuse *et al.*, 2010). Humans are the only natural hosts for measles virus, although other primates such as monkeys are also susceptible to measles and develop similar clinical diseases; these populations are not large enough to support sustained transmission (Rota and Bellini, 2003; Griffin, 2007).

Measles is an acute highly infectious disease characterized by prodromal period of fever, malaise, anorexia, cough, coryza, and conjunctivitis. During this prodromal period, Koplik's spots (small, bright red spots with a bluish-white center) appear on the buccal mucosa 1-2 days before onset of rashes and may be noticeable for an additional 1-2days afterwards (WHO, 2001). These spots are considered to be pathognomonic for measles diagnosis prior to onset of rashes (Moss and Griffin, 2009). The prodrome is followed by a characteristic erythematous maculopapular rash that appears first on the face and behind the ears, spreads to the trunk and extremities, and fades after 3-4days. People with measles are infectious for several days before and after the onset of rash(Shin-ichi *et al.*, 2004). In uncomplicated measles, clinical recovery begins soon after onset of rash, and results in viral clearance and lifelong immunity (James *et al.*, 2011). As a consequence of the immunosuppression induced by measles infection, and

the direct damage to the respiratory tract (loss of cilia), secondary bacterial, viral and parasitic infections may occur.

Transmission occurs predominantly via respiratory route, by direct contact, inhaling infected air or droplet emitted by coughing or sneezing from an infected person and occasionally transmitted through the conjunctivae (Griffin, 1990; Mbugua *et al.,* 2003). The incubation period of measles from exposure to onset of symptoms ranges from 10-12 days with 14 days' average (Walter *et al.,* 2004). Although there is no treatment, most infected individuals recover by themselves except when complications result.

In developed countries, complications such as otitis media, gastroenteritis, pneumonia, myocarditis and pericarditis occur in approximately 10% of measles cases, with encephalitis occurring in a very small subset of cases, while in developing countries, complication rate may reach 80% with pneumonia and gastroenteritis due to secondary infections as the most common fatal complications, especially in malnourished and immunocompromised children (Duke and Mgone, 2003; Griffin, 2007). Up to 15% of immunocompetent adults with measles may also experience pneumonia as a direct result of the measles virus (MV) infection, as opposed to a secondary infection. Childhood blindness associated with keratitis and corneal lesions and exacerbated by vitamin A deficiency, is a frequent complication of measles, especially in developing countries (Griffin, 2007; Mason, 2008).

Measles is a serious disease which usually affects susceptible children leading to epidemic (WHO, 2009).It occurs throughout the world and remains a serious and common disease in developing countries (CDC, 2005). Studies have shown that among the burden of vaccine preventable diseases measles is ranked first with 38% disease burden (Durbey and Choudhury, 2009; Onyiruika, 2011).

Very significant progress in measles control has been made over the past decade. Global measles deathswere reduced by 75% from 544,200 cases in 2000 to 145,700 cases in 2013 following an increase in routine measles vaccination coverage of up to 84%, with an estimated 15.6 million deaths prevented in this period (Perry*et al.,* 2015). Despite this global progress, endemic circulation persists in many developing countries (WHO, 2012). In Asia and Africa, the disease is the leading cause of childhood morbidity and mortality and an outbreak threat in the majority of countries, despite the availability of safe and effective vaccine for over four decades (Odegaa *et al.,* 2010; Ntshoe *et al.,* 2013; and Fatiregun *et al.,* 2014).

Measles affects about 20 million people a year, primarily in the developing areas of Africa and Asia (Caserta, 2013). No other vaccine-preventable disease causes as many deaths (Kabra and Lodhra, 2013). In 1980 2.6 million dead were recorded while in 1990 and 2014 545,000, and 73,000 respectively died from measles (GBD, 2016). Most of those who are infected and who die are less than five years old. The risk of death among those infected is usually 0.2% Atkinson, (2011) but may be up to 10% in those who have malnutrition.

Reports from developing countries shows that 20-80 percent of children who developed measles had received previous measles vaccination (Ojuawo and Bello, 2000; Audu *et al.,* 2004; and Fetuga *et al.,* 2007). Risk for illness and death from measles still exists in countries with variable routine vaccination coverage, as in Nigeria, where measles is a significant public health problem (CDC, 2009). In Africa, large measles outbreaks were reported by the Democratic Republic of the Congo, with over 103,000 cases, Nigeria, with 17,428 cases, Zambia, with 5, 397 cases, and Ethiopia, with 2, 902 cases(Salako and Sholeye, 2015). Occurrence of measles outbreaks in countries that have large volume of international travelers can lead to large outbreaks and associated death (Salako and Sholeye, 2015).

These occurrences may in no doubt be mainly connected with several setbacks including lack of political will to conduct high quality measles campaigns, competing health priorities, insufficient financial support to reach remote communities amongst other factors which result in resurgence of measles and have the capacity to place the recent gains in reducing measles mortality at risk (Grais *et al.,* 2007; Alemu *et al.,* 2004 and Adeboye *et al.,* 2011).

A study reported that Nigeria among other West African countries has faced high mortality from measles(Grais*et al.,* 2007) and remainedone of the 47 countries in the world with the highest burden of measles (Giusti *et al.,* 2013) where it is still responsible for the high number of vaccine preventable death. 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). However, activities are being stepped up by various health regulatory

agencies in a bid to control measles throughout the world, including Nigeria (Shena *et al.,* 2014).

Measles vaccination has been very effective in reducing the global measles disease burden. However, outbreaks can occur when inadequate vaccination coverage allows accumulation of a sufficient number of susceptible individuals in a population. Measles transmission is prevented by vaccination and in sub-Saharan Africa, it is recommended that the vaccine be given at 9 months of age, by which time the child would have lost passive immunity conferred by maternal antibodies. One dose of measles vaccine confers life-long immunity to approximately 85% in those vaccinated (Adeboye *et al.,* 2011).

Measles immunity involves humoral, cellular and mucosal responses. Cell-mediated immunity is required for recovery from measles, and humoral immunity in response to vaccination or natural infection is required for protection from infection or re-infection with measles (Duke and Mogne, 2003). In primary acute measles infection, IgM, IgG and IgA antibodies are produced. Serum IgM antibody concentrations peaks 7-10 days after the onset of rash and can persist for more than 1month after natural infection and vaccination. Transient production of serum and secretory IgA also takes place (Pederson *et al.,* 1986). Serum IgG antibody concentration peaks within 4 weeks and subsequently declines, but persist for life. Antibodies induced by vaccination peak at a lower concentration and do not persist as long as immunity after natural infection. Re-exposure to either wild type or vaccine strains of measles virus stimulates memory cells to

produce measles-specific responses in which IgG antibodies rise rapidly and peak about 12 days after re-exposure (Duke and Mogne, 2003).

Through effective implementation of WHO and UNICEF recommended control strategies, the estimated measles-related mortality decreased by 90% in the African Region between year2000 and 2008. In particular, a second dose of measles-containing vaccine (MCV) given during supplemental immunization activities played a vital role in raising population immunity levels, although routine vaccination coverage remains low (Manirakiza *et al.,* 2011; Salako and Sholeye, 2015).

However, in order to interrupt the endemic transmission of measles virus; a population immunity of ≥ 95% is recommended (Weldegebriel *et al.,* 2011). An outbreak can however be prevented in a country if 95% of children under 15 years of age are vaccinated.

Studies conducted in some parts of the country revealed the prevalence of 30.2% in Akwa Ibom (Bassey *et al.,* 2010), 32% reported in Ilorin, (Babaniyi *et al.,* 1995), 55% recorded in a study conducted in Lagos (Adetunji *et al.,* 2007), 15.6% reported in another study in Southwestern Nigeria (Omilabu *etal.,*1999), 8.0% in Bida (Adeboye *et al.,* 2011) , 32.%, 21.2% and 54% in a study conducted in Kaduna by(Chechet *et al.* 2014; Olaitan *et al.* 2015; Sheyin *et al.* 2016) respectively .

Measles case fatality is estimated to be between 3 to 5% in developing countries and may be as high as 10% during epidemics (Weldegebriel *et al.,* 2011), where

as in developed countries, less than one in 1000 children with measles dies (William, 2007; WHO, 2009). Measles case fatality ratios vary depending upon the age at infection, the nutritional status of the population, measles vaccine coverage, and access to health care (William, 2007).

Although measles virus is serologically monotypic, genetic and antigenic variation has been detected in wild type viruses. Genetic characterization of wild type measles viruses has focused on analysis of the genes coding for the H and/or N proteins, which contain up to 7% nucleotide variability. One of the most variable parts of the measles genome is the 450-nucleotide region, which codes for the carboxyl terminal 150 amino acids of the viral nucleoprotein, where nucleotide variability can approach 12% between wild type viruses (WHO, 2005).

Longitudinal analysis of MV strain diversity in Nigeria suggested that only a few of the previously described 1997–1998 variants had continued to circulate, but this was concomitant with a rapid restoration of genetic diversity, probably caused by low vaccination coverage and high birth rates (Kremer*et al.,* 2010). MV sequence data from Africa have been greatly expanded since characterization of the first endemic strains was reported(Rota *et al.,* 1994; Hanses *et al.,* 1999; Muller and Mulders 2002; El Mubarak *et al.,* 2002). Essential genetic baseline information is still missing from many countries (Rota *et al.,* 2009).

1.1 Statement of Research Problem

Measles is highly infectious, preventable disease, with potential for eradication, yet continues to be one of the present day scourges of the developing world, (Lagunju *et al.*, 2005; Salako and Sholeye, 2015; Sheyin *et al.*, 2016). Outbreaks continue to occur with 5-10% fatality rate among children (WHO, 2009. Elimination of measles has been achieved in some regions of the world; however, this has so far been unattainable in the African continent (CDC, 2010).

Nigeria is rated among one of the 47 countries in the world where the burden of measles is highest and where it is still responsible for the high number of death. The 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).

Measles can lead to lethal complications, such as pneumonia, croup, and diarrhea due to immunosuppression (Mason, 2008). It also causes various types of neurological diseases such as post-infectious encephalitis, measles inclusion body encephalitis (MIBE) and subacute sclerosing panencephalitis (SSPE) (Griffin, 2001; Rima and Duprex, 2006). Many that survive suffer lifelong disabilities such as blindness, deafness and irreversible brain damage (Simon *et al.*, 2012).

Sequence information from many developing countries with a persistently high measles incidence is limited. Although MV sequence data from Africa have been

greatly expanded since characterization of the first endemic strains was reported, (Rota *et al.,* 2009). Essential genetic baseline information is still missing from many countries. In Nigeria measles surveillance is still in its infancy, there is paucity of country wide data on circulating measles virus genotypes especially sequence analysis. In 2010, B3 genotypes with 2 separate clusters were reported in Lagos, Ibadan, Adamawa, and Borno (Kremer *et al.*, 2010).

1.2 Justification of the Study

The death toll of measles epidemics has been either under-reported or over-blown by different media. As researchers, we have the responsibility to give scientific and unbiased accounts of the measles situation in the study region. Furthermore, reports of measles prevalence vary disproportionately between the Northern and Southern parts of Nigeria, with a high incidence rate reported in Northern states (Orude, 2011; Sani, 2012).

However, as the measles incidence is high in many developing countries including Nigeria, surveillance of individual cases is necessary and is based on laboratory diagnosis using IgM and genotyping.

There are few reports on circulating wild type measles virus in Nigeria. In 2010, circulation of genotype B3 was reported in Lagos and Ibadan. The circulation of genotypes B3 with 2separate clusters was documented in Adamawa and Borno states, Northeast Nigeria (Kremer *et al.,* 2010). Moreover, there is lack of data on genotypic characterization of measles virus in the Northwestern region of Nigeria. Knowledge gained from this work could bridge the gap on the lack of information

on the strain of measles virus circulating in the region. Establishment of baseline molecular data and continuous monitoring of circulating viral genotypes is an important tool in measles control. Attempts should be made however to obtain specimens for viral (RT-PCR), for genetic characterization and strain banking. MV genotyping is an important tool of measles surveillance to document chains of transmission, discriminate between imported or indigenous viruses, and monitor the efficiency of vaccination campaign (Kessler *et al.*, 2010). However, continued analysis of measles virus strain is needed for understanding the molecular epidemiology of measles virus globally.

1.3 Aim of study

The aim of this research was to determine the Seroprevalence and genotypic characterization of measles virus among children 0-10 years in Northwestern Nigeria.

1.4 Objectives

The objectives of the study were to:

1. Determine the seroprevalence of measles virus IgM antibody among children 0-10 yearsusing Enzyme Linked Immunosorbent Assay (ELISA).
2. Determine the socio-demographic and risk factors associated with measles virus infection using self-structured questionnaire.
3. Confirm the presence of measles virus in IgM positive samples using nRT-PCR

4. Characterize the genotype of measles virus circulating in North-West Nigeria using sequencing.

5. Elucidate the multiple alignments of the measles virus sequences detected.

CHAPTER TWO

2.0 LITERATURE REVIEW

Measles, one of the most infectious viral diseases of humans affecting more than 95% of exposed individuals in the absence of vaccination, is spread by the respiratory route and remains a major cause of mortality in children, particularly in developing countries (Hope-Simpson, 1952; Van den ent *et al.*, 2011). Although other primates are also susceptible to measles and develop similar clinical disease, these populations are not large enough to support sustained transmission of the virus and thus humans are the only natural reservoir (Griffin, 2007). Measles, a highly infectious viral disease, is endemic in developing countries with a peak of transmission from October to March. The case fatality rate of measles in the developing countries stands at around 3-5%; this could be as high as 10% during epidemics. Although natural infection with measles virus confers life-long immunity, those vaccinated with the vaccine could get up to 10 years of protection (Saleh, 2016).

2.1 Historical Background of Measles Virus

Measles is a disease of human civilization – mathematical models indicate that a population size of 250 000 - 400 000 and an annual birth cohort of 5000 – 10 000 is needed in order to sustain circulation of the virus (Keeling and Grenfell, 1997).

Populations of this size did not exist until the development of urban civilizations in the Middle East, India and China, around the third millennium BC. Measles therefore appears to be a relatively new disease of humans, and on the basis of phylogenetic analysis of Morbillivirussequences, it is postulated that a zoonotic Morbillivirus(an ancestor of modern rinderpest virus) was transmitted to humans within the last 5,000 to 10,000 years, and this eventually evolved into measles virus (Moss and Griffin, 2009). However, another study estimated that divergence of MV from rinderpest virus may have occurred within the last 1000 years (Furuse *et al*., 2010). Similarly, carnivores could have contracted a *Morbillivirus* infection from their ruminant prey, this pathogen eventually evolving into canine distemper virus (Barrett and Rossiter, 1999).

Measles was already recognized as a disease distinct from smallpox in the ninth century, by Abu Becr (Rhazes of Baghdad) and he traced descriptions of the disease as far back as the sixth century. There are abundant historical records from Europe and the Far East that describe repeated epidemics of rash-related illnesses, and it was documented as a childhood disease in 1224 (Griffin, 2007). 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 1670, Thomas Sydenham described a measles outbreak in London, accurately detailed the clinical presentation of the disease, and highlighted pulmonary complications and increased severity in adults. In European literature, it was

named *morbilli* from the Latin “little diseases” to distinguish it from plague *il morbo*, however the term morbilli included several illnesses presenting with rash. In 1763, Sanvages defined morbilli as measles (from which the taxonomic nomenclature Morbilliviruswas derived), but called it *rubeola* (derived from Spanish) which led to subsequent confusion with rubella (Latin for ‘’little red”, commonly referred to as German measles) (Moss and Griffin, 2009). Epidemics of rash illnesses, associated with abrupt decreases in population size, were recorded in China, India and the Mediterranean region. 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. The European conquest of the New World decimated the indigenous populations, largely due to the introduction of diseases such as smallpox and measles (McNeill, 1976). These historical examples illustrate the extremely high mortality associated with the introduction of measles into virus-naïve populations.

Measles was shown to be caused by an infectious agent in 1757 when Francis Home, a Scottish physician, transmitted the disease to naïve individuals, while attempting immunization, by inoculation with blood from a person with the characteristic rash. Hektoen repeated this experiment using blood “free of bacteria” from acute measles cases to transmit the disease to volunteers in 1905. The next advance came through the astute observations of Peter Ludwig Panum

(Talbot, 1970). Panum was a Danish doctor, sent by his Government to investigate an epidemic of measles in the Faroe Island in 1846. The geographical situation of the Faroes was ideal for an epidemiological study: seventeen isolated islands, where the arrival of a boat was noted in the local calendar and provided an excuse for the gathering of the whole population (Axton, 1979). Visits from the Danish mainland were rare, but the dates were well documented. The epidemic originated in a single seaman from Copagen, who presumably left the city with no signs of measles. The sea journey to the Faeroes was sufficiently long for him to have become infectious on arrival, but not long enough for him to have recovered completely and have ceased excreting the virus (Axton, 1979). By following the course of the epidemic from village to village, and island to island, using the known movement of people and boats, Panum was able to establish four important facts (Axton, 1979):

i. The measles rash appears twelve to fourteen days after contact.
ii. Its greatest infectivity is during the late prodrome — three to four days before the rash appears.
iii. The disease is contagious, probably spread by droplets, and not miasmic in origin.
iv. The protection from an attack is life-long. The last epidemic of measles in the Faeroes had been in 1781, and Panum found that the only inhabitants immune were those over 64 years old, who had suffered from the disease as children during the earlier epidemic.

The viral etiology of measles was confirmed by Goldberger and Anderson in 1911 when the disease was transmitted to monkeys using filtered respiratory tract

secretions from measles patients, and the illness was then serially passed on to other monkeys (Griffin, 2007).

2.2 TheModern History of Measles

The modern history of measles begins with Thomas Sydenham's description of his son's attack (Dewhurst, 1966). He was the first clearly to separate measles from smallpox, and he recognized complications such as cancrum oris and encephalitis. At about the same time Mauriceau rejected the mother's blood theory and the infectious or contagious theory became generally accepted (Debus, 1970). Mauriceau also decried the use of red curtains in the sufferer's bedroom. He suggested green would be more appropriate, though probably more on aesthetic than on scientific grounds.

In 1954, Enders and Pebbles reported the isolation of measles virus in roller-tube culture of human and rhesus monkey and described the characteristic cytopathic effects that accompany measles virus replication. Adaptation of measles virus to growth in a variety of cell cultures permitted the detailed analysis of virus structure, replication, virus-cell interaction and vaccine development (Enders, 1963). 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). Licensed vaccines to prevent the disease became available in 1963(Bellini *et al.,* 1994; Yanagi, 2001).

2.3 Nature of Measles Virus Agent

Measles virus is a spherical, non-segmented enveloped with negative polarity and helical symmetry that lack virion neuraminidase activity and thus grouped into genus *Morbillivirus* of the family *Paramyxoviridiae* and order *Mononegavirales* (Rima *et al.,* 1995; Barrette and Rossitier, 1999; Griffin 2001).In addition to MV, *Morbillivirus* includes dolphin and porpoise morbillivirus, canine distemper virus, phocid distemper virus, peste des petits ruminant's virus, and rinderpest virus (RPV). Genetically and antigenetically, MV is most closely related to RPV, which is a pathogen of cattle (Sheshberadaran *et al.,* 1986). MV is assumed to have evolved in an environment where cattle and humans lived in close proximity (McNeil, 1976). MV probably evolved after commencement of livestock farming in the early centers of civilization in the Middle East. The speculation accords with mathematical analyses as mentioned above (Black, 1991 and Griffin, 2007).

MV is a single-stranded RNA genome measuring approximately 15.9kb.The MV particle is pleomorphic and is largely cell-associated with a virion size ranging from 50 to 510 nm. The surface projections of the viral envelope are composed of viral transmembrane haemagglutinin (H) and fusion (F) glycoproteins the matrix protein (M) lines the interior of the virions envelope (Nussbaum *et al.,* 1995). The helical nucleocapsid (up to 1.2 µm in length; diameter of 12-17 nm) is coiled within the envelope and consists of approximately 2500 copies of the

nucleoprotein (N) bound to the genomic RNA, and is associated with a small number of copies of the phosphoprotein (P) and large protein (L) (Wild *et al.*, 1991).

There is only one antigenic type of measles virus. Although studies have documented changes in the H glycoprotein, these changes do not appear to be epidemiologically important (WHO, 2005). Measles virus is rapidly inactivated by heat, sunlight, UV light, disinfectant, acidic pH, ether, and trypsin. It has a short survival time (less than 2 hours) in the air or on objects and surfaces depending on temperature and humidity.

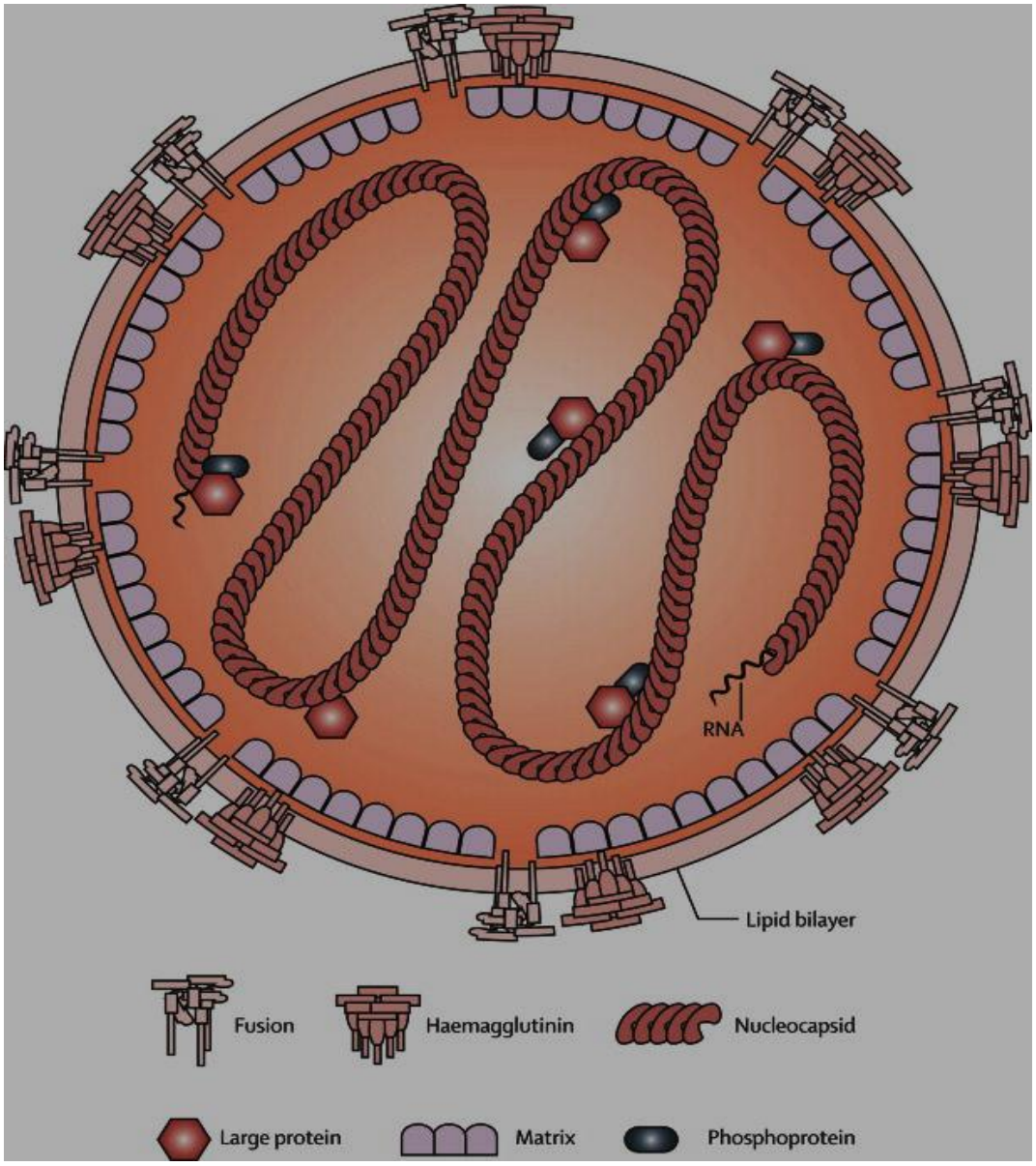


Figure 2.1: The structure of measles virus

Adopted from Nature review (2006).

2.3.1Measles virus genome organization

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 continuous, 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).

2.3.2Measles virus proteins

Measles virus has a linear non-segmented negative sense single-stranded RNA (-) ssRNA genome of 15,894 nucleotides (nt) which encodes six structural and two non-structural proteins. The structural proteins are the nucleoprotein (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (H) and large polymerase (L) proteins (Nussbaum *et al.,* 1995). The non-structural C and V proteins are both sub-products of the P gene: C results from an overlapping reading frame and V from an edited transcript. Each coding region is preceded and followed by untranslated regions, of which the longest (1,012 nt) is the non-coding region between the genes for the M and F proteins (M/F NCR) (Catteneo *et al.,* 1989).

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 (Moss and Griffin, 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; Taylor *et al.,* 1991). 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 and Bankamp*et al.,* 2014). The N protein also interacts with multiple cellular proteins (tenOever, 2002; Zhang *et al.,* 2002; Sato *et al.,* 2007). 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).

Transcription is more efficient at the 3' end of the genome, with a transcriptional gradient from N to L, the slope of which varies in different host cells (Horikami and Moyer, 1995). N occurs in the cytoplasm as a soluble N-P complex (Huber *et al.,* 1991). However, when P is present in reduced amounts (late in infection and in strains isolated from SSPE brain tissue) or entirely absent (e.g. when N is expressed from plasmids), N is an insoluble protein that migrates to the nucleus where it assembles into nucleocapsid-like structures without encapsidated viral RNA that can be seen as intranuclear inclusion bodies (Bohn *et al.,* 1990; Baczko *et al.,* 1992). The conserved amino-terminal portion of N is necessary for self-assembly into nucleocapsids and for RNA binding (Karlin *et al.,* 2002). The carboxyl-terminal portion of N is more variable, has an intrinsically disordered structure, and shares features with acidic activation domains of cellular transcription factors (Longhi *et al.,* 2003).

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 (Curran, 1995). 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 (Moss and Griffin 2007). The P gene also encodes two non-structural proteins, C and V. C is a basic protein, transcribed from an overlapping reading frame of the P-gene (Bellini *et al.,* 1985). V is produced by editing of the P mRNA during transcription by insertion of a non-templated guanosine residue that shifts the reading frame (Cattaneo *et al.,* 1989), therefore the amino terminal part of V is identical that of the P protein, while it's carboxyl-terminal 70 AA residues are unique. This carboxyl-terminal region of V is rich in cysteine residues and shares features with zinc-finger proteins (Baczko *et al.,* 1992).

Although neither C nor V are necessary for MV replication, they interact with cellular proteins, interfering with innate immune responses by inhibition of interferon signaling pathways (Valsamakis *et al.,* 1998; Palosaari *et al.,* 2003). A diverse range of host evasion activities, such as prevention of apoptosis, cell cycle alterations and inhibition of double stranded RNA signaling, have also been ascribed to the V-proteins of other members of the Paramyxoviridae (Lin and Lamb, 2000; He *et al.,* 2002; Wansley and Parks, 2002). These immune

suppression activities contribute to the pathogenicity of wild-type MV and to the attenuation in vaccine strains (Palosaari *et al.,* 2003).

The matrix protein (M; 335 amino acids) lines the inner surface of the viral envelope and participates in virion maturation (Wild *et al.,* 1995; Moss and Griffin, 2007). It plays a crucial role in virus assembly by interacting with the cytoplasmic tails of the H and F proteins. It binds the nucleocapsid and negatively regulates transcription, presumably by sequestering nucleocapsids to the plasma membrane (Suryanarayana *et al.,* 1994; Iwasaki *et al.,* 2009). Deletion of M increases cell-to-cell fusion and decreases production of infectious virus (Cathomen *et al.,* 1998). Defects in M are associated with persistent infections, which are characterized by a marked decrease in the amount of infectious virus released, accumulation of intranuclear and intracytoplasmic nucleocapsids and decreased virus-induced cytopathic effect (CPE) compared with lytic infections. Subacute sclerosing panencephalitis (SSPE) strains produce no infectious virus and persistent infection appears to be maintained by cell-to-cell transmission (Patterson *et al.,* 2001).

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 (Griffin, 2007). 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. 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 (Samuel and Shai,2001; Buckland*et al.,* 2001). The F2 subunit contains the signal peptide as well as all the predicted glycosylation sites necessary for stability of the mature fusion protein (Griffin, 2007 and Bankamp *et al.,* 2014). The F1 subunit, derived from carboxyl-terminus of F0, is anchored in the viral envelope, and has a cytoplasmic tail that contains 14 terminal amino acids which are highly conserved among the morbillivirus. The fusion peptide, a hydrophobic region of 25 amino acids at the amino-terminus of F1, interacts with the host cell membrane to induce fusion, is also highly conserved in the paramyxovirus family. However, F alone cannot induce fusion: interaction of H with the cellular receptor is necessary to bring the fusion peptide region of the F1 subunit into contact with the cell membrane (Richardson and Choppin, 1983; Bankamp *et al.,* 2014).

The hemagglutinin (H; 617 amino acids) protein is the second glycosylated surface-expressed protein. This type 2 glycoprotein mediates attachment to specific protein receptors on the host cell and is a required co-factor for fusion. There are 4 N-linked glycosylation sites between amino acids 168 and 200 and some wild-type isolates are additionally glycosylated at amino acid 416 (Hu *et al.,* 1994; Santibanez *et al.,* 2005 and Griffin, 2007). A cytoplasmic tail of 34 amino acids at the amino-terminus interacts with the M protein and contains sorting signals for intracellular localization (Naim *et al.,* 2000; Moll *et al.,* 2001 and Yanagi *et al.,* 2009).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. hSLAM is only expressed on a subset of immune cells (Aversa *et al.,* 1997; Tatsuo *et al.,* 2000;)All vaccine strains have acquired the ability to use CD46 as an alternative receptor during passage in various SLAM-negative cultured cells. The ubiquitous expression of CD46 explains the ability of vaccine strains to infect various human and monkey cell lines. Studies have revealed that another, presently unidentified receptor exists for wild-type MV on epithelial cells that form tight junctions (polarized epithelial cells)(Leonard *et al.,* 2008; Tahara *et al.,* 2008 and Shirogane *et al.,* 2010). The H protein is the major target for neutralizing antibodies induced by vaccination (Bankamp*et al.,* 2014). Interaction of H with a cellular receptor and F results in fusion of the viral and cellular envelopes, entry of the ribonucleocapsid into the cytoplasm and initiation of the viral replication cycle (Wild and Buckland, 1995).

The ability of MV to agglutinate monkey erythrocytes resides in a region of H that is close to, yet distinct from, the receptor binding sites, as antibodies that block haemagglutination can neutralize viral infectivity. Extensive glycosylation and oligosaccharide modifications are necessary for H to be correctly assembled as disulfide-linked homodimers that accumulate as tetramers on the viral envelope (Griffin, 2007). By comparison with the known 3-dimensional structure of the H-protein of Newcastle disease virus, structural models of MV H suggest a stem and six-β-sheet propeller structure. Receptor-binding sites in the predicted model

appear to map to β-sheets 4 (the CD46 binding site) and 5 (SLAM binding site) while neutralizing epitopes map to the top loops protruding from the blades of the propeller (Vongpunsawad *et al.*, 2004).

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 enzymatic activities required for transcription and replication, including nucleotide polymerization, mRNA capping, and polyadenylation .On the basis of amino acid sequence conservation among non-segmented negative strand viruses, 6 domains of higher conservation have been characterized in the L protein .Within these domains, several functional motifs have been identified (Ogino *et al.*,2005). The conserved domains may represent concatenated functional domains that are connected by less-conserved hinge regions 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 (Poch *et al.*, 1990; Cevik *et al.*, 2004).

2.3.3Measles virus cellular receptors

In 1993, human CD46, also known as membrane cofactor protein, was identified as a cellular receptor for the vaccine strains of MV (Dorig *et al.*, 1993; Naniche *et al.*, 1993). CD46 is a complement regulatory protein expressed on all nucleated cells and preferentially on the apical surface of polarized epithelial cells. Its main function is to protect cells from complement-mediated attack. However, the

ubiquitous distribution of CD46 does not explain the lymphoid tropism of MV, nor the fact that WT viruses grow poorly in Vero cells which do express CD46.

Signaling Lymphocyte Activation marker (SLAM) was identified in 1995 as a novel receptor of the immunoglobulin superfamily that is involved in the activation of T-cells and natural killer cells (Cocks *et al.*, 1995). Functional expression cloning experiments using extracts of B95-a cells, showed that SLAM (CD150) was a major cellular receptor for MV (Tatsuo *et al.*, 2000). Importantly, the Edmonston strain was found to use both SLAM and CD46 as receptors, indicating that SLAM acts as a cellular receptor for both B-cell line-isolated and laboratory-adapted strains of MV (Tatsuo *et al.*, 2000b; Yanagi *et al.*, 2002).

SLAM is only expressed on a subset of immune cells including immature thymocytes, mature dendritic cells, activated monocytes, activated T-cells and B-cells (Yanagi *et al.*, 2006). This distribution of SLAM coincides with the tropism of MV for cells of the immune system, and the utilization of SLAM as a receptor was thought to explain some of the pathology of MV infection, such as lymphopenia and immunosuppression

The lymphocytic B95-a cell line, although very sensitive for MV isolation because of SLAM expression, unfortunately persistently produces Epstein-Barr virus and therefore poses a risk of infection to laboratory staff. It is also difficult to distribute the cell line to laboratories undertaking MV isolation as there are shipping restrictions for infectious materials. For this reason, B95-a is no longer the preferred cell line for the isolation of MV, and has been replaced by the Vero-

hSLAM cell line (WHO, 2007). This is a genetically engineered Vero cell line transfected with a plasmid encoding the gene for human SLAM (hSLAM) (Ono *et al.,* 2001). The sensitivity of Vero-hSLAM cells for the isolation of WT MV is equivalent to that of B95-a cells (Kouomou and Wild, 2002). Vero hSLAM cells are also sensitive to infection by laboratory-adapted strains including vaccine viruses.

2.3.4Measles virus replication

Measles virus attaches to the host cell through the interaction of the viral H protein with a cellular receptor that may be hSLAM, or CD46 (Yanagi *et al.,* 2009). After the F protein mediates fusion of the virion with the cell membrane, the negative-stranded ribonucleoprotein (RNP) is introduced into the cytoplasm, where it acts as a template for both primary transcriptions of mRNAs and replication into positive-stranded antigenomic RNA (Fields *et al.,* 2007). The polymerase complex, consisting of L and P proteins, is delivered with the RNP. mRNA transcripts are capped, polyadenylated and terminated at the gene end signals. The full-length positive sense antigenomic intermediate acts as a template for the replication of full-length, negative sense genomic RNA.

During replication the gene end, intergenic trinucleotide and gene start signals are ignored, and both nascent positive and negative sense genomes are concomitantly encapsidated to produce RNPs (Rima and Duprex, 2009). F and H proteins insert into the plasma membrane, whereas M proteins line the inside of the membrane and interact with the cytoplasmic tails of the glycoproteins and the RNP (Wild and Buckland, 1995). The virion buds from the plasma membrane to complete the

replication cycle. The cell membrane is modified by attachment of N-linked carbohydrate chains of cellular origin before virus trans membranous proteins appear at the cell surface.

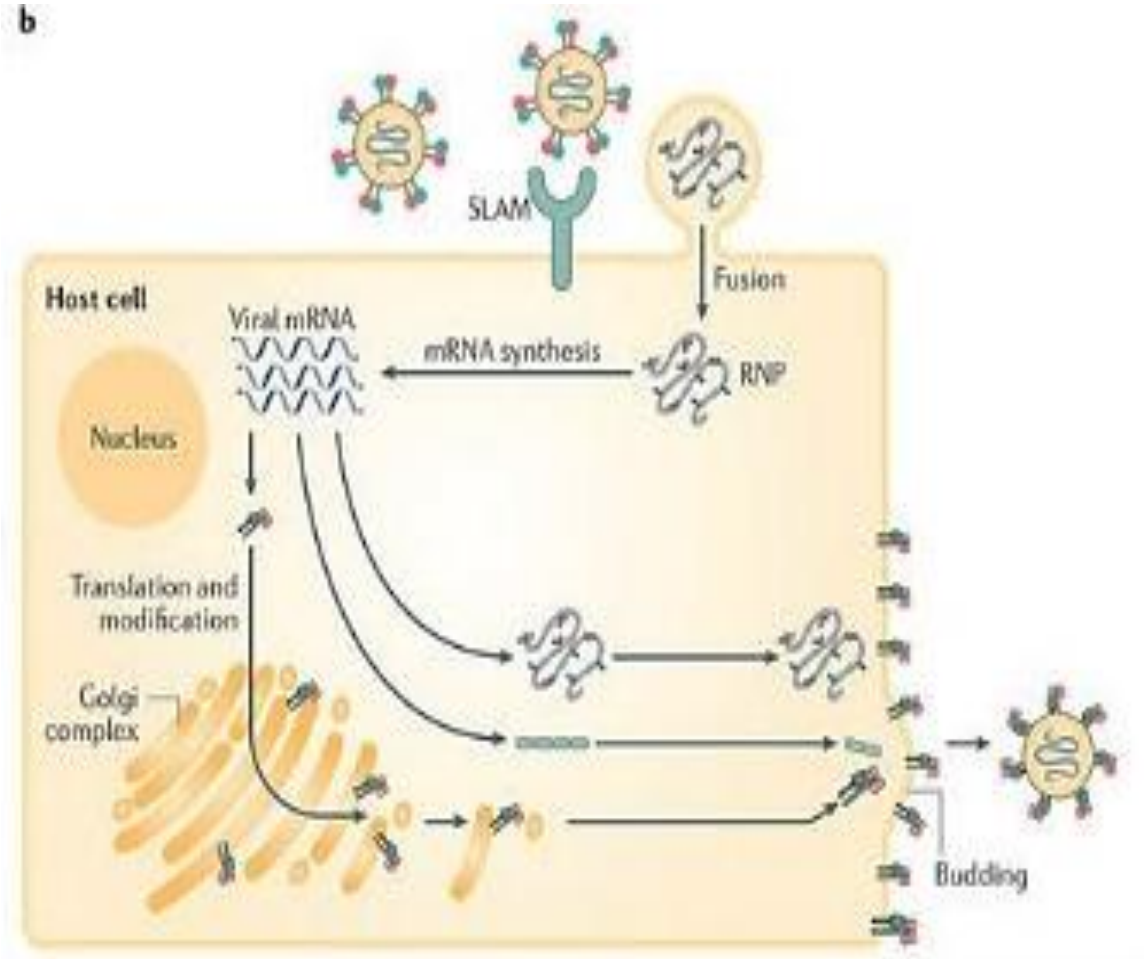


Figure 2.2: Measles virus replication cycle

Adopted from Nature Review (2013)

2.4 Classification of Antigenic Type of Measles Virus

Although MV is serologically monotypic, genetic and antigenic variation of WT viruses have been identified (Taylor *et al.*, 1991; Rota *et al.*, 1992).Before 1998, there was no uniform nomenclature or analysis protocol to describe the genetic characteristics of wild-type measles viruses. In 1998, the WHO made recommendations for a standard nomenclature for naming strains, describing genotypes, and conducting sequence analysis so that genetic data would be directly comparable between laboratories. These recommendations have been updated periodically since 1998 (WHO, 2001; 2003; 2005; 2006; 2007).

There is far less variation in the M, F and L genes (Rota *et al.*, 1994; Bankamp*et al.*, 1999) than in the N, P and H genes (Rota *et al.*, 1992; Rima *et al.*, 1997; Bankamp*et al.*, 2008). The maximum variation in the genome occurs in the 450 nucleotides encoding the 150 AAs of the carboxyl-terminus of the N protein, referred to as N-450 (Riddell *et al.*, 2005). As the most variable part of the genome, N-450 has been shown to vary up to 12% between WT strains of MV (Xu *et al.*, 1998). WHO recommends that the N-450 region is the minimum amount of sequence data required for genotyping of MV from cultured isolates or clinical specimens (Rota *et al.*, 2009).

Complete H gene sequences should be obtained from representative strains or if a new genotype is suspected. Phylogenetic analysis of the H gene sequences provides additional support for the genotype assignment while monitoring amino

acid substitutions that could affect antigenicity. F or molecular epidemiologic purposes, the genotype designations are considered the operational taxonomic unit, while related genotypes are grouped by clades. WHO currently recognizes eight clades designated A, B, C, D, E, F, G, and H. Within these clades, there are 24 recognized genotypes, designated A, B1, B2, B3, C1, C2, D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, E, F, G1, G2, G3, H1, and H2. Some clades contain only one genotype and, in such cases, the genotype designation is the same as the clade name. Other clades contain multiple genotypes and are designated by using the clade letter (in uppercase) and genotype number. Several of the genotypes—B1, E, F, G1, D1—appear to be extinct or inactive since representatives of these genotypes have not been isolated for at least 15 years.

However, the sequences of the inactive genotypes are maintained in the set of WHO reference sequences for completeness (WHO, 2005). With the exception of genotype F which is based only on sequences derived from a case of SSPE, all of the genotypes have an assigned reference strain chosen to represent the earliest isolation of virus from each genotype. Sequences from recent viral isolates are then compared to the set of WHO reference sequences, which are available from GenBank (WHO, 2005) and the WHO Strain Banks, to determine the genotype. WHO has established guidelines based on both molecular biologic and epidemiologic criteria for the designation of new genotypes (WHO, 2001b, 2003, 2005).

However, as a consistent naming convention underpins both the WHO measles database and MeaNS (and their interoperability), the criteria for naming are outlined below.

2.4.1Criteria for measles nomenclature

Measles virus RNA can be isolated from either viral isolates in cell culture or extracted directly from clinical material. Sequences should therefore be designated as either:

i. MVi – sequence derived from RNA extracted from measles virus isolate in cell culture.

ii. MVs – sequence derived from RNA extracted from clinical material. Other data to be included in the sequence name are:

iii. City or state/province where the case occurred. The full name should be given. Only use standard ASCII letters, and include spaces if needed.

iv. Country, ISO-3 letter designation (required). The full ISO-3 list can be accessed from the WHO and MeaNS databases.

v. Date of onset of rash by epidemiological week (1–53) and year (required). However, in the 2012 measles nomenclature update (WHO, 2012), the epidemiological week is calculated from the first Monday of each week, with week 1 being the first Monday of the year. For example, Sunday 1st January 2012 would be 52.11 and Monday 2nd January 2012 would be 1.12. If the rash onset is not known the date of specimen collection should be used. If onset and collection

dates are unavailable, the date the sample was received in the laboratory should be used. For historic samples where only the year is known, "0" should be used as the epidemiological week. If the year and month are known but not the epidemiological week or day, then the epidemiological week should be defined as the second full week in that month.

vi. Isolate/sequence number if >1 from the same epidemiological week and location (where required). MVi and MVs sequences from the different cases should have different isolate/sequence numbers where the date and location are identical.

vii. Genotype in square brackets (optional). For genotype assignment, sequencing of the minimum 450 nucleotide window of the nucleoprotein is required.

viii. Special designation for sequences derived from measles inclusion-body encephalitis (MIBE) or subacute sclerosing panencephalitis (SSPE) cases, or suspected cases with a history of recent vaccination and vaccine virus detected (WHO, 2001a; WHO, 2001b; WHO, 2003; WHO, 2005).

The following examples illustrate the current nomenclature:

MVi/ London. GBR/3.12/2 (D4)

MVs/New York. USA/17.11(G3) ((SSPE)

2.5 Pathology and Pathogenesis

Humans are the only natural host for measles virus, although numerous other species, including monkeys, dogs, and mice can be experimentally infected. Until recently, it was thought that the initial MV infection was established in the respiratory tract and oropharynx, with viral replication in the epithelial cells and

pulmonary macrophages, and subsequent spread to local lymphatic tissues where further replication occurs (Sakaguchi *et al.,* 1986; Shingai *et al.,* 2005). Replication within the lymph nodes results in the appearance of giant, multinucleate cells (containing up to 100 nuclei) of lymphoid or reticulo-endothelial origin, referred to as Warthin-Finkeldey cells. These cells are a consistent pathological feature of MV infection. Further dissemination to other sites (skin, conjunctivae, kidney, lung, gastrointestinal tract, genital mucosa and liver) is mediated by infected monocytes and results in a viraemic phase (White and Boyd, 1973). In these secondary sites, replication occurs primarily in endothelial cells, epithelial cells, monocytes and macrophages (Griffin *et al.,* 1994; Esolen *et al.,* 1995b and Takahashi *et al.,* 1996). This replication, together with the immune response, is responsible for the prodromal signs and symptoms. Giant, multinucleate epithelial cells are evident in nasal secretions and conjunctivae. MV infected epithelial cells are also shed into the urine (Lightwood and Nolan, 1970; Scheifele and Forbes, 1972).

Unfortunately, the hypothesis of initial infection of epithelial cells and subsequent viraemia mediated by infected monocytes is an unlikely sequence of events given that neither of these cell types express SLAM which is required for entry of WT MV (de Swart *et al.,* 2007). It was recently shown that the initial target cells infected by MV are macrophages and dendritic cells of the lower respiratory tract which express SLAM (Ferreira *et al.,* 2010; Leonard *et al.,* 2010; Lemon *et al.,* 2011). These cells then cross the respiratory epithelium and transport the infection to bronchus-associated lymphoid tissue, where there is

abundant viral replication (de Swart *et al.,* 2007) and subsequent systemic dissemination by viraemia (Lemon *et al.,* 2011). This was demonstrated using a recombinant MV clone (Takeda *et al.,* 2000) containing an enhanced green fluorescent protein (EGFP) inserted upstream of the N-gene. Expression of EGFP permitted the process of pathogenesis to be followed by fluorescence both in live monkeys (rhesus and cynomolgus macaques) as well as in tissue samples. Peripheral monocytes did not sustain productive MV infection (de Swart *et al.,* 2007).

Nevertheless, none of these studies was able to address how virions are able to infect epithelia from the basolateral (abluminal) surface, in order to replicate and exit from the apical (luminal) epithelial surface into the respiratory tract (Ludlow *et al.,* 2009). Several studies have alluded to the existence of a receptor on polarized epithelial cells that can be used by MV for attachment and subsequent fusion of membranes, for entry and for viral shedding (Leonard *et al.,* 2008; Tahara *et al.,* 2008; Yanagi *et al.,* 2009; Ludlow *et al.,* 2010). This receptor was recently identified as nectin-4 a member of the immunoglobulin superfamily of proteins, which is expressed on the adherens junctions of epithelial cells (Muhlebach *et al.,* 2011; Noyce *et al.,* 2011).

Another possible, but probably less important, route of MV entry is through MV infection of myeloid or lymphoid cells in the conjunctiva. The lamina propria of the conjunctiva is rich in DCs, Langerhans cells, macrophages, $CD4^+$ and $CD8^+$ T-cells and B-cells, providing a suitable site of replication for the virus. This infection and the ensuing MV-specific immune response may subsequently lead

to prodromal conjunctivitis (Seo *et al.,* 2010). In addition to the conjunctiva, MV has been shown to infect human corneal rim epithelial cells ex vivo. It has been reported that eye protection during contact with measles patients can reduce the risk of contracting infection by MV (Ludlow *et al.,* 2010) host invasion.

Infection of macaques with a mutant MV that was unable to bind to nectin-4 resulted in clinical symptoms of measles and detectable viremia, but the inability to infect respiratory epithelial cells via the nectin-4 receptor prevented this virus from being released and spread via respiratory secretions as aerosolized droplets (Leonard *et al.,* 2008; Noyce *et al.,* 2011). Wild-type MV is able to infect adenocarcinoma cell lines derived from the glandular epithelia of a variety of lung, breast and colon tumors, because these cell lines have high levels of nectin-4 expression. Blocking of this receptor prevents MV infection, thus confirming the role of nectin-4 for receptor mediated entry. As nectin-4 is a cellular marker of several types of cancer, MV is also being considered as an oncolytic agent to target cancerous cells (Takano *et al.,* 2009; Noyce *et al.,* 2011).

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).

2.6 Clinical Manifestation of measles

Infections in non-immune hosts are almost always symptomatic. After an incubation period of 8-12 days, measles is typically a 7-11 days' illness (with a prodomal phase of 2-4 days followed by an eruptive phase of 5-8 days (Jawetz *et al.,* 2010).

The prodromal phase begins days after exposure in susceptible persons and may resemble a severe upper respiratory tract infection. This phase is characterized by malaise, fever, anorexia, conjunctivitis and respiratory symptoms such as congestion, cough and coryza (Gershon, 2007). Other symptoms may include diarrhea, especially in infants, and generalized lymphadenopathy (CDC, 2009). Older children may complain of photophobia and occasionally of arthralgia (PAHO, 2005). Koplik spots which are pathogonomic of measles may appear toward the end of the prodrome, just before the appearance of the rash. Koplik spots (white spots on a red base on the inner lining of the mouth) usually occur on the mucosa opposite the second molars and begin to slough as the rash appears (Gershon, 2007) but occasionally occur on the conjunctiva, vaginal mucosa or the gastrointestinal mucosa (Steichan and Dauthevill, 2009).

Histological examinations showed that the characteristics of the Koplik's spots were similar to those of the skin rash and they may contain syncytia. The maculopapular rash of measles is usually identified approximately 14 days after exposure. It begins on the face, then progresses down the body to the extremities, including the palms and soles (Gershon, 2007; CDC, 2009), and lasts approximately five days. Patients tend to be most ill on the first or second day of

the rash. The rash fades in the same sequence it appears, from head to extremities. The rash can potentially be explained by infection of the dermal endothelial cells and keratinocytes, which are subsequently cleared by the virus-specific host cellular immune response (Griffin *et al.,* 2013).

Several studies reported that viral antigens were found in the corneal layer, spongiotic epidermal keratinocytes and even more in the dermal papillary layer. Skin lesions were characterized with spongiosis, cell necrosis and mononuclear cell infiltration of the epidermal keratinocytes (Takahashi *et al.,* 1996). The crucial role of the host immune response in the pathogenesis of the skin rash is illustrated by the fact that immunocompromised patients often do not develop skin rash following MV infection, although the course of a MV infection in these patients is typically severe and can be lethal (de Swart *et al.,* 2000).

Uncomplicated illness, from late prodrome to resolution of the fever and rash, lasts seven to 10 days (Heymann, 2008).The disease is usually more severe in infants and adults than in children. Measles is also often more severe in immunocompromised individuals and malnourished children, including those with Vitamin A deficiency (CDC, 2009). Uncomplicated recovery from measles is the norm in resource-rich areas; but serious complications of the respiratory tract (pneumonia) and central nervous system (CNS) (acute encephalitis) may occur (Gershon, 2007).

Other potential serious complications include myocarditis, pericarditis and hepatitis. Complications are more common among children younger than five

years of age and adults 20 years of age and older. Measles virus may directly cause croup, bronchiolitis and pneumonia. Secondary viral (Steichan, and Dauthevill, 2009) or bacterial invasion may also occur, resulting in complications such as pneumonia and otitis media. Measles associated with Vitamin A deficiency is a common cause of blindness in developing countries (Perry *et al.*, 2004).

Modified forms of measles with generally mild symptoms may occur in infants who still have partial protection from maternal antibody and, occasionally, in persons with only partial protection from the vaccine (PAHO, 2005). 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 acute 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).

Atypical measles may occur in persons who received inactivated ("killed") measles vaccine (KMV) and are subsequently exposed to wild-type measles virus. This is prevented by revaccination with live measles vaccine (CDC, 2009).In this case after an incubation period of 7-14 days a sudden onset of high fever with headache, abdominal pain and myalgia begins. In contrast to acute measles the rash develops on distal extremities and spreads centripetally. The eruption is

accentuated in the skin folds and may be macular, vesicular, petechial, or urticarial. The majority of cases develop a pneumonia. Occasionally marked hepatomegaly, hyperesthesia numbness or paresthesia are found (Polack *et al.*, 2000). 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).

2.7 Measles Complications

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

2.7.1 Respiratory complications of measles

2.7.1.1 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.

2.7.1.2Laryngotracheobronchitis

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 *Streptococcuspneumoniae, Haemophilus influenzae,Pseudomonas aeruginosa,*

Escherichia coli, and *Enterobacte*r species have also been identified (Ross *et al.*, 1992).

2.7.1.3Pneumonia

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 coinfection 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).

2.7.2Gastrointestinal complications of measles

Measles virus 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).

2.7.2.1 Diarrhoeain Measles

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 diarrhea, 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).

2.7.3Neurological complications in measles

2.7.3.1 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).

2.7.3.2 Post-infectious encephalomyelitis

Post-infectious encephalomyelitis (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).

2.7.3.3 Subacute sclerosing panencephalitis

Subacute sclerosing panencephalitis (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.

2.7.3.4 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 (Mustafa*et al.,* 1993).

2.7.4Ocular complications in measles

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).

2.8 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 (Walter *et al.*, 2004).

2.9 Factors affecting Measles morbidity and mortality rates

2.9.1Sex

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).

2.9.2Age

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).

2.9.3Crowding

Several studies from West Africa and Europe shows that children who develop measles 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).

2.9.4Measles in immunosuppressed patients

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(Walter *et al.,* 2004).

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).

2.9.5Malnutrition

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).

2.9.6Vitamin 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 (Walter *et al.,* 2004). In countries with high measles mortality, treatment with vitamin A once daily for 2 days (200,000 IU) for children more than 12 months of age or (100,000 IU) for infants less than 12 months) is associated with an less than 50% reduction in mortality (Dsouza and Dsouza 2002).The World Health Organization recommends vitamin A therapy for all children with measles (Walter *et al.,* 2004).

2.10 Persistent Infection with Measles

Natural infection with measles virus (MV) establishes lifelong immunity. Persistent infection with MV is likely involved in this phenomenon, as non-replicating protein antigens never induce such long-term immunity. Although MV establishes stable persistent infection *in vitro* and possibly *in vivo*, the mechanism by which this occurs is largely unknown(Amanna *et al.,* 2007; Wagar, 2011).Although a full explanation for life-long immunity against MV has remained elusive, the 1846 island studies conducted by Panum in the Faroes and the more recent work conducted in the United States, which both demonstrated life-long immunity after MV infection in the absence of reinfection, suggest the involvement of persistent MV infection in this phenomenon (Amanna *et al.,* 2007).

MV is highly cytopathic and commonly causes acute severe infection, resulting in measles and, for one out of 1,000 measles cases, acute demyelinating encephalitis. However, MV also causes sub-acute sclerosing panencephalitis (SSPE) at a rate of one out of 1,700–3,300 measles cases if the subject is younger than five years of age when he/she is infected with MV (Roos*etal.,* 1981). Measles inclusion body encephalitis (MIBE) is an additional central nervous system infection that occurs in immunocompromised patients. Thus, MV has the potential to cause persistent infection, although the occurrence of these diseases is very low and MV persistence has only been found in the brain in these cases. It seems likely that these diseases are induced after the long-term persistence of MV in the central nervous system; however, it cannot be excluded that MV persists elsewhere in the body. Furthermore, the persistent infection with MV in SSPE has been assumed to be a result of the appearance of mutant viruses, rather than being a natural property of wild type strains, because many mutations have been found in the MV genomes isolated from SSPE patients (Hirano *et al.,* 1993; Cattaneo *et al.,* 1989).

MV is known to establish persistent infection in culture (Rustigian *et al.,* 1979). The persistently infected cells show characteristic features, such as a disappearance of the cytopathic effect, a marked reduction in cell-free virion production, and a resistance to superinfection by closely related viruses (Rustigian *etal.,* 1979). Although common mutations responsible for persistent infection have not been determined, temperature-sensitive mutants and defective interfering (DI) particles were suggested to play a role in persistent infection through restricting viral replication (Fisher and Rapph 1979). Interferon (IFN) was also

suggested to be involved in persistent infection with MV, through restricting viral replication (Jacobson and McFarland 1982). Receptor down regulation and receptor redistribution may account for the disappearance of syncytium formation and the resistance to superinfection with the parent virus, but this down regulation or redistribution does not account for the resistance to superinfection with closely related canine distemper virus, which does not use the same receptor, and the reduction of cell-free virion release (Naniche *et al.*, 1993).

Despite the proposition of several possibilities, however, the mechanisms responsible for MV persistent infection are still under debate.

Griffin and colleagues provided the first evidence for more reproducible persistent MV infection with MV lacking viral mutations in human and primates. (Pan *et al.*, 2010). Additionally, MV strains isolated from the brain that are very similar to the epidemic MV strain were also reported in MIBE patients, probably due to the early onset of MIBE after a short period of persistence in immunocompromised patients (Hardie *et al.*, 2010;Tomomitsu *et a.*, 2016).

2.11 Epidemiology of measles

Measles virus is one of the most highly contagious, directly transmitted pathogens, and outbreaks can occur in populations in which fewer than 10% of people are susceptible. No latent or persistent measles virus infections result in prolonged contagiousness and there are no animal reservoirs that maintain virus transmission features that make eradication possible. Measles virus can only be

maintained in human populations by an unbroken chain of acute infections. (Gay, 2004).

Measles is spread by airborne droplets (sneezing or coughing) or direct (close personal) contact with nasal or throat secretions of infected persons. The virus may remain infective in droplet nuclei in the air for several hours especially under conditions of low relative humidity. It is spread less commonly by articles freshly soiled with nose and throat secretions (Heymann, 2008).

Infants become susceptible to measles virus infection when passively acquired maternal antibody is lost (Caceres *et al.,* 2000). Infants born to women with vaccine-induced immunity become susceptible to measles at a younger age than those born to women with naturally acquired immunity (Leuridan*et al.,* 2010). In the absence of vaccination, measles is a disease of young children (Simon *et al.*, 2012). Improved vaccination coverage reduces the likelihood of childhood exposure at earlier ages, exposing gaps in immunity and shifting the average age of infection. Under these conditions, all-ages incidence is reduced, however the relative proportion of cases is increased for both older age groups, where immunization gaps may have occurred and children under one year of age who are below the recommended age for vaccination (Zhang *et al.,* 2014; Wang*et al.*, 2015).

Evidence suggests that measles mortality might be higher in girls than boys. Among people of different ages and across different regions (primarily in the Americas and Europe), measles mortality in girls was estimated to be 5% higher

than in boys (Garenne, 1994). Although older historical data and surveillance data from the USA did not identify similar sex differences, if true, the higher mortality in girls' contrasts with most other infectious diseases, in which disease severity and mortality is higher in males. Supporting the hypothesis of sex differences in immune responses to measles virus was the finding that girls and not boys were at risk of delayed mortality after receipt of high-titre measles vaccine, which led to discontinuation of this vaccine (Halsey, 1993).

In the pre-vaccine era, outbreaks occurred in late winter and early spring in temperate climates, whereas in tropical climates outbreaks mainly occurred in the dry season with continual low-level measles transmission year-round (Rota *et al.*, 2016).This seasonal nature of measles reflects high levels of contact clustering or population density; in developed countries, this corresponds to the public school calendar, and in tropical climates this corresponds to yearly migration of agricultural workers (Bharti *et al.*, 2011).The size of the outbreaks varies with a periodicity of 2–5 years, during which susceptible individuals from multiple birth-cohorts accumulate in sufficient numbers to sustain large outbreaks.

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).

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).

The range of the basic reproduction number R_0 (a metric for transmissibility) for measles was estimated to be 9–18, exceeding that of other viral diseases (e.g., smallpox R_0 = 5–7 and polio R_0 = 4–13) Based on the high R_0, the level of population immunity (herd immunity) necessary to interrupt measles transmission is approximately 95% (Rota *et al.*, 2011; Thompson, 2016; Melissa *et al.*, 2017).Measles is often the first vaccine preventable disease detected following a breakdown in routine immunization activities, and as such is a leading indicator of the status of vaccination programs (Takahashi*et al.*, 2015).

The estimated global measles case burden exceeded 9.7 million cases in 2015 with 254,928 reported cases across all six regions of the WHO, with an estimated total of 134,200 (95% CI = 74,400–353,600) measles deaths (Patel *et al.*, 2016).One known limitation in measles case surveillance is that it is subject to

underreporting. Studies have demonstrated underreporting in outbreak settings; one study in Germany demonstrated an average of three times lower rates of reporting to the mandatory notification system compared to health insurance claim submissions by doctors (Six *et al.*, 2010 ; Takla *et al.*, 2014).

The Measles Strategic Planning (MSP) tool developed by the WHO for the analysis of immunization and surveillance data at a national level, also demonstrates underreporting in non-outbreak settings in countries across the globe. The MSP tool also allows estimation of measles mortality based on the number and age of reported cases, measles containing vaccine (MCV) coverage and country specific case fatality ratios (CFR) (Wofson *et al.*, 2009 and Simon *et al.*, 2011).Estimates of measles case fatality rates from 102 studies conducted between 1980 and 2008 indicated that the CFRs ranged from 0% to 40.16%, with a set of nationally representative studies showing a median CFR of 1.0%.

Estimates of CFR varied widely between geographic region and subnational locality of study and were inversely correlated with economic development. In developed countries, CFRs were determined to be approximately 0.05%, but CFRs have reached 6% in developing countries among children aged 1–4 years.Greater measles CFRs are observed in children during major humanitarian crises and are inversely correlated with access to vaccination. The current global incidence of measles is 36 cases/million populations, down from 146 cases/million populations in 2000 Measles incidence estimates declined by 75%

compared to 2000, and in 2015, 109 countries achieved an incidence of less than 5 cases/million populations (Perry *et al.*, 2014; Coughlin*et al.*, 2017).

Incidence varied by WHO region; the African, Eastern Mediterranean and European Regions reported increased incidence in 2014–2015 due to large measles outbreaks. The numbers of reported cases were 98,621 (incidence of 100/million) for the African region, 423 (0.6/million) for the region of the Americas, 21,335 (33/million) for the Eastern Mediterranean region, 25,974 (31/million) for the European Region, 29,109 (17/million) for the South-East Asian region and 65,176 (35/million) for the Western Pacific Region. The region of the Americas verified the elimination of measles in 2016, demonstrating the feasibility of elimination in low- and middle-income countries (Coughlin *et al.*, 2017).

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).

2.12 Global Molecular Epidemiology of Measles

Sequence analysis of measles virus genome has revealed that genotypes are widely distributed throughout the world, some are localized to specific regions where they are classified as endemic (WHO, 2001).Many MV genotypes show clear geographical distribution, while others appear to be more widely distributed(Rima *et al.,* 1995; Bellini and Rota, 1998;Santibanez *et al.,* 2002). In general, three patterns of measles genotype distribution have been described. In countries that still have endemic transmission of measles, the majority of cases are caused by several endemic genotypes that are distributed geographically. In these cases, multiple co-circulating lineages within theendemic genotype or genotypes are present. In countries that have eliminated measles, the small numbers of cases are caused by a number of different genotypes that reflect various sources of imported virus and suggest the lack of sustained transmission of an endemic genotype or genotypes. The third pattern occurs in countries or regions that have had very good measles control but are experiencing an increase in the numbers of susceptible individuals because of failure to maintain universally high vaccination coverage rates. In this situation, reintroduction of measles usually results in a large outbreak associated with a single genotype of virus with nearly identical sequences (Riddell *et al.*, 2005; Muller *et al.*, 2007).

Genotypes A, C1 and D1 were detected before the introduction of the measles vaccine. Analysis of sequences obtained from SSPE cases resulting from primary infection in the 1950s and 60s permitted the detection of genotypes C1, D1, E and F. However, there is no evidence that a specific genotype has a major probability

of causing SSPE: rather, the results are an indication of the efforts made to study this persistent complication of MV. Retrospective analysis of the sequences of isolates carried out during the 1970s showed continuous detection of genotypes C1 and D1 and the first detections of genotypes C2, D2, D4, and F. The remainder of the 23 genotypes were detected between 1980 and 2000; however, some (B1, D1, F and G1) have not been detected in the last 15 years and are therefore considered inactive (WHO, 2005).

Genotype A was found in acute cases of measles in the USA and South America, China, Japan, Russia, Finland and the United Kingdom from the 1950s to the 1990s (Bellini and Rota 1998; Xu *et al.*, 1998 and Santibanez *et al.*, 1999). Current vaccines are derived from old strains of this genotype. However, the detection of genotype A, in association with acute cases of measles would require detailed study, including more information on the genome detected in both specimens and isolates, in order to safely identify the vaccine-induced virus (Riddell *et al.*, 2005). Genotype B2, previously considered inactive, has recently been detected in South Africa (2002), Angola (2003) and the Democratic Republic of the Congo (2005) (Smit *et al.*, 2005). B3 is the endemic genotype in West and Central Africa and has been imported to various European countries in recent years (Hanses *et al.*, 1999; Kremer *et al.*, 2008) Genotype C1 was found in Northern Ireland, Japan, the USA, Spain and Germany in cases of SSPE and in Canada, Japan and Germany in acute cases of measles (Rima *et al.*, 1995; Nakayama *et al.*, 1995; Tipples *et al.*, 2004; Riddell *et al.*, 2005).

Genotype C2 has circulated around Europe, where it has remained endemic in different countries, but has not caused outbreaks since 2004. In addition, between 1995 and 1997, exports to Canada from France and Germany (Tipples *et al.*, 2004)and to the USA from Italy, Austria, Greece and Germany, were detected and the genotype was also identified in Australia in 1990 and 1991 (Chibo *et al.*, 2002) and Morocco in 1998 and 1999 (Alla *et al.*, 2002)Genotype D1 was endemic in Australia in the pre vaccination era (Chibo *et al.*, 2002) and was also detected in the United Kingdom in the pre-vaccine era but has not been found since 1986. Genotype D2 was endemic in Southern Africa from the end of the 1970s until 2000 and also caused a large outbreak in Ireland in 1999-2000 (Troung *et al.*, 1999; Coughlan *et al.*, 2002; Muwonge *et al.*, 2005).

Genotype D3 is endemic in Papua New Guinea and, possibly, the Philippines, give the imports to the USA associated with the Philippines. (Miki *et al.*, 2002; Riddell *et al.*, 2005). Genotype D4 is widely distributed and has been associated with multiple outbreaks in the Indian subcontinent and East and Southern Africa and an outbreak in Quebec (Canada) in 1989. Genotype D4 has recently been found in several European countries, including Germany (2005 and 2006), Croatia (2003-2004), Denmark (2006), France (2006), Italy (2006), Romania (2004-2007) and Russia (2000-2006) among others and in Asian countries like Syria and Iran in 2003 and Indian Ocean Islands such as Mayotte (2005-2006) and the Seychelles (2006) (Kremer *et al.*, 2008).

Genotypes D2 and D4 co-circulated in Southern Africa from the beginning of the 1970s until the end of the 1990s (Riddell *et al.,* 2005). Genotype D5 is endemic in Cambodia. Genotype D6 has spread widely in Europe and may have been endemic, together with genotype C2, since the 1990s. In 2005-2006, it was detected in 17 European countries, mainly with respect to a large outbreak in the Ukraine. Currently, genotype D6 is endemic in Turkey and the Russian Federation (Kremer *et al.,* 2005). Genotype D7 circulated in the UK and Australia during the 1980s, and more recently has been found in several European countries, although it seems to be missing from Europe since 2004. It has recently been detected in India (Vaidya *et al.,* 2008). Genotype D8 seems to co-circulate with genotype D4 in the Indian subcontinent (Wairagkar *et al.,* 2002) and Ethiopia (Nigatu *et al.,* 2001).

Genotype D9, described for the first time after its importation to Australia from Indonesia (Bali) in 1999, was isolated during the outbreak in Colombia and Venezuela in 2000-2001 and was associated with an outbreak in Japan in 2004 .Genotype D10 was described for the first time in outbreaks in Uganda in 2000-2002 (Muwonge *et al.,* 2005). Group G consists of three genotypes, of which the original, G1, has not been detected since 1983; the other two (G2 and G3) are associated with transmission chains and imports from Indonesia and Malaysia (Rota *et al.,* 2000).

Group H contains two genotypes that predominate in Asia. Genotype H1 is endemic in China and is divided into two clusters that circulate throughout the

whole country,it was also detected in the epidemic in Korea in 2000-2001 and, since 2000, has been the predominant genotype in Japan Its circulation in Mongolia has also been verified (Na *et al.,* 2001; Zhou *et al.,* 2003 and Zhang *et al.,* 2007). Genotype H2, first described in China, was recently associated with imports from Vietnam (Liffick *et al.,* 2001 and Kremer *et al.,* 2007). Therefore, some MV genotypes are associated with a specific geographic region while others are more widely distributed. Group B predominates in Sub-Saharan and Central Africa, group G in Southeast Asia, and group H in China and Southeast Asia. Group D, however, is more widely distributed and is found in East Africa, parts of Europe and the Indian subcontinent (Mosquera *et al.,* 2010).

2.12.1Molecular epidemiology of measles in Africa

Historically, African measles viruses have been shown to be genetically diverse. Clade B viruses were endemic in the central and western parts of sub-Saharan Africa, and were subdivided into three genotypes: B1, B2 and B3 (Hanses *et al.,* 1999; Truong *et al.,* 1999; El Mubarak *et al.,* 2002; Rota and Bellini, 2003). Genotype B1 is considered inactive as these viruses were last detected in 1983. Genotype B2 was believed to be inactive for the same reason, but was shown to be circulating in Angola resulting in importation into South Africa in 2002 and in the Central African Republic (Smit *et al.,* 2005). More recently, genotype B2 has been detected in samples from the Democratic Republic of the Congo (DRC), Angola and Zambia (Rota *et al.,* 2011). Genotype B3 was divided into two sub genotypes: cluster 1 virus (B3-1) were widely distributed in sub-Saharan Africa,

while cluster 2 viruses (B3-2) had a more limited distribution and were only detected in western Africa (Kouomou *et al.,* 2002; Mulders *et al.,* 2003).

By 2010, genotype B3-1 appeared to be endemic to most of Africa (Rota *et al.*, 2011; Rota *et al.,* 2016). In contrast, some clade D viruses (genotypes D2, D4 and D10) circulated endemically in the southern and eastern parts of the African continent (Mbugua *et al.,* 2003; Muwonge *et al.,* 2005) with genotype D4 reported from as far north as Ethiopia (Nigatu *et al.,* 2001). However, genotypes D4 and D10 have not been detected since 2006 (Rota *et al.,* 2011). Genotype C2 viruses were detected in an outbreak in Morocco in 1998-1999 which suggests that northern Africa may be linked to European transmission patterns rather than to African patterns (Alla *et al.,* 2002).

2.13 Measles Immunity

There is only one antigenic type of measles virus. Infection confers lifelong immunity. Most so-called second attacks represent errors in diagnosis of either the initial or the second illness.The generation of immune responses to a viral infection is primarily dependent on the dynamic interactions between viral factors and host determinants. Viruses are subject to control by the innate immune system before an adaptive immune response is generated, and like many other human viruses, MV encodes proteins that function to evade or delay the innate immune response.

In particular, the V and C proteins encoded by the P-gene of WT MV strains function as interferon antagonists (Naniche *et al.,* 2000; Ohno *et al.,* 2004)

whereas these proteins in attenuated, vaccine strains are unable to significantly suppress interferon pathways and signaling. Gene-expression profiling experiments have revealed significant cytokine suppression at the transcriptional level for viruses encoding the functional WT P-gene compared to attenuated vaccine strains (Haralambieva *et al.*, 2010).

MV-specific immune responses are essential for recovery from measles, and may be directly responsible for a number of the clinical manifestations of measles and its complications. Immune responses can be detected during the prodrome period and are well developed by the time the rash appears. Marked immune activation is evidenced by T- and B-cell activation and proliferation, as well as increased levels of cytokines. MV-induced immune suppression occurs concurrently with immune activation, resulting in impaired delayed-type hypersensitivity skin test responses to recall antigens, in reduced humoral and CMI responses to new antigens and in lowered natural killer cell function thus increasing susceptibility to secondary infections (Moss *et al.*, 2004).

Measles patients exhibit a marked neutropenia, i.e. decreased levels of polymorphonuclear neutrophils (Kim *et al.*, 2002). Neutrophils display defects in chemotaxis, adherence, metabolic burst and killing capacity, but have normal serum opsonic activity (Toraldo *et al.*, 1992).

Measles immunity involves humoral, cellular and mucosal responses. Cell-mediated immunity is required for recovery from measles, and humoral immunity is associated with protection from infection or re-infection. Whereas recovery in

patients with hypogammaglobulineamia can be uneventful, measles in individuals with severe congenital or acquired cases of fatality (Duke and Mgone, 2004).

Humoral immunity, in response to vaccination or natural infection, is associated with prevention of infection or re-infection with measles. In primary acute measles infection, IgM, IgG and IgA antibodies are produced. Serum IgM antibody concentrations peak 7-10 days after the onset of rash and can persist for more than 1month after natural infection and vaccination. Transient production of serum and secretory IgA also takes place (Duke and Mgone 2004) serum IgG antibody concentration peak within 4 weeks and subsequently decline, but persist for life. Immunity after natural measles is usually life-long. Antibodies induced by vaccination peak at a lower concentration and do not persist as long as immunity after natural infection. Re-exposure to either wild type or vaccine strains of measles virus stimulates memory cells to produce measles-specific responses in which IgG antibodies rise rapidly and peak about 12 days after re-exposure (Panua, 1992). However, long term protection does not require re-exposure because the immunological memory causes a continuous production of antibody 1 and circulating measles virus-specific T cells (Wu *et al.,* 1990)

Antibodies are produced against most of the viral proteins, proportionally to the protein abundance, thus most antibodies recognize various epitopes of the viral nucleoprotein (Graves *et al.,* 1984). Neutralizing antibodies are primarily directed against the H-protein, and to a lesser extent, against the fusion protein (de Swart *et al.,* 2005).

2.14 LaboratoryDiagnosis of Measles

Measles is readily diagnosed by clinicians familiar with the disease, particularly during outbreaks. Koplik's spots are especially helpful because they appear before the rash and are pathognomonic. Clinical diagnosis is difficult in regions where the incidence of measles is low because other pathogens are responsible for most illnesses with presenting symptoms of fever and rash, particularly rubella virus in countries that have not introduced rubella vaccination(Moss and Strebel, 2011). The WHO clinical case definition for measles is a person with fever and maculopapular rash (i.e., non-vesicular) and cough, coryza, or conjunctivitis (WHO, 2011). In addition, many measles cases in previously vaccinated or immunosuppressed individuals do not meet the clinical case definition. Therefore, confirmation of measles virus infection must be made using laboratory-based methods. As disease prevalence falls, the laboratory plays an increasingly important role in differentiating measles from other diseases with similar clinical presentations (Featherstone *et al.,* 2011).

2.14.1 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 (Rota *et al.,* 1998).

2.14.2Immunofluorescence

Direct and indirect immunofluorescence have been used extensively to demonstrate isolation and identification of virus antigens in cells from

nasopharyngeal swab specimen.This technique can also be applied the urine as such cells may be present in the urine 2-5 days after the onset of the rash. Although like the mump, measles is also excreted in the urine, this route is likely to play a significant role in the spread the infection (de Swart *et al.,* 2000).

Nasopharyngeal and conjuctival swabs, blood samples, respiratory secretion, and urine collected from a patient during the febrile period are appropriate source for viral isolation. Monkey or human kidney cells or lymphoblast cell line are optimal for isolation attempts. Continuous cells line such as vero cells can be used, although they are not as efficient as primary cell lines. The presence of measles can be confirmed by haemadsorption. 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 (Rota *et al.,* 1998). Measles virus grows slowly; typical cytopathic affects (multinucleated giant cells containing both intracellular and intracytoplasmic inclusion bodies) take 7-10 days to develop. Shell vial culture test can be completed in 2-3 days using fluorescent antibody staining to detect measles antigen he inoculated cultures. However, virus isolation is technically difficult (Jawetz *et al.,* 2010)

2.14.3Measles serologic techniques

Serologic confirmation of measles infection depends on a fourfold rise in antibody titer between acute-phrase and convalescent-phrase sera or on demonstration of measles specific IgM antibody in a single serum specimen drawn between 1 and 2 weeks after the onset of rash. ELISA, HI, and Neutralization test all may be used to measure measles antibody, though ELISA is

the most practical method (Jawetz *et al.,* 2010; Bellini and Helfand 2003).The presence of IgG antibodies to measles virus in a single serum specimen is evidence of previous infection or immunisation, which cannot be distinguished serologically. Measles-virus-specific IgM antibodies might not be detectable until 4 days or more after rash onset and usually fall to undetectable concentrations within 4—8 weeks of rash onset (Helfand *et al.,* 1997) Oral fluid assays have been used to detect both IgM and IgG antibodies to measles virus. A point-of-care diagnostic test for measles is needed, similar to a rapid diagnostic test for malaria, which ideally could be done with oral fluid samples (Warener *et al.,* 2010)

Dried blood spots and oral fluid appears to be useful alternatives to serum for detection of measles antibody in areas where serum samples are difficult to collect and handle. The major part of the immune response is directed against the viral nucleoprotein. Patients with SSPE display an exaggerated antibody response, with titers 10-to-100-fold higher than those seen in typical convalescent sera (Jawetz *et al.,* 2010).

2.14.4Antigen and nucleic acid detection

Measles antigen can be detected directly in epithelial cells from respiratory secretion, the nasopharynx, conjunctiva, and urine. Antibodies to the nucleoprotein are useful because that is the most abundant viral protein in the infected cells. Detection of viral RNA by RT-PCR is a sensitive method that can be applied to a variety of clinical sample for measles diagnosis.Detection of measles virus RNA by reverse transcriptase-PCR amplification of RNA extracted from clinical specimens can be done with primers targeted to highly conserved

regions of measles virus genes. Primers that span a variable region combined with nucleotide sequencing allow the identification and characterization of measles virus genotypes for molecular epidemiological studies and can distinguish wild-type and vaccine measles virus strains. Molecular epidemiology can be used to identify sources of importation in regions that have eliminated measles. For example, the D4 genotype endemic to Romania was responsible for the measles outbreak in Indiana, USA, after importation (Rota *et al.*, 2009).

2.15 Treatment of Measles

Vitamin A is effective for the treatment of measles and can result in a reduction in morbidity and mortality. WHO recommends administration of once daily doses of 200 000 IU of vitamin A for 2 consecutive days to all children aged 12 months or older who have measles (WHO, 2000; Duke and Mgone, 2003). Lower doses are recommended for younger children: 100 000 IU per day for children aged 6-12 months and 50 000 IU per day for children younger than 6 months. In children with clinical evidence of vitamin A deficiency, a third dose is recommended 2-4 weeks later. Vitamin A has profound and diverse effect on immune system and the integrity of epithelial barriers, and its beneficial effect on measles is certainly mediated in part by the effect on the system (Moss *et al.*, 2004).

There is no specific antiviral therapy for people with measles, although ribavirin, interferon α, and other antiviral drugs have been used to treat severe measles, particularly measles virus infections of the CNS. Secondary bacterial infections are a major cause of morbidity and mortality after measles and effective case management involves prompt treatment with antibiotics (Reuter *et al.*, 2010).

Antibiotics are indicated for people with measles who have clinical evidence of bacterial infection, including pneumonia and otitis media. *Streptococcus pneumoniae* and *Haemophilus influenzae* type b are common causes of bacterial pneumonia after measles, and vaccines against these pathogens will probably lower the incidence of secondary bacterial infections after measles (Kabra *et al.*, 2008).

2.16 Prevention of Measles

The best means of preventing measles is active immunization with measles vaccine. Measles antibodies are transferred from mothers who have been vaccinated against measles or who have been previously infected with measles to their children while they are still in the womb. Such antibodies will usually give newborn infants some immunity against measles, but such antibodies are gradually lost over the course of the first six months of life. Infants under one year of age whose maternal anti-measles antibodies have disappeared become susceptible to infection with the measles virus(Leuridane*et al.*, 2012).

The recommended age of first vaccination varies from 6 to 15 months and is a balance between the optimum age for seroconversion and the probability of acquiring measles before that age. The proportion of children who develop protective concentrations of antibody after measles vaccination are about 85% at age 9 months and 95% at 12 months. Two doses of measles vaccine are needed to achieve sufficiently high levels of population immunity to interrupt transmission (Gay, 2004).

In developing countries where measles is endemic, WHO recommends that the first dose of measles vaccine be administered at age 9 months although countries in which the risk of measles is low often provide the first dose at age 12—15 months(WHO, 2009). The vaccine should be given whether the child is HIV-infected or not. The vaccine is less effective in HIV-infected infants than in the general population, but early treatment with antiretroviral drugs can increase its effectiveness (Helfand *et al.*, 2008).

A second dose of the vaccine is usually given to children between the ages of four and five, to increase rates of immunity. Two strategies to administer the second dose of measles vaccine are through the primary health-care system or mass immunization campaigns, called supplementary immunization activities, an approach first developed by the Pan American Health Organization for South and Central America and modelled after polio eradication strategies(de Quadros *et al.*, 1996).These campaigns are used to deliver other health interventions, including insecticide-treated bed nets, vitamin A, anthelmintic drugs, and other vaccines, such as rubella vaccine which contribute to the reduction of child deaths from other causes (UNICEF, 2007).

The duration of vaccine-induced immunity is at least several decades if not longer (Amanna *et al.*, 2007). Secondary vaccine failure rates are estimated to be about 5% at 10-15 years after immunization, but are probably lower when vaccination is given after 12 months of age (Kremer *et al.*, 2006).Decreasing antibody concentrations do not necessarily imply a complete loss of protective immunity,

because a secondary immune response usually develops after re-exposure to measles virus, with a rapid rise in antibody titers without overt clinical disease.

Standard doses of licensed measles vaccines are safe in children and adults who are immunocompetent. Fever to 39·4°C occurs in about 5% of seronegative vaccine recipients and 2% of vaccine recipients develop a transient rash. Transient thrombocytopenia has been reported with a median incidence of 2·6 cases per 100 000 doses of measles-mumps-rubella (MMR) vaccine. Much public attention has focused on a purported association between MMR vaccine and autism after a case series in 1998 suggested that the MMR vaccine might cause a syndrome of autism and intestinal inflammation (Mantadakis *et al.,* 2010). Several comprehensive reviews and epidemiological studies found no evidence of a causal relation between MMR vaccination and autism (Madsen *et al.,* 2002; deStefano,2007).

The ideal measles vaccine would be inexpensive, safe, heat stable, immunogenic in neonates and very young infants, and administered as a single dose without a needle or syringe(Griffin *et al.,* 2008). The age at vaccination would ideally coincide with other vaccines in the Expanded Programme on Immunization schedule to maximize compliance and share resources.

2.17 Measles Vaccine

The first live attenuated measles vaccine was licensed in 1963. An early candidate vaccine, the Edmonston B vaccine strain, was derived by serial passage of the Edmonston strain in human and monkey kidney cells and chicken embryo

fibroblasts but proved to be insufficiently attenuated (Griffin and Pan, 2009).A second, more attenuated generation of measles vaccines (Schwarz, Edmonston-Zagreb, AIK-C and Moraten strains) all belong to the Edmonston lineage and are currently used. Additionally, the three live-attenuated strains CAM-70, Leningrad-16, and Shanghai-191 were generated from non-Edmonston WT progenitors (Griffin and Pan, 2009).

All measles vaccines are in genotype A. Sequences of measles vaccine strains do not differ greatly from each other; several conserved nucleotide changes between vaccine and WT strains have been documented and the functional consequences of these differences have been observed (Bankamp *et al.,* 2011). At the same time, the live attenuated measles vaccine was developed in the 1960s a formalin-inactivated vaccine was also developed. However, this vaccine resulted in enhanced disease following exposure to WT virus, leading to atypical measles, in which vasculitis and pneumonitis were associated with immune complex formation and deposition in tissues (Griffin and Pan, 2009).

Studies in non-human primates demonstrated that the immune response to the formalin-inactivated vaccine was characterized by the absence of cytotoxic T cells and by antibodies of low avidity due to the lack of affinity maturation. These antibodies waned quickly and could neutralize vaccine virus which used CD46 as a receptor but failed to neutralize WT virus which used CD150, the inactivated vaccine was initially licensed in 1963, but withdrawn in 1967 (Polack *et al.,* 1999; Griffin and Pan 2009). Another vaccine preparation that was determined to be

unacceptable due to adverse events was vaccination with high titer (equivalent to or greater than 40,000 plaque-forming units (pfu)), live-attenuated measles vaccine which was explored in the 1990s. Initial studies showed that use of high titer vaccine led to comparatively higher seroconversion rates in younger infants and could partially overcome maternally acquired antibodies, which hinder the development of an effective immune response. However, antibody levels in high titer vaccinated younger infants remained lower than those of infants vaccinated with standard dose at nine months (Coughlin *et al.,* 2017). The recommendation to use high titer vaccine was withdrawn by the WHO after studies revealed increased mortality within 2–3 years following measles vaccination, especially in young girls. Theories for the increased mortality rate in girls included increased immunosuppression by the high titer vaccine leaving children more susceptible to secondary infections, and the change in sequence of vaccine delivery (Aaby *et al.,* 2017).Some studies have linked increased mortality in girls with receipt of an inactivated vaccine following measles vaccination (Aaby *et al.,* 2016).

The current MV vaccine is safe and highly effective at providing protection against measles. The goal of the current vaccination schedule is to provide optimal vaccine efficacy, ideally by administration to an infant following decay of maternal antibody, and before the likelihood of the child being exposed to MV (Coughlin *etal.,* 2017). WHO recommends administration of the first dose of measles-containing vaccine (MCV) at nine months in measles-endemic regions and at 12–15 months in non-endemic regions (Griffin and Pan 2009; Coughlin *etal.,* 2017). During an outbreak of measles, the first dose may be given as early

as six months to protect infants under one year of age, with a follow-up dose at 9–12 months After a single dose of MCV at nine months, 85% of children develop protective immunity to measles, and when the first dose is given at 12 months, 95% of children are protected from measles (Griffin and Pan 2009; Hales*et al.*, 2016).

A two-dose vaccination strategy is recommended to achieve the 95% coverage within a community to provide sufficient herd immunity to interrupt transmission by this highly contagious pathogen. The second dose also targets vaccinees that did not achieve an adequate immune response following a single dose of MCV. When countries have sufficient health system infrastructure, the administration of the second dose of MCV may be achieved as part of routine immunization and school entry requirements. In other countries, supplemental immunization activities (SIAs) are important to achieve high vaccination coverage and to immunize children that may have been missed during routine immunization or previous SIA activities (Rota *et al.*, 2009; Coughlin *et al.*, 2017).

2.17.1Novel vaccination strategies

Despite the availability of an effective vaccine delivery system, several alternative vaccination strategies are under investigation. The motivations behind the desire to improve upon the current vaccine delivery method include eliminating or reducing the cold chain requirements, concerns regarding injection safety and contaminated waste disposal, and decreasing the need for medically trained personnel to administer the vaccine.The current live attenuated measles vaccine

rapidly loses potency once reconstituted. Within one hour of reconstitution if the vaccine is stored at 20 °C, it will lose approximately half of its titer; if stored at 37 °C, it will lose nearly all of the 10^3 plaque forming units required for adequate vaccination (Griffin and Pan, 2009). An example of the result of cold chain breakdown was demonstrated in the Marshall Islands, in which secondary vaccine failures resulted from a compromised cold chain and lead to the first outbreak of measles in more than two decades (Hales *et al.*, 2016). Furthermore, alternative routes of immunization are being explored to determine if the inhibitory effects of maternal antibody may be overcome to allow for immunization of infants at a younger age, particularly in regions of high endemicity (Rota *et al.*, 2016).

The alternative routes of vaccination currently under evaluation are vaccination via the respiratory route and the intradermal route by micro needles which are described below. Other alternative vaccination devices such as needle-free disposable cartridge jet injectors have also been evaluated with intent to eliminate the logistics of sharps usage as currently administered by the subcutaneous route (Mitagotri, 2006; Coughlin *et al.*, 2015).

Respiratory vaccination via the aerosol route has been evaluated with two vaccine formulations, nebulized liquid and dry power vaccine. Respiratory delivery of measles vaccine has been studied for 30 years and the WHO Measles Aerosol Project was established with the goal to license one respiratory mode of measles vaccine delivery. Understanding the average volume of inhaled vaccine delivered to the deep lung is important to determine the dosage received upon

aerosolization. Studies suggest that an effective immune response is achieved when the vaccine reaches the deep lung and, therefore, aerosolized particles must be <5 µM in size (Hales *et al.,* 2016).

Evaluation of liquid nebulization in the macaque animal model demonstrated that tidal volume, the lung volume representing total volume of air displaced during inhalation and expiration during normal breathing, is similar to that of children. The studies also showed that the effect of breathing parameters (i.e., tidal volume, breaths/min, and inspiratory to expiratory ratio) on dose inhalation differs with the various nebulizer devices. Testing of a nebulizer device used extensively in Mexico to deliver liquid aerosolized vaccine demonstrated that approximately 30% of the aerosolized vaccine is of the <5 µM particle size needed to reach the deep lung, and that about 20%–30% of the dose is likely inhaled (Wong-chew *et al.,* 2011; Macoughlin *et al.,* 2016).Aerosol delivery generates an immune response with no adverse reactions.

In clinical trials, aerosol delivery boosted the immune response in school aged children, but initial results in infants were less promising. Several studies showed that infants under 12 months of age responded to vaccine but did not meet the strict non-inferiority threshold compared to subcutaneous delivery. However, increasing the exposure time of infants to the nebulized vaccine demonstrated comparable immunity to subcutaneous injection (Griffin, 2014; Diaz-ortega *et al.*, 2014; Low *et al.,* 2015). Infants vaccinated for an increased time duration (1–2.5 min of exposure instead of 30 s) by aerosol delivery of the vaccine demonstrated

higher antibody responses compared to vaccination at the reduced exposure time (Whittle *et al.*, 1984). When the time of aerosol vaccination was increased to 2.5 min, greater than 95% of infants seroconverted with protective levels (>120 mIU/mL) of antibody as measured by plaque reduction neutralization assay (Wong-chew *et al.*, 2011). The use of aerosol vaccination as a primary immunization is less effective when given at the shorter 30s exposure time, however, studies suggest that increasing the exposure time of aerosol delivery of a primary dose can increase the efficacy of this alternative route of immunization. Aerosol delivery is effective to provide a second dose of vaccine at the shorter 30 s exposure time, and MCV2 delivery via aerosol performed as well and often better than subcutaneous delivery as measured by the antibody response (Dilraj *et al.*, 2000; Bennett *et al.*, 2002). Measles vaccine formulations perform unequally when delivered by aerosol. The Edmonston-Zagreb strain appears to be the most immunogenic, withstanding aerosolization without loss of vaccine titer, and the immunogenicity as measured by antibody titer was comparable to subcutaneous injection. Additional alternative vaccine delivery methods are newer in development and because of their additional benefits such as cold-chain elimination prospects, pre-clinical data is included for discussion.

Measles vaccine is also being studied for aerosol delivery in a dry powder formulation. The dry powder vaccine is reconstituted in vivo by moisture in the respiratory tract. The advantages to the dry powder formulation are that it has greater thermo stability and is not dependent on maintenance of the cold chain (de Swart *et al.*, 2007; Griffin, 2014). In the initial studies, the dry powder

formulation did not perform as well as subcutaneous delivery of measles vaccine because the large particle size of the powder limited access of particles to the deep lung. Improvements in the production of dry powder vaccine preparations by CO_2 assisted nebulizer with bubble dryer (CAN-BD) produced a smaller particle size of 3–5 µM, the size required to reach the deep lung (de Swart *et al.*, 2007). Subsequent studies with the improved preparation methods have evaluated two devices for the delivery of dry powder vaccines, the Puffhaler (Aktiv-Dry, Boulder, CO, USA) and the BD Solvent (Becton-Dickinson, Franklin Lakes, NJ, USA), both have been shown to be equally effective in delivering measles dry powder vaccines in a macaque model (Griffin, 2014). A study of delivery of dry powder MV vaccine using both devices in macaques demonstrated an immune response as effective as subcutaneous delivery of MV vaccine. The animals produced effective humoral and cellular immune responses that protected animals from challenge and were characterized by high avidity antibodies, the production of IgA in the respiratory mucosa and the activation of memory cells.The dry powder vaccine formulation evaluated in phase 1 clinical trials was immunogenic and produced no adverse events in seropositive adult males (Lin *et al.*, 2011; Chaudari *et al.*, 2014).

One of the most promising alternative delivery technologies is the microneedle patch. Microneedles have been under development since the mid-1990s and the most recent formulations used to evaluate measles vaccine delivery include dissolving micro needles. Dissolving microneedles can be formulated from inert material such as polymers and sugars including trehalose, sucrose, arginine and

hepatagluconate in which vaccine is encapsulated into the tip of the needle. These materials allow more effective delivery upon patch application, increase the stability of the vaccine and are approved for human use (Kim *et al.,* 2012; Mistilin *et al.,* 2016). The microneedles dissolve in the skin, releasing the vaccine and eliminating the need for sharps or biohazardous waste disposal. An additional benefit to microneedle patch usage is that it may be administered by minimally trained individuals (Edens *et al.,* 2015).

Studies in macaques have demonstrated an equivalent antibody and cellular response following vaccination with measles microneedle patches and subcutaneous vaccination with no significant reactivity at the site of patch placement (Edens *et al.,* 2015). This new delivery method will not be available in the near future because development of microneedle vaccines for measles and rubella is still in the preclinical phase. Candidate vaccines will need to be evaluated in clinical trials and successfully negotiate regulatory pathways. Though the microneedles will be fabricated with the live attenuated vaccines that are currently produced by vaccine manufacturers, a plan for large scale commercial production of microneedles under GMP condition will be required.

2.18 Measles Control and Prospects of Eradication

Measles is on a very short list of potentially eradicable diseases. Following smallpox eradication in the 1970s, the second human disease targeted for eradication is polio. Many lessons learned during the polio eradication efforts have helped in the efforts to control measles(Hampton *et al.,* 2016).Furthermore,

the eradication of a related *Morbillivirus* of cattle, rinderpest virus, supports the biological feasibility of measles eradication (Mariner *et al.,* 2012). MV has no animal reservoir, a highly effective and safe vaccine is available, and a surveillance network has been established to identify and respond to cases and outbreaks. Molecular data demonstrate that chains of measles transmission are frequently interrupted by vaccination (Rota *et al.,* 2016).

In 2010, the World Health Assembly (WHA) identified goals for measles control that were to be achieved by 2015. These included coverage with MCV1 of 90% at the national level and 80% in all districts, reported measles incidence of less than 5/million population, and 95% measles mortality reduction compared to 2000 (WHO, 2011;Strebel *et al.,* 2011). Though significant improvements have been made, with an estimated 20.3 million deaths prevented by measles vaccination in the last 15 years, global control efforts have lagged and the global community has not met the WHA goals (Patel *et al.,* 2016).

As of 2014, estimated global coverage with MCV1 is 85%, and as of 2015, only 61% of countries have reached >90% MCV1 coverage. Coverage with MCV2 also increased significantly globally, with six countries (Angola, Malawi, Mozambique, Nepal, Sierra Leon, and Zimbabwe) adding a routine MCV2, bringing global MCV2 coverage to 61% (Perry *et al.*, 2015; Patel *et al.*, 2016). In 2015 alone, SIAs vaccinated 184 million individuals, with most of these efforts also providing other childhood health interventions including vitamin A supplementation, rubella vaccination, and deworming interventions. More than

half of all unvaccinated children reside in six countries, the Democratic Republic of Congo, Ethiopia, India, Indonesia, Nigeria, and Pakistan (Perry *et al.*, 2015; Patel *et al.*, 2016).

Though the 79% decrease in global measles deaths between 2000 and 2015 fell short of the WHA goal of 95% reduction, the impact on mortality in children under the age of five has been significant (Perry *et al.*, 2015; Patel *et al.*, 2016). With an estimated 23% of the decrease in mortality in children under the age of five from 1990 to 2008 accounted for by measles reduction, measles control efforts have significantly contributed to the fourth United Nations Millennium Development Goal, to reduce overall childhood mortality by two-thirds compared to 1990 (Strebel *et al.*, 2011; Rota*et al.*, 2016).

The greatest challenge to eradicate measles is to increase vaccine coverage in all countries. This will require strengthening of the public health infrastructure. The development of new strategies to deliver measles vaccines will help to improve coverage. In many regions, the cold-chain requirement of the measles vaccine is difficult to maintain and financially burdensome. Improved vaccine formulations that offer greater thermostability such as the microneedle patch may lead to improved coverage.

Furthermore, the availability of medically trained personnel to administer subcutaneous vaccinations is limited in many countries; therefore, a vaccine delivery technique such as the micro needle patch that does not require medically trained personnel could contribute significantly to improving global MCV

coverage. All three of the discussed novel vaccination strategies, aerosol, dry powder and micro needle patches have been estimated to reduce cost per dose due to improved thermo stability, reduction of sharps waste disposal, reduced need for trained personnel, and reduced wastage of unusable vaccine (Garrison *et al.*, 2011; Adhikhari *et al.,* 2016).The dry powder and micro needle vaccines have the additional advantage of being available in single dose packages which reduces wastage.

Vaccine hesitancy or exemption can lead to relatively large outbreaks even within regions with high vaccination coverage (CDC, 2013; Phadke *et al.,* 2016).Many countries need to improve case-based surveillance for measles and rapid confirmation of cases because of the constant threat of measles importations .Therefore, elimination efforts require not only the establishment of surveillance capacity but also the ongoing maintenance of laboratory capacity within the GMRLN .Finally, a measles eradication goal will require a significant commitment of financial resources from both governmental and non-governmental organizations(Rota *et al.,* 2016).

In 2010, an expert advisory panel stated that measles can and should be eradicated (WHO, 2011; Strebel *et al.,* 2011; Rota *et al.,* 2016). Measles has been verified as eliminated from the region of the Americas and the remaining five WHO regions have adopted measles elimination goals. Five out of the six regions have established regional elimination verification commissions Sustained efforts to improve vaccine coverage and to maintain adequate surveillance will help to

reduce measles incidence globally, allowing all children worldwide to avoid this previously inevitable childhood disease.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location

Nigeria is divided into six geopolitical regions; this includes North-West, North-Central, North-East, South-West, South-East, and South-South. The Northwestern region of Nigeria where this study was conducted consist of Kano state, Katsina state, Zamfara state, Sokoto state, Kebbi state, Jigawa state and Kaduna state. This region of Nigeria lies between longitude 4^o and 10^o East of the Greenwich meridian and latitude 9^o and 13^o North of the equator with a typical tropical continental climate and Northern dry savannah vegetation with alternating humid to wet and cool to hot dry season (April to September and October to March respectively). The seasons are governed by the movement of the inter-tropical discontinuity, a zone where warm, moist air from the Atlantic converges with hot, dry and often dust-laden air from the Sahara known locally as the harmattan. Temperatures are high throughout the year, averaging from 25^o to 28^oC (77^o to 82^oF).

Northern Nigeria typically experiences greater temperature extremes than the south. Rainfall is especially variable over distance and time. The inhabitants of the region are mostly subsistence farmers who cultivate cereal such as rice, cotton

and onionsand animal husbandry produce livestock. The North-West region has a total population of about 35,786,944 (NPC, 2006), Three states were randomly selected for the purpose of this study and these includes:

3.1.1Kano state

Kano state has Kano as its capital, it is located at latitude 12^{o} 03' N and longitude 8^{o} 34'E and altitude 486.5M (1595ff). The state is characterized by two seasons, the rainy and dry seasons. The rainy season usually begins from May and ends in September with heavier rainfall in July and August, October to about May marks the dry season with little or no rainfall (Mukhtar, *et al.,* 2010). Kano state borders Katsina state to the North-West, Jigawa state to the North-East, Bauchi state to the South-East and Kaduna state to the South-West. The state has 44 Local Government Areas with three senatorial districts. Kano state has been a commercial and agricultural state, which is known for the production of groundnuts as well as for its solid mineral deposits. The state has 20,131 square kilometers (7,773sq mi) of cultivable land and is the most extensively irrigated state in the country. According to the 2006 census figures Kano state had a population of 9,383,682 (NPC, 2006).

3.1.2Zamfara state

The State with Gusau as its capital is located at latitude 12^{o} 10' N and longitude 6^{o} 15' E. The state shares boundaries with Sokoto state and Niger republic in the North, Katsina state in the East, Kebbi state in the West, Kaduna and Niger states in the South. There are 14 Local Government Areas in the state. The vegetation of the state is mainly Sudan and savannah grassland. There is a vast land with

scattered community settlement, agricultural land, and two main rivers (River Bunsuru and River Ka).About 80% of the population lives in rural communities.

The state has two seasons namely: the rainy and dry seasons: the rainy season commences from May to November, while the dry season starts from December to April. The state has a population of 3,278,873 according to NPC, (2006) with Hausa and Fulani being the main ethic groups. Islam is the predominant religion in practice. The main activities of the people are crop production and animal husbandry.

3.1.3Katsina state

Katsina state has Katsina as its capital. The state is located at latitude 12^{o} 59' N and longitude 7^{o} 36' E.The state is bounded in the East by Kano and Jigawa states in the West by Sokoto state, in the South by Kaduna state and in the North by the Niger Republic. Katsina state has an estimated population of 5,792,578 (NPC, 2006). Katsina State consists of 34 Local Government Areas. The state is the center of an agricultural region producing groundnuts, cotton, hides, millet and guinea corn, and also have mills for producing peanut oil and steel with an area of 24,194km^2 (9,341 sq. mi).

3.2 Study Design

The research was a descriptive cross sectional study, and was conducted throughout the period of one year (November 2014-December 2015) which covers both outbreak and sporadic cases within the study period in all the selected states. Three hospitals were selected from each state by using simple random

sampling and each state is stratified into three strata, by stratified sampling. Each stratum represent a senatorial zone. Simple random sampling was used to select one local government from a zone. Hospitals were randomly selected to represent a local government in each zone. In Kano state Hasiya Bayero Specialist Hospital (Kano municipal), General Hospital Gwarzo, and General Hospital Wudil were selected. In Katsina state general hospital Daura, General Hospital Funtua and Turai Yar'adua Hospital (Katsina municipal) were selected, while General Hospital Anka, King Fahad women and children Hospital and Kauran Namoda General Hospital were selected in Zamfara state.

3.3 Study population

Children 0-10 years of age with suspected measles case attending selected hospitals in the study locations were included in the study. WHO definition of measles was adopted; which is any person with fever of 38^{o}C or greater, and maculopapular rash, and any one of the following: cough, coryza, or conjunctivitis and any person in whom the health professional suspected measles was also included (WHO, 2012).

3.4 Ethical Clearance Approval

Ethical clearance (Appendix I, II, and III) was obtained from the ethical committee of the Ministries of Health of the selected states. The selected hospitals in each state were visited to discuss the feasibility of the study. The consent and cooperation of the management, clinicians (pediatricians) and laboratory staff was also obtained.Consent of parents and/or adult care givers of children under study were obtained (Appendix V).

3.5 Inclusion and Exclusion Criteria

The inclusion criteria included all children aged 0-10years of both sexes, suspected measles cases characterized by appearance of maculopapular rashes and consented for the research.

The exclusion criteria included: children above 10 years of bothsexes, non-consenting respondents and absence of any clinical symptoms suspected to be measles.

3.6 Questionnaire Administration

Data were collected using a self-structured questionnaire which was administered by the researcher and trained research assistants who were adequately briefed by the researcher. Information such as previous history of measles vaccination, natural infection, demographic variables, clinical data, and risk factors were collected. Information was collected from the parent or adult care givers of the children under study (Appendix IV).

3.7 Determination of Sample Size

A prevalence rate of 32.2% as reported from a study conducted in Kaduna (Chechet *et al.*, 2014) was used to determine the sample size using the equation below (Sarmukaddam and Garad, 2006).

$$n = \frac{Z^2 pq}{L^2}$$

Where n' is the sample size

'Z' is the standard normal distribution at 95% confidence interval = 1.96

'P' is the prevalence rate, which is taken to be 32.2 =

'q' is 1 – p = 1 –0.322 = 0.68

'L' is the allowable error, which is taken as 5% = 0.05

Therefore;

$$n = \frac{(1.96)^2 \quad x \quad 0.32 \quad x \quad 0.68}{(0.05)^2} = 334.42$$

Therefore, n = 334 is the number of sample to be collected. However, in order to have a good representation of the target population a total of 752 samples were collected for the study which was shared across the selected states.

3.8 Collection of Blood Sample and Processing

A total of 752 samples were collected for the study across the nine selected Local Government Areas (LGAs) of the selected states, (Kano, Katsina and Zamfara). In Kano state 297 samples were collected while in Katsina and Zamfara states 231 and 224 were collected respectively. Blood samples were collected by experienced medical laboratory scientists who were recruited throughout the duration of sample collection. Three milliliter of blood sample was collected aseptically from each patient by venipuncture using a sterile disposable syringe and dispensed into sterile labeled containers. Serum sample was separated into sterile plain containers before being transported to the laboratory in a cold pack.

The samples were stored at 2^{o}C until assay time. All serology tests were carried out in the Department of Microbiology, Ahmadu Bello University Zaria.

3.9 Collection of Nasopharyngeal Swab

Nasopharyngeal swabssamples were obtained by firmly rubbing the nasopharyngeal passage and throat with sterile cotton nasal swabs (Miraclean Technology Co.) to dislodge epithelial cells, this was carried out by experienced laboratory scientist. The swabs were then placed in sterile viral transport medium (Phosphate Buffered Saline, (PBS) in labeled screw capped tubes and transported to the laboratory on ice box, and there after kept at -20^{o}C until required for analysis.

3.10 Detection of Measles IgM Antibody

Using commercially available Enzyme immunosorbent assay (Measles IgM ELISA, Diagnostic Automation and Cortez, Calabasas, CA, USA), serum samples were assayed for specific IgM antibody against the measles virus. All assays were carried out according to the manufacturer's instructions.

3.10.1Procedure for assay of measles IgM antibody using ELISA

All samples, microtitre plates and reagents were brought to room temperature (28^{o}C) prior to commencement of the assay. The coated strips were placed in a holder and labeled. The first well A-1 was used for reagent blank, A-2 and A-3 for positive and negative controls respectively and A-4 and A-5 were used for calibrators. Three microliter (3µl) of the test samples, positive control, negative

control and calibrators, was added to 240µl of the serum diluents and mixed thoroughly to make 1:80 dilutions.

One hundred (100µl), each of the diluted controls, calibrators and 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 and 100µl of the diluent was then added into reagent blank well. The microtitre well were incubated at room temperature (28^{o}C), for 30minutes. After incubation, liquids from all wells were aspirated, thereafter 300µl of diluted wash buffer was added to each well: this procedure was repeated two more times for a total of three washes. After the final wash, the plates were blotted on tissue paper to remove all liquid from the well.

One hundred microlitre(100µl) of enzyme conjugate was added to each well including reagent blank, the plates were incubated for 30 minutes at room temperature. Excess enzyme conjugate was removed by washing three times with the buffer and 100µl of chromogen/substrate solution was then dispensed into each well including reagent blank and incubated at room temperature for 15 minutes. A blue color was formed in some wells. Finally, 100µl of stop solution was added, and the plates were tapped gently to mix the contents of the wells. After the addition of stop solution, the wells develop yellow coloration whose intensities vary. The intensity of the color is proportional to the concentration of the IgM antibody. The optical density was read using microplate reader at 450 nm wavelength; the result was printed out by a printer attached to the ELISA machine (Appendix VI).

3.10.2Interpretation of ELISA results

Results were interpreted by following the manufacturer's guide. The calculations involved in arriving at the final result are:

a. Mean absorbance of the calibrators (C)

$$= \frac{\text{A-2 + A-3}}{2} = C$$

Where A-2 and A-3, stand for calibrator 1 and 2

Mean absorbance of calibrator = C

b. Correction factor (X-factor, was supplied by the manufacturer) = 0.50

c. Cut-off value (COV) = mean absorbance of calibrator x correction factor

= C x 0.50

COV = Y

d. Sample absorbance is divided by the cut-off value to get the value for each patient sample

If $\frac{\text{Sample absorbance}}{\text{Cut-off value(Y)}} \leq 0.90$ = Negative

If $\frac{\text{Sample absorbance}}{\text{Cut-off value(Y)}}$ 0.91-1.09 = Equivocal

If $\frac{\text{Sample absorbance}}{\text{Cut-off value(Y)}} \geq 1.10$ = Positive

Cut-off value(Y)

3.11Molecular Characterization of MV N Gene from ELISA IgM Positive Samples

3.11.1RNA extraction

MeaslesvirusRNA was extracted from throat swab of forty (40) serologically confirmed measles cases using QIAamp viral RNA Mini Kit (Qiagen, The Netherlands).This was placed at -20^{o}C till further use. The extraction was performed following manufacturer's instructions.

The viral transport media containing the swab was vortexed briefly to dislodge any viral particle into the medium. Thereafter 1.2 ml of the suspension was transferred into a 1.5 ml micro centrifuge tube and centrifuge for 2 minutes at 2300 x g to pellet the cellular material. After centrifugation the supernatants was discarded and the pellets re-suspended in 200 µl of nuclease free water. To another micro centrifuge tube, 200 µl of the suspension was added into 400 µl binding buffer (buffer VB) containing carrier RNA, followed by pulse vortexing for 5seconds to yield a homogenous mixture. This was then incubated at room temperature for 10 minutes to ensure complete lysis. Potentially infectious agents and RNAse were inactivated in buffer VB. The tube was centrifuged briefly to sediment drops from the inside of the lid.

Exactly 100 µl of isopropanol was added to the sample, followed by slight vortexing for 5 seconds, the tube was spun down for 10 seconds to ensure that no

liquid remain on the walls of tube. The binding column was fitted into the collection tube and the solution was carefully transferred into the binding column. The lid was closed and centrifuged at 6000 x g for 1 minute. Following centrifugation, the binding column was transferred into a new collection tube and the tube containing the filtrate was discarded. Each binding column was closed in order to avoid cross- contamination during centrifugation. The binding column was carefully opened, and 500µl washing buffer W1 was added, the lid was closed and centrifuged at 8000rpm for 1 minute.

After the centrifugation binding column was transferred into a clean collection tube and the tube containing the filtrate was discarded. The binding column was then carefully opened, and 500µl of buffer was added, and the cap was closed and centrifuged at 6000 x g for 1 minute. After the centrifugation binding column was transferred into a clean collection tube and the tube containing the filtrate was discarded, and it was centrifuge at full speed 9000 x g for 1 minute to remove ethanol completely, making sure that there is no droplet hanging from the bottom of the binding column.The binding column was placed in a sterile 1.5ml micro centrifuge tube, and was carefully opened and 50µl of elution buffer was added and incubated at room temperature for 1 minute to allow buffer to permeate the column, this was allowed to stands for additional 15 minutes to increase RNA yield. Finally, it was centrifuge at 6000 x g for 1 minute. The eluted RNA was used for amplification.

3.11.2 Reverse transcription of MV RNA to cDNA

Following extraction of RNA, Specific cDNA of MV nucleoprotein was synthesized by reverse transcription (RT) with Moloney murine leukemia virus reverse transcriptase (Invitrogen, Merelbeke, Belgium) and primer MVR1(Table 3.1). A total of 1µg of extracted RNA was transferred to a sterile RNAse free microcentrifuge tube. The following reaction mixture were added: 1µg template RNA, 2µl measles primer, 1µl 10mM dNTP mix, 2µl of 10x RT buffer, 1µl each of RNAse inhibitor and reverse transcriptase and finally addition of nuclease free water to the total volume of 20µl. These was incubated at 42^{o}C for one hour, subsequently the enzyme was inactivated at 80^{o}C for 5 minutes.

3.11.3Amplification of MV N-gene by polymerase chain reaction nRT-PCR.)

3.11.3.1First Round N-gene Amplification

Following reverse transcription, specific primers targeting the MV gene were used to amplify a 644 bp fragment with first-round primers MVF1 and MVR1 (Table 3.1) using Platinum Taq DNA polymerase (Invitrogen). Master Mix was set up using the volumes of reagents as follows: Nuclease-free water 23 µl, 10x reaction buffer 5 µl, 25 mM $MgCl_2$, 1.5 µl, 10 mM mix of dNTPs, 1.5µl, measles primers MVF1 and MVR1 2µl each, nuclease-free water was used as negative control. This was properly mixed by inversion, and pulse spun to get drops off the lid, followed by addition of 5 µl template cDNA to correctly labeled tubes. The PCR cycling program consisted of denaturation for 4 min at 94 °C, followed by

40 cycles of 30 seconds at 94°C, 30 seconds at 60 °C and 45 seconds at 72°C, with final extension for 7 min at 72°C.

3.11.3.2Second Round N-gene Amplification

A second round of semi nested PCR was performed on first round PCR product to amplify a 528bp fragment of N-gene using primers MVF2 and MVR1 (Table 3.1) to increase specificity. The PCR cycling condition consisted of denaturation for 4 minutes at 94^{o}C, followed by25 cycles of 30 seconds at 94^{o}C, 30 seconds at 60^{o}C and 45 seconds at 72^{o}C, with final extension for 7 minutes at 72^{o}C.

Table 3.1: Primers Used for PCR Amplification and Sequencing of Measles Virus N gene

Primer	Primer Sequence(5'-3')	Position(nt)	Amplicon size	References
MVF1	TACCCTCTCTGCTCT GGAGCTATGCC	985-1008	644bp	Chibo *et al.*, 2000
MVR1	AACAATGATGGAGG GTAGGCG	1629-1609	644bp	Chibo *et al.*, 2000
MVF2	GATGGTAAGGAGGTC	1101-1121	528bp	Chibo *et*

3.11.4Agarose gel electrophoresisof PCR products

Agarose gel of 1% was prepared by mixing 40ml Tris acetate EDTA (TAE) buffer (pH 7.9) in a glass bottle followed by addition of 1gram of agarose and boiled in a microwave oven for 3 minutes. After boiling, the volume was made up to 60 mls with distilled water and cooled to about 50^{o}C. Two microliter (µl) of ethidium bromide was added, swirled and the gel poured into gel casting tray with a comb in place. The gel was allowed to solidify at room temperature for 30minutes and the comb removed. The casting tray was submerged in the TAE running buffer in an electrophoresis apparatus. Ten microliter (10 µl) of each PCR product and 5µl of the 100 base-pair (bp) molecular size ladder was mixed with

loading dye and loaded into separate wells of the gel. The first and last wells of the gels were loaded with the 100 bp molecular size ladder and 100volts was applied for 30minutes using Bio-Rad power pack (powerpac TMHC). The power supply was turned off after 30minutes. The gel was removed and examined using Bio-Rad Gel Doc Molecular Imager System (Gel Doc TMXRT). The amplicon size was determined using a standard 100bp DNA ladder (Invitrogen Carlsbad, CA, USA). The result was documented.

3.11.5Sequencing and phylogenetic analysis.

The RT-PCR products were purified using a QIAquick PCR purification kit (QIAGEN) following the manufacturer's instructions. Purified PCR products were sequenced in the forward and reverse directions using a cycle sequencing reaction with an ABI Prism Big Dye Terminator cycle sequencing kit (Applied Biosystems, Perkin Elmer). Sequencing reactions were performed using 3 µl purified PCR products, 2 µl Big Dye reaction mix (Applied Biosystems, Foster City, CA, USA) and 2 µl of 20 µM of MV primers. Unincorporated Big Dye terminators were removed from the reactions by precipitation method using 0.3 M sodium acetate and 50% ethanol.

The relatedness of 450 bp fragment of the N gene of the eight (8) measles virus strains was assessed; sequences wereedited and multiplealignment of the nucleoprotein was done using CLC Mainwork Bench (version 7.8.2) software. Phylogenetic and molecular evolutionary analyses were performed using Molecular Evolutionary Genetic Analysis (MEGA Version 4) software package (Tamura *et al.,* 2007). The phylogenetic tree was constructed using the Neighbor-

Joining method and bootstrap analysis of 1000 replicates. Genotype assignment was performed by sequence comparison with the measles virus reference strains from NCBI Genbank data base and identical sequences revealing genotype B3 were obtained.

Sequences were named according to the current WHO recommendations for nomenclature (WHO, 2001a; WHO, 2001b; WHO, 2003; WHO, 2012), hence all sequences may have city name, and sequence names include the epidemiological week of onset of rash. However, as this information was frequently missing, either the date of specimen collection or even the date of sample receipt in the laboratory was used. A database of WHO-designated reference sequences for each genotype was obtained from GenBank. MV strains were designated according to their clade (A–H) and genotype (1–7). An MV strain of clade B genotype 3 is referred to as B3. Prototype genotypic sequences included in this analysis for comparative purposes are indicated by block capitals.

3.12 Analysis of Results

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 21.0 software program. Pearson's Chi-square (χ^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.

CHAPTER FOUR

4.0 RESULTS

4.1 Seroprevalence of measles IgM Antibodies in parts of North Western Nigeria.

Out of seven hundred and fifty-two (752) suspected measles cases screened for the presence of measles virus IgM antibodies, three hundred and thirty-four (334) samples were positive, indicating an overall seroprevalence of 44.4% (Figure 4.1). The distribution of measles IgM antibodies among children with respects to states in North Western Nigeria is presented in Table 4.1. The finding revealed that Kano State had the highest seroprevalence of 47.8%, followed by Zamfara State with a seroprevalence of 43.3%, while Katsina State was the least with a seroprevalence

of 41.1%. However, the result shows no significant difference (χ^2 =2.512; 0.285 P> 0.05) between measles IgM antibody seroprevalence and the States.

The result of seroprevalence of measles IgM antibodies was analyzed in respect to some demographic characteristics of the study subjects as shown in Table 4.2. Measles IgM antibody was detected in all age groups, with the highest seroprevalence of 53.2% recorded in age groups 1-24months, and the lowest of 25.7 % in age group 73-96months. The result showed decrease seropositivity with age and the association between measles IgM antibodies and age was statistically significant (χ^2=20.1, P=0.001). Of the seven hundred and fifty-two(752) samples analyzed, 373 were males with IgMseroprevalence of 42.1% while 379 females were among the population analyzed with a seroprevalence of 46.7% though the difference was not statistically significant (χ^2= 1.619, P= 0.203, OR=0.901, CI=0.768-1.058).

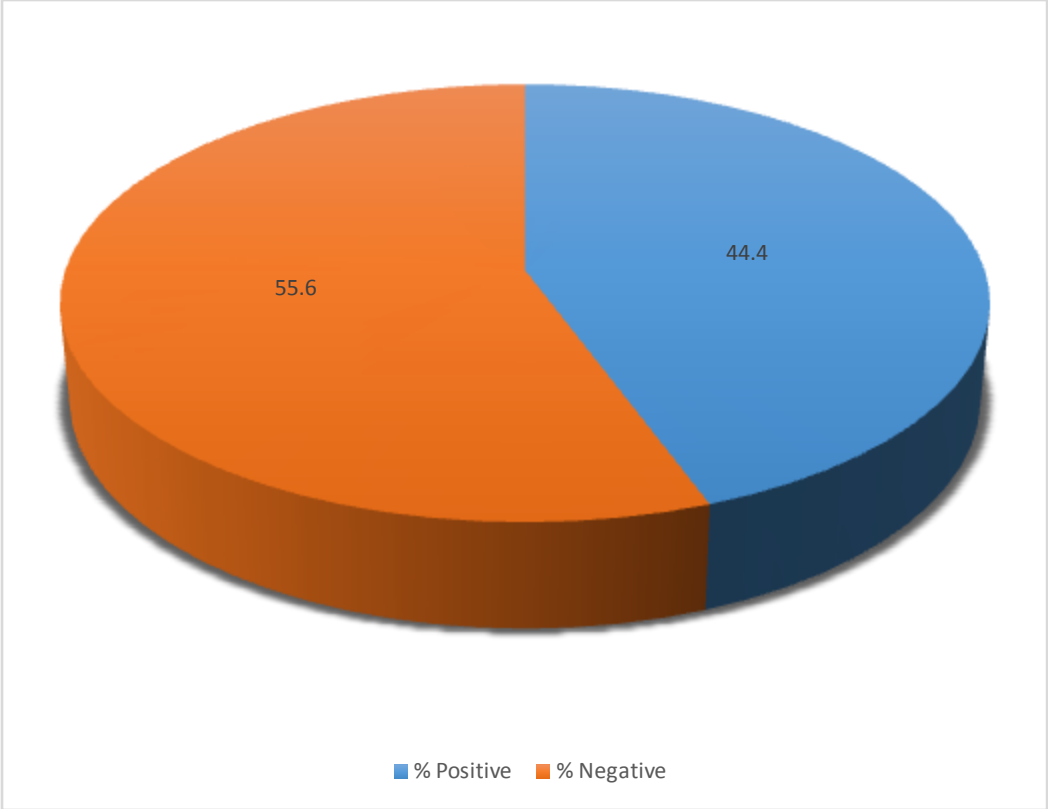


Figure 4.1: Seroprevalence of measles IgM Antibodies among children 0-10 years in some parts of North Western Nigeria.

Table 4.1: Distribution of Measles Virus IgM Antibodiesamong Children 0-10 Years of Age According to States in theNorth Western Nigeria.

States	No tested	No positive (%)	Chi-square value	p-value
Kano	297	142 (47.8)	2.512	0.285
Katsina	231	95 (41.1)		
Zamfara	224	97 (43.3)		
Total	752	334(44.4)		

KEY:

P-value significant at ≤ 0.05.

(%) = Percent

The distribution of measles IgM antibodies with respect to parents employment types (Table 4.2) revealed that children of unemployed parents had the highest seroprevalenceof 57.1% followed by civil servants 41.4% and the least was

among self-employed 37.6%, there was significant association between the measles IgM seroprevalence and employment type of parents (χ^2=22.584, P=0.001).

Meanwhile seroprevalence of measles IgM antibodies was found to be highest50.4% among children of tertiary educated parents followed by non-educated parents 47.4% and the least was observed among children with primary educated parents 34.2%. The result revealed that parents educational status was significantly associated with IgM seroprevalence (χ^2 =15.161, P=0.025),

Table 4.2: Distribution of Measles IgM Antibodies with Respect to Some Demographic Characteristics among Children Aged 0-10 in Partsof North Western Nigeria

Demographic Factors	No tested	No positive (%)	Chi-square value	P-value	OR	95% CL
Age (months)						
1-24	357	186 (53.2)	20.1	0.001		
25-48	252	100 (39.7)				
49-72	94	32 (34.0)				
73-96	35	9 (25.7)				
97-120	14	7 (50)				
Sex						
Male	373	157 (42.1)	1.619	0.203		0.768-1.058
Female	379	177 (46.7)				
Fathers Employment Type						
Civil servant	152	63 (41.4)	23.871	0.001		
Self employed	367	138 (37.6)				
unemployed	233	133 (57.1)				
Education type of father						
Primary	117	40 (34.2)	9.348	0.025		
Secondary	270	114 (42.2)				
Tertiary	230	116 (50.4)				
Informal	135	64 (47.4)				

KEY: P-value significant at ≤0.05

OR= Odd ratio

CI= confidence interval

The seroprevalence of measles IgM antibodies with respect to vaccination status and previous infection with measles is presented in(Table 4.3). The result revealed a higher seroprevalence of 46.3% among unvaccinated children

compared to 41.6% seroprevalence observed among vaccinated individuals. Meanwhile a prevalence of 27.3%, was found among those whose vaccination status was not ascertained. There was no statistical significance difference between the seroprevalence of measles IgM antibodiesand children's vaccination status (χ^2=2.555, P=0.279).

On the relationship between measles IgM seroprevalence and the number of times of vaccination, the lowest seroprevalence was observed in children who received vaccination once 9(41.6%), compared to those that receivedvaccination more than once (60.0%). There was however no statistical difference between the seroprevalence of measles IgMantibodies and the number of times vaccination was given (χ^2=1.950, P=0.160, OR= 0.474, CI= 0.562-0.909).

Children with previous infection history had lower prevalence 281(33.8%), than those with no infection history 51(47.1%), the study established a statistical association between measles IgM antibodies seroprevalence and previous history of measles infection (χ^2 =8.678, P=0.013).

Table 4.3: Seroprevalence of Measles IgM Antibodieswith Respect to History of Vaccination and Measles Infection

Vac/inf history	No tested	No positive (%)	χ^2	P-value	OR
Vaccination					
Yes	294	124 (41.6)	2.555	0.279	
No	447	207 (46.3)			
Unknown	11	3 (27.3)			
Number of vaccination					
Once	279	116 (41.6)	1.950	0.160	0.474
More than once	15	9 (60.0)			
History of Previous exposure					
Yes	151	51 (33.8)	8.678	0.013	
No	597	281 (47.1)			
Unknown	4	2 (50.0)			

KEY:P-value significant at ≤ 0.05,

OR = Odds Ratio

(+)= Positive

CI= Confidence Interval

%= Percent

χ^2= Chi-square

The result of (Table 4.4) showed the distribution of measles IgM antibodies with respect to some clinical features. Children presented with rashes, fever, cough,

runny nose and red itchy eyes had higher measles IgM antibodies seroprevalence of 50.6%, 48.2%, 48.7%, 53.9% and 56.5% respectively compared to children without symptoms with a seroprevalence of 28.6%, 27.2%, and 28.3%, 23.5% and 25.2% respectively. There was statistical association between measles IgM antibodies prevalence and the presence of all presenting symptoms ($P \leq 0.05$).

The result of measles IgMantibody seroprevalence with respect to clinical diagnosis revealed that children who were clinically diagnosed with measles had slightly lower measles IgM antibody seroprevalence (44.0%) compared to those who were not diagnosed (44.7%). There was no significant difference between measles IgM antibody seroprevalence and clinical diagnosis (χ^2=0.035, P=0.851, OR=0.972, CI=0.835-1.161).

Table 4.4:Seroprevalence of Measles IgM Antibodies With Respect To Some Clinical Features among Children 0-10 Yearswith Suspected Measles Cases

Clinical features	No tested	No positive (%)	Chi-square value	P-value	OR	95% CL
Measles Diagnosis						
Yes	291	128 (44.0)	0.035	0.851	0.972	0.835-1.161
No	461	206 (44.7)				
Rash						
Yes	539	273 (50.6)	29.960	0.001	2.557	1.408-2.221
No	213	61 (28.6)				
Fever						
Yes	616	297 (48.2)	19.916	0.001	2.491	1.330-2.361
No	136	37 (27.2)				
Cough						
Yes	593	289 (48.7)	21.204	0.001	2.408	1.327-2.235
No	159	45 (28.3)				
Runny nose						
Yes	518	279 (53.9)	60.166	0.001	3.799	1.794-2.926
No	234	55 (23.5)				
Red itchy eyes						
Yes	462	261 (56.5)	70.796	0.001	3.860	1.812-2.780
No	290	73 (25.2)				

KEY: P-value significant at ≤ 0.05

OR = Odds Ratio

CI= Confidence interval

Possible risk factors associated with measles IgM antibody prevalence were determined as presented in table 4.5. Higher seroprevalence of (44.7%)was

observed in children with contact history compared to those without (44.0%). There was no statistical significant difference between contact history and measles IgM seroprevalence (χ^2=0.030, P=0.862, OR=0.974, CI=0.834-1.164).Similarly, children who lived in crowded environment had the highest measles virus IgM seroprevalence of 46.5% compared to those who live in less crowded environments 43.4%. There was statistical association between the seroprevalence of measles IgM antibodies and exposure to crowd (χ^2=6.298, P=0.012, OR=0.603, CI=0.574-0.953). Children with travel history had lower seroprevalence 31.0% compared to those without 45.2%. There was no statistically significant difference between seroprevalence of measles IgM antibody and travel history(χ^2=3.266, P=0.071, OR=0.543, CI=0.433-1.083).

The distribution of measles virus IgM with respect to vitamin A supplement intake(Table 4.6), reveals that children who received vitamin A supplement had slightly higher seroprevalence of (45.2%), compared to those that did not receive vitamin A (43.8%). There was no statistical association between intake of vitamin A supplement and the presence of measles IgM antibody (χ^2=0.153, P=0.696, OR= 0.944, CI=0.536-0.).

Table 4.5:Seroprevalence of Measles IgM Antibodies with Respect to other Risk Factors among Children 0-10 Year with Suspected Measles

Risk factors	No tested	No positive (%)	Chi-square value	p-value	OR	95% CL
Travel history						
Yes	42	13 (31.0)	3.266	0.071	0.543	0.433-1.083
No	710	321 (45.2)				
Contact history						
Yes	477	213 (44.7)	0.030	0.862	0.974	0.834-1.164
No	275	121 (44.0)				
Crowd						
Yes	624	290 (46.5)	6.298	0.012	0.603	0.574-0.953
No	128	44 (43.4)				

KEY: P-value significant at ≤ 0.05

OR = Odds Ratio

(%) = Percent

CI= Confidence Interval

Table 4.6: Seroprevalence of Measles IgM Antibodieswith Respect toVitamin A Supplement Intake among Children 0-10 Years with Suspected Measles Cases

Vitamin A supplement	No tested	No positive (%)	Chi-square value	p-value	OR	95% CL
Yes	352	159 (45.2)	0.153	0.696	0.944	1.753-2.354
No	400	175(43.8)				

KEY: P-value significant at ≤ 0.05,

OR = Odds Ratio

CI= Confidence Interval

(%)= Percent

The distribution of measles virus IgM in children with respect to hospitals location is shown in (Table 4.7). In Kano State highest seroprevalence was recorded among children who attended General Hospital Gwarzo 58(61.1%)

followed by General Hospital Wudil 48(44.4%) and the children from Hasiya Bayero had the least seroprevalence 36(38.7%). The results indicate a statistical association between measles IgM antibody and hospitals located in Kano State (P=0.006).

Similarly, children from General Hospital Daura recorded the highest measles IgM antibody seroprevalence 39(54.9%), followed by Turai Yar'adua Hospital and General Hospital Funtua, with a seroprevalence of 29(38.2%) and 27(31.8%) respectively. There was statistical association between seroprevalence of measles IgM antibody and Hospital location in Katsina State (P=0.011).

Meanwhile in Zamfara state children attending Kauran Namoda General Hospital recordedhighest measles IgM seroprevalence 40(52.6%), compared to those from Anka General Hospital and King Fahad women and children Hospital which recorded a seroprevalence of 21(39.6%) and 36(36.8%) respectively. There was no statistically significant difference between measles IgM antibody prevalence and location of hospitals in Zamfara state (P=0.124).

Table 4.7: Seroprevalence of MeaslesIgM Antibodies According to Hospital Location in Parts of North Western Nigeria

Location/hospitals	No tested	Number of positive (%)	Chi-square value	p-value
Kano				
Hasiya Bayero	93	36 (38.7)	10.248	0.006
Wudil	108	48 (44.4)		
Gwarzo	95	58 (61.1)		
Zamfara				
Anka	57	21 (36.8)	4.182	0.124
Kaura Namoda	76	40 (52.6)		
King Fahad	91	36 (39.6)		
Katsina				
Daura	71	39 (54.9)	8.949	0.011
Turai	76	29 (38.2)		
Funtua	85	27 (31.8)		

KEY: P-value significant at ≤ 0.05.

4.2 Molecular Identification of Measles IgM from seropositive Samples

Out of forty randomly selected measles IgM ELISA positive samples subjected to nRT-PCR and genotyping, only eight (8) of the samples showed visible bands.

The results of gel electrograph of the first round PCR products amplified with MVF1 and MVR1 primers is presented in (Plate I and II). Lane one is 100 base pair (bp) molecular weight ladder ML, lane M3, 4,5,6,7, and 8, 10 and 12 are samples for measles virus IgM positive, showing distinct bands of 644bp fragment of N gene amplified, while lane M1 and 2 are RT-PCR products generated with the same primers showing no distinct bands, indicating an absence of N-gene fragment from measles IgM positive samples. NC indicate a negative control.

The result of plate III illustrate an agarose gel electrograph of the semi nested PCR products amplified with MVF2 and MVR1 primers using the RT-PCR products obtained with MVR1 and MVF1. Lane one is 100 base pair (bp) molecular weight ladder ML, lanes M3, 4, 5, 6, 7, 8, 10 and 12 are sample for measles virus IgMpositive, showing distinct bands of 528bp fragment of N- gene amplified, while lanes M1, 2, 9, 11, had no bands indicating an absence of measles N-gene from measles IgM positive samples.

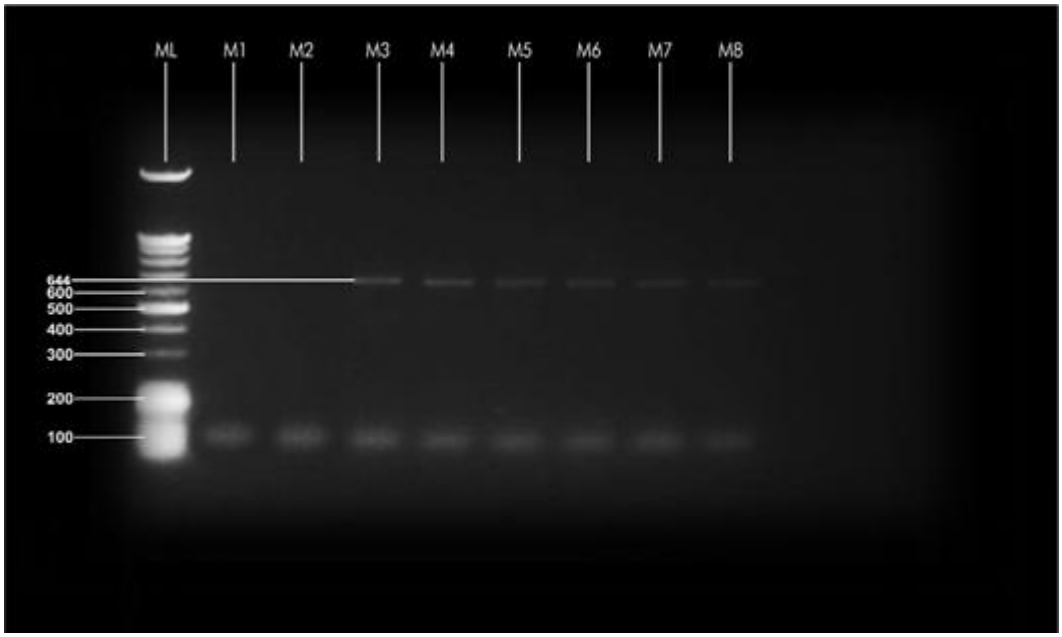


Plate I: Agarose gel electrograph of first round PCR products generated with MVF1 and MVR1 primers. Distinct bands of 644bp fragment of N gene amplified.

Key: Lane **ML** = 100bp DNA ladder, Lane **M3, 4,5,6,7, and 8** = 644bp amplicons of N-gene**NC=** Negative control

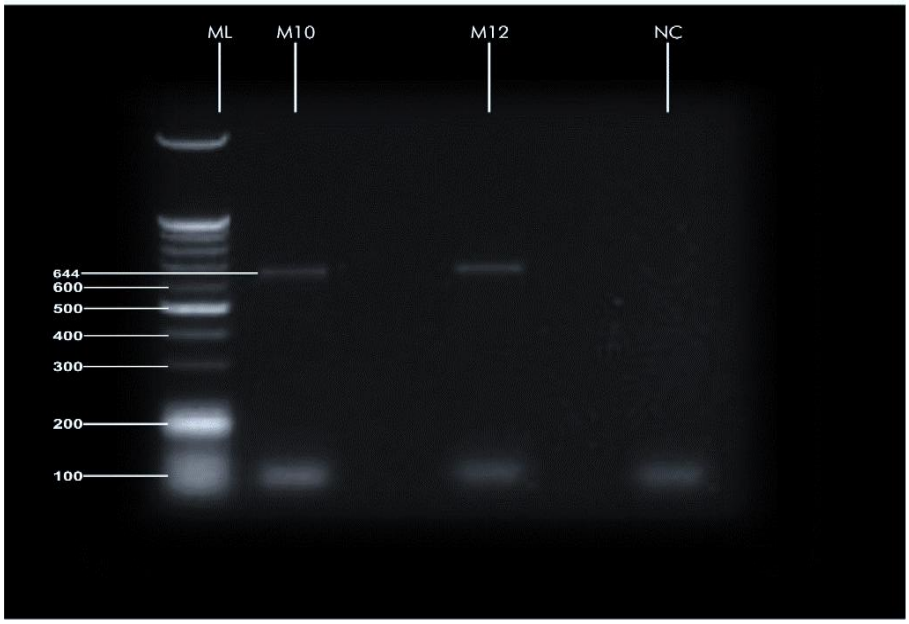


Plate II: Agarose gel electrograph of first round PCR products generated with MVF1 and MVR1 primers. Distinct bands of 644bp fragment of N gene amplified.

Key: ML = 100bp DNA ladder, **M10 and 12 =** 644bp amplicons,**NC=** Negative control

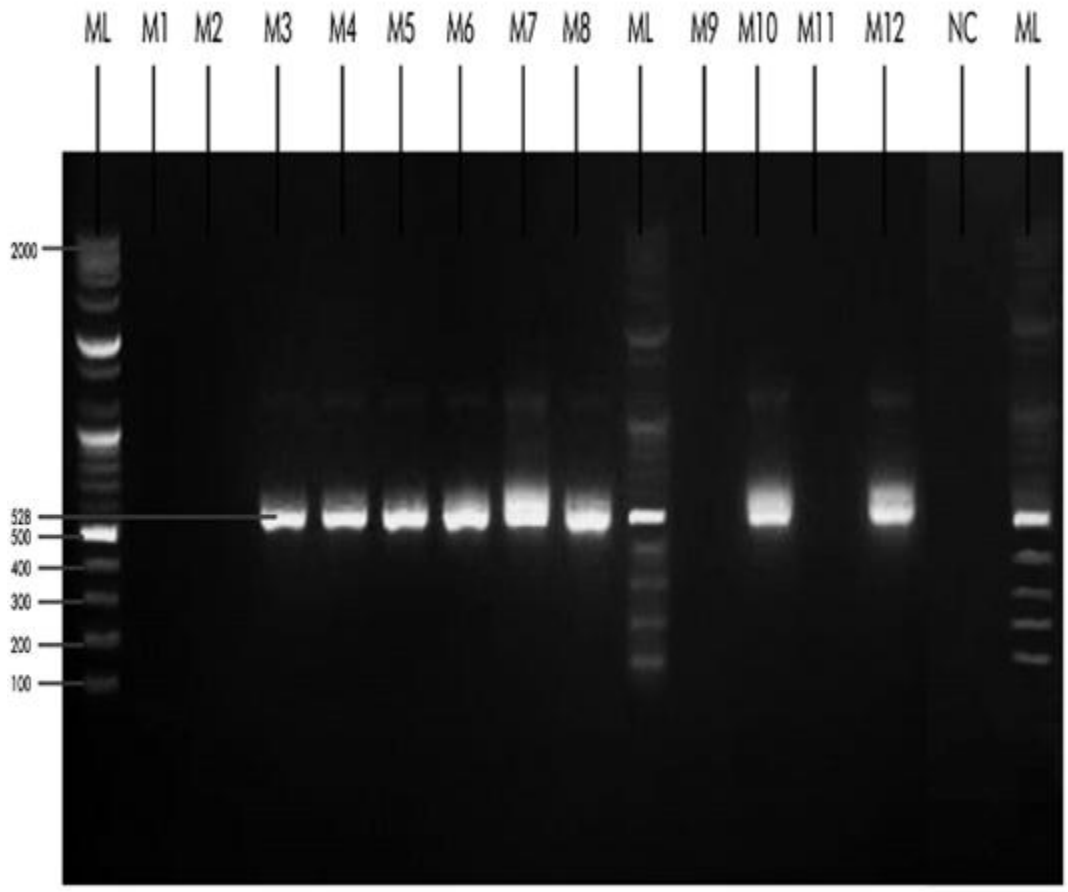


Plate III: Gel electrophoresis of semi-nested PCR products generated with MVF2 and MVR1 primers using the RT-PCR products obtained with MVF1 and MVR1. Distinct bands of 528bp fragment of N gene amplified.

Key: - ML = 100bp DNA ladder, **M3, 4, 5, 6, 7, 8, 10, 12 =** 528bp amplicon, **M1, 2, 9, 11,** = absence of amplifiable genes. **NC=** Negative control

4.3 Nucleic acid sequencing and Phylogenetic analysis

The results of table 4.8 shows the names of all the measles virus genotypes according to WHO nomenclature, this comprises all the isolates from the different hospitals locations within the study area, their names according to WHO nomenclatures and the sample codes.

Table 4.9 revealed the result of measles virus sequences compared to isolates deposited at NCBI GenBank. The BLAST results further shows that all sequences (Appendix VII) belong to genotype B3, with the nucleotide sequence homologyof 99%. The result of multiple alignment of the predicted amino acid sequences of the variable 450bp fragment of carboxyl-terminal region of the nucleoprotein of the B3 viruses is shown in (Appendix VIII), by comparison to reference strain of genotype B3. Multiple alignments of the sequences revealed the number of differences between each unique sequence and an ancestor obtained through the blast analysis.Although the N-gene sequences of all the strains were closely related, multiple sequence alignment revealed nucleotides substitution in the N-gene of some of the specimen positive for N-gene amplification.

Eight MV strains sequenced were assigned to genotype B3 on the basis of their MVN-HVR sequences (Table 4.9). Phylogenetic analysis enables the assignment of all genotypes listed in Table 4.9 into previously identified genotypes (Figure 4.2). The assignment of these eight viruses to an apparently novel genotype within clade B, based on N gene analysis, was confirmed by sequence comparison from Gen bank. Analyses are based on 450 nt sequence encoding the COOH-terminal

150 nt of the N gene. The numbers indicated at the node represent bootstrap values of 1000 replicates in percent.

The comparison strain have its GenBank accession number indicated in parentheses while the strains from present study were italicized. Comparison sequence was from virus isolated in Romania (KX 987226.1). Sequences from Kano were found to be closely related to one another indicating a single transmission chain, while sequences from other states shows divergence indicating independent source of infection. However, all strains are closely related to the B3 viruses.The sequences of the newly detected B3 viruses were very closely related to each other and to the B3 reference strain.

Table4.8: Lists of Measles Virus Sequences According to WHO Nomenclature

Sample code	Hospital location	Sequence name
M3	KFWCH	MVs/Zamfara.NGA/19.15
M4	KGH	MVs/Zamfara.NGA/32.15
M5	HBSH	MVs/Kano.NGA/48.14
M6	GGH	MVs/Kano.NGA/20.15
M7	HBSH	MVs/Kano.NGA/15.15
M8	KFWCH	MVs/Zamfara.NGA/16.15
M10	GHD	MVs/Katsina.NGA/21.15
M12	TYSH	MVs/Katsina.NGA/50.14

KEY-: KFWCH- King Fahad women and children hospital, HBSH – Hasiya Bayero specialist hospital, KGH- Kaura-namoda general hospital, TYSH- Turai Yar'adua specialist hospital, GHD- General Hospital Daura, GGH- Gwarzo general hospital

Table 4.9: Measles virus sequences amplified by PCR that had 99% similarity with measles virus isolates deposited at the NCBI GenBank.

Sequence Code	Description	Max Score	Total Score	Query Cover (%)	E Value	Ident (%)	ACCESSION
M3	Measles virus genotype B3 strain MVs/Dolj.ROU/41.16/[B3] nucleoprotein (N) gene, partial cds	640	640	100	1e-179	99	KX987226.1
M4	Measles virus genotype B3 strain MVs/Dolj.ROU/40.16/[B3] nucleoprotein (N) gene, partial cds	640	640	100	1e-179	99	KX987225.1
M5	Measles virus genotype B3 strain MVi/Calais.FRA/01.16[B3], complete genome	640	640	100	1e-179	99	KX838946.2
M6	Measles virus genotype B3 strain MVs/Niger.NGA/10.13/2[B3] nucleoprotein (N) gene, partial cds	640	640	100	1e-179	99	KX950930.1
M7	Measles virus genotype B3 strain MVs/Niger.NGA/19.13/[B3] nucleoprotein (N) gene, partial cds	640	640	100	1e-179	99	KX949613.1
M8	Measles virus genotype B3 strain MVs/Niger.NGA/21.13/[B3] nucleoprotein (N) gene, partial cds	640	640	100	1e-179	99	KX949612.1

M10	Measles virus genotype B3 strain MVs/Sokoto.NGA/20.13/[B3] nucleoprotein (N) gene, partial cds	640	640	100	1e-179	99	KX949610.1

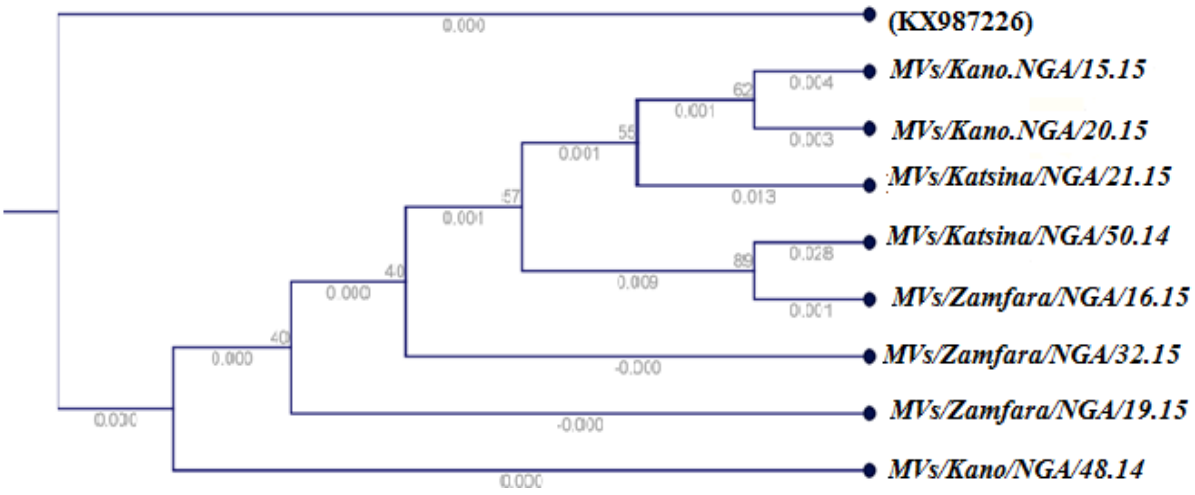


Figure 4.2: Neighbor joining tree showing evolutionary relationship between genotypes of Measles virus (nucleoprotein gene) in parts of Northwest Nigeria.

NB: Accession number of sequence of reference strains of measles virus from the Genbank database is indicated in parentheses. The numbers indicated at the node represent bootstrap values of 1000 replicates in percent.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

In this study, measles virus specific IgM antibody was evaluated among suspected cases. Consequently 332 (44.4%) of the 752 study subjects had detectable levels of measles which is an indication of recent measles infection. This result indicate that measles remains endemic in this part of the globe.

The result of the present study is higher than 32.2% and 21.2% reported in Kaduna (Chechet *et al.*, 2014; Olaitan *et al.*, 2015) respectively. It also contradicts findings from other parts of the country with much lower prevalence of 8.0% in Niger state, North Central Nigeria, (Adeboye *et al.*, 2011). While Bassey *et al.* (2010); Chukwuemeka and Hycient(2013) reported 30.2% in Akwa Ibom, South-South and 16.5% in Abia Southeastern Nigeria respectively. Variation in vaccination coverage was observed to be the major reason for the differences in the prevalence. Earlier study also reveals the prevalence of 32% and 15.6% in Lagos and Ilorin (Babaniyi *et al.*, 1995; Omilabu *et al.*, 1999) respectively.

Our result however agrees with a prevalence of 41% reported by WHO, (2010) in some African countries. Higher prevalence recorded in this study might be as a result of our sampling criteria which include only suspected measles cases that presented with febrile rashes. However, higher prevalence was reported by

Adetunji *et al.* (2007), Chukwu *et al.* (2009); Sheyin *et al.* (2016) with a prevalence of 55% in Lagos 71.1% and 54.1% respectively in studies conducted within Kaduna state. Similarly, our findings contrast reports from other African countries such as Ghana and Egypt by Tayil *et al.* (1998) and Ishiwada *et al.* (2001), with a prevalence of 86.1%, 73.7%, respectively, lower values of 15% was also recorded in Uganda (Frederick *et al.*, 2011).

Measles infection is widespread throughout the three states showing varying prevalence with Kano recording the highest prevalence compared toKatsina and Zamfara States respectively. The differences in measles IgM antibody prevalence observed might be as a result of the differences in population density which is higher in Kano compared to Katsina and Zamfara States. Our result also observed same pattern of measles distribution across the States this might be based on the fact that the three states shares boundary with each other and have similar features and cultural practices.

The distribution of measles infection varies slightly with the age of the children, with the number of seropositive individuals observed to decrease with age. This finding agrees with those of similar studies conducted in Calabar, Lagos, Ibadan , Akwa Ibom and Abuja (Etuk *et al.,* 2003; Adetunji *et al.,* 2007; Fetuga *et al.*, 2007; Bassey *et al.,* 2010; Ahmed *et al.*, 2010).These early presentations of measles could be due to depletion of maternal antibody in the lower age group as well as development of acquired immunity by the children of higher age group due to exposure to the virus in the environment, it could also be attributed to low vaccine coverage during childhood immunization. Measles was seen mostly in

children below the age of 2 years, with previous report showing that 80% of the affected children were below this age as reported in other studies (Bassey *et al.*, 2010; Ahmed *et al.*, 2010).

The age specific prevalence is inconsistent with the report from other parts of the world such as Saudi Arabia in which older children seem to be at risk of contacting the diseases with a peak age of 5-15 years as reported by Kamel, (2002). Fikre *et al.*, (2003) reported largest population of susceptible children to be in the age group of 15-19 years. While Sharma, (1998) reported 42.2% of measles among older children 10-14 years, Salpathy *et al.*, (1990), recorded 10.4% among 15 years and above in Bengal. However, the fact that measles was found in older age group in the present study might be an indication that routine immunization of single vaccine might be insufficient to interrupt measles transmission and a booster dose may be required at a later age.

The prevalence of measles IgM in relation to gender in this study showed a higher prevalence in female population (46.1%) as compared to the male counterpart (42.1%), though the difference was no statistically significant. This finding agrees with previous studies which documented measles infection being higher in females than males (Gdalevich, 2002; Bassey *et al.*, 2010; Olaitan *et al.*, 2015).Other studies in Kaduna Nigeria however reported higher prevalence in males than females (Chukwu *et al.*, 2009; Sheyin *et al.*, 2016). Altynkaynak *et al.* (2005) and Shakrokh*et al.*(2012) reported higher prevalence in males than females in Turkey and Iran respectively.

Higher prevalence in females observed in the present study might be possibly due to chance as the difference was not statistically significant. However, Aaby *et al.* (1995), have documented lower rates of immunization of females than males with evidence suggesting that measles mortality might be higher in girls than boys. Among people of different ages and across different regions (primarily in the Americas and Europe), measles mortality in girls was estimated to be 5% higher than in boys (Garenne, 1994).

Seroprevalence of measles infection was found to be highest among children whose parents have tertiary education (50.4%) and lowest among those with primary school education (34.2%). The trend in measles prevalence with paternal level of education observed in this study contradicts a study in Iran (Shakrokh *et al.,* 2012) which reported low education level as a factor for measles infection with a prevalence of 68.8% in parent with no literacy. Contrary to the result of our study is also a report by Fowlkes *et al.,* (2011) and Masuet-Aumatell*et al.* (2015) who documented no association between measles prevalence and educational level of parents. This might be due to the endemic nature of measles virus infection in the study location as such individuals have equal chances of acquiring measles infection.

Paternal employment type and measles virus IgM seropositivity were found to be statistically associated, with the children from unemployed parents having the highest seroprevalence of (57.1%) and the least (37.6%) was recorded among children of civil servants. This finding contradicts that of Holmes *et al.* (1996),

who reported that children of working class parents were more susceptible to infection.

In this study, measles infection as confirmed by IgM sero-positivity was observed to be 41.6% in children who had been previously vaccinated against measles. This contradicts 17.4%, 14.8%, 22.1% and 18.8% reported by Adeboye *et al.* (2010), Bassey *et al.*(2010), Onyiruika, 2011; Duru *et al.*(2014) respectively, though higher rates were reported from other Nigerian studies (Fetuga *et al.*,2007; Adetunji *et al.,* 2007;Ahmed *et al.,* 2010). Earlier studies also reported much higher prevalence of 80% (Ojuawo and Bello, 2000). Similar findings from studies conducted in Kaduna reported lower rates among vaccinated children 7.6% and 10.0% (Chechet *et al.,* 2014;Olaitan *et al.,* 2015). This study also contradictsMalawian measles seroprevalence study which reported lower prevalence of 23% among vaccinated children (Yamaguchi *et al.,* 2002).

The high prevalence reported among children who contracted measles despite measles vaccination may be due to primary vaccine failure which may result from break in the cold chain, poor host immune response or wrong techniques of vaccination. Another plausible explanation could have been inaccuracy of the history of vaccination. It is however; difficult to identify which of these factors either individually or in combination would have been implicated as the cause of the observed prevalence in the vaccinated subjects.

In other studies, Adu *et al.* (1992), Bautesta *et al.* (2001) and Nnebe-Agumadu, (2005) documented persistence of maternal measles antibody interfering with

seroconversion especially in children less than one year. Saffar *et al.*(2006) documented secondary vaccine failure due to waning immunity after the passage of years.Although there was no verification by inspection of vaccination cards in this study nevertheless measles infection after vaccination is well documented (Gans *et al.,* 2001).

Many epidemiological studies have shown that measles disease in previously vaccinated children runs a milder course. Aaby *et al.* (1986); Van den Ent *et al.*(2009) and Aaby *et al.* (2012) suggested that previously vaccinated children had partial immunity which though not enough to prevent the disease, was enough to modify its severity.

In the present study 46.1% of measles IgM seropositive children did not receive vaccination. Higher seroprevalence was also documented in previous Nigerian studies among unvaccinated children (Fetuga *et al.,* 2007; Bassey *et al.,* 2010; Onyiruika, 2011; Duru*et al.,* 2014) with (70.5%, 47.4%, 77.9% and 81.2%) respectively. Vaccination coverage of measles vaccine is currently put at 62% according to WHO/UNICEF, (2009). This study revealed coverage rate of less than 50% which is probably due to the fact that children included in the study were those with suspected measles cases and not the generality of the population. Lack of vaccine at the health centers being a major reason for failure to vaccinate children. A similar reason was given by Etuk *et al.,* (2003) in Calabar, Adetunji *et al., (*2007) in Oshogbo, and Onyiriuka, (2011) in Benin. Also some mothers gave their reasons as the child being ill during vaccination.

The highest prevalence was observed among thosethat received vaccine once (33.8%), compared with those that received more than one dose (%). This finding agrees with the report of Linnemann *et al.* (1982), who found out that 40% of children who had received two doses of measles vaccine are less likely to have measles. This study pointed out that only subjects who had been vaccinated more than once had significantly lower chance of acquiring measles when compared to those vaccinated once as reported by (Shakrokh *et al.,* 2012). Various studies on the advantages of an early 2 dose measles vaccine administration in African countries with high measles burden have been conducted (Carly *et al.,* 1999; Aaby *et al.,* 2010; Nije-Jobe *et al.,* 2012). Carly and his coworkers in Guinea-Bissau reported that the administration of the measles vaccine to children twice in infancy; at 6 and 9 months resulted in high vaccine coverage, high antibody levels and a better outcome compared to their cohorts on single dose of vaccine administration at 9 months. Other authors have also reported that the administration of 2 doses of the measles containing vaccine (MCV) in early infancy was beneficial to overall child survival (Aaby *et al.,* 2012).

Measles IgM seroprevalence was found to be higher in children without previous history of measles infection (47.1%) compared to those with previous infection history (33.8%).These findings are comparable with those of Kandpal *et al.* (2010), which documented 5% seropositivity after measles infection due to the false history of measles and the possibility parent mistaken any other eruptive fever to be measles. An association between history of measles and presence of IgM was previously reported in Bolivian study (Cutts *et al.,* 1995) with

prevalence of 12.3% and 2.56% respectively for those with no previous measles history and those with history respectively this is also in agreement with the present research.

The statistical association between the prevalence of measles IgM and contact with infected person was not significant, even though our study reported a considerable population of seropositive children with contact history (44.7%) as compared to (44.0%) without contact. This contradicts the findings of Shakrokh *et al.,* (2012) who reported a strong association between measles prevalence and contact history (77.6%). This could be due to the nature of viral transmission which apart from direct contact with infected person could be transmitted through droplet emission.

Travel history as a risk factor was also found not to be statistically associated with measles IgM seroprevalence. Lower seroprevalence of (31.0%) was recorded among children with travel history while the value was higher (45.2%) amongst those without the history, this agrees with a published work of Shakrokh *et al.* (2012). This could be attributed to the endemic nature of the virus in our community and as such importation of measles cases are unlikely to occur.

Measles virus IgM seroprevalence was statistically associated with exposure to crowd, as the high prevalence of (46.5%) was recorded among those children found to be exposed to crowded environments. Overcrowding has been documented by many researchers as a predisposing factor in the acquisition of the measles infection considering the nature of transmission of the virus.

There was no statistically significant association between measles IgM antibodyseroprevalence and administration of vitamin A supplement. As this study revealed 45.2% measles IgM seropositivity among those who received at least one dose of vitamin A routinely as compared to 43.8% seropositive among those who were not given. This is contradict a report in South Africa by Mcmorrow *et al.,* (2009). Vitamin A deficiency is a recognized risk factor for severe measles and recommended for children with measles in areas with vitamin A deficiencyand this was reported to reduce the number of death from measles by 50% (WHO, 2016).

In assessing clinical symptoms of measles, statistically significant relationship was established between presence of measles virus IgM and all the presenting measles symptoms, with the prevalence higher than 40% in all (fever, cough, coryza, rash and conjunctivitis). This agrees with the work of Chechet *et al.*(2014) and Olaitan *et al.* (2015). It also agrees with the WHO clinical case definition that is any person presenting with a history of fever (39-41^{o}C) lasting 3 days or more and generalized maculopapular rash with one of the above symptoms.Ojuawo and Bello, (2000), also reported that fever, cough and conjuctival infection were observed in 100% and 96.2% patients with suspected measles respectively.

The study however presents an extensive genetic characteristics and Phylogenetic analysis of measles virus in some parts of Northwest Nigeria. To our knowledge this is the first study that genetically characterized measles virus in the context of regional measles epidemics in the study area, detecting the circulating genotype in the area from November 2014 to December 2015. The nucleotide sequence

analysis revealed a complete nucleotide identity among strains in the N gene, indicating that all the viruses belong to the same genotype B3, no other genotype was detected and all were closely related to viruses found in the country between 1997- 2005 (Hanses*et al.*,1999; Kremer*et al.*, 2010).

This suggest uninterrupted transmission of endemic MV in the country, since population immunity in Nigeria is generally not sufficient enough to prevent transmission of measles virus, and was probably the reason why further transmission of same strains continue. In agreement with our finding was the reports of Xu *etal.* (1998); Hanses *et al.* (1999); Liffick *et al.* (2001) and El-Mubarak*et al.* (2002), who reported that in countries that have endemic transmission of measles, a limited number of viral genotype are usually detected whereas in countries where endemic transmission of measles has been interrupted, variety of genotypes are detected which reflects the multiple sources of importation.

All the eight sequences were found to be closely related withNiger strain with Accession number KX950930.1, the reference sequence for genotype B3, which is clearly the endemic genotype in most of the African countries with the exception of the North African countries, but other countries on these continents also have other genotypes in circulation. B3 was the most frequently reported genotype from Africa to the WHO Global Sequence database, and was also found circulating in Malawi, Liberia and Mauritania in 2010 (Rota *etal.*, 2011). The detection of single genotype in this study is not surprising since 100% of the studied populations are of Nigerian origin and travelling within Nigeria is more

frequent than to the other part of the world. This also agrees with the finding of Zhang *et al.,* (2007) who revealed a similar uniformity for single genotype H1 circulating in China without obvious geographic restriction.

Genotype B3 has been divided into 3 clusters as reported in most studies (Haddad- Boubaker *et al.,* 2010). Therefore, the sequence from this study were compared to the sequences of other viruses that were available in the Genbank Data Base (Table 4.9). The result show that all the viruses in this study belongs to B3 cluster 1. This results agrees with those of Hanses *et al.* (1999); Kouomou *et al.* (2002)and Govandji,-Vasilache *et al.* (2006) who discovered B3.1 as predominant circulating genotype in someWest African countries (Ghana and Nigeria), Central African Republic (Cameroon) and East African (Kenya and Tanzania). Even though another B3 variant B3.2 was earlier reported to be circulating in Nigeria (Hanses *et al.,* 1999), it was not discovered in the present study. Similarly cluster B3.3 which was also described to have circulated in Northern Africa Tunisia and Libya (Haddad- Boubaker *et al.,* 2000) was also not detected in this study.

The strains characterized showed the highest degree of Identity 98-99% with most strain previously circulating in some parts of the country with the following accession numbers KX949610.1, KX950930.1 from Sokoto, and Niger state respectively. All sequences clustered with measles virus genotype B3 demonstrate genetic homogeneity among the B3 viruses detected in the present study, it however indicates close relationship between recent and the previously circulating B3 viruses in the country (Hanses *et al.,* 1999 and Kremer *et al.,* 2010).

Phylogenetic analysis revealed that two of the Kano genotypes MVs/ Kano.NGA/15.15 and MVs/ Kano.NGA/20.15 are closely related, indicating the same transmission chain, and they are the most closely related virus with the reference strainshowing a very high bootstrap value of 67%, indicating a very close relationship with the reference strain. However, the two Kano strains were distantly related to another genotype from same state, MVs/Kano.NGA/48.14.MV, this indicate different transmission chain, even though they are form the same state. Strains Katsina, MVs/Katsina/NGA/50.14 and Zamfara, MVs/Zamfara/NGA states however are closely related to the reference strain with a high bootstrap value of 89% indicating an evidence of genotype exportation from African countries to other parts of the world and vice versa (Chibo *et al.,* 2000; Santinabez *et al.,* 2002; Chironna *et al.,* 2007; Rota *et al.,* 2011).

This study has insufficient epidemiological data to identify a link between all cases infected with the slightly different B3 variants, indicating that the strains were independently transmitted and showing how easily measles virus spreads among susceptible individuals. However, only minor sequence differences were obtained. The fact that most isolates from this study were identical to those previously identified in Niger, and Sokoto suggests continuous circulation of the same variant all over the country. However, the contribution of other African countries cannot be excluded, because molecular surveillance is still largely lacking in Africa.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. The study reaffirm that measles remain one of the major cause of childhood illnesses with an overall IgM seroprevalence of 44.4%.

2. Measles IgM antibody seroprevalence was found to be associated with age (P=0.001)parent'seducational level (P=0.004) and employment type (0.001) as socio-demographic factors.

3. Overcrowding remains a risk factor in the spread of measles with a prevalence of (46.5%) and history of previous measles exposure as risk factor with a prevalence of (47.1%) was also significantly associated with measles IgM antibody prevalence.

4. The persistence of measles and its burden in our communities are worrisome as high prevalence was observed in both vaccinated (41.6%) and unvaccinated (46.3%)children.

5. The study also describes molecular characteristics of clade B measles virus circulating in parts of the North Western Nigeria. It also confirmed the endemic circulation of measles virus B3 subtype 1, which is a known circulating genotype indicating sustained pattern of transmission.

6. Phylogenetic analysis revealed a close relationship with those genotypes circulating in other parts of the country, some African countries and other parts of the world.

6.2 Recommendations

Based on the results of this study on the seroprevalence and genetic variability of measles virus among children 0-10 years in some parts of the North West, we recommend thus:

1. Community and population bases prevalence should be carried out in order to shed more light on other infection dynamics.

2. It is important to embark on molecular epidemiological study of measles virus in other parts of the country in order to monitor the transmission pattern.

3. Public enlightenment and awareness programs for parents emphasizing the importance of vaccination to their children

4. Molecular epidemiological surveillance should also be intensified to monitor the circulating strain and possible changes in genotype that might occur over time.

5. There is need to expand the length of the sequencing window to include other regions of the MV genome, to generate more phylogenetically meaningful sites.
6. Further studies with samples from a wider area are required to determine the spread and genetic diversity of these strains and ascertain their relationship to the global B3 strains.

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APPENDICES

Appendix I: Ethical Approval, Kano State

KANO STATE

HOSPITALS MANAGEMENT BOARD

BOARD HEADQUARTERS
P.M.B 3540, Post Office Road, Kano

HMB/GEN/488/VOL. I

2/2/1436AH = 24/11/2014

Maryam Aliyu Yakasai
Department of Microbiology,
Ahmadu Bello University,
Zaria.

PROVISIONAL ETHICAL CLEARANCE

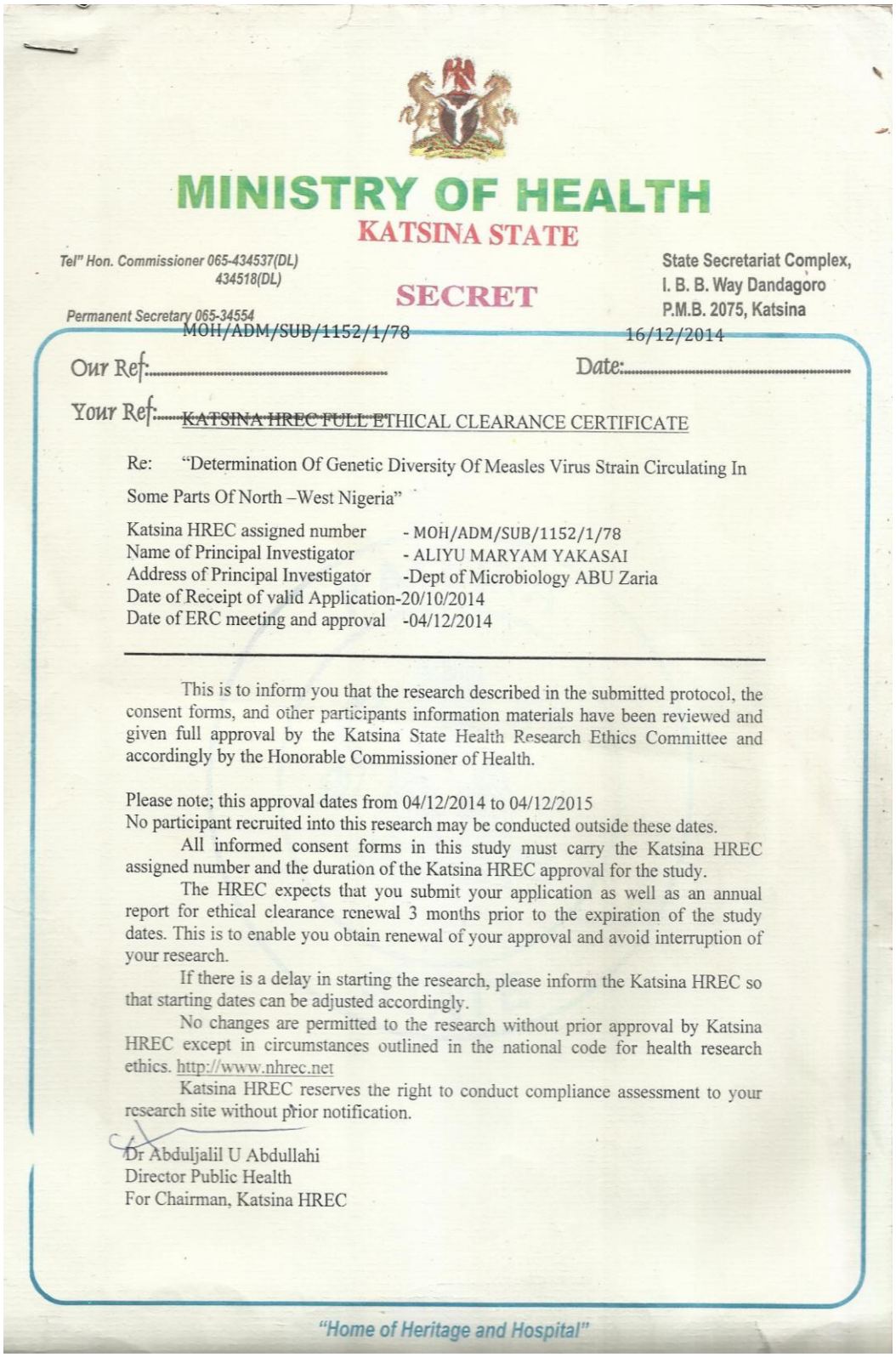
Sequel to conduct a research title "DETERMINATION OF GENETIC DIVERSITY OF MEASLES VIRUS STRAINS CIRCULATING IN SOME PART OF NORTH WEST". In the light of the above, I am mandated to convey provisional clearance to proceed on your study based on the following conditions.

i. That the consent of all participants must be obtained by filling in consent form.
ii. That you should liase with the Management of the facility for appropriate guidance.
iii. That any publication related to the study should be brought to the knowledge of the Ethical Committee for approval.
iv. That a copy of your finding should be submitted for documentation, record and final approval, please.

Best regards.

BASHIR G. IBRAHIM
Senior Assistant Secretary
FOR: EXECUTIVE SECRETARY

Appendix II: Ethical Approval, Katsina State



MINISTRY OF HEALTH

KATSINA STATE

Tel" Hon. Commissioner 065-434537(DL)
434518(DL)

Permanent Secretary 065-34554

State Secretariat Complex,
I. B. B. Way Dandagoro
P.M.B. 2075, Katsina

SECRET

Our Ref: MOH/ADM/SUB/1152/1/78 Date: 16/12/2014

Your Ref: ~~KATSINA HREC FULL ETHICAL~~ CLEARANCE CERTIFICATE

Re: "Determination Of Genetic Diversity Of Measles Virus Strain Circulating In Some Parts Of North –West Nigeria"

Katsina HREC assigned number - MOH/ADM/SUB/1152/1/78
Name of Principal Investigator - ALIYU MARYAM YAKASAI
Address of Principal Investigator -Dept of Microbiology ABU Zaria
Date of Receipt of valid Application-20/10/2014
Date of ERC meeting and approval -04/12/2014

This is to inform you that the research described in the submitted protocol, the consent forms, and other participants information materials have been reviewed and given full approval by the Katsina State Health Research Ethics Committee and accordingly by the Honorable Commissioner of Health.

Please note; this approval dates from 04/12/2014 to 04/12/2015
No participant recruited into this research may be conducted outside these dates.

All informed consent forms in this study must carry the Katsina HREC assigned number and the duration of the Katsina HREC approval for the study.

The HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to the expiration of the study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is a delay in starting the research, please inform the Katsina HREC so that starting dates can be adjusted accordingly.

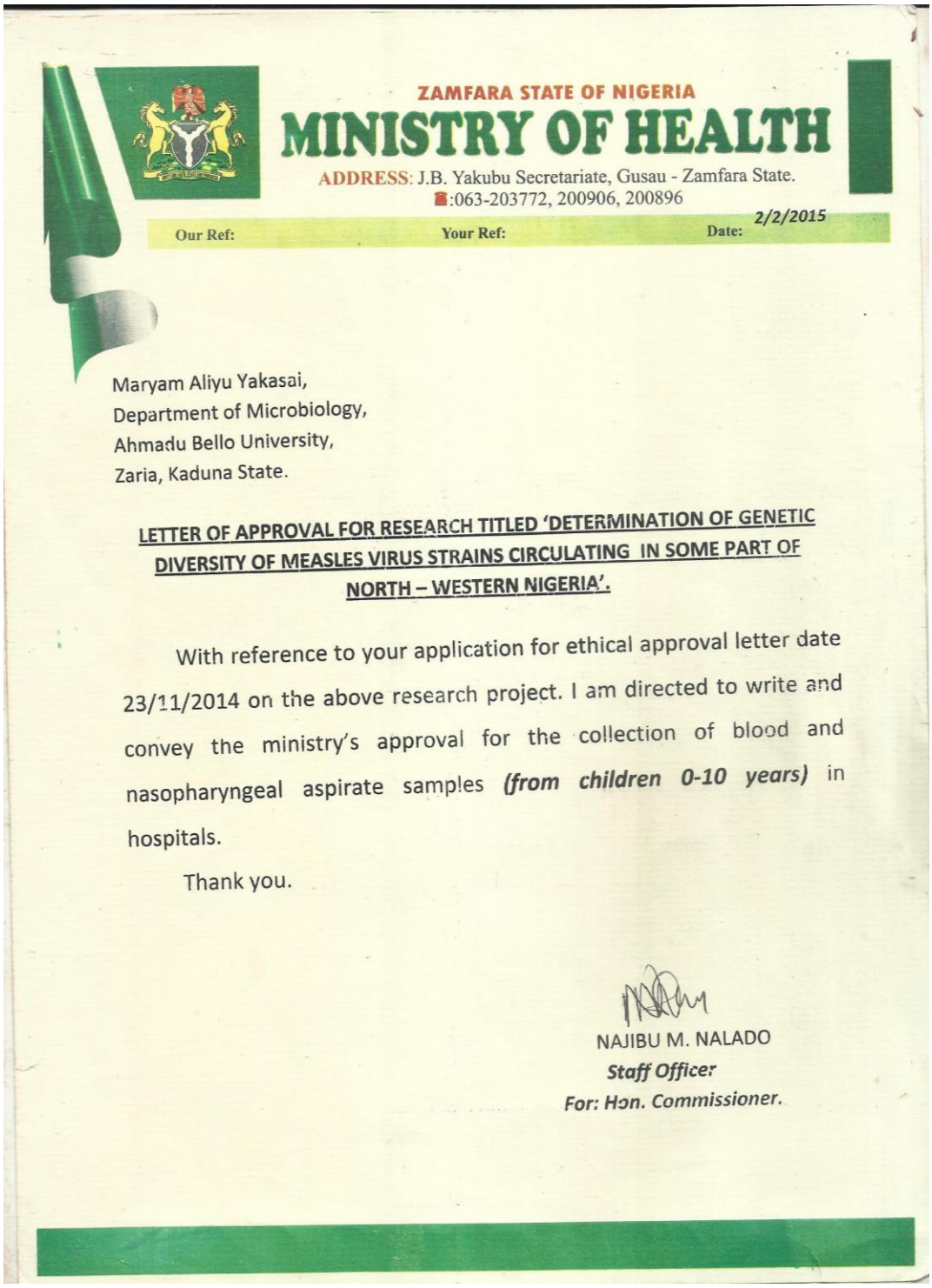
No changes are permitted to the research without prior approval by Katsina HREC except in circumstances outlined in the national code for health research ethics. http://www.nhrec.net

Katsina HREC reserves the right to conduct compliance assessment to your research site without prior notification.

Dr Abduljalil U Abdullahi
Director Public Health
For Chairman, Katsina HREC

"Home of Heritage and Hospital"

Appendix III: Ethical Approval, Zamfara State



ZAMFARA STATE OF NIGERIA

MINISTRY OF HEALTH

ADDRESS: J.B. Yakubu Secretariate, Gusau - Zamfara State.
☎:063-203772, 200906, 200896

Our Ref: Your Ref: Date: 2/2/2015

Maryam Aliyu Yakasai,
Department of Microbiology,
Ahmadu Bello University,
Zaria, Kaduna State.

LETTER OF APPROVAL FOR RESEARCH TITLED 'DETERMINATION OF GENETIC DIVERSITY OF MEASLES VIRUS STRAINS CIRCULATING IN SOME PART OF NORTH – WESTERN NIGERIA'.

With reference to your application for ethical approval letter date 23/11/2014 on the above research project. I am directed to write and convey the ministry's approval for the collection of blood and nasopharyngeal aspirate samples ***(from children 0-10 years)*** in hospitals.

Thank you.

NAJIBU M. NALADO
Staff Officer
For: Hon. Commissioner.

Appendix IV: Informed Consent Form

Serial No…….. *Hospital No……..*

This informed consent form is basically for a Ph.D research entitle '**Seroprevalence and genetic variability of circulating strains of measles virus among children 0-10 years in parts of North-Western Nigeria'**. You are being requested to allow your child to participate in the study, because we believe you may be able to contribute to our understanding of the disease. Besides reviewing biodata and medical information of the child, blood and nasopharyngeal/nasal swab samples will be collected for laboratory test. The result obtained thereby may be used in any way to improve the understanding and management of the disease in our community. You are assured that all data concerning your child 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.

I……do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the researcher. I understand that blood and nasopharyngeal aspirate/swab of my child will be taken for laboratory test. I therefore give this consent voluntarily without being subjected to any pressure.

Sign/right thumb print

…….. ……
Parent/guardian Date

Appendix V: Questionnaire

Section A: BIODATA Serial No._____

1. Reference no_____
2. Age of the child (Years): _____
3. Sex Male [ ] Female [ ]
4. State of residence _____
5. Occupation of parents

   [ ] Civil Servant [ ] Self-employed [ ] Unemployed [ ] others
6. Educational Status of parents

   [ ] Primary [ ] Secondary [ ] Tertiary [ ] Informal [ ] None

Section B: IMMUNITY STATUS

7. Any history of measles vaccination? Yes [ ] No [ ] Not sure

   If no state, the reasons_____

   If yes, how many times? _____
8. Any previous history of measles infection? Yes [ ] No [ ] Don't know
9. Any complication? Yes [ ] No [ ]
10. Has the child been given Vitamin A? Yes [ ] No[ ]

Section C: RISK FACTORS

11. Has this person traveled outside Nigeria (within the last 21 days)?

    Yes [ ] No [ ]
12. Has this person come in contact with an infected person?

    Yes [ ] No [ ]
13. Has this person traveled through an airport or any place where large groups of people from various areas may be within the last 21 days? Yes [ ] No[ ]

Section D: CLINICAL INFORMATION

14. Did physician diagnose this person with measles? Yes [ ] No [ ]

15. What are the case symptoms?

(a) Rash: Yes [ ] No [ ]…… if yes date of onset_____

(b) Fever: Yes [ ] No [ ]……..if yes date of onset_____

(c) Cough: Yes [ ] No [ ]

(d) Runny nose: Yes [ ] No [ ]

(e) Red itchy eyes or drainage from the eyes: Yes [ ] No [ ]

Section E: LABORATORY RESULTS

16. Measles IgM level _____

17. Genotype of Measles _____

Appendix VI:Measles IgM ELISA Datasheet

Analysis Report

Plate No.:1
1st Item:MEASLES IGM
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.100

	1	2	3	4	5	6
Hole	S	S1	S2	S3	S4	S5
OD	0.212	3.405	0.168	0.340	0.718	0.085
Quant	3.405	0.168	0.340	0.718	0.085	
Quali						

Hole	S12	S13	S14	S15	S16	S17
OD	0.000	0.000	0.000	0.000	0.000	0.000
Quant	0.000	0.000	0.000	0.000	0.000	0.000
Quali						

Hole	S24	S25	S26	S27	S28	S29
OD	0.038	0.085	0.093	0.010	0.076	0.016
Quant	0.038	0.085	0.093	0.010	0.076	0.016
Quali						

Hole	S36	S37	S38	S39	S40	S41
OD	0.419	0.053	0.552	0.091	0.080	0.674
Quant	0.419	0.053	0.552	0.091	0.080	0.674
Quali						

Hole	S48	S49	S50	S51	S52	S53
OD	0.000	0.039	0.041	0.052	0.058	2.945
Quant	0.000	0.039	0.041	0.052	0.058	2.945
Quali						

Hole	S60	S61	S62	S63	S64	S65
OD	0.017	0.008	0.000	0.002	0.022	3.131
Quant	0.017	0.008	0.000	0.002	0.022	3.131
Quali						

Hole	S72	S73	S74	S75	S76	S77
OD	0.092	0.168	2.707	0.048	0.066	0.061
Quant	0.092	0.168	2.707	0.048	0.066	0.061
Quali						

Hole	S84	S85	S86	S87	S88	S89
OD	0.027	0.012	0.010	0.010	0.027	0.029
Quant	0.027	0.012	0.010	0.010	0.027	0.029
Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	0.156	0.115	0.131	0.225	0.137	0.153
Quant	0.156	0.115	0.131	0.225	0.137	0.153
Quali						

Hole	S18	S19	S20	S21	S22	S23
OD	0.000	0.000	0.000	0.000	0.000	0.000
Quant	0.000	0.000	0.000	0.000	0.000	0.000
Quali						

Hole	S30	S31	S32	S33	S34	S35
OD	0.049	0.476	0.062	0.141	1.750	0.411
Quant	0.049	0.476	0.062	0.141	1.750	0.411
Quali						

Hole	S42	S43	S44	S45	S46	S47
OD	1.568	0.059	0.862	0.092	0.039	0.088
Quant	1.568	0.059	0.862	0.092	0.039	0.088
Quali						

Hole	S54	S55	S56	S57	S58	S59
OD	0.169	0.086	0.088	0.077	0.084	0.107
Quant	0.169	0.086	0.088	0.077	0.084	0.107
Quali						

Hole	S66	S67	S68	S69	S70	S71
OD	0.391	0.119	0.017	0.256	0.082	0.096
Quant	0.391	0.119	0.017	0.256	0.082	0.096
Quali						

Hole	S78	S79	S80	S81	S82	S83
OD	0.058	0.025	0.058	0.051	0.052	0.119
Quant	0.058	0.025	0.058	0.051	0.052	0.119
Quali						

Hole	S90	S91	S92	S93	S94	S95
OD	0.060	0.028	0.017	0.017	0.007	0.038
Quant	0.060	0.028	0.017	0.017	0.007	0.038
Quali						

Test date:2015-08-17 Test time:22:19:21
Print date:2015-08-17 Print time:22:20:03
User: Verifier:

Analysis Report

Plate No.:2
1st Item:MEASLES IGM
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.100

	1	2	3	4	5	6
Hole	S	S1	S2	S3	S4	S5
OD	0.243	0.185	0.287	0.177	0.194	0.182
Quant	0.185	0.287	0.177	0.194	0.182	
Quali						

Hole	S12	S13	S14	S15	S16	S17
OD	0.027	0.036	0.010	0.007	0.018	0.023
Quant	0.027	0.036	0.010	0.007	0.018	0.023
Quali						

Hole	S24	S25	S26	S27	S28	S29
OD	1.097	0.827	0.311	0.402	0.188	0.256
Quant	1.097	0.827	0.311	0.402	0.188	0.256
Quali						

Hole	S36	S37	S38	S39	S40	S41
OD	0.252	0.522	0.277	0.216	0.228	0.231
Quant	0.252	0.522	0.277	0.216	0.228	0.231
Quali						

Hole	S48	S49	S50	S51	S52	S53
OD	0.238	0.234	0.245	0.251	0.380	0.278
Quant	0.238	0.234	0.245	0.251	0.380	0.278
Quali						

Hole	S60	S61	S62	S63	S64	S65
OD	0.672	2.995	0.095	0.878	2.905	1.519
Quant	0.672	2.995	0.095	0.878	2.905	1.519
Quali						

Hole	S72	S73	S74	S75	S76	S77
OD	0.560	0.132	0.157	0.181	0.169	0.187
Quant	0.560	0.132	0.157	0.181	0.169	0.187
Quali						

Hole	S84	S85	S86	S87	S88	S89
OD	0.087	0.144	0.095	0.351	0.128	0.098
Quant	0.087	0.144	0.095	0.351	0.128	0.098
Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	0.174	0.204	0.204	0.188	0.223	0.440
Quant	0.174	0.204	0.204	0.188	0.223	0.440
Quali						

Hole	S18	S19	S20	S21	S22	S23
OD	0.009	0.105	0.002	1.734	2.945	0.852
Quant	0.009	0.105	0.002	1.734	2.945	0.852
Quali						

Hole	S30	S31	S32	S33	S34	S35
OD	1.482	0.129	0.203	0.180	0.158	0.186
Quant	1.482	0.129	0.203	0.180	0.158	0.186
Quali						

Hole	S42	S43	S44	S45	S46	S47
OD	0.266	0.233	0.187	0.212	0.217	0.243
Quant	0.266	0.233	0.187	0.212	0.217	0.243
Quali						

Hole	S54	S55	S56	S57	S58	S59
OD	0.458	0.439	0.468	0.481	0.449	1.082
Quant	0.458	0.439	0.468	0.481	0.449	1.082
Quali						

Hole	S66	S67	S68	S69	S70	S71
OD	3.441	1.692	1.544	0.596	0.428	0.630
Quant	3.441	1.692	1.544	0.596	0.428	0.630
Quali						

Hole	S78	S79	S80	S81	S82	S83
OD	0.286	0.118	0.081	0.116	0.088	0.105
Quant	0.286	0.118	0.081	0.116	0.088	0.105
Quali						

Hole	S90	S91	S92	S93	S94	S95
OD	0.082	0.080	0.051	0.167	0.103	0.096
Quant	0.082	0.080	0.051	0.167	0.103	0.096
Quali						

Test date:2015-08-17 Test time:22:20:33

Analysis Report

Plate No.:1 (#1)
1st Item:MEASLES IGM
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.100

	1	2	3	4	5	6
Hole	B	S1	S2	S3	S4	S5
OD	0.166	0.061	0.061	0.053	0.072	0.118
Quant		0.061	0.061	0.053	0.072	0.118
Quali						
Hole	S12	S13	S14	S15	S16	S17
OD	0.527	0.000	0.000	0.000	0.364	0.000
Quant	0.527	0.000	0.000	0.000	0.364	0.000
Quali						
Hole	S24	S25	S26	S27	S28	S29
OD	0.009	0.000	0.000	2.748	0.000	0.098
Quant	0.009	0.000	0.000	2.748	0.000	0.098
Quali						
Hole	S36	S37	S38	S39	S40	S41
OD	1.483	0.014	0.061	0.025	0.024	0.012
Quant	1.483	0.014	0.061	0.025	0.024	0.012
Quali						
Hole	S48	S49	S50	S51	S52	S53
OD	0.124	0.853	0.011	0.184	0.028	0.018
Quant	0.124	0.853	0.011	0.184	0.028	0.018
Quali						
Hole	S60	S61	S62	S63	S64	S65
OD	2.743	2.716	0.392	3.802	0.329	0.079
Quant	2.743	2.716	0.392	3.802	0.329	0.079
Quali						
Hole	S72	S73	S74	S75	S76	S77
OD	1.772	1.208	1.604	2.364	3.382	2.936
Quant	1.772	1.208	1.604	2.364	3.382	2.936
Quali						
Hole	S84	S85	S86	S87	S88	S89
OD	1.080	1.555	0.927	3.455	2.587	2.493
Quant	1.080	1.555	0.927	3.455	2.587	2.493
Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	0.111	0.139	0.102	0.117	0.122	0.150
Quant	0.111	0.139	0.102	0.117	0.122	0.150
Quali						
Hole	S18	S19	S20	S21	S22	S23
OD	0.000	0.000	0.000	0.000	0.000	0.000
Quant	0.000	0.000	0.000	0.000	0.000	0.000
Quali						
Hole	S30	S31	S32	S33	S34	S35
OD	0.030	0.044	0.030	0.030	0.043	0.066
Quant	0.030	0.044	0.030	0.030	0.043	0.066
Quali						
Hole	S42	S43	S44	S45	S46	S47
OD	0.054	0.102	0.064	0.089	0.073	0.092
Quant	0.054	0.102	0.064	0.089	0.073	0.092
Quali						
Hole	S54	S55	S56	S57	S58	S59
OD	0.068	0.088	0.073	0.126	3.381	3.326
Quant	0.068	0.088	0.073	0.126	3.381	3.326
Quali						
Hole	S66	S67	S68	S69	S70	S71
OD	2.194	2.716	2.153	3.640	2.062	2.944
Quant	2.194	2.716	2.153	3.640	2.062	2.944
Quali						
Hole	S78	S79	S80	S81	S82	S83
OD	2.419	2.741	4.00*	3.445	3.280	3.445
Quant	2.419	2.741	*	3.445	3.280	3.445
Quali						
Hole	S90	S91	S92	S93	S94	S95
OD	2.550	1.857	0.196	0.356	0.666	2.725
Quant	2.550	1.857	0.196	0.356	0.666	2.725
Quali						

Test date:2015-08-18 Test time:21:40:14
Print date:2015-08-18 Print time:21:40:55
User: Verifier:

Analysis Report

Plate No.(13)
1st Item:MEASLES IGM
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.100

	1	2	3	4	5	6
Hole	B	S1	S2	S3	S4	S5
OD	0.207	2.595	0.165	0.642	0.673	0.403
Quant		2.595	0.165	0.642	0.673	0.403
Quali						
Hole	S12	S13	S14	S15	S16	S17
OD	0.179	0.000	0.000	0.000	1.247	0.000
Quant	0.179	0.000	0.000	0.000	1.247	0.000
Quali						
Hole	S24	S25	S26	S27	S28	S29
OD	2.015	0.233	0.173	0.161	1.067	2.594
Quant	2.015	0.233	0.173	0.161	1.067	2.594
Quali						
Hole	S36	S37	S38	S39	S40	S41
OD	2.982	2.842	0.376	1.010	3.007	0.761
Quant	2.982	2.842	0.376	1.010	3.007	0.761
Quali						
Hole	S48	S49	S50	S51	S52	S53
OD	2.804	0.792	1.651	0.914	0.082	0.038
Quant	2.804	0.792	1.651	0.914	0.082	0.038
Quali						
Hole	S60	S61	S62	S63	S64	S65
OD	0.269	0.171	0.024	0.091	0.030	0.129
Quant	0.269	0.171	0.024	0.091	0.030	0.129
Quali						
Hole	S72	S73	S74	S75	S76	S77
OD	3.132	0.124	0.311	0.252	0.160	0.113
Quant	3.132	0.124	0.311	0.252	0.160	0.113
Quali						
Hole	S84	S85	S86	S87	S88	S89
OD	0.061	0.119	0.366	0.056	0.068	0.137
Quant	0.061	0.119	0.366	0.056	0.068	0.137
Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	0.118	0.218	0.082	0.128	0.114	0.161
Quant	0.118	0.218	0.082	0.128	0.114	0.161
Quali						
Hole	S18	S19	S20	S21	S22	S23
OD	0.015	0.000	0.370	0.000	0.000	0.000
Quant	0.015	0.000	0.370	0.000	0.000	0.000
Quali						
Hole	S30	S31	S32	S33	S34	S35
OD	3.402	1.928	0.811	2.723	0.696	1.513
Quant	3.402	1.928	0.811	2.723	0.696	1.513
Quali						
Hole	S42	S43	S44	S45	S46	S47
OD	3.007	0.924	0.403	1.389	2.842	1.439
Quant	3.007	0.924	0.403	1.389	2.842	1.439
Quali						
Hole	S54	S55	S56	S57	S58	S59
OD	0.115	0.095	0.072	0.096	0.167	0.153
Quant	0.115	0.095	0.072	0.096	0.167	0.153
Quali						
Hole	S66	S67	S68	S69	S70	S71
OD	0.036	0.019	0.036	0.045	0.697	0.104
Quant	0.036	0.019	0.036	0.045	0.697	0.104
Quali						
Hole	S78	S79	S80	S81	S82	S83
OD	0.057	0.183	0.180	0.071	0.231	0.120
Quant	0.057	0.183	0.180	0.071	0.231	0.120
Quali						
Hole	S90	S91	S92	S93	S94	S95
OD	0.095	0.032	0.018	0.028	0.023	0.039
Quant	0.095	0.032	0.018	0.028	0.023	0.039
Quali						

Test date:2015-08-18 Test time:21:39:03

Analysis Report

Plate No.:
1st Item:MEASLES IGM
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.100

	1	2	3	4	5	6
Hole	B	S1	S2	S3	S4	S5
OD	0.241	3.125	3.404	3.215	1.933	1.717
Quant		3.125	3.404	3.215	1.933	1.717
Quali						

Hole	S12	S13	S14	S15	S16	S17
OD	0.296	0.000	0.000	0.000	0.000	0.000
Quant	0.296	0.000	0.000	0.000	0.000	0.000
Quali						

Hole	S24	S25	S26	S27	S28	S29
OD	0.052	0.114	0.058	0.026	0.034	0.041
Quant	0.052	0.114	0.058	0.026	0.034	0.041
Quali						

Hole	S36	S37	S38	S39	S40	S41
OD	0.040	0.051	0.091	0.062	0.039	0.026
Quant	0.040	0.051	0.091	0.062	0.039	0.026
Quali						

Hole	S48	S49	S50	S51	S52	S53
OD	0.081	0.063	0.123	0.053	0.055	0.088
Quant	0.081	0.063	0.123	0.053	0.055	0.088
Quali						

Hole	S60	S61	S62	S63	S64	S65
OD	0.482	0.030	0.028	2.682	0.025	0.459
Quant	0.482	0.030	0.028	2.682	0.025	0.459
Quali						

Hole	S72	S73	S74	S75	S76	S77
OD	1.337	0.204	0.114	1.345	1.020	0.128
Quant	1.337	0.204	0.114	1.345	1.020	0.128
Quali						

Hole	S84	S85	S86	S87	S88	S89
OD	1.692	0.032	0.073	0.051	0.318	0.024
Quant	1.692	0.032	0.073	0.051	0.318	0.024
Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	3.125	3.771	3.330	3.771	0.118	0.287
Quant	3.125	3.771	3.330	3.771	0.118	0.287
Quali						

Hole	S18	S19	S20	S21	S22	S23
OD	0.000	0.000	0.584	0.002	0.000	0.000
Quant	0.000	0.000	0.584	0.002	0.000	0.000
Quali						

Hole	S30	S31	S32	S33	S34	S35
OD	0.153	0.040	0.031	0.048	0.210	0.040
Quant	0.153	0.040	0.031	0.048	0.210	0.040
Quali						

Hole	S42	S43	S44	S45	S46	S47
OD	0.171	0.098	0.055	0.042	0.050	0.101
Quant	0.171	0.098	0.055	0.042	0.050	0.101
Quali						

Hole	S54	S55	S56	S57	S58	S59
OD	0.055	0.099	0.072	0.056	0.157	0.109
Quant	0.055	0.099	0.072	0.056	0.157	0.109
Quali						

Hole	S66	S67	S68	S69	S70	S71
OD	0.206	0.040	0.177	0.006	0.400	0.071
Quant	0.206	0.040	0.177	0.006	0.400	0.071
Quali						

Hole	S78	S79	S80	S81	S82	S83
OD	3.015	0.237	0.577	1.354	0.205	1.638
Quant	3.015	0.237	0.577	1.354	0.205	1.638
Quali						

Hole	S90	S91	S92	S93	S94	S95
OD	0.101	0.143	3.074	2.879	3.074	3.464
Quant	0.101	0.143	3.074	2.879	3.074	3.464
Quali						

Test date:2015-08-18 Test time:22:42:50
Print date:2015-08-18 Print time:22:44:29
Verifier:

Analysis Report

Plate No.:6
1st Item:MEASLES IGM
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.030

	1	2	3	4	5	6
Hole	B	S1	S2	S3	S4	S5
OD	0.030	2.562	0.148	0.873	4.00*	4.00*
Quant		2.562	0.148	0.873	*	*
Quali						

Hole	S12	S13	S14	S15	S16	S17
OD	3.081	1.246	1.444	0.419	1.636	2.312
Quant	3.081	1.246	1.444	0.419	1.636	2.312
Quali						

Hole	S24	S25	S26	S27	S28	S29
OD	1.714	0.266	1.852	0.325	2.200	2.876
Quant	1.714	0.266	1.852	0.325	2.200	2.876
Quali						

Hole	S36	S37	S38	S39	S40	S41
OD	3.006	0.784	2.864	0.356	0.527	0.168
Quant	3.006	0.784	2.864	0.356	0.527	0.168
Quali						

Hole	S48	S49	S50	S51	S52	S53
OD	3.399	1.339	2.573	0.603	3.202	3.069
Quant	3.399	1.339	2.573	0.603	3.202	3.069
Quali						

Hole	S60	S61	S62	S63	S64	S65
OD	0.375	0.153	2.048	0.113	0.130	1.658
Quant	0.375	0.153	2.048	0.113	0.130	1.658
Quali						

Hole	S72	S73	S74	S75	S76	S77
OD	3.399	0.748	1.356	0.410	0.880	2.788
Quant	3.399	0.748	1.356	0.410	0.880	2.788
Quali						

Hole	S84	S85	S86	S87	S88	S89
OD	0.643	1.204	2.630	2.887	3.617	2.630
Quant	0.643	1.204	2.630	2.887	3.617	2.630
Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	4.00*	0.373	4.00*	1.539	4.00*	4.00*
Quant	*	0.373	*	1.539	*	*
Quali						

Hole	S18	S19	S20	S21	S22	S23
OD	0.004	0.379	2.760	2.841	2.213	1.395
Quant	0.004	0.379	2.760	2.841	2.213	1.395
Quali						

Hole	S30	S31	S32	S33	S34	S35
OD	2.792	3.073	1.925	2.054	2.944	2.596
Quant	2.792	3.073	1.925	2.054	2.944	2.596
Quali						

Hole	S42	S43	S44	S45	S46	S47
OD	0.346	0.160	2.355	2.057	3.006	0.951
Quant	0.346	0.160	2.355	2.057	3.006	0.951
Quali						

Hole	S54	S55	S56	S57	S58	S59
OD	2.920	2.766	3.798	2.904	1.795	3.798
Quant	2.920	2.766	3.798	2.904	1.795	3.798
Quali						

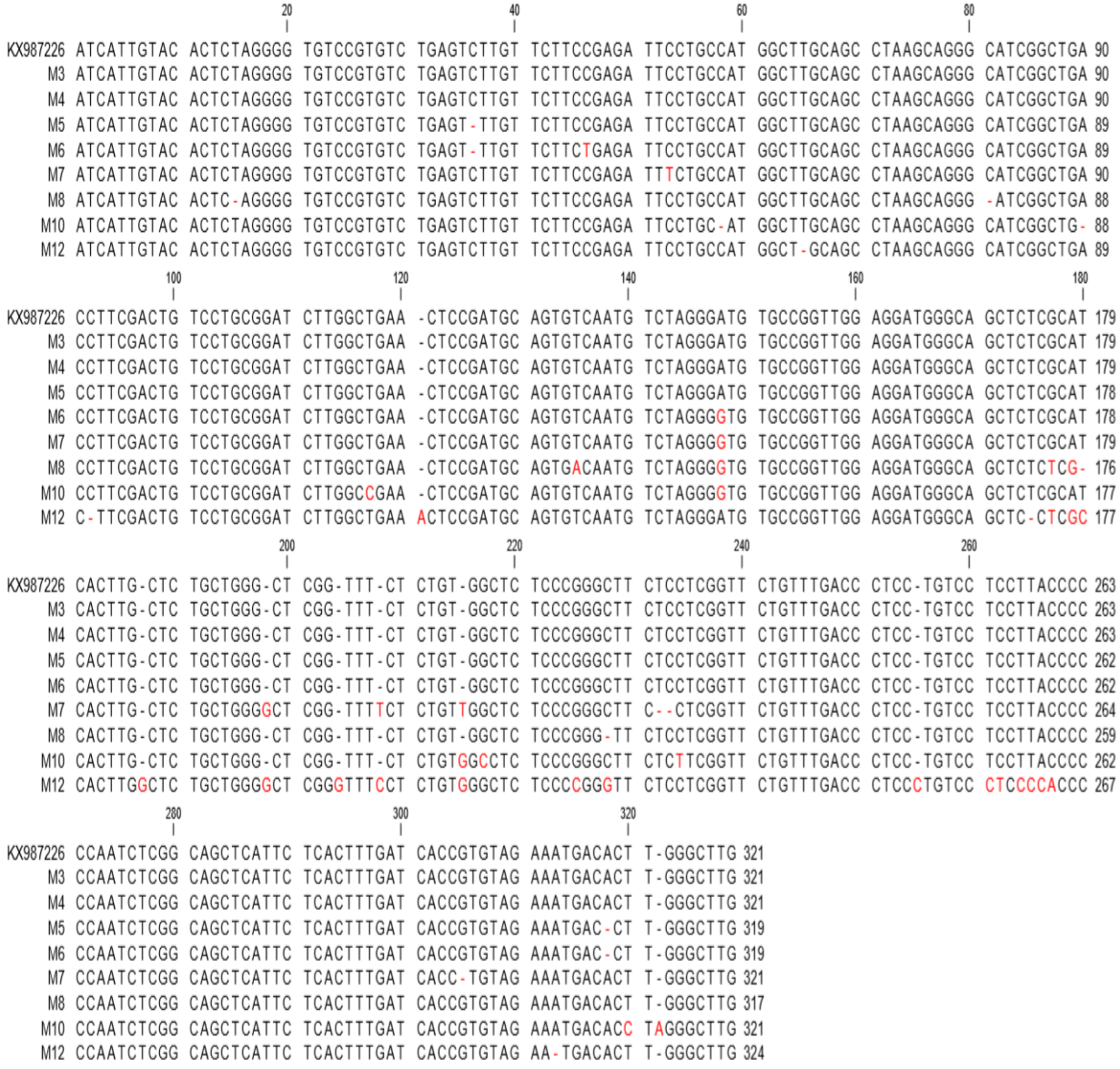
Hole	S66	S67	S68	S69	S70	S71
OD	2.088	1.883	0.860	2.204	1.759	3.065
Quant	2.088	1.883	0.860	2.204	1.759	3.065
Quali						

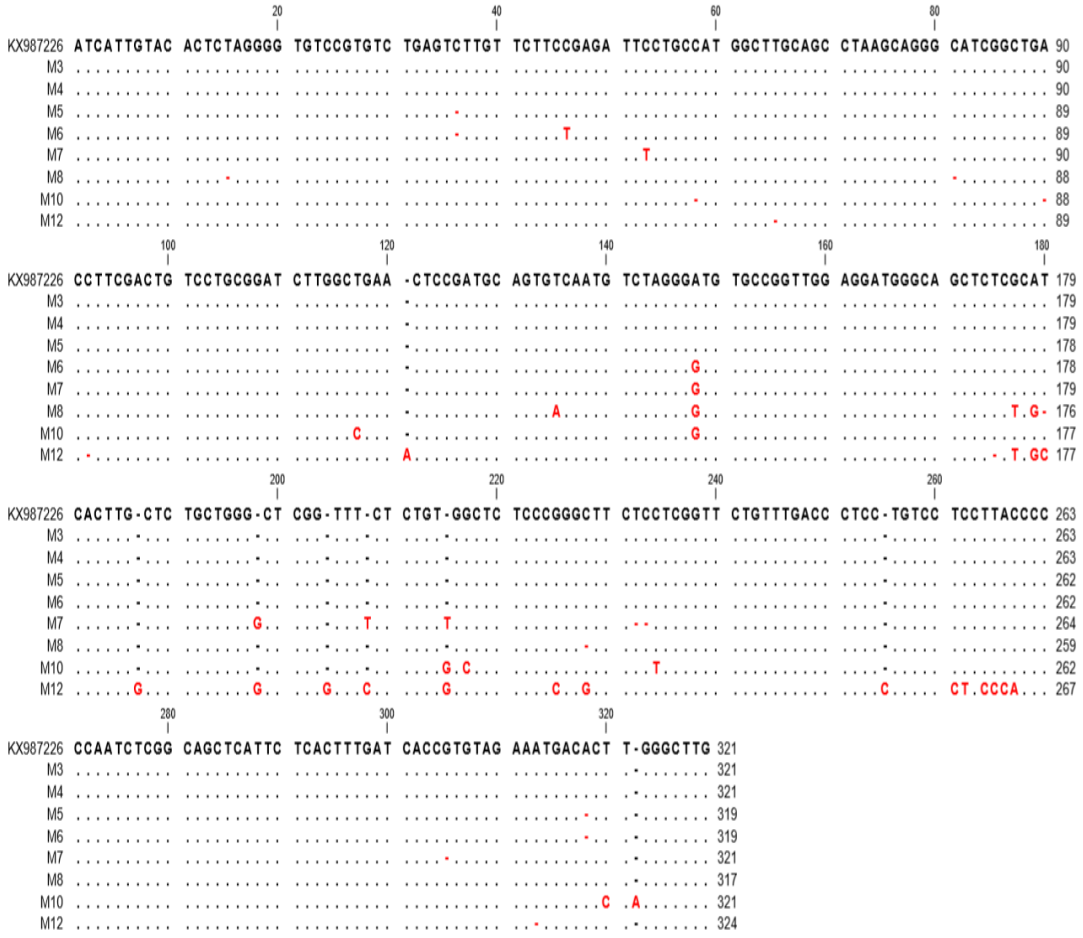
Hole	S78	S79	S80	S81	S82	S83
OD	0.741	0.810	3.171	2.104	1.430	1.251
Quant	0.741	0.810	3.171	2.104	1.430	1.251
Quali						

Hole	S90	S91	S92	S93	S94	S95
OD	2.733	3.078	3.078	2.126	1.967	0.303
Quant	2.733	3.078	3.078	2.126	1.967	0.303
Quali						

Test date:2015-08-19 Test time:21:22:06
Print date:2015-08-19 Print time:21:22:48

Appendix VII:Multiple Alignment of the sequences of nucleoprotein N-gene by comparison to reference strain of genotype




20 40 60 80
KX987226 ATCATTGTAC ACTCTAGGGG TGTCCGTGTC TGAGTCTTGT TCTTCCGAGA TTCCTGCCAT GGCTTGCAGC CTAAGCAGGG CATCGGCTGA 90
M3 90
M4 90
M5 89
M6 89
M7 90
M8 88
M10 88
M12 89
100 120 140 160 180
KX987226 CCTTCGACTG TCCTGCGGAT CTTGGCTGAA -CTCCGATGC AGTGTCAATG TCTAGGGATG TGCCGGTTGG AGGATGGGCA GCTCTCGCAT 179
M3 179
M4 179
M5 178
M6 178
M7 179
M8 176
M10 177
M12 177
200 220 240 260
KX987226 CACTTG-CTC TGCTGGG-CT CGG-TTT-CT CTGT-GGCTC TCCCGGGCTT CTCCTCGGTT CTGTTTGACC CTCC-TGTCC TCCTTACCCC 263
M3 263
M4 263
M5 262
M6 262
M7 264
M8 259
M10 262
M12 267
280 300 320
KX987226 CCAATCTCGG CAGCTCATTC TCACTTTGAT CACCGTGTAG AAATGACACT T-GGGCTTG 321
M3 321
M4 321
M5 319
M6 319
M7 321
M8 317
M10 321
M12 324

Appendix VIII: Sequences of Measles Virus N-gene Generated from PCR products

M3

ATCATTGTACACTCTAGGGGTGTCCGTGTCTGAGTTTGTTCTTCCGAGATTCCTGCCATGGCTTGCAGCCT
AAGCAGGGCATCGGCTGACCTTCGACTGTCCTGCGGATCTTGGCTGAACTCCGATGCAGTGTCAATGTCT
AGGGATGTGCCGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGCTGGGCTCGGTTTCTCTGTGGC
TCTCCCGGRCTTCTCCTCGGTTCTGTTTGACCCTCCTGTCCTCCTTACCCCCCAATCTCGGCAGCTCATTCTC
ACTTTGATCACCGTGTAGAAATGACCTTGGGCTTGTCTGGGTCCAACTGCTCTGCTGACCCTGTCCTCAGT
AGTA

M4

ATCATTGTACACTCAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTCCTGCCATGGCTTGCAGCCT
AAGCAGGGATCGGCTGACCTTCGACTGTCCTGCGGATCTTGGCTGAACTCCGATGCAGTGACAATGTCTA
GGGGTGTGCCGGTTGGAGGATGGGCAGCTCTCTCGCACTTGCTCTGCTGGGCTCGGTTTCTCTGTGGCTC
TCCCGGGTTCTCCTCGGTTCTGTTTGACCCTCCTGTCCTCCTTACCCCCCAATCTCGGCAGCTCATTCTCACT
TTGATCACCGTGTAGAAATGACACTTGGGCTTGTCCTGGGTAACCTGCTCTGCTGATCCCTGTCCTCCAGT
AGTA

M5.
ATCATTGTACACTCTAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTCCTGCCATGGCTTGCAGCC
TAAGCAGGGCATCGGCTGACCTTCGACTGTCCTGCGGATCTTGGCTGAACTCCGATGCAGTGTCAATGTC
TAGGGATGTGCCGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGCTGGGCTCGGTTTCTCTGTGG
CTCTCCCGGGCTTCTCCTCGGTTCTGTTTGACCCTCCTGTCCTCCTTACCCCCCAATCTCGGCAGCTCATTCT
CACTTTGATCACCGTGTAGAAATGACACTTGGGCTTGTCTGGGTCCAACTGCTCTGCTGACCCTGTCCTCA
GTAGTA

M6.

ATCATTGTACACTCTAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTCCTGCCATGGCTTGCAGCC
TAAGCAGGGCATCGGCTGACCTTCGACTGTCCTGCGGATCTTGGCTGAACTCCGATGCAGTGTCAATGTC
TAGGGATGTGCCGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGCTGGGCTCGGTTTCTCTGTGG
CTCTCCCGGGCTTCTCCTCGGTTCTGTTTGACCCTCCTGTCCTCCTTACCCCCCAATCTCGGCAGCTCATTCT
CACTTTGATCACCGTGTAGAAATGACACTTGGGCTTGTCTGGGTCCAACTGCTCTGCTGACCCTGTCCTCA
GTAGTA

M7.

ATCATTGTACACTCTAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTTCTGCCATGGCTTGCAGCC
TAAGCAGGGCATCGGCTGACCTTCGACTGTCCTGCGGATCTTGGCTGAACTCCGATGCAGTGTCAATGTC
TAGGGGTGTGCCGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGCTGGGGCTCGGTTTTCTCTGT
TGGCTCTCCCGGGCTTCCTCGGTTCTGTTTGACCCTCCTGTCCTCCTTACCCCCCAATCTCGGCAGCTCATT

CTCACTTTGATCACCTGTAGAAATGACACTTGGGTTGTCTGGGCCAACTGCTCTGCTGACCCTGTCCTCAG
TAGTA

M8

ATCATTGTACACTCTAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTCCTGCATGGCTTGCAGCCT
AAGCAGGGCATCGGCTGCCTTCGACTGTCCTGCGGATCTTGGCCGAACTCCGATGCAGTGTCAATGTCTA
GGGGTGTGCCGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGCTGGGCTCGGTTTCTCTGTGGCC
TCTCCCGGGCTTCTCTTCGGTTCTGTTTGACCCTCCTGTCCTCCTTACCCCCCAATCTCGGCAGCTCATTCTC
ACTTTGATCACCGTGTAGAAATGACACCTAGGGCTTGTCTGGGTCCAACTGCTCTGCTGATAATGTCCAG
TAGTA

M10.

ATCATTGTACACTCTAGGGGTGTCCGTGTCTGAGTTTGTTCTTCTGAGATTCCTGCCATGGCTTGCAGCCT
AAGCAGGGCATCGGCTGACCTTCGACTGTCCTGCGGATCTTGGCTGAACTCCGATGCAGTGTCAATGTCT
AGGGGTGTGCCGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGCTGGGCTCGGTTTCTCTGTGG
CTCTCCCGGGCTTCTCCTCGGTTCTGTTTGACCCTCCTGTCCTCCTTACCCCCCAATCTCGGCAGCTCATTCT
CACTTTGATCACCGTGTAGAAATGACCTTGGGCTTGTCTGGGTCCAACTGCTCTGCTGATCCTGTCCTCAG
TAGTA

M12

ATCATTGTACACTCTAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTCCTGCCATGGCTGCAGCCT
AAGCAGGGCATCGGCTGACTTCGACTGTCCTGCGGATCTTGGCTGAAACTCCGATGCAGTGTCAATGTCT
AGGGATGTGCCGGTTGGAGGATGGGCAGCTCCTCGCCACTTGGCTCTGCTGGGGCTCGGGTTTCCTCTG
TGGGCTCTCCCCGGGTTCTCCTCGGTTCTGTTTGACCCTCCCTGTCCCTCCCCACCCCCAATCTCGGCAGCT
CATTCTCACTTTGATCACCGTGTAGAATGACACTTGGGCTTGTCTGGGTCCAACTGCTCTGCTGACCCTGT
TCCAGTAGTA