SEROPREVALENCE AND MOLECULAR CHARACTERIZATION OF MEASLES VIRUS AMONG CHILDREN 0 – 12 YEARS OF AGE IN SOME STATES IN NORTH WESTERN NIGERIA

BY

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AUGUST, 2017

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A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

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DECLARATION

I declare that the work in this thesis entitled "**Seroprevalence and Molecular Characterization of Measles Virus among Children 0 – 12 Years of Age in some States in North Western Nigeria"** has been carriedout me in the Department of Microbiology Zaria. Kaduna, Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any institution.

Khadijat, ABDULFATAI	__________________	___________________
Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "**Seroprevalence and Molecular Characterization of Measles Virus among Children 0 – 12 Years of Age in some States in North Western Nigeria"** by ABDULFATAI Khadijat meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its scientific contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to my wonderful parents, Alhaji and Hajiya Abdulfatai, Olayiwola Jimoh, May Allah (SWT) continues to bless them always, My beloved husband, Bar. Nurudeen Muhammad, and my lovely children, Maryam, Muhammad and Umar.

ACKNOWLEDGEMENT

All praise is to Almighty Allah, the most beneficent, the benediction and salutation of Allah (S.W.T) is upon our noble prophet Muhammed (S.A.W). Thanks to Allah for counting me admirably to be alive and giving me the knowledge, capability and ability to reach the end of this professional programme in my academic pursuit.

I wish to express my profound gratitude to my diligent, able and highly esteemed supervisors, Prof. O.S. Olonitola, Prof. M. Aminu-Mukhtar and Prof. E.D. Jatau for their patient, painstaking supervision and constructive criticism, despite their commitment in guiding me throughout the process of this research work. May Almighty God bless them and grant them the desires of their hearts.

I also want to thank Dr. Tajudeen Bolajoko of Sokoto specialist hospital, I can never say thank you enough for all your support and assistance given to me during sampling, God will reward you abundantly. I must also remember my friend and colleague Dr. Maryam of Rigasa General Hospital, Muhammad Bello of Sokoto specialist hospital (lab) for his helpand assistance during sampling, Mr. Elisha of Yusuf Dansoho hospital for his helpand assistance during sampling analysis, thanks a lot.

I wish to express my sincere gratitude to my Darling Husband and my heart throb for his love, care, moral, financial support and his encouragement. May Allah bless him abundantly and keep us together for our children and family. I will forever be grateful to you my sweet heart.

My sincere thanks and appreciation goes to my beloved parent for their support, most especially my mother for her support morally, intellectually, financially as well as proper upbringing and parental care from my infancy up to date. May Allah bless them and grant them Aljannatult Firdaus (Amin).

My sincere gratitude goes to my big sister, I can’t thank you enough and I can feel the pain of a single parent in you, may Allah grant your husband soul peace and Aljannatul Firdaus, may your children bring you joy. Allah is your strength.

My sincere gratitude goes to my younger ones, Qasim, Abdulkabir, Tajudeen, Habibullah and Lateefat. Wishing you all a brighter future and Allah's guidance.

My sincere gratitude also goes to the Ministry of Health for Kaduna State, Sokoto State and Jigawa State and all thosewho participated in this research. Thanks to you all.

ABSTRACT

A serological survey and molecular characterization of measles virus among children 0 – 12 years of age was carried out in some states in North Western Nigeria. A total of 450 blood samples were collected and analyzed for measles virus IgM and IgG using Elisa kit obtained from Diagnostic Automation/ Cortez Diagnostics, California USA and the results was interpreted according to the manufacturer's instruction. The data was analyzed using statistical package for social sciences (SPSS) Version 21. Out of the 450 samples analyzed 158(35.10%) were seropositive for IgM and 385 (85.60%) were seropostive for IgG. In relation to the age group for measles virus IgM, children in age group 1 – 5 years had the highest seroprevalence of 47.80%(96/201), followed by children who were < 1 years with the seroprevalence of 31.25%(25/80). Children in age group 11 – 15 years had the least seroprevalence of 11.90%(08/67). For measles virus IgG, in relation to the age group, children in age group 1 – 5 years had the highest seroprevalence of 97.07%(195/201), followed by those in age group 6 – 10 years with the seroprevalence of 93.14%(95/102). Children who were <1years had the least seroprevalence of 50%(40/80). There was statistically significant association between age and presence of measles virus IgM and IgG ($P < 0.05$). In relation to sex for IgM, male children had the highest seroprevalence of 35.80%(83/232) while female children had lower seroprevalence of 34.40%(75/218). For IgG antibody in relation to sex, male children had the highest seroprevalence of 85.31%(198/232) compared to female children who had seroprevalence of 85.77%(187/218). These difference was however not statistically significant ($P = 0.761 > 0.05$, $P = 0.896 > 0.05$) respectively. Seroprevalence of measles virus IgM in relation to socio-demographic factors showed that there was statistically significant association between level of parents education ($P = 0.000$), parent's occupation ($P= 0.000 < 0.05$), parent socio-economic status ($P = 0.000$) and nutritional status of children ($P = 0.002 < 0.05$) and the presence of measles virus. Analysis of the results in respects to vitamin A supplement intake showed a statistically significant association between measles virus infection and intake of vitamin A ($P = 0.001 < 0.05$). The analysis of results in relation to symptoms of Measles Virus Infection also showed a significant association between measles infection and symptoms of measles except for diarrhoea ($P = 0.059 > 0.05$), vomiting ($P = 0.077 > 0.05$) and conjunctivitis ($P = 0.114 > 0.05$). Analysis of the results in respect to vaccination status of children showed a significant association between presence of IgM and vaccination status of children ($P = 0.000 < 0.05$). This

study found age, nutritional status of the children, parent socio-economic status, parent occupation, parent education, vaccination status of children and vitamin A supplement intake as important demographic and risk factors of measles virus infection in children. Ten (10) IgM positive samples from each state were randomly selected and subjected to RT PCR, out of which 21(66.7%: 21/30) showed a band with an amplicon size of 527bp. Genotype B3 strain was shown to be the circulating strain of measles virus by sequencing in the study area. The BLAST information obtained from the queried sequences from the GenBank corresponded with genotype B3 strain from Genbank. The phylogenetic relatedness of measles virus in this study with those in the GenBank revealed a common homology. This study found 85.6% of the children in the study area to possess IgG antibody to measles virus. This implies that they were immune to measles. However, 14.4% had acute infection (IgM) which implies lack of vaccination or an incomplete course of vaccination which made them susceptible to measles virus infection. It is therefore recommended that measles immunization program should be improved on in order to reach all eligible children

ABBREVIATION OF TERMS

-	aa	–	Amino Acid
-	ACIP	_	Advisory Committee on Immunization Practices
-	APC	–	Antigen Presenting Cell
-	CD46	–	Cluster of differentiation 46
-	CD8	–	Cluster of Differentiation of T – cell8
-	$_C$DNA	–	Complementary Deoxyribo Nucleic Acid
-	CDV	–	Canine Distempe Virus
-	CPE	_	cytopathic effects
-	DC	–	Dendritic Cell
-	CD	–	Cluster of Differentiation
-	DSH	–	Dutse Specialist Hospital
-	ELISA	_	Enzyme Linked Immunosorbent Assay
-	EdMV	–	Edonston Measles Virus
-	FP	–	Fusion Protein
-	GFP	–	Green Flourescent Protein
-	GHD	–	General Hospital Dutse
-	HP	–	Hemagglutinin Protein
-	Hslam	–	Human Slam
-	IFN	–	Interferon
-	IgG	–	Immunoglobulin G
-	IgM	–	Immunoglobulin M
-	IL – 2	–	Interleukin-2
-	IL	–	Interleukin
-	Kb	–	Kilobase
-	KDa	–	Kilo Dalton
-	KMV	_	Killed virus Measles Vaccine
-	L	–	Large
-	LN	–	Lymphnode
-	LP	–	Large Protein
-	MCP	–	Member Cofactor Protein

- MHC – Major histocompatibility Complex
- mProtein – Matrix protein
- MMR _ Measles, Mumps and Rubella
- MMRV _ Measles, Mumps and Rubella Vaccine
- mRNA – Messenger Ribonucleic Acid
- MV – Measles Virus
- MVFP – Measles Virus Forward Prioners
- NC – Nucleocapsid
- N Protein – Nucleic acid Protein
- NK _ Natural killer cells
- PAGE – Polyacylamide Gel Electrophoresis
- PBMC _ Peripheral Blood Mononuclear Cells
- PCR – Polymerase Chain Reaction
- PI – Post Infection
- PRRs – Pattern Recognition Receptors
- RdRP – RNA Dependent RNA Polymerase
- RGH – Rigasa General Hospital
- RNA – Ribonucleic acid
- RNC – Ribonucleocapid
- RNP – Ribonucleoprotein
- RPV – Rinder Pest Virus
- SCR – Short Concensure Repeat
- SDSPAGE – Sodium Dedecy / Sulfate Polysacrylamide Gel Electrophoreses
- SLAM – Signalling Lymphocytic Activation Molecule
- SSH – Sokoto Specialist Hospital
- SSPE – Subacute Sclerosing Panencephalitis
- ST – Short interfering
- STAT – Signal Transducers and Activator of Transcription
- Th2 – T – Helper 2 lymphocyte
- THI – T – Helper lymphocyte
- TMB _ Tetramethyibenzidine

- UNICEF _ United National International Children's Emergency Fund
- VIA – Through
- WCWC – Women Welfare Centre
- WHO _ World Health Organization
- YDMH – Yusuf Dantsoho Memorial Hospital

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CHAPTER ONE

1.0 INTRODUCTION

Measles is an acute viral infectious disease that is highly contagious. First described in the 7thcentury as a universal infectious disease of childhood in prevaccination era which is common and often fatal in developing countries (Bellini *et al.,* 2005).

Measles virus belongsto the genus *Morbillivirus* in the family *Paramyxoviridae* is an enveloped virus with a non-segmented, negative-strand RNA genome (Griffin, 2001). It is 100 – 200 nm in diameter and two of it membrane envelope proteins are important in the pathogenesis, which are the F (fusion) protein, which is responsible for fusion of virus to host cell membranes, viral penetration, and hemolysis, and the H (hemagglutinin) protein, which is responsible for adsorption of virus to cells (Bellini *et al.,* 2005).

The World Health Organization (WHO) currently recognizes 8 measles clades designated A, B, C, D, E, F, G, and H. Within these clades, there are 23 recognized genotypes, designated A, B1, B2, B3, C1, C2, D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, E, F, G1, G2, G3, H1, and H2, and one provisional genotype which is d11 (WHO, 2015).

Measles virus genome contains six genes that encode the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin (H) and large (L) proteins, respectively. The genomic RNA is encapsulated with the N protein and together with RNA-dependent, RNA polymerase composed of the L and P proteins, forms a ribonucleoprotein complex. The M protein that lines the inner surface of the envelope plays a role in virus budding (Cathomen *et al.*, 2011) and transcription regulation (Suryanarayana *et al.*, 2008). The P gene encodes an additional protein that is the V and C proteins by a process of RNA editing and by an alternative translational initiation in a different reading frame, respectively. The functions of the V and C proteins are not understood completely, but some of their functions are concerned with their activities as interferon (IFN) antagonists (Shaffer *et al.,* 2003).

In order to infect a cell, a virus must first bind to a cellular receptor on the surface and enter the cell. The presence of such a receptor determines whether the cell is susceptible to the virus. However, whether a cell is permissive for the replication of a particular virus (at post-entry steps) depends on other intracellular components found only in certain cell types. Cells must be both susceptible and permissive to allow a successful viral infection. Two molecules, CD46 (also called membrane cofactor protein) and signalling lymphocyte activation molecule (SLAM; also called CD150), have been identified as receptors for Measles virus. (Seki *et al.,* 2006).

Measles is a highly infectious disease characterized by fever, respiratory symptoms, redness of the eyes and a maculopapular rash. Complications are common and may be quite serious. The rash usually starts on the head and then spreads to the rest of the body. Fever can persist, reaching extremely high temperatures, rash can last for up to a week, and coughing can last about 10 days (Jawestz *et al.,* 2007).

The causative agent of measles virus is generally transmitted by aerosolized secretions deposited on upper-respiratory-tract mucosal surfaces. Exposure leads to local respiratory tract replication; infection of regional lymphoid tissues then occurs followed by viremia and systemic dissemination as revealed by the characteristic skin rash. Most children recover uneventfully from the illness, but serious complications can occur, including pneumonia and involvement of the central nervous system (Parks *et al.*, 2009). Airflow studies demonstrated that droplet nuclei generated in the examining room used by the source patient were dispersed throughout the entire office suite. Airborne spread of measles from a vigorously coughing child was the most likely mode of transmission (Bloch *et al.*, 2006).

Measles is a systemic infection and the primary site of infection is the respiratory epithelium of the nasopharynx. Two to three days after invasion and replication in the respiratory epithelium and regional lymphnodes, a primary viremia occurs with subsequent infection of the reticuloendothelial system. Following further viral replication in regional and distal reticuloendothelial sites, a second viremia occurs 5–7 days after initial infection. During this viremia, there may be infection of the respiratory tract and other organs. Measles virus is shed from the nasopharynx beginning with the prodrome until 3–4 days after rash onset (Gerber *et al.*, 2009).

Humans are the natural host of the virus. Approximately 30% of reported measles cases have one or more complications. Complications of measles are more common among children younger than 5 years of age and adults 20 years of age and older

(Nester *et al.,* 2010). Diarrhea was reported in 8% of measles cases, making this the most commonly reported complication of measles. Otitis media was reported in 7% of cases and occurs almost exclusively in children. Pneumonia (in 6% of reported cases) may be viral or superimposed bacterial, and is the most common cause of death. Acute encephalitis occurs in approximately 0.1% of reported cases. Onset generally occurs 6 days after rash onset (range 1–15 days) and is characterized by fever, headache, vomiting, stiff neck, meningeal irritation, drowsiness, convulsions, and coma. Measles illness during pregnancy results in a higher risk of premature labor, spontaneous abortion, and low-birth weight infants (Sugerman *et al.,* 2010).

In industrialized countries, measles occurs in 5-10 year old children, whereas in developing countries it commonly infects children under 5 years of age. Measles rarely causes death in healthy people in developed countries. However, in malnourished children in developing countries where adequate medical care is unavailable, measles is a leading cause of infant mortality (CDC, 2009). Measles virus infects approximately 30 million people annually with a mortality of 197,000, mainly in developing countries. Measles is the fifth leading global cause of mortality among children under 5 years of age (WHO, 2009).

Prevention of measles infection rests on successful immunization with currently available live, attenuated vaccine. The immunity produced by the vaccine lasts many years and is probably life-long. The recommended age of administration in infant immunization programmes is 9 to 15 months. Vaccine efficacy is 85% at 9 months of age and increases to 90–95% at 12–15 months of age. Measles virus is highly transmissible. High routine immunization coverage can reduce measles incidence but will not prevent accumulation of susceptible individuals, which can lead to outbreaks if virus is introduced into a population where the number of susceptible individual are above the critical threshold for that population (WHO, 2010).

The use of vitamin A in treatment has been investigated. A systematic review of trials into its use found no significant reduction in overall mortality, but it did reduce mortality in children aged less than two years (Souza *et al.*, 2002)

Longitudinal analysis of measles virus strain diversity in Nigeria suggested that only a few of the previously described variant 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. Measles virus sequence data from Africa have been greatly expanded since characterization of the first endemic strains was reported (Rota *et al*., 1994). Essential genetic baseline information is still missing from many countries (Rota *et al*., 2009)

Five out of six WHO regions have set goals to eliminate measles, and at the 63rd World Health Assembly in May 2010, delegates agreed to a global target of a 95% reduction in measles mortality by 2015 from the level seen in 2000, as well as to move towards eventual eradication. However, no specific global target date for eradication has yet been agreed to as of May 2014 (WHO, 2015).

The case fatality rate of measles in the developing countries is around 3-5%; this could be as high as 10% during epidemics (WHO, 2015, Odega *et al.,* 2010). Although natural infection with the measles virus confers life-long immunity, those vaccinated with the vaccine would get up to 10 years of protection from the measles virus. However, it is interesting to note that about 85% of children vaccinated with a dose of the measles vaccine could also get life-long immunity. In spite of the global efforts to vaccinate all eligible children as early as 9 months of age as the maternal immunity wanes out around that age, it is disheartening to mention that measles remains one of the leading causes of death among the under-fives, especially in the Sub-Saharan African continent (Odega *et al.,* 2010).

Measles elimination strategies are currently defined on the basis of past experience. A common principle to all measles strategies currently being implemented is the need to maintain the number of susceptible individuals in the population below a certain critical number required to sustain transmission of the virus (Vitek *et al.,* 1999).

1.1 Statement of the Research Problem

Despite the availability of an effective vaccine, measles continues to be one of the leading causes of childhood morbidity and mortality in many regions of the world. Precise incidence estimates are difficult to obtain because of heterogeneous surveillance systems and probable under reporting (Stein *et al.*, 2003).

In spite of the increase in measles immunization coverage aimed at building population immunity against the disease, measles outbreaks still occur especially in the developing countries; this ranks measles top among the burden of vaccine-preventable diseases across the globe with worst picture seen in the developing countries that are not unconnected to malnutrition and overcrowding (Fatiregun and Odega, 2013). The sporadic measles outbreaks seen are often fatal with high morbidity and mortality in those under the age of 5 years. Interrupting transmission of this fatal disease requires an effort to achieve population immunity of at least 95% (Odega *et al.,* 2010). This calls for the need to review the existing strategies for the control of this fatal but preventable disease.

Currently, Nigerian is one of the countries in the world where the burden of measles is highest and where 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 (Mohammed *et al.,* 2011)

In Nigeria, measles case-based surveillance started in response to measles catch-up supplementary immunization activities (SIAs) in the Northern part in the last quarter of 2005. However, the measles case-based surveillance was subsequently implemented across the country after the supplementary immunization activities (SIAs) in the Southern states in late 2006. It is interesting to mention that a total of 1,346 suspected measles cases were reported since January 2007 with 196 laboratory-confirmed by the laboratory and or epidemiological linkage. Of these confirmed cases, 62% were 1-4 years and 23% aged 5-14 years. There is a need to emphasize that the pattern of measles in Nigeria is predominantly among the younger un-immunized population due to immunity gaps as a result of inadequate routine measles coverage among others (Umeh and Ahaneku, 2013).

It is disheartening to note that Nigeria is among the 45 countries that account for 94% of the global deaths due to measles (Odega *et al.,* 2010). While there is paucity in the literature in Nigeria on the population-based prevalence of measles, several studies from tertiary hospitals show the prevalence of measles from pediatric admissions stands at around 1.3-5.1% (Fatiregun *et al.,* 2014). Furthermore, the reported case fatality rations (CFRs) for this disease in Nigeria showed some variations ranging from 1.9% to 12.4%.In a Southern Nigerian city, it was observed that measles accounted for 3.1% of all pediatric admissions in the hospital; this figure is higher than the 2.3% reported in 1998 at a tertiary health center in the same city (Fatiregun *et al.,* 2014). Etuk *et al.*(2015) also reported a rise in the prevalence of measles in a tertiary health center, in a neighboring state.

Measles is a vaccine preventable disease whose incidences and prevalence in a community should decline with an appropriate immunization programme. However, it continues to be one of the present day scourge of the developing world (Lagunju *et al.,* 2005). 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). A disease with such a high rate of morbidity and mortality requires proper attention in order to achieve control.

As of 2014, measles vaccine was widely used throughout Africa, and measles incidence was at an historic low however, outbreaks continue to occur, and case fatality rates among young children can be as high as 5%-10% during outbreaks (WHO, 2016). With an estimated 28,000 measles – related deaths still occurring each year. Measles remains a major public health problem in Africa (WHO, 2016).

In Nigeria, an effective measles vaccine has been available for over 40years. However, the global goal of achieving vaccine coverage rate of 90% and reducing mortality rate by 95% is far from becoming a reality; measles still remains a serious problem in Nigeria (WHO, 2015).

1.2 Justification of the Study

Studying seroprevalence and molecular characterization of measles virus in some states in north western Nigeria is to obtain the prevalence of measles epidemic in our society which has become a great concern especially in country nearing the measles elimination goal.

In Nigeria measles surveillance is still in its infancy.There is paucity of country's wide data on circulating measles virus strain. In 2010, B3 strains with 2 separate clusters were reported in Lagos, Ibadan, Adamawa and Borno, (Jacques *et al.*, 2010). To the best of our knowledge no such official reports are available on molecular characterization of measles virus from Northwestern Nigeria. This study will provide a baseline molecular data on the circulating measles virus strain. The introduction of enhanced diagnostic test for IgM detection such as the IgM EIA, result of which may be positive for patient with vaccine failure, measles surveillance can also help to measure vaccination programs by documenting the interruption of transmission of the endemic viral strains (Rota *et al.*, 2009).

However, continued sampling of measles virus strains is needed for a more completed understanding of their evolving global distribution.

1.3 Research Hypothesis

1. What is the seroprevalence of measles virus among children 0 – 12years of age in north western Nigeria?
2. What are the factors responsible for this infection?
3. Are there any changes in the measles virus strain in the study area?
4. Is there any links between outbreak and transmission?

1.4 Aim

The aim of this study was to determine the seroprevalence and molecular characterization of measles virus among children 0 – 12years of age in some states in north western Nigeria.

1.5 Objectives

The specific objectives of this study were to :

1. Determine the seroprevalence of measles virus infection among children in north western Nigeria using ELISA.
2. Determine the correlation between measles virus infection and vaccination status of children in the study population.

3. Determine the possible risk factors and obtain socio demographic data that might be associated with measles virus infection using questionnaire.
4. Confirm the presence of the measles virus using Reverse transcription polymerase chain reaction (RT-PCR)
5. Determine the measles virus genotype from the already confirmed RT – PCR measles virus using sequencing technique and determine the evolutionary relationship between plylogenetic analysis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Background of Measles Virus

Measles virus is a member of the genus *Morbillivirus,* which belongs to the family *Paramyxoviridae(*Griffin, 2007). In addition to Measles virus, *Morbillivirus* includes dolphin and porpoise morbillivirus, canine distemper virus, phocid distemper virus, peste des petits ruminants virus, and rinderpest virus (RPV) (McCarthy *et al*., 2005). Genetically and antigenetically Measles virus is most closely related to RPV, which is a pathogen of cattle (Sheshberadaran *et al*., 2000). Measles virus is assumed to have evolved in an environment where cattle and humans lived in close proximity. Measles virus probably evolved after commencement of livestock farming in the early centers of civilization in the Middle East. FrancisHome in 1757 demonstrated that measles was caused by an infectious agent by takingblood from measles patients and transmitting the disease to naive individuals. Priorto this measles was recognized as an independent disease by the English physician,Sydenham, in the 17th century and the Arabian physician, Rhazes, in the 9th centurydistinguished measles from smallpox (Premenko *et al.*, 2003).

Rhazes credited El Yehudi, aHebrew physician of the first century for the initial description of measles.In 1846 a Danish medical student Peter Panum described a measles outbreak in anisolated population living on the Faroe Islands (Panum *et al.*, 1940). His description was the first todocument important aspects of measles such as transmission via respiratory tract dropletsand that spreading occurred during the prodromal period. He also documented a 2-weekincubation period, increased mortality among infants and those older than 60 years. Hestated the attack rate was 100% of susceptible and those exposed had persistentimmunity. This was the first scientific observation of long-lived immunologic memory. Plotz cultivated measles in vitro in 1938). Peebles and Enders in 1954propagated measles virus in tissue culture (Enders *et al.*, 1954) and by serial passage they went on to develop a live attenuated measles vaccine This vaccine has had a significant impact on the control and virtual elimination of measles in several regions of the world. Measles remains the leader among vaccine preventable diseases in having the highest mortality rate. Measles is one of six infectious diseases that cause 90% of infectious disease deaths annually worldwide. Due to low vaccine coverage in developing nations and that it is the most contagious disease known to man infecting over 90% of susceptible persons, measles has survived mass vaccination strategies and continues to be a public health problem and a major childhood killer in developing nations (Enders *et al.,* 1960).

2.2 Structure of the Virus

Measles viruses are enveloped non-segmented negative stranded RNA virus with spherical geometries. The diameter is around 150 nm and genomes are linear, around 15-16kb in length. The genome codes for eight (8) proteins (Griffin *et al.,* 2007). The particle consists of a lipid envelope made up of the hemagglutinin (H) and fusion (F) proteins (Figure 2.1). The viral ribonucleoprotein (RNP) complex includes the viral RNA associated with the nucleoprotein (N) and the viral polymerase complex including the L protein and the phosphorilated P protein.

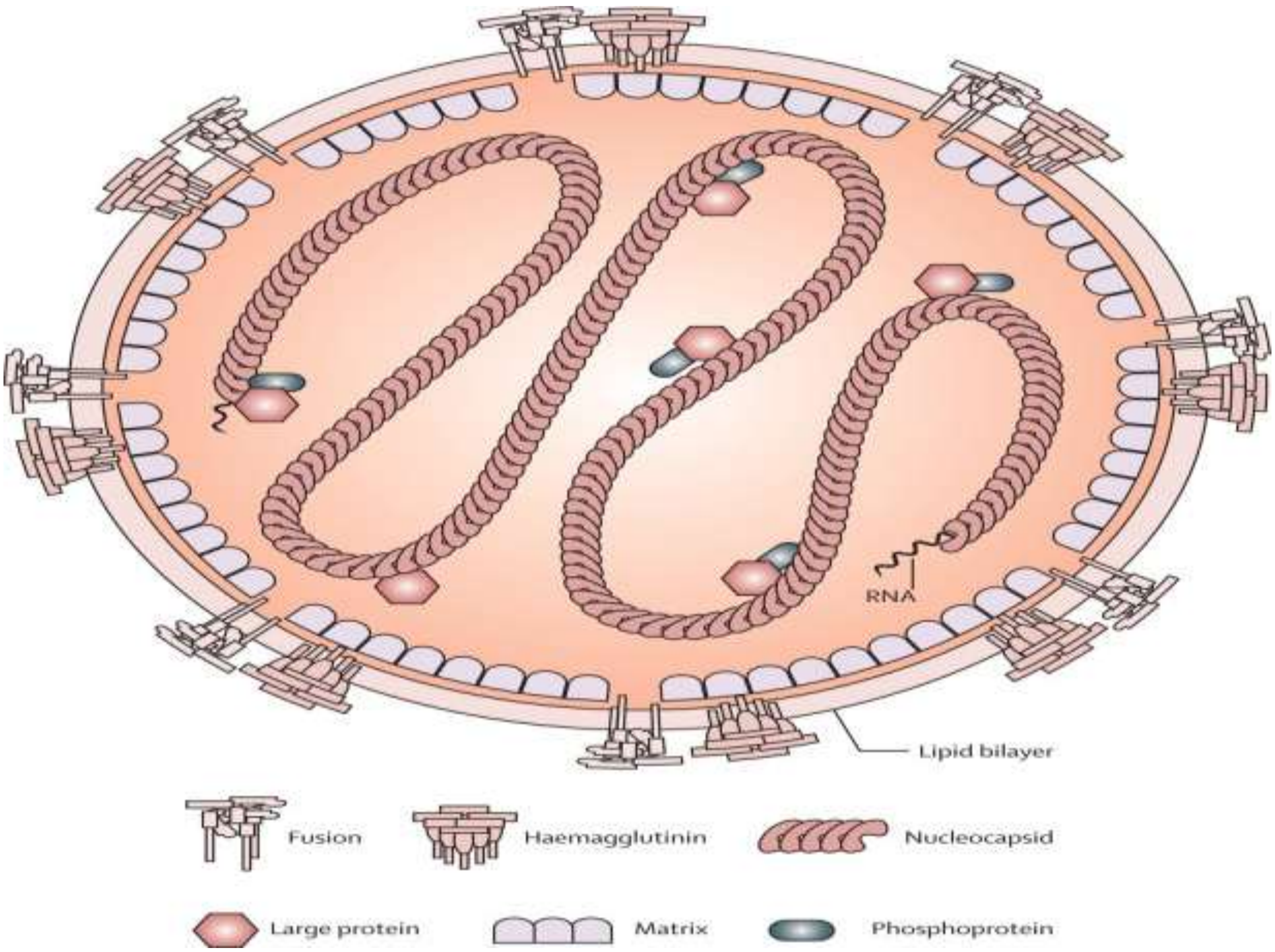

Fig. 2.1; Measles virus structure

(Adopted from www.thelancet.com)

The six structural genes are arranged linearly on the viral. RNA with no overlap in the following order 3 ’ NP, P, matrix (M), F, H and L. 3 nonstructural proteins C, R and V are expressed from the P gene. The functions of these non structural genes have not been elucidated but there is some evidence that they share in the regulation of transcription (Liston *et al.,* 1995). The N protein is the most abundant measles virus mRNA because the non-segmented genome is transcribed as a gradient so that mRNA abundance decreases in relation to distance from the 3’ end of the genome. Every N protein binds 6 nucleotides of the genomic or antigenomic RNA and encapsidation is essential for both replication and transcription. N protein also binds to the P protein and the putative viral RNA polymerase L protein to form the replicase complex.

The P gene encodes the V and C proteins in addition to the P protein. The V protein shares the same translation start site but has a different carboxyl terminal domain due to RNA editing. The C protein, in contrast, has the same mRNA as P protein but is translated from an alternate initiator codon. Both V and C proteins are important for defense against the host innate immune responses but are not essential for measles virus replication. The M protein is part of the viral envelope and interacts with both the nucleocapsid inside and with the cytoplasmic tails of the glycoproteins (H and F) on the envelope of the virion (Perryand Halsey 2004)

2.3 Genome of Measles Virus

The genome of measles virus is non-segmented negative-sense RNA with 15–19 kilobases in length and contains 6–10 genes. Extracistronic (non-coding) regions which include A 3’ leader sequence, 50 nucleotides in length, which acts as a transcriptional promoter and A 5’ trailer sequence, 50–161 nucleotides long. Each gene contains transcription start/stop signals at the beginning and end, which are transcribed as part of the gene (Amarasinghe *et al.*, 2017).

Gene sequence within the genome is conserved due to a phenomenon known as transcriptional polarity in which genes closest to the 3’ end of the genome are transcribed in greater abundance than those towards the 5’ end. After each gene is transcribed, the RNA-Dependent RNA polymerase pauses to release the new mRNA when it encounters an intergenic sequence.

Evidence for a single promoter model was verified when viruses were exposed to UV light. UV radiation can cause dimerization of RNA, which prevents transcription by RNA polymerase. If the viral genome follows a multiple promoter model, the level inhibition of transcription should correlate with the length of the RNA gene. However, the genome was best described by a single promoter model. When measles virus genome was exposed to UV light, the level of inhibition of transcription was proportional to the distance from the leader sequence. That is, the further the gene is from the leader sequence, the greater the chance of RNA dimerization inhibiting RNA polymerase (Figure 2.2).

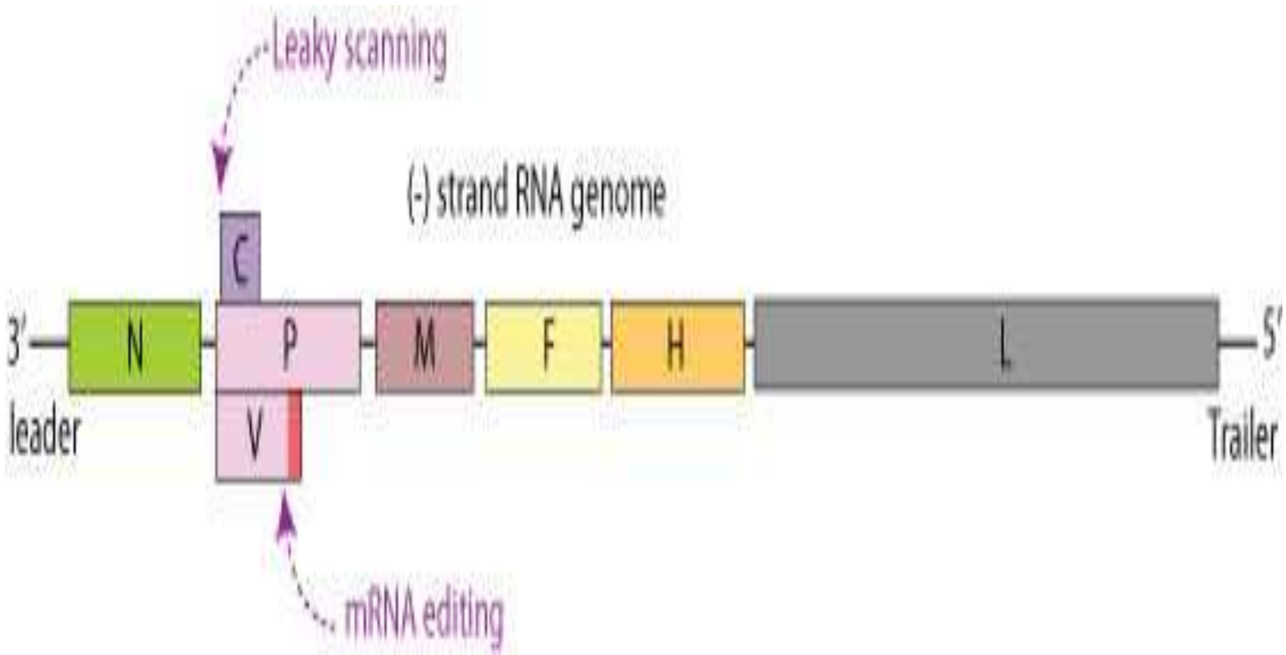

Fig. 2.2; Measles virus Genome organization

(Adopted from www. viralzoneexpasy.org)

The virus takes advantage of the single promoter model by having its genes arranged in relative order of protein needed for successful infection. For example, nucleocapsid protein, N, is needed in greater amounts than RNA polymerase, L. The gene sequence is Nucleocapsid – Phosphoprotein – Matrix – Fusion – Attachment – Large (polymerase) (Wong and Goetz, 1993)

2.4 Measles Virus Proteins

Measles virus is a member of the genus Morbillivirus in the family Paramyxoviridae , is an enveloped virus with a non-segmented, negative-strand RNA genome (Griffin, 2001). The genome contains six genes that encode the nucleocapsid (N), phospho- (P), matrix (M), fusion (F), haemagglutinin (H) and large (L) proteins, respectively. The genomic RNA is encapsidated with the N protein and, together with RNA-dependent RNA polymerase composed of the L and P proteins, forms a ribonucleoprotein complex. Measles virus proteins include:

2.4.1 The H protein

The hemagglutinin (H) protein is a 617 amino acid (aa) long, 78 kDa glycoprotein spike that spans the viral envelope. Its primary function is receptor-binding at the cell surface which facilitates subsequent viral entry. The ability of H to hemagglutinate red blood cells is one of the hallmarks of *morbillivirus* cellular tropism.

As a type II transmembrane protein, the C-terminus of H extends into the luminal space, while the Nterminus remains in the cytoplasm. Hemagglutinin proteins form homodimers via disulfide bonds and further associate to form tetramers. The crystal structure of H has been solved (Hashiguchi *et al***.,** 2007), allowing for closer examination into the interactions of H and host cell receptors as well as interactions between H and F.

2.4.2 The F protein

The fusion protein of measles virus is a 553 amino acid, type 1 transmembrane glycoprotein translated as an inactive precursor, This 60 kDa precursor is enzymatically cleaved by the protease Furin in the trans-Golgi network, resulting in two fragments; a 41 kDa Fi and 18 kDa F2. Once joined by disulfide bonds, the fusogenically active F protein is obtained. Viral attachment mediated by the binding of H to the target cell receptor provides the activation energy required to trigger the F protein (Navaratnarajah *et al.,* 2008). Following insertion of the fusion peptide into the target membrane, a conformational change in the F protein brings the target membrane and viral envelope in close apposition, allowing for membrane fusion. Cell to cell fusion is a hallmark of measles virus infection, leading to the formation of giant multinucleated cells called syncytia (sing, syncytium) (Richardson *et al.,* 2009). Synthetic oligopeptides with sequence similarities to the highly conserved N-terminus of Fi can inhibit measles virus fusion activity (Richardson *et al.,* 1980). The H and F proteins contain specialized basolateral sorting signals which allow the proteins to be transported to the basolateral cell surface where they mediate syncytium formation and promote cell-to-cell spread of the virus (Moll *et al.,* 2001). In polarized epithelial cells however, the actions of the measles virus matrix protein (M) can shuttle H and F to the apical membrane, thereby allowing for apical release of the virus.

2.4.3 The M protein

The matrix protein of measles virus is 335 amino acid in length (37 kDa) and associated closely with the intracellular surface of the host cell plasma membrane. Lining the inner surface of the viral envelope, M is found associating with viral nucleocapsids and serves to anchor the transmembrane glycoproteins. M interacts with the cytoplasmic tails of H and F, modulating both targeting and fusion capabilities. Upon deletion of the M protein, the production of infectious virus is impaired and cell-to-cell fusion is enhanced (Cathomen *et al.,* 2011).

2.4.4 The N protein

The nucleocapsid protein (N) is encoded by the first gene transcribed from the measles virus genome. This 525 amino acid, 60 kDa proteins can self-assemble into a helical structure that serves to shield the viral genome from enzymatic breakdown. Given the close association of viral RNA and the nucleocapsid, this ribonucleoprotein (RNP) complex, in conjunction with the P and L proteins, serves as the replicative unit (Longhi, 2009). It is his RNP complex that is thought to be transmitted via cell-to-cell contacts in cases of Subacute Sclerosing Panencephalitis (SSPE) (Ehrengruber *et al.,* 2002). The N protein has also been shown to interact with cellular proteins at the cell surface, to inhibit B cell antibody production (Ravanel *et al.,* 2008).

2.4.5 The L protein

The large protein (L) is encoded by the last gene transcribed of the measles virus genome, and is found in fewest numbers. This large protein at 2,183 aa, 250 kDa, functions in cohort with the P protein as an RNA-dependent RNA polymerase (Longhi, 2009). The L protein serves both to replicate the measles virus genome and to transcribe viral mRNAs

2.4.6 The P protein

The polymerase cofactor phosphoprotein (P) is 507 amino acid in length with a molecular weight of 72 kDa. Its main function is in tethering the large polymerase to the ribonucleoprotein (RNP) complex for viral transcription (Griffin, 2007). P also acts as a chaperone protein for newly synthesized nucleoproteins, preventing inappropriate self-assembly (Huber *et al.*, 2009).

2.4.7 The V protein

Sharing the methionine initiator and the N-terminal 231 amino acid of P is the V protein of measles virus, with a molecular weight of 40 kDa. The insertion of a non-coding amino acid results in a frame shift mutation, replacing the C-terminal sequence with a stretch of amino acids displaying zinc-binding propertiesand a premature stop codon. The V protein is also responsible for innate immune suppression via inhibition of phosphorylation processes, preventing downstream signalling through alpha and beta interferon (Takeuchi *et al.*, 2003).

2.4.8 The C protein

Originating from an overlapping open reading frame of the phosphoprotein gene, the non-structural C protein, at 186 amino acid and 21 kDa, is believed to have an active role in immunosuppression of target cell immune responses. With similar action to the V protein, the C protein perturbs signalling through type 1 interferons, alpha and beta (Shaffer *et al.*,2003). Neither V nor C proteins is essential in measles virus replication in Vero (African green monkey kidney) cells deficient in type 1 interferon secretion.

2.5 Measles Virus cellular Receptors

Measles virus receptors play a very important role in its pathogenesis and these receptors are:

2.5.1 The CD46/MCP

Early efforts to identify a measles virus receptor resulted in the generation of a monoclonal antibody that inhibits measles virus infection (Naniche *et al.*,1992). This antibody was applied to HeLa cell lysates, immunoprecipitating out two proteins with molecular weights of 57 and 67 kDa (Naniche *et al.*,1993). Two distinct approaches were applied to identify this viral receptor. The first approach used the knowledge that rodent cells were not permissive to measles virus infection. By generating rodent-human hybrid cell lines, it was discovered that only the mouse cells harbouring the q arm of chromosome 1 infected with measles virus. Taken together, it was predicted that cluster of differentiation 46 (CD46) was likely the elusive measles virus receptor.

The expression of human CD46 in Chinese hamster ovary cells conferred permissiveness to measles virus infection, establishing CD46 as a receptor for the commonly cultured laboratory strain of measles virus (Edmonston). (Dorig *et al.,* 1993). The second approach involved sequencing of the amino terminus of the proteins precipitated with the monoclonal antibody, and confirmed its identity as that of CD46 (Naniche *et al.*,1993).

This type 1 transmembrane glycoprotein was first discovered on the surface of peripheral blood mononuclear cells, initially termed gp45-70 given the unusually broad mobility pattern on Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), 45-70kDa. gp45-70 was shown to act as a membrane cofactor in factor 1-mediated proteolytic inactivation of the complement proteins, C3b and C4b (Liszewski *et al.*,1991). The complement cascade is a major player in the innate immune system, protecting the host from invading pathogens, acting through the membrane attack complex. gp45-70 was then later renamed membrane cofactor protein (MCP) and given the designation, CD46. Encoded by a 45,000 base gene consisting of 14 exons, CD46 is susceptible to multiple isoforms due to alternative splicing (Liszewski *et al.,* 1991), consistent with the protein's mobility pattern on SDSPAGE. CD46 is characterized by 4 extracellular, amino terminal short consensus repeats (SCR) and is ubiquitously expressed in the nucleated cells of the body. Measles H utilizes SCR1 and SCR2 for viral attachment. Sequence alignments of CD46 showed that the New World Monkeys from South America have a deletion in their SCR1 region which prevents viral attachment and infection of most cells derived from these primates with the Edmonston strain of measles virus; this is in contrast to the Old World Monkeys of Africa and humans which possess an intact CD46 (Hsu *et al*,1997). New World Monkeys infected with clinical isolates of measles virus showed a lymphotropic disease resulting in immunosuppression, clear evidence for a second measles virus receptor (Hsu *et al.,* 1997).

2.5.2 The CD150/SLAM

Following the initial isolation of the Edmonston strain of measles virus, and its propagation in Vero cells, difficulties arose in culturing wild type isolates of the virus from clinical samples, given poor infection and growth *in vitro.* As such, most measles virus research focused on attenuated laboratory strains. In 1990, an Epstein-Barr virus-transformed marmoset B-lymphoblastoid cell line, B95-8, and its adherent derivative B95a, were shown to be 10,000-fold more permissive to wild type measles virus (wt.MV) isolates compared to growth in Vero cells (Kobune *et al.*,1990). Expression of cDNAs from B95a and B95-8 cells in non-susceptible 293T and Chinese hamster ovary cells, resulted in the identification of CD 150 as a receptor for wt.MV. CD 150 or Signalling Lymphocytic Activation Molecule (SLAM) is a 70 kDa lymphotropic protein expressed on activated lymphocytes, mature dendritic cells, macrophages, and platelets (Yanagi *et al.*,2009). Coincidentally, the SLAM gene is found on the q arm of chromosome 1, as is the gene for CD46. SLAM belongs to the immunoglobulin superfamily by means of its two extracellular domains, V and C2. Its cytoplasmic tail is the site of phosphorylation and protein-protein interactions. SLAM functions by interaction with another SLAM on an adjacent cell via their V domains, the same domain required for binding of measles H. Following phosphorylation, downstream effectors are triggered resulting in the production of the cytokine interleukin-4 in CD4+ T cells (Yanagi *et al.*,2009). The production of interleukin-12, tumour necrosis factor a, and nitric acid is also regulated by SLAM in response to bacterial lipopolysaccharide in the context of mouse macrophages (Figure 2.3) (Wang *et al.*,2004).

A more recently identified epithelial receptor, nectin-4, is exploited by both wildtype and vaccine strains to facilitate cellular entry (Muhlebach *et al.,* 2011). Nectin-4 is an adherens junction protein, originally described as poliovirus-receptor-like-4 (PVRL-4), and plays an important role in measles virus pathogenesis by ‘shedding’ the virus in epithelial airways through the course of infection (Leonard *et al.,* 2008*)*

2.6 Pathogenesis of Measles Virus Infection

Measles virus infects the respiratory epithelial cells as primary targets via the inefficient SLAM and CD46 independent pathway, but it may well not explain the fact that measles is one of the most contagious diseases. Thus, it is more likely that the primary targets of Measles virus are SLAM cells of the immune system in the respiratory tract, such as monocytes, DCs and lymphocytes, rather than epithelial cells. This contention is supported by the finding that almost all CD14 monocytes in tonsils express SLAM in humans (Farina *et al.,* 2004).

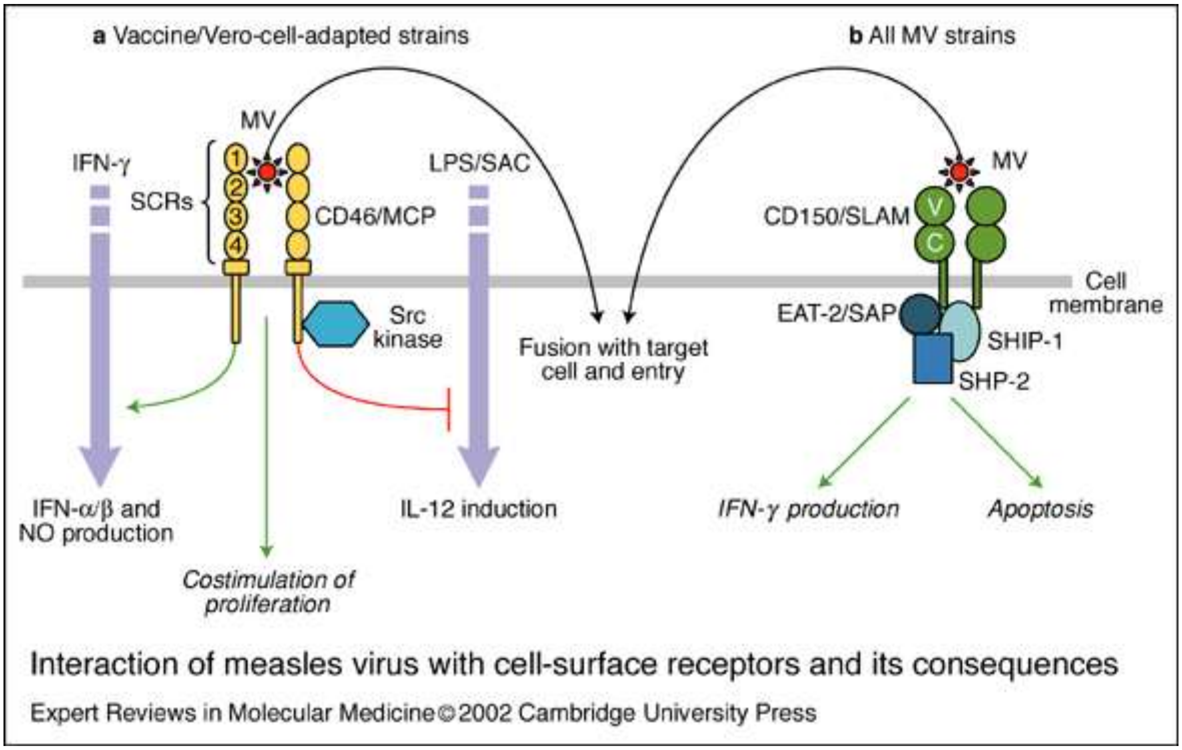

Fig. 2.3; Measles virus receptor interaction

(Adopted from www.cambridge.org)

Skin rash is thought to be caused by the T-cell response to Measles virus infected cells in capillary vessels because it does not appear in children with T-cell immunodeficiency (Griffin, 2007). Permar *et al.*, (2003) showed that rhesus monkeys whose CD8 + T cells were depleted exhibited a more extensive rash, suggesting that CD4 T cells, not CD8 T cells, are responsible for the development of the rash. The cells recognized by CD4 T cells are probably class II major histocompatibility complex (MHC) molecule-expressing cells of the immune system, rather than endothelial cells. However, endothelial cells can be infected by Measles virus via the SLAM- and CD46-independent pathway and inflammatory reactions may induce class II MHC molecules on them. Thus, determination of the targets of the CD4 T-cell response will have an important implication in the mechanism of rash formation in measles.

The mechanisms underlying severe immunosuppression and lymphopenia, characteristic of measles, are not well understood. Infection and subsequent destruction of $SLAM^+$ cells of the immune system may account for these immunological abnormalities. The finding that memory T cells express high levels of SLAM (Cocks *et al.*, 1995) well explains why patients with measles show suppressed delayed-type hypersensitivity responses, such as the tuberculin skin test .Furthermore, binding of the H protein on Measles virus particles or infected cells to SLAM on the cell surface may affect SLAM signal transduction of immune cells positively or negatively by mimicking the natural ligand. Although such an effect of the Measles virus SLAM interaction on signalling has not been demonstrated, expression of the

Measles virus H protein was found to down regulate SLAM from the cell surface (Erlenhoefer*et al.*, 2001).

Other mechanisms of Measles virus induced immunosuppression have also been proposed. Schneider-Schaulies and colleagues have shown that the cell-surface contact of Measles virus glycoproteins via an unidentified molecule (not SLAM) induces inhibition of lymphocyte proliferation *IN VITRO* (Erlenhoefer *et al.*, 2001), CD46 cross-linking by Measles virus inhibits IL-12 production by monocytes and suppression of IL-12production was indeed observed in measles patients (Atabani*et al.*, 2001).

The identification of Measles virus receptors made it possible to develop small-animal models for Measles virus infection by using transgenic technologies. CD46- and SLAM-transgenic mice have been produced by using cDNAs with various promoters or genomic genes, allowing Measles virus growth to some degree. (Atabani*et al.*, 2001).

2.7 Replication of Measles Virus

Measles virus contains non segmented single-stranded RNA genome of negative polarity and replication occurs entirely in the cytoplasm. The H protein contacts the receptors whereas the F protein mediates fusion. After the viral membrane fuses with the cellular plasma membrane at the neutral pH, the helical nucleocapsids are released into the cytoplasm (Figure 2.4).

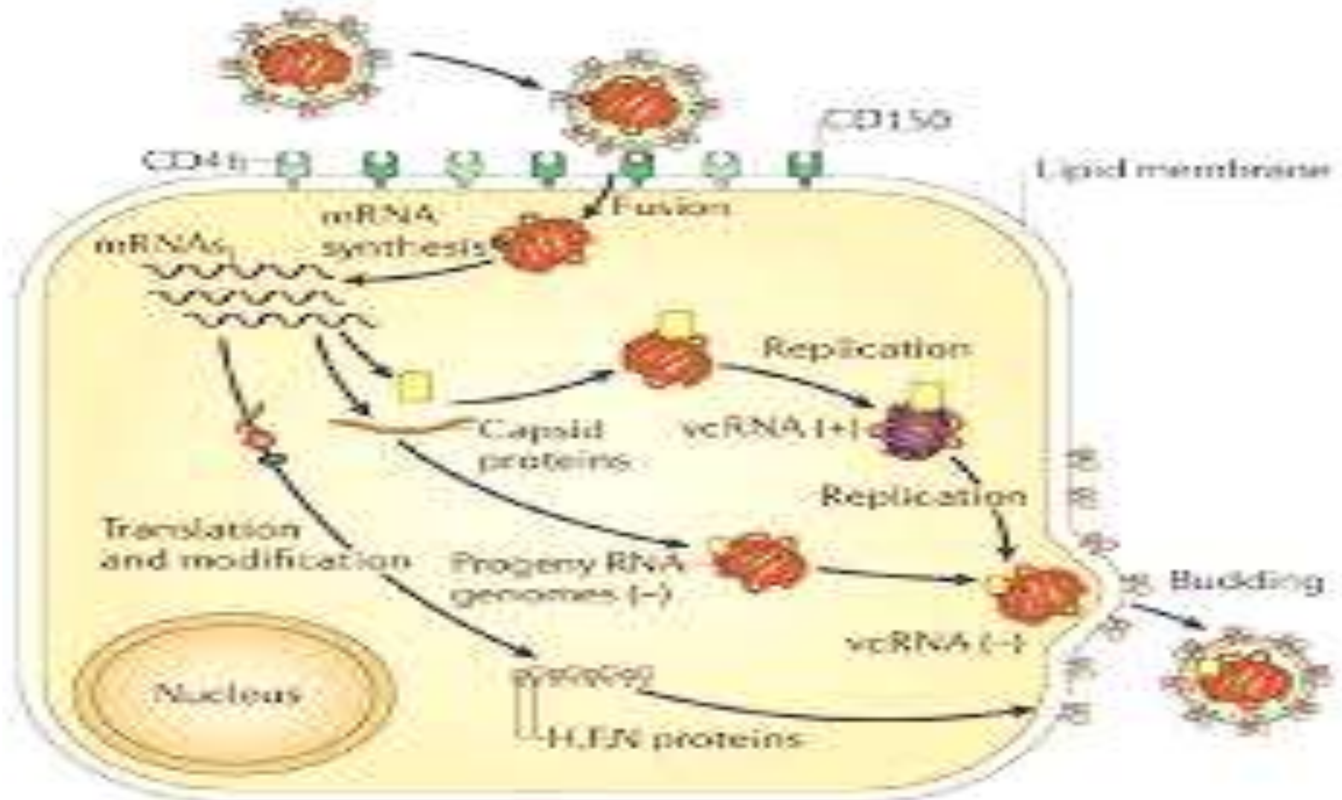

Fig. 2.4; Measles virus replication cycle

(Adopted from Moss *et al.,* 2002)

The uncoating process takes place by disrupting of the M-N contacts. Early in virus infection, before the viral translation products accumulate to high levels, the viral RNA-dependent RNA polymerase (vRdRp) activity is restricted to the production of a leader RNA and mRNAs from the incoming virion nucleocapsid in what is called primary transcription. At later times following infection, this input Ribonucleoprotein (RNP) is used as a template to produce (+) sense antigenomes which are in turn used as templates to produce new (-) sense genomic RNA. After primary transcription and translation, when abundant progeny genomes have been produced, they can serve as additional templates in what is called the secondary transcription to produce much higher levels of viral mRNA transcripts (Lamb *et al.,* 2007).

The processive viral polymerase transcribes the N-encapsidated genome RNA into 5' capped and 3' polyadenylated mRNAs. Beginning at the 3' end of the genome, the polymerase transcribes the genes in a sequential and polar manner by terminating and reinitiating at each end of the gene junctions. This sequential "stop-start" mechanism continues across the viral genome in a 3' to 5' direction. Polyadenylation occurs by reiterative copying of four to seven uridylates in the gene end sequence, followed by release of the mRNA. Initiation and capping of the downstream mRNA is specific by the gene-start sequence following the short intergenic region. The RdRp sometimes fails to reinitiate and this leads to a gradient of mRNA abundance that decreases according to the distance from the genome 3'end with N mRNA being the most abundant and the L mRNA the least abundant (Lamb *et al.,* 2007).

Paramyxovirus particles are formed by a budding process. Buds emerge from the plasma membrane in locations where the viral components are assembled. Assembly is thought to require coordinated localization of multiple virus proteins that are preferently incorporated into nascent viral particles, whereas most of the host proteins are excluded. The assembly of the envelope is at the cell surface and in polarized epithelial cells.

2.7.1 Measles Virus Entry and Spread

Measles virus is transmitted by aerosol droplets and until recently initial replication was believed to occur in the epithelia of the respiratory tract followed by viremia mediated by infected monocytes or macrophages (Griffin, 2007). From the respiratory tract the virus spreads to the lymphatic tissues, where viral amplification occurs and then to other organs like skin, conjunctiva, kidney, lung, gastrointestinal tract, respiratory and genital mucosa, and the liver (Rima and Duprex, 2006). A classical study of the infection of Canine distemper virus (CDV), a related morbillivirus of dogs showed that the virus was present in the bronchial lymphnode and tonsils, one day post infection and it appeared in the lungs only 3 to 6 days later. Moreover, in a ferret model of Canine distemper virus (CDV) pathogenesis (Von *et al.,* 2004) primary replication occurred in the lymphatic tissue of the oral cavity and of the respiratory tract. Viral replication in epithelial cells, sustaining lung and bladder invasion, was found only in later stages of infection (Von *et al.,* 2004). In addition, experimentally infection of macaques with a recombinant wild type Measles virus expressing Green Flourescent Protein (GFP) resulted in predominant infection of SLAM positive lymphocytes and Dendritic Cell (DC) and an occasional infection of epithelial cells at later stages of infection (De Swart *et al.*, 2007).

2.8 Classification of Measles Virus

Measles viruses can be classified into four major types namely: classical, modified, and atypical and congenital measles.

2.8.1. Classic Measles

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

2.8.1.1 Incubation Period

The incubation period begins after measles virus entry via the respiratory mucosa or conjunctiva. The virus replicates locally, spreads to regional lymphatic tissues, and is then thought to disseminate to other reticuloendothelia sites via the bloodstream. The incubation period of measles is usually 10 days with a range generally of eight to ten days. Infected individuals are characteristically asymptomatic during the incubation period, although some have been reported to experience transient respiratory symptoms, fever, or morbiliform rash (Cherry, 2009).The dissemination of measles virus due to viremia, with associated infection of the endothelial, epithelial, monocyte, and macrophage cells, may explain the variety of clinical manifestations and complications that can occur with measles infection.

A second viremia occurs several days after the first, coinciding with the appearance of symptoms signaling the beginning of the prodromal phase (Bernstein *et al.,* 1998).

2.8.1.2 Prodrome Phase

The prodrome phase is defined by the appearance of symptoms which typically include fever, malaise, and anorexia, followed by conjunctivitis, coryza, and cough. The severity of conjunctivitis is variable and may also be accompanied by lacrimation or photophobia (Cherry, 2009). The respiratory symptoms are due to mucosal inflammation from viral infection of epithelial cells. Fever is typically present; the pattern may be variable. Various fever patterns have been described; fever as hight as 40^0C can occur. The prodrome usually last for two to three days but may persist for as long as eight days (Griffin and Bellini, 1996).

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

2.8.1.3 Exanthem

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

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

2.8.1.4 Recovery and Immunity

Cough may persist for one to two weeks after measles infection. The occurrence of fever beyond the third to fourth days of rash suggests a measles-associated complication. Immunity after measles infection is thought to be life long, although there are rare reports of measles re-infection. A measles surveillance program conducted in mid 1960s, for example, identified measles in a 16years old female with a prior history or measles at age eight. A rise in anti-measles IgG but not IgM was noted, suggesting an anamnestic response (Schaffiner *et al.*, 1968).

Measles infection can cause transient immunosuppression due to suppression to T-cell responses. Anergy may be present before the appearance of the exanthem and for several weeks after measles infections. This is exemplified by reports of tuberculosis reactivation in the setting of recent measles infection (Gershon, 1995).

2.8.2 Modified Measles

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

2.8.3 Atypical Measles

Atypical measles occurs in individuals who were vaccinated with the original killed virus measles vaccine (KMV) between 1963 and 1967 and who have incomplete immunity. The KMV unfortunately sensitizes the patient to the measles virus but does not offer any protection. After exposure to the measles virus, a mild or subclinical prodrome of fever, headache, abdominal pain and myalgia precede a rash that begins on the hands and feet and spread centripetally. The eruption is accentuated in the skin folds and may be macular, vesicular, petechial, or urticarial. Despite its severity, atypical measles is generally self-limiting, although pulmomary abnormalities may persist. People having this type of measles do not appear to transmit virus to others (Norby and Oxman, 1990).

2.8.4 Congenital Measles

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

2.9 Immune Response to Measles Virus Infection

Measles virus infection induces an efficient immune response leading to viral clearance and long life immunity against re-infections. It gives rise to a non-specific activation of the immune system characterized by a spontaneous proliferation of peripheral blood mononuclear cells (PBMC) and an up regulation of activation associated cell surface markers. Along with this immune activation, Measles virus induces a transient but severe immunosuppression which increases the susceptibility to opportunistic secondary bacterial and viral infections, mainly in immunocompromised individuals. Different components of the immune response are elicited by Measles virus infection. The first immune response to be activated by Measles virus infection is the innate immune response followed by the development of Measles virus specific adaptive immune responses(Lievano and Helfand, 2004).

2.9.1 Innate Immunity

Innate immune responses are the first line of host defense against the invasion of pathogens. For many virus infections, activation of the innate immune system contributes to the initial control of virus and subsequent activation of the adaptive immune system. Mechanisms involved in the innate immune response include production of cytokines and chemokines, activation of natural killer (NK) cells and macrophages, and activation of the complement cascade. The innate response to Measles virus infection has been studied in many cell culture systems, but the role of the innate response during natural measles has not been well characterized. The innate immune system recognizes viral components through pattern-recognition receptors

(PRRs). This recognition triggers a signal transduction cascade that activates transcriptional responses that culminates in the production of pro inflammatory cytokines and interferon (Lievano and Helfand, 2004).

2.9.2 Adaptive Immunity

Measles virus specific neutralizing antibodies play a key role in preventing re-infection after natural exposure to measles virus. However, the role of antibodies in viral clearance remains unclear since individuals with deficient antibody production are still able to resolve the infection, and depletion of B cells in experimentally infected monkeys does not affect viral clearance.

Nevertheless, it was reported that failure to mount an adequate antibody response to measles virus infection carries a poor prognosis in children. Antibodies are initially detected at the onset of the rash. During acute measles IgM are predominant and this is followed by a switch to IgG2 and IgG3, whereas during recovery IgG3 seems to decrease and IgG1 and IgG4 increase.

Antibodies are produced to all measles virus proteins, with antibodies to the N protein being initially most abundant. Absence of this antibody is an indicator of sero-negativity. The majority of neutralizing antibodies are specific for the H protein although antibodies to F contribute to virus neutralization as well (Von *et al.*, 2004). Measles virus specific neutralizing antibodies binding to infected cells alter intracellular virus replication and this way may contribute to control of infection.

2.9.3 Cellular Immunity

The role of cellular immunity in recovery from measles virus infection has been appreciated from humans presenting immune abnormalities. Children with impaired T cell immunity frequently die of progressive measles while infection is cleared in individuals with agammaglobulinaemia. The cellular immune response is present at the onset of the rash. Expansion of cytotoxic CD8 T cells and production of IFN-γ were observed after MV infection and the establishment of CD8 T cell memory as well (Jaye *et al.,* 1998).

In addition, CD4 T responses are activated during measles virus infection and the synthesis of cytokines is increased. Acute measles generates a Th1 response profile, characterized by IFN-γ and IL-2 production while the recovery phase is characterized by a skew towards a Th2 response resulting in high levels of IL-4 and IL-10 and low IFN-γ levels. The cytokine response after measles virus infection influences disease outcome. In the acute phase of measles, IFN-γ, neopterin and IL-2 rise where as at the time of the rash there is an increase in IL-2, in soluble CD8 and CD4. In the recovery phase, when the rash starts to fade, there is an elevation in the levels of IL-4, IL-10 and IL-13 persisting for several weeks (Jaye *et al.,* 1998).

2.10 Clinical manifestation of Measles Virus

Measles is the fifth leading cause of mortality in the world. Data from the WHO, 2015 indicate that measles virus is responsible for 10% of deaths worldwide in children less than 5 years of age. Worldwide, 40 million cases and 800 000 deaths still occur every year; over half these deaths occur in Africa (De Quadros, 2004). The measles fatality rate increases tenfold or more in developing countries due to younger age o f infection, malnutrition and overcrowding. Measles is a highly contagious viral infection. After an incubation period o f 10 to 12 days, the infected person experiences fever, malaise, sneezing, rhinitis, congestion, conjunctivitis and cough. Koplick spots develop on the inside o f the mouth and a distinctive maculopapular rash usually appears about 4 to 5 days after exposure. The rash begins at the hairline, then extends over the face and upper neck (Plaza and Nuovo, 2005). Over the next 3 days, the rash gradually proceeds downward and outward, eventually reaching the hands and feet.

2.11 Epidemiology

In 2011, the WHO estimated that there were about 158,000 deaths caused by measles, about 430 deaths every day. Mortality in developed countries is about 1 in 1,000 cases (0.1%). In populations with high levels of malnutrition and a lack of adequate healthcare, mortality can be as high as 10%. In cases with complications, the rate may rise to 20 to 30%(WHO, 2012). According to the 2011 United Nations Millennium Development Goals report, "the combination of increased immunization coverage and the opportunity for second-dose immunizations led to a 78% drop in measles deaths worldwide. These averted deaths represent one quarter of the decline in mortality from all causes among children under five.

Even in countries where vaccination has been introduced, rates may remain high. In Ireland, vaccination was introduced in 1985. There were 99,903 cases that year. Within two years, the number of cases had fallen to 201, but this fall was not sustained. Measles is a leading cause of vaccine-preventable childhood mortality. Worldwide, the fatality rate has been significantly reduced by a vaccination campaign led by partners in the Measles Initiative: the American Red Cross, the United States Centers for Disease Control and Prevention (CDC), the United Nations Foundation, UNICEF and the WHO. Globally, measles fell 60% from an estimated 873,000 deaths in 1999 to 345,000 in 2005. Estimates for 2008 indicate deaths fell further to 164,000 globally, with 77% of the remaining measles deaths in 2008 occurring within the Southeast Asian region (De Quadros, 2004).

In 2007 there were 12,132 cases in 32 European countries: 85% occurred in five countries: Germany, Italy, Romania, Switzerland and the UK. 80% occurred in children and there were 7 deaths (McNeil, 2013). Five out of six WHO regions have set goals to eliminate measles, and at the 63rd World Health Assembly in May 2010, delegates agreed a global target of a 95% reduction in measles mortality by 2015 from the level seen in 2000, as well as to move towards eventual eradication. However, no specific global target date for eradication has yet been agreed to as of May 2010 (McNeil, 2009).

In 2014, a review by the Centers for Disease Control reported a total of 911 cases of measles from 2001 to 2011, with an annual median number of 61 cases and concluded that "the elimination of endemic measles, rubella, and CRS has been sustained in the United States. However, in 2015, a measles outbreak occurred in the U.S. and spread rather farther than it should have, because misguided ideas about anti-vaccination and vaccination delaying have decreased the community immunity afforded by proper public health programs. In 2015, a U.S. woman died of pneumonia, as a result of measles. She was the first fatality in the USA from measles since 2003. The woman had been vaccinated for measles and was taking immune suppression drugs for another condition. The drugs suppressed the measles immunity, the woman became infected with measles, did not develop a rash, and contracted pneumonia which caused her death (WHO, 2016).

Between October 2014 and March 2015, a measles outbreak in the German capital of Berlin resulted in at least 782 cases. From January 4 to April 2, 2015, there were 159 reported cases of measles to the CDC. Of those 159 cases, 111 (70%) were determined to have come from an earlier exposure in late December 2014. This outbreak was believed to have originated from the Disney theme parks in California. The initial exposure to the virus was never found. There have been cases associated with this outbreak in seven states, Mexico, and Canada. Of the cases 48% were unvaccinated and 38% were unsure of their vaccination status.

In the Naga Self-Administered Zone in a remote northern region of Myanmar, at least 40 children died during a measles outbreak in August 2016 that was probably caused by lack of vaccination in an area of poor health infrastructure (WHO, 2016).

2.11.1 Measles in Nigeria (EPI)

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

An increase in measles resurgence was reported in Nigeria from 383 cases in 2008 to 2,542 in 2009 in 9,510 in 2010 (WHO, 2009). About 19,184 measles cases were suspected in 2015, out of which 17,248 were confirmed to be measles, with an annual incidence rate 18.2 per 100,000 children (WHO, 2015). Several deaths have been reported in 2016 in Kebbi and Kaduna States, Northern Nigeria. These show that measles burden is still heavy and severe in Nigeria (Weldegebriel *et al.,* 2016). Other states that were involved include Katsina, Yobe, Nassarawa, Bauchi, Taraba, Benue, Zamfara with varying number of deaths. This outbreak has been linked to drop in immunization due to vaccine shortages in regional health clinics and widespread suspicion of the vaccine. Many parents have declined to vaccinate their children against measles as they believe the vaccine is harmful. Suboptimal routine coverage and a wide internal gap between the follow up campaigns may likely led to an accumulation of children susceptible to measles (Weldgebriel *et al.*, 2016).

2.12 Complications of Measles Virus

Measles virus infects multiple organ systems and targets epithelial, reticuloendothelial, and white blood cells, including monocytes, macrophages, and T lymphocytes (Cherry *et al.*, 2008). 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 (Vieth *et al.*, 2001). Measles virus infection leads to a decline in CD4 lymphocytes, starting before the onset of rash and lasting for up to 1 month, and resulting in suppression of delayed-type hypersensitivity as measured by energy to skin test antigens, including tuberculosis antigen. Many of these complications are caused by disruption of epithelial surfaces and immunosuppression (Vieth *et al.*, 2001). Rates of complications from measles vary by age and underlying conditions.

2.12.1 Respiratory Complications

Measles virus respiratory complications includes Otitis Media, Laryngotracheobronchitis, Pneumonia and Measles Pneumonia in Immunocompromised Patients

2.12.1.1 Otitis Media

Otitis media is the most common complication of measles reported in the United States and occurs in 14% of children 5 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.

2.12.1.2 Laryngotracheobronchitis

Laryngotracheobronchitis or "measles croup" was noted in 9%–32% of US children hospitalized with measles (Butler *et al.*, 1993) The majority of affected children were 2 years old. In one-third to one-half of such cases, culture of samples from the trachea yields positive results for bacterial pathogens, with a purulent exudate and evidence of secondary bacterial tracheitis, pneumonia, or both. The most commonly cultured organism is *Staphylococcus aureus,* although *Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Escherichia coli,* and *Enterobacter* species have also beenidentified (Manning *et al.*, 1991). In a series of 6 children intubated becauseof measles croup, viral cultures revealed that one child wascoinfected with adenovirus and another with herpes simplexvirus (HSV). Laryngotracheobronchitis was the secondmost common cause of death in US children hospitalized withmeasles, after pneumonia (Butler *et al*., 1993)

2.12.1.3 Pneumonia

Measles infects the respiratory tracts of nearly all affected persons. Pneumonia is the most common severe complication of measles and accounts for most measles-associated deaths (Hussey and Clements, 1996) in studies of unselected hospitalized children with measles, 55% had radiographic changes of bronchopneumonia, consolidation, or other infiltrates; 77% of children with severe disease and 41% of children with mild disease had radiographic changes. (Henneman *et al.*, 1996) In recent years, pneumonia was present in 9% of children 5 years old with measles in the United States in 0%–8% of cases during outbreaks and in 49%–57% of adults (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. 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 (Hussey and Clements, 1996). Other bacteria (e.g., *Pseudomonas* species, *Klebsiellapneumoniae,* and *E. coli*) are less common causes of severe pneumonia associated with measles. In studies of young adult military recruits with pneumonia associated with measles, *Neisseria meningitidis* was a probable cause in some cases (Henneman *et al.*, 1996). Pneumomediastinum and mediastinal emphysema have beenreported as complications of measles in several countries. 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.

2.12.1.4 Measles Pneumonia in Immunocompromised Patients

Among immunocompromised persons, diffuse progressive pneumonitis caused by the measles virus is the most common cause of death (Nakano *et al.*, 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 2 weeks after the first onset of symptoms. Other patients have had reappearance of rash and pneumonitis after long intervals following"classical" measles (Nakano *et al.*, 1996).

2.12.2 Gastrointestinal Complications

Measles probably infects the intestinal tracts of most persons with measles. A gastric biopsy obtained the day before rash onset from a 44-year-old man revealed characteristic giant cells that were positive for measles by immunologic staining, and 8 of 10 children exposed to the man subsequently developed 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. This complication includes diarrhea (Greenberg *et al.*, 1991)

2.12.2.1 Diarrhea

In the United States, 8% of all reported measles cases during 1987–2000 were complicated by diarrhea. Rates were higher in those 5 or 13 years old. Among hospitalized persons with measles in the United States, 30%– 70% had diarrhea (Henneman *et al.*, 1996). Feachem and Koblinsky found that 15%–63% of measles cases from community based studies from developing countries in the prevaccine era were complicated by diarrhea and that 9%–77% of all diarrheal deaths were measles-associated. Stools of children with measles associated diarrhea usually have the same bacteria as those of children with diarrhea not associated with measles. Measles-associated diarrhea 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 (Wong and Geotz, 1993).

Dehydration was found in 32% of hospitalized patients in California (Wong and Geotz, 1993). Morley first described the high rates of gastrointestinal complications that occurred after measles in developing countries: mouth sores, decreased food intake, protracted diarrhea, weight loss, and precipitation of severe protein calorie malnutrition (Wong and Geotz, 1993). Noma (cancrum oris), a progressive oral lesion that destroys orofacial tissue, has been noted after measles in Africa and India. In young adults, measles is associated with hepatitis, hypocalcemia, and elevation of creatinine phosphokinase levels (Wong and Geotz, 1993).

2.12.3 Neurological Complications

Measles virus Neurological complications includes Febrile seizures, Post infectious Encephalomyelitis, Subacute sclerosing panencephalitis (SSPE), Measles Encephalitis in Immunocompromised Patients

2.12.3.1 Febrile seizures

Febrile seizures occur in 0.1%–2.3% of children with measles in the United States and England (Henneman *et al.*, 1996). 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.

2.12.3.2. Post infectious Encephalomyelitis

Post infectious Encephalomyelitis (PIE) occurs in 1–3 per 1000 infected persons, usually 3–10 days after onset of rash (Stratton, 2001). 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. Although measles virus was found in cerebrovascular endothelial cells in a person who died during the first few days of rash the virus usually is not found in the central nervous systems of persons with PIE. 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 33% of survivors have lifelong neurological sequelae, including severe retardation, motor impairment, blindness, and sometimes hemiparesis (Wong and Geotz, 1993).

2.12.3.3 Subacute sclerosing panencephalitis (SSPE)

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. 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 (Wong and Geotz., 1993). Subacute sclerosingpanencephalitis (SSPE) occurs on average in 1 per 8.5 million persons, who develop measles in the United States, but the rate appears to be higher in some other countries. Factors responsible for persistence of measles virus in these persons are not known, nor is it known whether measles virus persists in otherwise normal hosts. Geographic clustering of SSPE occurs in several countries, and there is an increased incidence in children residing in rural areas. In 2 studies, children with SSPE had more close exposure to birds than did control subjects. These data suggest that as-yet-undefined environmental factors, most likely another infectious agent, contribute to this disease (Halsey *et al*, 2001).

2.12.3.4 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 5 weeks to 6 months after acute measles. The illness begins with mental-status changes and seizures in the absence of fever; 180% of deaths occur within weeks (Halsey *et al.*, 2001).

2.12.4 Ocular Complications

Conjunctivitis occurs in most persons with measles, and inflammation of the cornea (keratitis) is common. In a study of 61 Turkish military personnel with measles, 57% had keratitis detected by slit lamp examination. 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 (Niedermeyer *et al.*, 2000).

2.13 Factors Affecting Measles Morbidity and Mortality Rates

The factors affecting Measles virus Morbidity and Mortality rates includes Sex, Age, Malnutrition and Vitamin A deficiency.

2.13.1 Sex

Historically, males have had higher case-fatality rates than did females. An analysis of vital statistics data from several countries (primarily in the Americas and Europe) for the years 1950–1989 suggests that women and girls may have slightly higher mortality rates after measles than do men and boys but recent surveillance data from the United States and United Kingdom show equal rates of complications for men and women. Pregnant women have an increased risk of complications, including death, following measles (Niedermeyer *et al*., 2000).

2.13.2 Age

Complication rates, including mortality, from measles are highest in children 5 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. Okada *et al*. (1995) found that young infants and adults have more severe and a longer duration of lymphopenia after measles than do children.

2.13.3 Crowding

Several studies from West Africa and Europe show that children who develop measles after within household exposure have higher case-fatality rates than do children who are exposed to measles outside the household. This phenomenon is most likely secondary to a higher inoculum from more intensive and prolonged exposure compared with more casual exposures outside the home. In Bangladesh, Koenig *et al.* (1993) found that children who lived in a house of 18.6 m^2 had 2.6 times the risk of dying from measles as that of children who lived in houses of 137 m^2. In the United

States, however, no relationship between crowding and measles case-fatality rates has been found.

2.13.4 Malnutrition

Malnourished children have impairments in multiple aspects of the immune system, prolonged excretion of measles virus, and higher measles case-fatality rates. Measles contributes to the development of malnutrition because of protein-losing enteropathy, increased metabolic demands, and decreased food intake. Children who have measles early in life have significantly lower mean weights for age than do children of the same age who do not develop measles (Wong *et al.*, 1993).

2.13.5 Vitamin A deficiency

Children with clinical or subclinical vitamin A deficiency in many developing countries have increased case-fatality rates. Measles and other illnesses are associated with reductions in serum retinol concentrations and may induce overt vitamin. A deficiency Hospitalized US measles patients frequently have deficiencies in vitamin A; these children are more likely to have pneumonia or diarrhea after measles In countries with high measles mortality, treatment with vitamin A once daily for 2 days (200,000 IU for children 12 months of age or 100,000 IU for infants 12 months) is associated with an 50% reduction in mortality. The World Health Organization recommends vitamin A therapy for all children with measles. For hospitalized children 2 years old with measles in the United States, the American

Academy of Pediatrics recommends a single dose of vitamin A (200,000 IU for children 12 months; 100,000 IU for those 12 months) (Barnard, 2004).

2.14 Prognosis

The majority of people survive measles, though in some cases, complications may occur. Possible consequences of measles virus infection include bronchitis, sensorineural hearing loss and in about 1 in 10,000 to 1 in 300,000 cases panencephalitis, which is usually fatal. Acute measles encephalitis is another serious risk of measles virus infection. It typically occurs two days to one week after the breakout of the measles rash and begins with very high fever, severe headache, convulsions and altered mutation. A person with measles encephalitis may become comatose, and death or brain injury may occur (Halsey *et al.*, 2001).

2.15 Diagnosis of Measles Virus

Clinical diagnosis of measles virus requires a history of fever of at least three days, and with one of the three symptoms (cough, coryza and conjunctivitis). Observation of the Koplik's sport is diagnosis of measles (Taylor *et al.,* 2002). However, as the vaccination program progresses, atypical forms of measles have emerged and laboratory diagnosis may be required. Laboratory confirmation of measles infection requires isolation of measles virus, detection by Polymerase Chain Reaction (PCR) or detection of Measles IgM by serological testing (Helfand *et al.,* 1997).

2.15.1 Microscopy

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

2.15.2 Virus Isolation

Measles virus can be isolated from a variety of sources, e.g. throat or conjunctival washing, sputum, urinary sediment cells and Iymphocytes. Primary human kidney (HEK) cells are the best, although primary monkey kidney can be used as well. Continuous cell lines such as vero cells can also be used although they are not as efficient as primary cell lines. A cytopathic effects (CPE) develops between two to fifteen days, and consist of either a broad syncytium or a stellate form with inclusion bodies visible. The presence of measles can be confirmed by haemadsorption. In acute measles, the isolation rate is difficult and the success rate is low. Isolation is most likely to be successful from material taken in the prodrome phase but not in the later stage after the rash has developed. Therefore isolation should only be attempted in complicated cases such as suspected Subacute sclerosing panencephalitis (SSPE) where the Iymphocytes may carry the virus, and in immunocompromised individuals developing pneumonia (WHO, 2000).

2.15.3 Measles Culture and Immunofluorescence

Measlse virus may be identified by cell culture from blood, conjunctival swab, throat swab, nasophryngeal swab or aspirate if taken within five days of the onset of the rash, and from urine for up to ten days after the onset of rash. Throat swabs and nasal swabs are suitable for culture. Antigen testing by immunofluorescence may also be performed on respiratory or urine samples collected during the prodrome or within five days after rash onset. Nasopharyngeal aspirates or nasopharyngeal swabs are the preferred samples for antigen detection by immunofluorescence (WHO, 2012).

2.15.4 Serology

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

2.15.5 Measles IgM

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

75% of measles cases three days after rash onset, rising to approach 100% after seven days (WHO, 2000).

2.15.6 Measles IgG

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

2.15.7 Polymerase Chain Reaction

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

2.16 Treatment of Measles Virus

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

Some patients will develop pneumonia sequel to the measles. Other complications include ear infections, bronchitis, and encephalitis. Acute measles enecphalities has a mortality rate of 15%. While there is no specific treatment for measles encephalitis, antibiotics are required for bacterial pneumonia, sinusitis, and bronchitis that can follow measles infection. The following antiviral drugs may also be prescribed.

2.16.1 Ribavirin

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

2.16.2 Vitamin A

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

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

2.16.3 Immune Globulin (Human)

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

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

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

2.17 Prevention of Measles Virus

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

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

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

2.17.1 Measles, Mumps and Rubella Vaccine

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

An improved measles vaccine was developed in 1968. Vaccines for the mumps and rubella became available in 1967 and 1969 respectively. The three vaccines (for measles, mumps and rubella) were combines in 1971 to become the measles-mumps-rubella (MMR) vaccine. It was first developed by Maurice Hilleman. It is a mixture of live attenuated virus of the three diseases, administered via injection in 1973. Merek's further attenuated combination of Measles-mumps-rubella vaccines was licensed and as of July 2012, it is the only measles-containing vaccine available in the U.S (Edmonston-Enders strain, formerly called "Moraten") (CDC, 2012).

Vaccination with MMR vaccine provides significant improvement in the efficiency of paediatric immunization through the administration of three vaccines in a single injection, reducing cost while increasing immunization coverage against the three diseases. MMR vaccine is usually administered at 12-15months and 45years of age in the U.S (Jefferson *et al.*, 2005).

Measles vaccine produces an inapparent or mild, non-communicable infection. Measles antibodies develop in approximately 95% children vaccinated at 12 months of age and 98% of children vaccinated at 15 months of age. Seroconversion rates are similar for single-antigen measles vaccine, MMR and MMRV. Approximately 2%-5% of children who receive only one dose of MMR vaccine is generally administered to children around the age of one year, with a second dose before starting school, between ages four and six years in the United States, the second dose is a dose to produce immunity in the small number of persons (2-5%) who fail to development measles immunity after the first dose. In the United States, the vaccine was licensed in 1971 and the second dose was introduced in 1989. It is widely used around the world. Since introduction of its earliest versions in the 1970s, over 500million doses have been used in over 60 countries. It is usually considered a childhood vaccination. However, it is also recommended for use in some cases of adults with HIV. MMR vaccine failure may occur because of passive antibody in the vaccine recipient, damaged vaccine, incorrect records, or possibly other reasons. Most person fail to respond to the first dose will respond to a second dose. Studies indicate the more than 99% of persons who receive two doses of measles vaccine (with the first dose administered no earlier that the first birthday) develop serologic evidence of measles immunity (CDC, 2010).

2.17.2 Inactivated Vaccine

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

2.17.3 Live Vaccine

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

2.17.4 Adverse Reactions Following Measles Vaccination

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

Fever is the most common adverse reaction following MMR vaccination. Although measles, mumps, and rubella vaccines may cause fever after vaccination, the measles component of MMR vaccine is most often assoicated with this adverse reaction. After MMR vaccination, 5%to 15% of susceptible persons develop a temperature of 103^0F ($39,4^0$C) or higher, usually occurring 7 to 12 days after vaccination and generally lasting 1or 2 days. Most persons with fever are otherwise asymptomatic.

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

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

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

Arthralgias and other joint symptoms are reported in up to 25% of susceptible adult women given MMR vaccine. This adverse reaction is associated with the rubella component. Allergic reactions following the administration of MMR or any of its component vaccines are rare. Most of these reactions are minor and consist of a wheal and flare or urticaria at the injection site. Immediate, anaphylactic reactions to MMR or its component vaccines are extremely rare. Allergic reactions including rash, pruritus and purpura have been temporally associated with mumps vaccination, but there are uncommon and usually mild and of brief duration (CDC, 2012).

2.17.5 Measles Vaccine and Autism

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

2.18 Control of measles virus

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

Disease also can be modified within three days of exposure by reducing the dose of immunoglobulin, immunoglobulin may protect recipient for about four weeks.

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

2.18.1 Post Exposure Prophylaxis

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

2.18.2 Immunocompetent Patients and Staff

- Non-immune, immunocompetent, non-pregnant adults should be offered MMR irrespective of time since exposure.

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

2.18.3 Immunocompromised Patients

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

2.18.4 Pregnant Patients/Staff

- The measles immune status of pregnant patients should ideally be determined prior to admission to hospital.
- Non-immune pregnant patients or staff should be considered for use of immunoglobulin if within six days of exposure.
- Pregnant patients or pregnant staff who have had at least one previous dose of MMR vaccine do not require immunoglobulin(CDC, 2012).

2.18.5 Infants

- In exposed infants between six and twelve months of age, if immunocompromised, use of immunoglobulin should be discussed with the clinical team caring for that patient if within six days of exposure. If immunocompetent, MMR vaccine should be offered if within 72 hours of exposure. Alternatively, if within six days of exposure, use of immunoglobulin should be discussed with the clinical team caring for the patient(CDC, 2012).
- In exposed less than six months of age, if the mother is non-immune and exposure occurred less than six days earlier, use of immunoglobulin should be discussed with the clinical team caring for that patient.
- Premature infants less than 28 weeks gestation should be considered non-immune irrespective of maternal immune status.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area, the North Western region of Nigeria (Figure 3.1) has a population of about 35,786,944(National Population Census, 2006) and consist of six states, Sokoto, Kebbi, Kano, Zamfara, Jigawa, Katsina and Kaduna state. For the purpose of this research, three state will be selected which will include Sokoto, Jigawa and Kaduna state using suspected measles virus samples.

3.1.1 Sokoto State

Sokoto State is located in the extreme northwest of Nigeria, near to the confluence of the Sokoto River and the Rima River. As at 2005 it has an estimated population of more than 4.2 million. Sokoto City is the modern-day capital of Sokoto State (and its predecessor, the Northwestern State). Sokoto State is in the dry Sahel savannah, surrounded by sandy savannah and isolated hills. With an annual average temperature of 28.3 °C (82.9 °F), Sokoto is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40 °C (104.0 °F) and the dryness makes the heat bearable. The warmest months are February to April when daytime temperatures can exceed 45 °C (113.0 °F). The rainy season is from June to October during which showers are a daily occurrence. The showers rarely last long and are a far cry from the regular torrential rain known in wet tropical regions. From late October to February, during the cold season, the climate is dominated by the Harmattan wind blowing Sahara dust over the land.

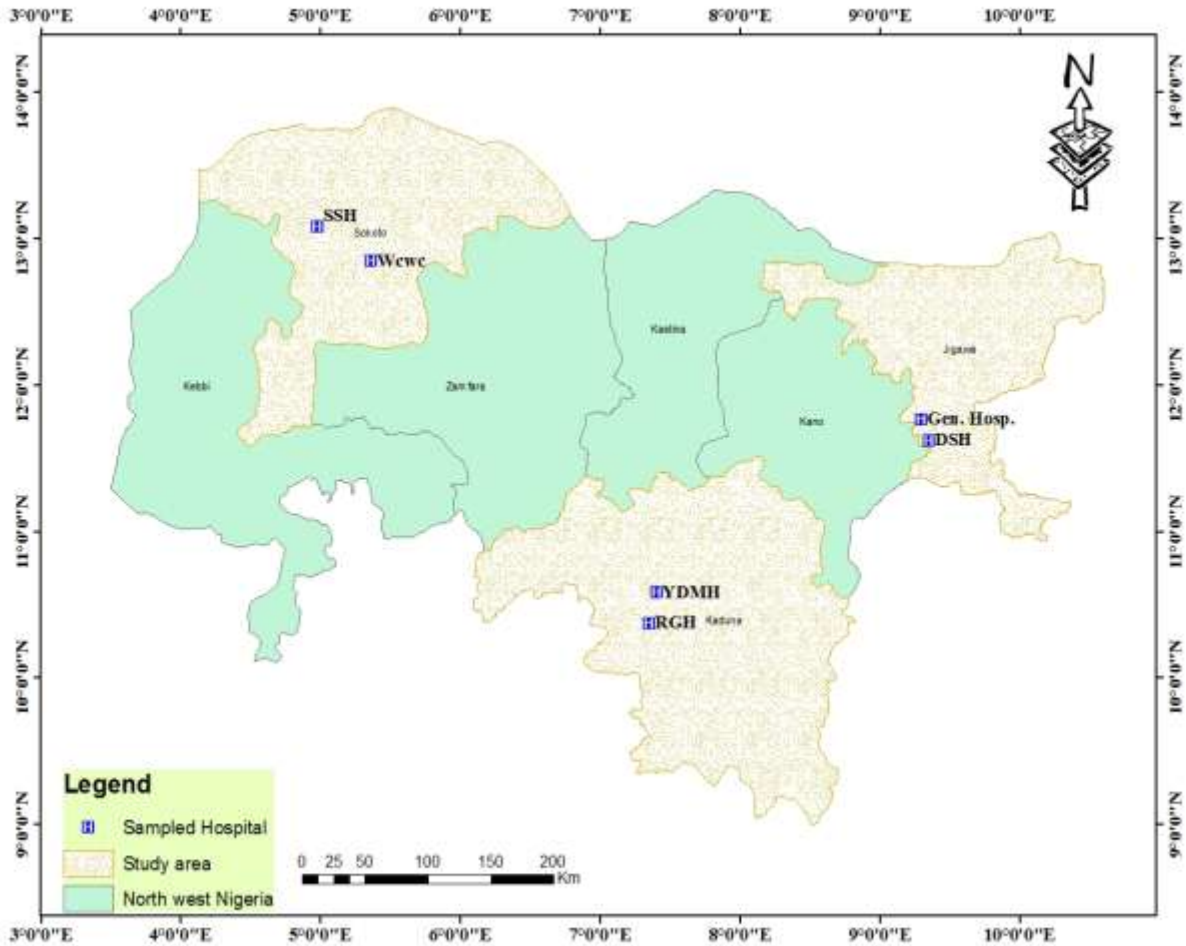

Figure 3.1: Map of North Western Nigeria showing the selected state and the sampling areas

3.1.2 Jigawa State

Jigawa State is one of thirty-six states that constitute Federal Republic of Nigeria. It is situated in the north-western part of the country between latitudes 11.00°N to 13.00°N and longitudes 8.00°E to 10.15°E. Kano State and Katsina State border Jigawa to the west, Bauchi State to the east and Yobe State to the northeast. To the north, Jigawa shares an international border with Zinder Region in The Republic of Niger.

3.1.3 Kaduna State

Kaduna State, north central Nigeria, is politically classified as belonging to the now 'North - West' zone of the current six (6) Geo - political zones of Nigeria. It is populated by about 59 to 63 different ethnic groups if not more with the exactitude of the number requiring further verification through a genuine field work [Hayab, 2014]

3.2 Study Design

This study was a descriptive cross-sectional study which covers both the outbreak and the sporadic cases within the study period. Two hospitals were selected from each state, in Sokoto State, samples were collected from the Women and Children Welfare Clinic and Sokoto Specialist Hospital, in Jigawa State, samples were collected from General Hospital Dutse and Danmasara Specialist Hospital while samples for Kaduna state were collected from Yusuf Dantsoho Memorial Hospital and Rigasa General Hospital.

3.3 Study Population

Children under 0-12 years of age with suspected measles symptoms like, fever of 38^0 C or greater, cough, redness of the eyes and maculopapular rash, attending selected hospitals in the study area were included.

3.4 Ethical Approval and consent

Ethical clearance for this study (Appendices I - III) was obtained from the ethical committee of the State Ministry of Health in each state on the selected hospitals and consent (Appendix IV) was obtained from the care givers of the children with suspected measles cases. The purpose of the study and procedure was explained to the parents, caregivers, the management and staff of respective hospitals.

3.5 Inclusion and Exclusion Criteria

The inclusion criteria for this study were children age 0-12 years of both sexes with suspected measles cases and whose parents consented.

The exclusion criteria were children that are >12years of age of both sex, non consenting parents and non measles cases.

3.6 Questionnaire Administration

Children under 0 -.12 years of age were studied. Prior to sample collection, the parents or care givers of the children under study were given a letter of introduction seeking their consents and a detailed structured questionnaire (Appendix V) was administered to capture data on demographic and risk factors such as Age, Sex, previous history of measles vaccination and recurrent cases.

3.7 Sample Size

A prevalence of 32.2% as reported in a study conducted at Kaduna state by Chechet *et al*. (2014) was used to determine the sample size, using the equation below (Sarmukaddam and Gerad, 2006) at 95% confidence level

$N=\frac{Z^2 pq}{L^2}$ Where

N = sample size

Z = standard normal distribution at 95% confidence interval = 1.95

p = prevalence rate which is taken as 32.2% = 0.322,

q = (l-P): 1 - 0.322 = 0.68

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

Therefore,

N = $(l.95)^2$ x 0.32 x 0.68

$(0.05)^2$

N = 334.42 samples

The sample size calculated was 334.42, which is the minimum number of samples to be used for this study. Therefore 450 samples were collected and used for the study.

3.8 Sample Collection and Processing

The hospitals selected were visited after obtaining ethical approval. Samples were collected using a sterile disposable syringe, 2ml of venous blood was collected aseptically from each clinical case children with the assistant of an experienced laboratory technician and dispensed into a sterile labeled plain specimen bottles and transported safely to the Department of Microbiology laboratory, Ahmadu Bello University, Zaria in a cold park after which the samples were then centrifuge in order to separate the serum using a clean Pasteur pipettes and stored frozen at -80^{o}C until is needed for analysis.

3.9 Samples Analysis

Serum samples from all samples measles cases were serially diluted. One (1) ml of serum sample was transferred into 9 ml of sample diluent to make a 10% serum dilution. The children serums were screened for measles virus antigens using IgM and IgG enzyme-linked immunoassay (ELISA) kit at the Department of Microbiology, Ahmadu Bello University. The serum samples were transferred to the DNA LABS Limited, Kaduna, Nigeria for further analysis.

3.10 Detection of Measles Virus IgM and IgG Using Enzyme-Linked Immunosorbent Assay (ELISA)

Serum samples were analyzed for the presence of measles virus using commercially available Enzyme Linked Immunosorbent Assay (ELISA) test kit obtained from

(Diagnostics Automation U.S.A). All assays were performed according to the manufacturers' instruction.

3.10.1 Principle of ELISA for the Detection of Measles Virus

Enzyme Linked Immunosorbent Assay (ELISA) relies on the ability of biological materials (e.g. antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When the antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase with wash buffer solution. This is followed by the addition of goat anti-human IgG or IgM globulin conjugated with horseradish peroxidase which will bind to the antibody antigen complexes. The excess conjugate is removed by washing with the wash buffer, followed by the addition of chromogen and substrate tetramethy ibenzidine (TMB) for 10 minute at room temperature. If specific antibody to the antigen is present in the patient's serum, a blue coloration develops. When the enzymatic reaction is stopped with IMH_2SO4 the contents of the wells turns yellow. The colour, which is indicative of the concentration of antibody in the serum, was then read on a suitable ELISA micro well plate reader.

3.10.2 Assay Procedure of ELISA for the Detection of Measles Virus

The samples, microtiter wells and reagents were brought to room temperature (25^0C-28^0C) before the analysis was carried out. Wells for samples and control were inserted into the microtiter well holder. 100µl each of the dilutions were dispensed into appropriate wells, and incubated at room temperature for 30minutes. After incubation, the wells were washed three times with washing buffer, excess washing solution were removed by gentle tapping on a tissue cloth, this was followed by the addition of 100µl enzyme conjugate and incubation at room temperature for 30 minutes, the same initial washing steps was followed to remove excess conjugate, the substrate were then dispensed into the wells and incubated for 20mins at room temperature, finally the reaction was stopped by addition of 100µl stop solution and the resulting reaction was read at 450nm using ELISA reading machine.

3.10.3 Interpretation of Result

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

Cut – off Calibrator Value = Correction Factor X Mean Calibrator O.D

ISR Value = O.D Obtained for patient's serum

Cut – off Calibrator Value.

ISR Value C 0.90 was considered as negative which means that no significant level of detectable antibody to measles was present in the sample. ISR Value within 0.91- 1.09 was considered as equivocal and the sample was tested. ISR Value > 1.10 was considered as positive, indicating significant level of detectable IgG and IgM antibody to measles in the sample, which was indicative of current or recent infection.

3.11 Detection of Measles Virus Using Reverse Transcription Polymerase Chain Reaction (RT - PCR)

3.11.1 RNA Extraction

Total measles virus RNA from clinical samples was obtained using the QIAamp Viral RNA kit (Qiagen Hilden Germany) according to manufacturer's protocol. All extractions were carried out at DNA LABS LIMITED Kaduna. Blood samples from confirmed IgM measles cases were obtained by centrifugation for 2mins at 2300rpm. RNA were extracted from pellet using the spin-column procedure. Two hundred microlitre of each positive blood sample was transferred into a clean 15µl microlitre tube. Five hundred microlitre (500µl) of lysis buffer AVL was dispensed into each clean tube and incubated at room temperature for 10 minutes. The samples were mixed by pulse – vortexing for one minute. The sample mixtures were transferred into spin columns and centrifuged at 12000rpm for five minutes. The spin columns were transferred into a clean 2µl collection tube and the filtration was discarded. Five hundred microlitre (500µl) of wash buffer AWI was added to each spin column and centrifuged at 12000rpm for two minutes. The filtrate was discarded. The empty spin columns were centrifuged at 10000rpm for two minutes. The spin columns were transferred into new clean tubes and 60µl of elution buffer AVE was added into each spin column and allowed to stand at room temperature (25^{o}C) for 2 minutes. Centrifugation was done at 10000 rpm for two minutes to elute the RNA from the spin columns into the tube. A double elution was done by adding 60µl of elution buffer AVE into each spin column and centrifuged after two minutes at 10000rpm for two minutes. RNA samples were stored at – 80^0C until required for use.

3.11.2 RT-PCR Amplification

Specific cDNA of Measles Virus nucleoprotein was synthesized by a reverse transcription using Superscript III Reverse Transcriptase (Invitrogen, Merelbeke, Belgium). The Measles Virus cDNA was amplified by forward and reverse PCR using primers (Table 3.1) MN5 (5′ - GCC ATG GGA GTA GGA GTG GAA C - 3′) and MN6 (5′ - CTG GCG GCT GTG TGG ACC TG - 3′) for the first round and primers Nf1a (5′ - CGG GCA AGA GAT GGT AAG GAG GTC AG - 3′) and Nr7a (5′-AGG GTA GGC GGA TGT TGT TCT GG-3′) for the second round. Both PCRs were performed in a total volume of 25 µL that contained 1.8 mmol/L $MgCl_2$, 1× PCR buffer, 0.2 mmol/L dNTPs, 0.5 U Platinum Taq (Invitrogen), and 0.8 µmol/L forward and reverse primer (Eurogentec, Seraing, Belgium). One microliter of cDNA or 5 µL of first-round product (diluted 50× in water) was added as template. Cycling conditions was denatured at 94°C for 2 min; 35 (first round) or 30 (second round) cycles of amplification at 94°C for 30sec, 55°C (first round) or 58°C second round) for 1 min, and 72°C for 1 min; and a final extension at 72°C for 5 min

Table 3.1: Primers Sequence used in Measles Virus Amplification and Sequencing

Target gene	Primers sequence Amplicon size	References
MN 5	5′ GCCATGGGAGTAGGAGTGGAAC 3′528bp	Jacques *et al.*, 2010
MN 6	3′ CTGGCGGCTGTGTGGACCTG 5′527bp	Jacques *et al.*, 2010
Nf1a	5′CGGGCAAGAGATGGTAAGGAGGTCAG3′527bp	Jacques *et al.*, 2010
Nr7a	3′AGGGTAGGCGGATGTTGTTCTGG 5′527bp	Jacques *et al.*, 2010

3.11.3 Detection and Identification of the Amplified PCR Products

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

3.12 Sequencing

Sequencing reaction was prepared in a 0.2mL thin-walled tube with 2 tubes per template DNA for a forward sequencing primer and a reverse sequencing primer reaction. All reagents were kept on ice box while preparing the sequencing reactions, 8µl of sterile water and 2µl of diluted DNA (at 100ng/ul) were added to each sequencing tube. The tubes were spinned briefly. The DNA water was preheated and mixed at 96^0C for 1 min in the thermo cycler and then cools on ice box.8µl DTCS

Quick Start master was added and mixed to each tube followed by 2µl of T-7 forward sequencing primer added to one of the tubes for a template and 2µl of SP6 reverse sequencing primer as added to the other tube for a template.

The reaction components were mixedthoroughly. The liquid at the bottom of the tube was concentrated and briefly centrifuging before thermo cycling with the following conditions; 96^{0}C for 20 seconds, 50^{0}C for 20 seconds and 60^{0}C for 4 minutes

3.12.1 Sequencing Clean up

PCR products were purified by using the Jetquick PCR product Purification Spin Kit (Genomed, Lohne, Germany). Twenty-five cycles of cycle sequencing (2-min elongation) were performed by using a BigDye Terminator version 6.1 Cycle Sequencing kit (Applied Biosystems, Nieuwerkerk, the Netherlands) with Nf1a or Nr7a primers (0.5 μmol/L) and 10 ng of purified PCR product. The product was sequenced for measles virus genotyping using an ABI 3130 Genetic Analyzer (Applied Biosystems).

3.13 Data Analysis

Data generated from questionnaire and laboratory analysis results were entered into Microsoft excel. The data obtained were analyzed with SPSS (statistical package for social science) version 21.0 software program. Pearson's Chi – Square (x^2) was used to test the significance of variables at 95% confidence interval and p – value < 0.05

was considered significant. Bar chart was used to visually display the results of where applicable.

CHAPTER FOUR

4.0 RESULTS

4.1 Measles Virus in Children

Out of the 450 blood samples screened for the presence of measles virus IgG and IgM antibody in children 0 – 12 years of age 385(85.6%: 385/450) were positive for measles virus IgG and 158 (35.17%: 158/450) were positive for measles virus IgM antibody (Table 4.1), There was statistically significant association between the prevalence of measles virus infection and selected hospitals IgG (x^2= 32.458, df=5, p= 0.02), IgM (x^2=80.371, df= 5, p= 0.000).

The seroprevalence of measles virus IgG and IgM antibody in children with respect to selected states is shown in Table 4.2. Children in Sokoto state had the highest seroprevalence of IgM IgG and IgG (50.7%:76/150; 88%: 132/150) compared to children in Kaduna with a lower seroprevalence of IgM and IgG (21.3%:32/150; 82%: 123/150). There was statistically significant association between prevalence of measles virus infection and selected states (x^2=28.637, df= 2, p= 0.000).

Measles virus was detected in all age groups with the highest IgG prevalence of 97.0% (195/201) and IgM prevalence of 47.8% (96/201) occurring in children of age group 1 – 5 years old. Children who were <1 years old had the least prevalence of 52.0% (40/80) for measles IgG antibody and children in age group 11 – 15 years old had the least prevalence for measles virus IgM antibody, (11.9%, 08/67). This result is presented in Figure 4.1.

Table 4.1: Seroprevalence of Measles IgM and IgG Antibody in the Selected Hospitals within the North Western Nigeria

Hospitals	N	IgM Positive (%)	χ^2	P-Value	IgG Positive (%)	χ^2	P- Value
YDMH	75	25 (33.30)	80.371	0.000*	68 (90.70)	32.458	0.000*
RGH	75	07(09.30)			55 (73.30)		
DSH	75	25 (33.30)			56 (74.70)		
GHD	75	25 (33.30)			74 (98.70)		
WCWC	75	19 (25.30)			62 (82.70)		
SSH	75	57(76.00)			70 (93.30)		
Total	450	158 (35.10)			385 (85.60)		

IgM: χ^2 =80.371, df=5, p=0.000* IgG: χ^2 =32.458, df=5, p=0.000*

KEY: n = Number of subjects Tested, (%ve) = Percentage positive, χ^2=Chi-Square, df= Degree of freedom, p=P-value, YDMH, Yusuf Dantsoho Memorial Hospital, RGH = Rigasa General Hospital, DSH = Danmazara Specialist Hospital, GHD = General Hospital Dutse, WCWC = Women and Children Welfare Center and SSH = Sokoto Specialist Hospital.

Table 4.2: Seroprevalence of Measles IgM and IgG antibody within the states in the North Western Nigerian

State	N	IgM n(%)	IgG n(%)
Sokoto	150	76(50.7)	132(88.0)
Jigawa	150	50(33.3)	130(86.6)
Kaduna	150	32(21.3)	123(82.0)
Total	**450**	**158(35.1)**	**385(85.6)**
χ^2		**28.637**	**2.410**
P – Value		**0.000**	**0.0002**

Key: N= Number Examined, n= Number Positive, %=Percentage Prevalence, IgM= Immunoglobulin M, IgG=Immunoglobulin G, χ^2=Chi-Square value

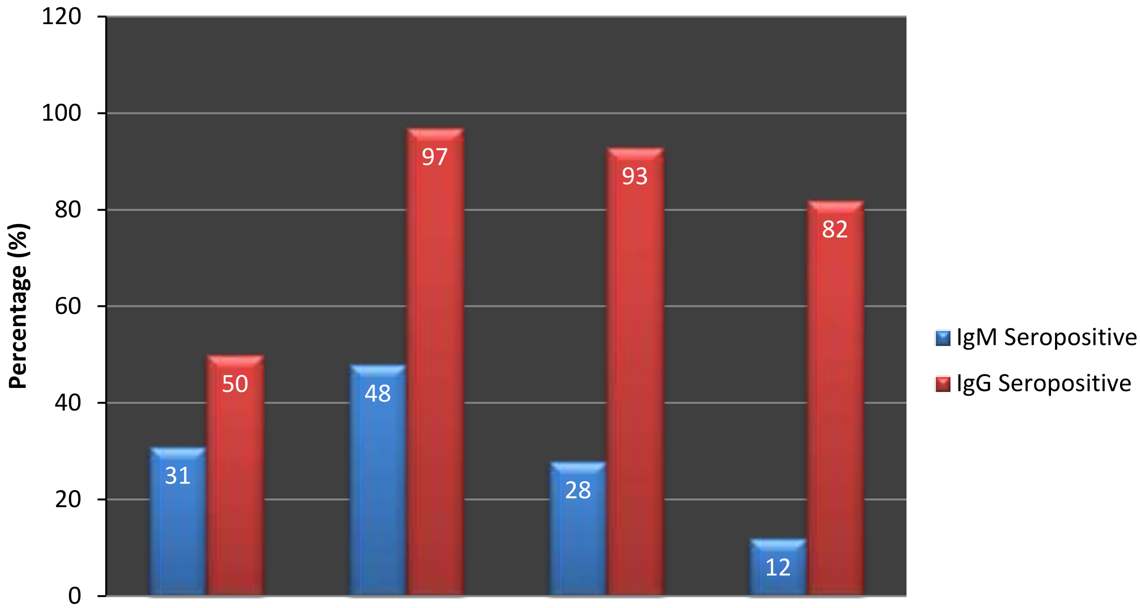

Fig. 4.1: Seroprevalence of Measles IgM and IgG Antibodies in Relation to Age Group within the Selected Hospitals in North western nigeria

There was statistically significant association between measles virus infection and age of the children (x^2= 108.592, df =3, p=0.000) and IgM (x^2 = 32.427, df =3, p=0.000).

The seroprevalence of measles virus IgG and IgM with respect to sex of the children was observed to be higher in male children with the prevalence of 85.8% (199/232) for measles IgG and 35.8% (83/232) for measles IgM respectively and lowest in female children with the prevalence (85.3%:186/218), (34.4%:75/218) respectively. There was no statistically significant association between measles virus infection and sex of the children (x^2= 0.017, df=1, p=0.896), (x^2= 0.093, df = 1, p= 0.761), as shown in Figure 4.2.

The result was also analyzed according to some demographic factors and presented in Table 4.3. Seroprevalence of measles virus IgM infection in children with respect to parent's educational status showed that parents with no formal education had the highest seroprevalence of 62.8% (71/113) compared to children whose parents had tertiary education with the prevalence of 6.6%(04/61). There was a statistically significant association between measles virus and educational status of parents (x^2= 108.671, df=4, p=0.000).

The result also revealed that there was a statistically significant association between parents occupation and the presence of measles virus IgM infection (x^2= 21:361, df=1, p=0.000). Highest prevalence of 43.2%(121/280) was observed in children whose parents were civil servant and the least prevalence (21.7%:37/170) in children whose parents were self employed.

The distribution of measles virus infection with respect socio economic status, children of parents with low socio economic status had the highest seroprevalence (43%:101/235) compared to children of parents with high socioeconomic status with the least prevalence (20.7%:24/116) There was a statistically significant association between measles virus infection and parent socio economic status (x^2= 17.111, df =2, p= 0.000).

The result also revealed a statistically significant association between measles virus infection and the nutritional status of children (x^2=12.444, df = 2, p=0.002) highest prevalence was recorded in children with poor nutritional status (40.8%:98/240) and children with normal nutritional status had the least seroprevalence (22%:26/118). This result is presented in Table 4.3.

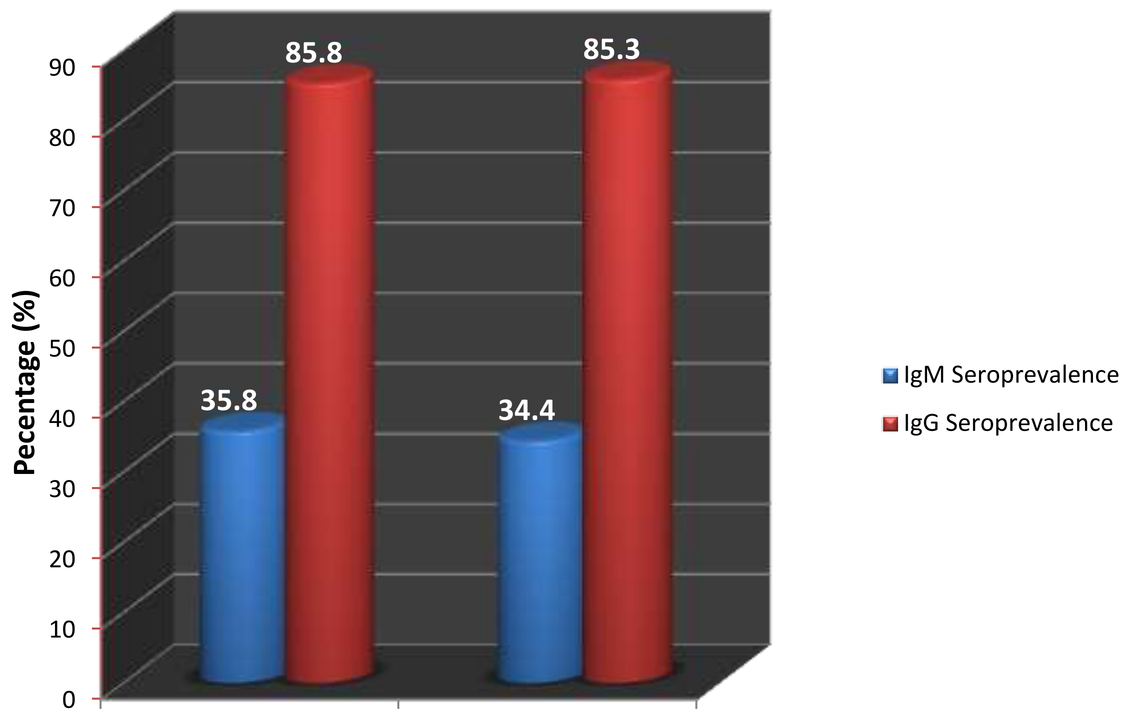

Fig.4.2: Seroprevalence of Measles IgM and IgG Antibodies in Relation to Gender in the Selected Hospitals

Table 4.3: Seroprevalence of Measles IgM Antibody in Relation to Some Socio – demographic Factors

Factor	Number Tested	Seropositive (%)	χ^2	P – value
Parent's educational Status				
Primary	115	61 (53.0)	108.677	0.000
Secondary	161	22 (13.7)		
Tertiary	61	04 (06.6)		
Non – Formal	113	71 (62.8)		
Parent's Occupation				
Self Employed	170	37(21.8)	21.361	0.000
Civil Servants	280	121(43.2)		
Parent Socio –Economic Status				
High Status	116	24 (20.7)	17.111	0.000
Middle Status	99	33 (33.3)		
Low Status	235	101 (43.0)		
Nutritional Status of child				
Normal	118	26 (22.0)	12.444	0.002
Average	92	34 (36.9)		
Poor	240	98 (40.8)		
Total	**450**	**158(35.1)**		

Key: % Percentage, P level of Significance

Seroprevalence of measles virus infection in relation to vitamin A intake presents a statistically significant association between measles virus infection and the children intake of vitamin A as supplement (x^2= 34.939, df =2, p=0.001). Highest prevalence was observed in children with not sure of taking vitamin A supplement (48.4%:91/188) and lowest prevalence was observed in children who were given vitamin A supplement (13.9%: 14/101). This result is presented in Table 4.4.

Seroprevalence of measles virus IgM antibody in relation to clinical symptoms of infection (Table 4.5) Children that had fever had a higher prevalence of 27.4% (78/285) compared to children who did not have fever (14.6%:24/165) There was a statistical significant association between fever and the presence of measles virus infection in children (x^2 = 9.803, df =1, p=0.002).

Children that were coughing had a high prevalence (29.7%:65/219) compared to children that were not coughing with the least prevalence (10.8%:25/231). There was statistically significant association between measles virus infection and the presence of coughing in children (x^2 = 24.987, df=1, p=0.000)

Children who had diarrhea had a higher prevalence (28.1%:81/295) compared to children who did not have diarrhoea with a lower prevalence of 20% (31/155). There was no statistically significant association between measles virus infection and the presence of diarrhoea (x^2=3.555, df=1, p=0.059).

Children that were vomiting had a high prevalence of 25.4% (75/295) compared to children that were not vomiting (18%:28/155). There was no statistically significant association between vomiting and the presence of measles virus infection (x^2=3.118, df=1, p=0.077).

Children that had rash on their body had a high prevalence of 80.2% (81/101) compared to children who did not have rash (17.8%:62/349). There was statistically significant association between measles virus infection and the presence of rash (x^2= 140.836, df=1, P=0.000)

Children with conjunctiva had a high prevalence of 21.4% (21/98) compared to children without conjunctiva (140.8%:52/352) There was a statistically significant association between measles virus infection and the presence of conjunctiva (x^2=2.499, df=1, p=0.004) (Table 4.5}

Seroprevalence of measles virus infection in relation to vaccination status of the children shows that children who are not vaccinated had a high prevalence of 61.7% (84/141) compared to children who are vaccinated with a low prevalence of (22.9%:71/309). There was statistically significant difference between measles virus infection and children vaccination status (x^2=63.728, df=1, p=0.000). This result is presented in Table 4.6.

Table 4.4: Seroprevalence of Measles IgM Antibody in Relation to Vitamin A Supplement Intake

Vit.ASupplement	N/ Tested	N/positive (%)	χ^2	P-Value
Yes	101	14 (13.9)	34.939	0.001
No	161	53 (32.9)		
Not Sure	188	91 (48.4)		
Total	**450**	**158(35.1)**		

Key: Vit – Vitamin, % - Percentage, N- Number

Table 4.5: Seroprevalence of Measles IgM Antibody in Relation to Symptoms of Disease

Symptoms	Response	Number of Subject Tested	Number positive (%)	χ^2	P – Value
Fever	Yes	285	78 (27.37)	9.803	0.002
	No	165	24 (14.55)		
Cough	Yes	219	65 (29.68)	24.987	0.000
	No	231	25 (10.82)		
Diarrhoea	Yes	295	83 (28.14)	3.555	0.059
	No	155	31 (20.00)		
Vomiting	Yes	295	75 (25.42)	3.118	0.077
	No	155	28 (18.06)		
Rash	Yes	101	81 (80.19)	140.836	0.000
	No	349	62 (17.76)		
Conjunctiva	Yes	98	21 (21.42)	2.499	0.114
	No	352	52 (14.77)		

Key:- % = Percentage, P = Level of Significances

Table 4.6: Seroprevalence of Measles IgM Antibody in Relation to VaccinationStatus of the Children

Vaccination Status	Number Tested %	N/positive (%)	Prev	χ^2	P - value
Vaccinated	309(68.7)	71 (22.9)	15.8	63.728	0.000
Non Vaccinated	141(31.3)	87 (61.7)	19.3		
Total	**450**	**158 (35.1)**	**35.1**		

Key:- % = Percentage, P = Level of Significances, Prev = Prevalence, N = Number

4.2 Measles Genotypes in Children Blood Samples

Out of the 450 blood samples screened for the presence of measles virus IgM antibody in children 0 – 12 years of age, 158 (35.1%) were positive for measles virus IgM antibody. Out of 158 IgM positive samples, 76, 50 and 32 were IgM ELISA positive in Sokoto, Jigwa and Kaduna States respectively. Thirty (30) IgM positive samples were randomly selected, 10 each from the States were subjected to RT- PCR. Out of these ten (10) IgM positive samples from each state, 10 samples selected from Sokoto State, 6 samples selected from Jigawa State and 4 samples from Kaduna State showed bands for measles virus. (Plate I, II, and III). Four (4) IgM positive measles virus's samples from the studied states were sequenced, 2, 1 and 1 sample from Sokoto, Jigawa and Kaduna State respectively. All the 4 sequenced were genotype B3 strain. The distribution of measles virus in the respective states in the study area is also presented in Tables 4.7, 4.8 and 4.9.

The BLAST information obtained from the Query sequences and the closest corresponding sequences from the genbank revealed that samples S3, S4, J21 and K24 correspond with genotype B3 sequences from the genbank. This result is presented in Table 4.11.

Phylogenic tree were prepared using Mega 7 version software and the neighbor – joining method with 1000 bootstrap replicates. Sequences from North Western Nigeria Measles virus strains ware compared with the sequences of B3 genotype on Genbank. This is presented in figure 3.

Plate I: Agarose Gel Electrophoresis of RT-PCR products of Measles virus IgM Positive Samples from Sokoto States with an Amplicon size of 527bp

Key: Lane M 100bp DNA Ladder; Lane 1- 10 amplicons products; Lane + = Positive Control; Lane - = Negative Control

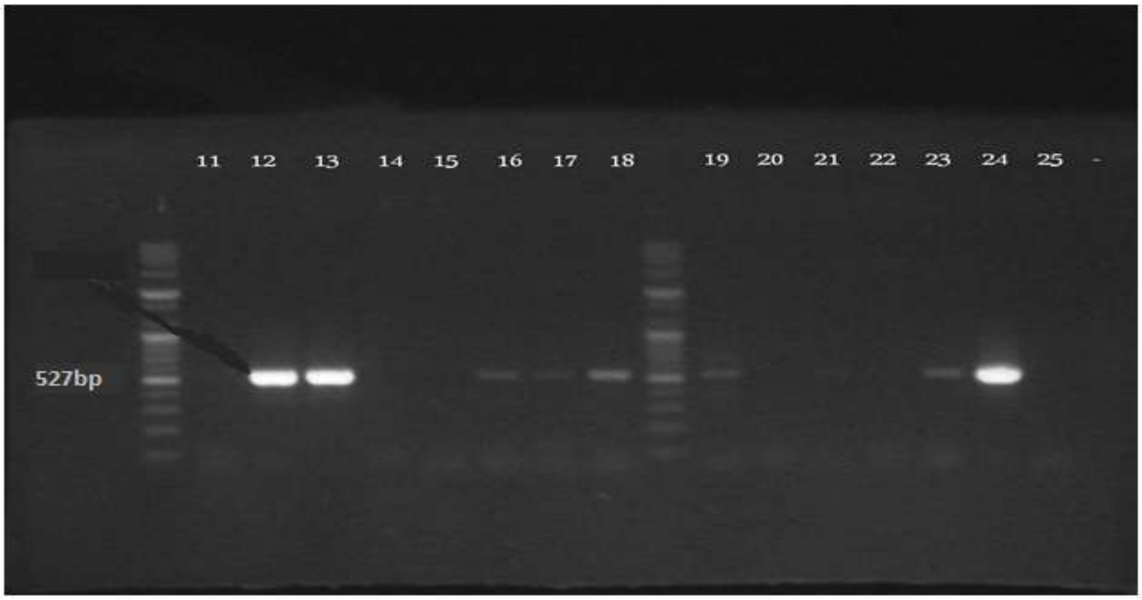

Plate II: Agarose Gel Electrophoresis of RT-PCR products of Measles virus IgM Positive Samples from Jigawa and Kaduna States with an Amplicon size of 527bp

Key: Lane M 100bp DNA Ladder; Lane 11, 14, 15, 20, 21, 22 and 25 were negative; Lane 12, 13, 16, 17, 18, 19, 23 and 24 shows amplicons of Measles virus; Lane - = Negative Control

Plate III: Agarose Gel Electrophoresis of RT-PCR products of Measles virus IgM Positive Samples from Kaduna State with an Amplicon size of 527bp

Key: Lane M 100bp DNA Ladder; Lane 26, 27 and 29 were negative; Lane 28 and 30 shows amplicons of Measles virus; Lane + = Positive Control; Lane - = Negative Control

Table 4.7: Detection of Measles Virus from IgM Positive Samples in Sokoto State

S/N	Sample Number	RT-PCR
1	S3	+
2	S4	+
3	S19	+
4	S24	+
5	S25	+
6	S26	+
7	S50	+
8	S51	+
9	S70	+
10	S78	+

Table 4.8: Detection of Measles from IgM Positive Samples in Jigawa State.

S/N	Sample Number	RT- PCR
1	J5	-
2	J10	+
3	J21	+
4	J29	-
5	J66	-
6	J76	+
7	J82	+
8	J92	+
9	J98	+
10	J102	-

Key: J=Samples in Jigawa State

Table 4.9: Detection of Measles Virus from IgM Positive Samples in Kaduna State

S/N	Sample Number	RT- PCR
1	K2	-
2	K3	-
3	K8	+
4	K24	+
5	K28	-
6	K36	-
7	K40	-
8	K43	+
9	K63	-
10	K76	+

Key: K= Samples Collected from Kaduna

Table 4.10: Percentages of Measles Virus from IgM Positive Samples

State	n	% occurrence
Sokoto	10	10(10)
Jigawa	10	6(60)
Kaduna	10	4(40)
Total	**30**	**20(66.7)**

Key: n= Number of IgM Positive Sample; Number in Parentheses= Percentage % = Percentage

Table 4.11: Closest Affiliates of Measles Virus Sequence Obtained Compared with those from GenBank

Seq	Accession No	Max Score	Total Score	Query length	E – value	Identity
S3	KX838946.2	764	764	442	0.0	98%
S4	KX949610.1	722	722	443	0.0	98%
J21	KX949603.1	773	773	436	0.0	99%
K24	KT732217.1	785	785	450	0.0	99%

The query sequences covers almost 100%

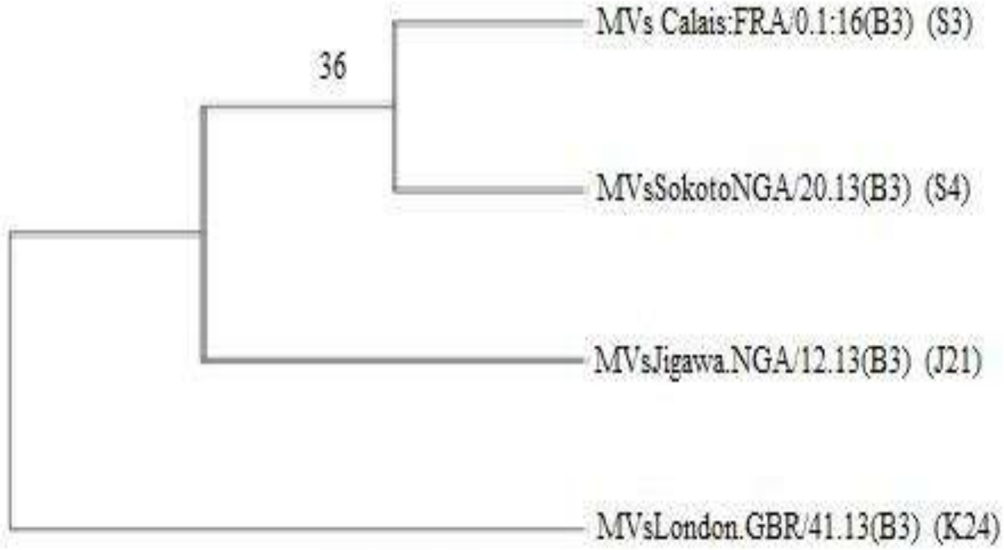

Fig. 4.3: Neeighbour - Joining Dendogram of Measles Virus Gene Sequences

CHAPTER FIVE

5.0 DISCUSSION

This study showed that Measles remain a highly infectious disease characterized by fever, respiratory symptoms, redness of the eyes and a maculopapular rash in children. This can be attributed to the pathogenesis and pathology of the measles virus. The high positive rate of measles IgG antibodies compared to IgM may be due to previous infection or vaccination of the children. 35.1% of sero positive cases of measles IgM antibodies in this study were from children who may have been unimmunized with the measles vaccine or had an incomplete course of the routine vaccination. The result agrees with 32.2% recorded in Kaduna state, Nigeria (Chechet *et al*., 2014), and 34% reported in Abia state, Nigeria (Nguku *et al*., 2012) However the prevalence contradicts 19% reported in Oshogbo, Osun state (Adetunji *et al.*, 2010), 21.4% reported in Senegal and 62.8% reported in India (Wairagkar *et al.*, 2011).

The highest and lowest seroprevalence of measles IgG and IgM antibodies seen in Sokoto and Kaduna States respectively, This may be attributed to geographical and environmental factors, most especially the humidity of the area. This agrees with the finding of James *et al*. (2011) on the geographical variation in the epidemiology of measles in Africa.

The high presence of IgG antibody to measles virus in age group 1 – 5 years may be due to previous exposure to the virus through vaccination or infection. It could also be as a result of the closeness of the age group to the vaccination age.

The high prevalence of IgM antibody to measles virus in the same age group 1 – 5 years may be due the inability of the IgG antibody to prevent against second infections.The high prevalence of IgM observed is in agreement with the prevalence of 55% recorded in a studyin Lagos (Adetunji *et al*., 2007), and 69.61% reported in Bolivia (Automatell*et al*., 2013).

The reason for the observed decline in the seroprevalence of IgM with age may be attributed to the proper measles vaccine immunization coverage in Nigeria. Accelerated measles control programme including improved provision of a second dose of measles vaccine at supplementary vaccination activities in certain countries of the world and case based surveillance with laboratory confirmation may have reduced measles associated morbidity and mortality (Manirakiza *et al*., 2012).

This study also revealed that children < 1year in the North Western Nigeria were more susceptible to measles virus infection. This may be due to failure to received vaccine against measles infection or the early waning of transplacentally acquired immunity which made the pre-vaccination age infants more vulnerable. Positive cases of measles decreases as the age of the children increased This may be attributed to the physiological status of the individual child and variation in prevailing environmental factors (Akramuzzaman *et al*., 2010), in addition, immunity acquired by older children over the years as a result of sub clinical infections might provide the required protection against measles (Patel *et al*., 2009).

It was also observed from this study that children 1 – 5 years of age were more likely to be infected with measles virus. This is in agreement with previous study which shows that 58% of infants loose there protective antibodies by four months and 97% between seven and nine months (Oyedele *et al.,* 2005, Nishoe *et al.*, 2013, Ismail *et al.*, 2014). The second peak age of presentation of measles virus occurred in the second year of life (Onyiruka *et al*., 2011, Ahmed *et al*., 2010), the reasons for this disparity is unclear but the finding of about 31.25% in all affected children age less than 1 year (< 1) in this research has been attributed to a rapid declined in maternally acquired antibodies which some author reported was due to the presence of other infections, (Fetuga *et al*, 2007) and malnutrition (Odoemele *et al.*, 2008).

The prevalence of IgM antibodies against measles virus observed in age groups 6-10 and 11-15 years may be due to a shift of the infection to older age groups if past immunization efforts had been weak implying that older unimmunized children may still be susceptible to new infection. This agrees with the finding of Wichmann *et al.* (2009) who reported a shift in the distribution of measles infection to older children.

The high seroprevalence of measles virus IgG antibodies in relation to gender may be attributed to previous vaccination or incomplete course of routine vaccination in both sexes.

There was no statistically significant association between measles virus infection and gender of the children. This result is in agreement with previous study in Nigeria (Automatell*et al.*, 2013) which reported that measles antibody is marginally higher in male than in their female.

Seroprevalence of measles virus IgM in relation to socio demographic factors showed a significant association between parent levels of education and measles virus. The high seroprevalence in children of parents with non formal and primary education may be due to the parent's ignorance on the need for measles vaccination. Studies had shown that educated women are more concerned with the immunization of their children and proper immunization schedules than uneducated women (Muhammed and Danish, 2014). This is also similar with the findings of Automatell*et al.* (2013) where seroprevalence of measles virus was higher in children of mothers with less than secondary educational level but lower in children of mothers with highly educational level.

There was also a significant association between parent occupation and measles virus infection, Children of parents who are self employed had the lower seroprevalence while children of parents who are civil servant had the highest seroprevalence. These differences could be as result of the time spent on child care since civil servant spend more time at work than on other things and may possibility miss the routine immunization of their children. This finding agrees with a study carried out in United State where children of working class parents were more susceptible to infection (Holmes *et al.*, 1996).

A significant association was observed between parent socio economic status and measles virus infection. Parents with low economic status had the highest seroprevalence followed by parents with Middle economic status and parent with high economic status had the least seroprevalence. This agrees with the finding of Saleh.(2016). Trends of measles in Nigeria, there are great number of parents in Nigeria who do not utilize vaccination services due to lack of awareness and poor socio economics status.

The nutritional status of children was also assessed and there was significant association between the Nutritional status of children and measles virus infection Children with poor nutritional status has the highest seroprevalence followed by children with average nutritional status and the least seroprevalence as observed in children with Normal Nutritional status. This agrees with finding of Augoye ,.(2012) which is corroborated by the claim of about a quarter (27%) parents in Nigeria, that their children go without food for a day, according to a survey conducted by charity save the children's group

Seroprevalence of measles IgM antibody in relation to Vitamin A Supplement intake shows significant associations between vitamin A supplement intake and measles virus infection. Children that were giving Vitamin A supplement had the least seropositive and children with no intake of vitamin A supplement had a higher seroprevalence. Concurrent vitamin A deficiency increases rates of complications. This agrees with the finding of Okada *et al.* (2012) who reported that vitamin A supplementation, and antibiotic therapies for secondary infections have reduced measles-associated deaths in the developing world.

Seroprevalence of measles IgM in relation to symptoms of Disease, this study observed that all symptoms, fever, cough, conjunctiva and rash had a significant association with measles virus infection. Diarrhea and vomiting was however not statistically signification. This agrees with WHO (2013) on clinical case definition of measles virus symptoms, any child presently with a history of fever (38 to 40^{o}C) lasting three days or more and generalized Rash with one of the following, Cough or Conjunctivitis. It also agrees with the finding ofNester*et al.* (2010) who reported that Measles is a highly infectious disease characterized by fever, respiratory symptoms, redness of the eyes and a maculopapular rash. Complications are common and may be quite serious. The rash usually starts on the head and then spreads to the rest of the body. Fever can persist, reaching extremely high temperatures, rash can last for up to a week, and coughing can last about 10 days.

Seroprevalence of measles virus in relation to vaccinated and non vaccinated children shows a statistically significant association between measles virus infection and vaccination. Vaccinated children had the least seroprevalence while t unvaccinated children had the highest seroprevalence. This agrees with the finding of Aaby *et al*. (2012) on the policy of measles vaccination at 9 months was based on various studies on sero-conversion after measles vaccination at different ages. Expanded Programme on Immunization recently recommended by WHO is that all children should receive 2 doses of measles containing vaccine(MCV); the first dose during the routine immunization programme and the second dose either through routine services or through Supplemental immunization activities(SIAs) (Danet and Fermon, 2013)

Twenty (66.7%) of the 30 IgM positive samples were positive for measles virus gene. This agree with the finding of Mohammoud *et al. (*2014), Zhang *et al*. (2012), who reported the incidences of measles virus in Africa and China respectively. This could be due to long period of sampling before analysis because the virus may not be able to survive a long period of time before screening, but the IgM antibody will still be present in the sample no matter the period it stayed before screening

This result also shows that RT – PCR is a sensitive and specific assay for detecting measles virus RNA in a variety of clinical samples. This agrees with the finding of Jangu *et al.* (1995) who detected measles virus RNA in clinical samples using RT-PCR.

The entire four measles virus were subjected to sequencing and BLAST. All the four were genotype B3 strain. This agrees with the finding of Michaela *et al.* (2005) who reported that Genotype B3 was first detected in 1993 in Gambia but have subsequently been detected in cases from Cameroon, and Central Africa and has been imported into numerous countries including France, Germany and the USA. Genotype B3 is clearly the endemic genotype in most parts of the African Continent where it is widely distributed (WHO, 2012).

The BLAST information obtained from the queried sequences and the closest corresponding sequences from the GenBank revealed that samples S3 and S4 from Sokoto State corresponded with genotype B3 from Genbank, Sample J21 and K24 from Jigawa and Kaduna State respectively also corresponded with B3 strain in the GenBank.

The phylogenetic relatedness of measles virus in this study with those in the GenBank revealed a common homology. Measles virus S3 and S4 on the same leavenode were genetically highly related to one another and related to those gotten from Calais (FRA/01:16) and Sokoto (NGA/20.13), but vary with those on a neighbouring clade positions. Measles virus J21 is genetically closely related to Measles virus S4 on the neighboring leave node while Measles virus K24 is genetically closely related to Measles virus J21 on the neighboring clade position. This could be due to evolutionary relatedness, this could be that, they all emolliated from the same ancestor.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The burden of measles in Nigeria remains high despite global efforts targeted at elimination, with infants and the unvaccinated being the most susceptible. The findings in the study confirmed the presence of Measles virus in children (0-12years of age) in the North Western Nigeria, with seroprevalence comparable to the rates obtained in other parts of the country. The seroprevalence of 35.1% was obtained for IgM antibody and 85.6% was obtained for IgG. This is an indication that measles is endemic in the North Western Nigeria and still poses a public health problem, despite the availability of a safe and effective vaccine. The results obtained from this study confirmed that serological has a high sensitivity and specificity and are suitable for routine use, therefore Elisa techniques should be employed for routine use due to the high sensitivity and specificity of the techniques.

This study recognized age, sex, vaccination, vitamine A intake and nutritional status of child, important demographic information. The findings in this study confirmed the presence of measles virus in children 0 – 12 years of age in North Western Nigeria with the prevalence of 35.1% for IgM antibody and 85.6% for IgG antibody and This study further identified the importance of vaccination against measles virus as non vaccinated children had the highest prevalence of 61.7% while vaccinated children had a lower seroprevalence of 22.9%.

The study revealed Vitamin Supplement intake as an important prophylaxis, children with Vitamin A supplement intake had the prevalence of 13.9% while children with no vitamin A intake had the highest prevalence of 32.9%. The measles virus strain detected in this study by sequencing and phylogenetic analysis was genotype B3 Strain and the phylogenetic analyses in this study revealed a close relationship of the querry sequences with the one obtained from France, Newcastle, London and North central part of Nigeria (Niger).

6.2 RECOMMENDATIONS

It is necessary to employ the following measures so as to prevent and reduce the rate of measles virus infection in any part of the country.

1. A revision of the administration of the measles vaccine in the Nigerian EPI to an early second dose schedule in infancy should be advocated. Governments’ commitment to improving vaccine potency and availability should be made a priority to ensure increased vaccine coverage and prevent missed opportunities. Health education of mothers as well as training and retraining of the health workers on childhood measles should be emphasized at all levels.
2. It is important for health providers and policy makers to recognize the health implication of this measles virus and review the vaccination age of infants and also, intensify vaccination campaign programs at all time.
3. Diets rich in Vitamin A is recommended for children above breastfeeding age, as this will boost immunity to measles virus infection and other related diseases.

4. The administration of antiviral agent should be reviewed with the aim of finding out better and more effective agent that could stand the test of time in the treatment of measles virus infection, particularly in children.
5. Molecular epidemiological severance is recommended from time to time in order to accelerate measles control and eliminate endemic virus strain.

REFERENCES

Aaby P., Samb B., Simondon F., Coll Seck A.M., Knudsen K., Whittle H. (2012). Non-specific beneficial effect of measles immunization: analysis of mortality studies from developing countries. *Medicine Journal,* 311: 481–485.

Abramson, O., Dagan, R., Tal. A., and Sofer, S. (1995). Severe complications of measles requiring intensive care in infants and young children *Archive of Pediatrics of Adolescent Medicine,* 149:1237

Adeboye, M., Adesiyaun, O., Adegboye, A., Eze, E., Abubakar, U., Ahmed G., Usman, A., Amos, S., and Rotimi, B.F. (2011). Measles in a tertiary institution in Bida, Niger State, Nigeria: Prevalence, Immunization Status and Mortality Pattern. *Oman Medical Journal,* 26(2) 114-117.

Adetunji O.O., Olusola E.P., Ferdinad F.F., Olorunyomi O.S., Idowu, J.V., and Ademola O.G., (2010). Measles among hospitalized Nigerian Children. *The Internet Journal of Paediatrics and Neonatology*, 7 (1) pp 88-102

Ahmed PA, Babaniyi IB, Otuneye AT (2010). "A review of childhood apoptosis of uninfected lymphocytes in acute measles patients. *ArchiveBulletin of World Health organizatios,* 70(4) pp 457-460.

Akramuzzaman, S.M., Cutts, F.T., Hossain, M.J., (2010*).* Measles Vaccine Effectiveness and risk factor for measles in Dhaka, Bangladesh. *Bulletin of World Health Organization,* 80 (10) 776 – 782.

Amarasinghe, Gaya K.; Bào, Yīmíng; Basler, Christopher F.; Bavari, Sina; Beer, Martin; Bejerman, Nicolás; Blasdell, Kim R.; Bochnowski, Alisa; Briese, Thomas (2017)*"Taxonomy of the order Mononegavirales. Archives of Virolog*y, (10) 3311 – 3320

Atabani, S. F., Byrnes, A. A., Jaye, A., Kidd, I. M., Magnusen, A. F., Whittle, H. & Karp, C. L.(2001). Natural measles causes prolonged suppression of interleukin-12 production. *Journal Infectious Disease,* 184, 1-9

Augoye, J. (2012). Nigerian children among the most malnurished - Survey. The Punch Newspaper February, 2012. Children among the most malnourished survey volume 14 pp 19

Automatell, C.M., Ramon – Torrell, J.M., Rituerto, A.C Navarro, M.B Gamboa Mdel, R.D., Rodriguez, S.L. (2013). Measles in Bolivia a honeymoon period vaccine *Journal of Immunology*, 31:2097 – 2102.

Bernstein, D.I. Reuman, P.D., and Schiff, G.M. (1998). Rubeola (measles) and subacute sclerosing panencephalitis virus. In: Gorabach, S.L., Bartlett, J.G., Blacklow, N.R. (Ed). *Infectious Diseases, West Bengal Saunders,*philadelphia, Pp. 2135.

Bellini, W. J., Englund, G., Rozenblatt, S., Arnheiter, H. & Richardson, C. D. (2005). Measles virus P gene codes for two proteins. *Journal of virology,* 53, 908-919.

Bloch A.B, Orenstein W.A, Ewing W.M, Spain W.H, Mallison G.F, Herrmann K.L, (2006). Measles outbreak in a pediatric practise: Airborne transmission in an office setting. *International Journal of Pediatrics,* 75(4): 676 - 683.

Butler JC, Havens PL, Sowell AL, (1993) Measles severity and serum retinol (vitamin A) concentration among children in the United States. *Journal of Virology,* (6) 243-268

Cathomen, T., Mrkic, B., Spehner, D., Drillien, R., Naef, R., Pavlovic, J., Aguzzi, A., Billeter, M. A. & Cattaneo, R. (2011). A matrix-less measles virus is infectious and elicits extensive cell fusion: consequences for propagation in the brain. *TheEuropean Molecular Biology Organization Journal,* 17 3899-3908.

Centres for Disease Control and Prevention. (2009). Use of combination measles, mumpls, rubella, and varicella vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity and Mortality Weekly Report*, 59 (No. RR-3):1-12.

Centers for Disease Control and Prevention. (2012). Measles- Epidemiology and prevention of vaccine preventable diseases. *The Pink Book,* 12th ed. Reviewed May 7, 2012.

Chechet J., Ella E.E and Ige, S.O., (2014). Seroprevalence of measles in children 5-12 years from selected primary schools in Giwa Local Government Area, Zaria, Kaduna State. *Scientific Journal of Microbiology,* 3 (2): 19-24

Cherry, J.D. Feigin, R.D., Cherry, J.D., Demmle-Harrison, G.J., (2009). Measles virus. In:., *Textbook of Pediatric Infectious Disease,* 6th ed., Saunders, Philadelphia. PP. 2427.

Cocks, B. G., Chang, C.-C. J., Carballido, J. M., Yssel, H., de Vries, J. E. & Aversa, G.(1995). A novel receptor involved in T-cell activation. *Nature*, 376, 260–263.

Danet C, and Fermon F (2013). Médecins San Frontiéres. Management of a *Diseases and Immunity,* 3(6) 112-6.

De Quadros, C. A. (2004) Global Measles Eradication and Vaccines: preventing disease Health. Scientific and Technical Publications, Washington, 2004, 35-42.

De Swart, R. L., M. Ludlow, L. de Witte, Y. Yanagi, G. van Amerongen, S. McQuaid, S. Yuksel, T. B. H. Geijtenbeek , W. P. Duprex, and A. D. M. E. Osterhaus. (2007). Predominant infection of CD150+ lymphocytes and dendritic cells during measles virus infectious Pathogens *Journal of Virology,* 3: e178.

D'Souza, R.M. and D'Souza, R. (2002). Vitamin A for preventing secondary infrctions in children with measles-a systematic review. *Journal of Tropical Pediatrics,* **48**(2): 72-77.

Ehrengruber, M. U,, Ehler, E,, Billeter, M. A. and Nairn, H. Y. (2002). Measles virus spreads in rat hippocampal neurons by cell-to-cell contact and in a polarized fashion. *Journal of Virology,* 76 5720-5728.

Enders, G. (1996). Paramyxoviruses, In: Baron, S. (Ed) Vitamin A for preventing secondary infections in children with measles-a systematic review. *Medical Microbiology.* NCBI Bookshelf, Galveston. PP. 59.

Enders JF, P. T. (1954). Propagation in tissue cultures of cytophatic agents from patients with measles. *Proceedings of Society of Experimental Biological Medicine,* 86:277-286.

Enders, J., S. Katz, M. Milovanovic, and A. Holloway. (1960). Studies on an attenuated measles-virus vaccine: I Development and preparation of the vaccine: techniques for assay of effects of vaccination. *English Journal of Medicine,* 263:153-159.

Erlenhoefer, C., Wurzer, W. J., Loffler, S., Schneider-Schaulies, S., ter Meulen, V. & Schneider-Schaulies, J.(2001). CD150 (SLAM) is a receptor for measles virus but is not involved in viral contact-mediated proliferation inhibition. *Journal of Virology,* 75, 4499–4505.

Etuk I.S, Ekanem E.E, and Udo J.J. (2015) Comparative analysis of measles morbidity and mortality in Calabar during the expanded programme on immunisation and the national programme on immunisation eras. Niger *Journal of Paediatric,* (30) 81-5.

Fatiregun A.A, and Odega C.C (2013). Representativeness of suspected measles cases reported in a southern district of Nigeria. *Journal of Tropical Medicine,* (6) 131-4.

Fatiregun A.A, Adebowale A.S, and Fagbamigbe A.F (2014). Epidemiology of measles in Southwest Nigeria: An analysis of measles case-based surveillance data from 2007 to 2012. *Tropical Medical Hygiene*, (108) 133-40.

Farina, C., Theil, D., Semlinger, B., Hohlfeld, R. and Meinl, E. (2004). Distinct responses of monocytes to Toll-like receptor ligands and inflammatory cytokines. *International Immunology*, 16 799–809.

Fetuga M.B, Njokanma O.F, Ogunfowora O.B, Runsewe-Abiodun (2007). global measles control and elimination. *Weekly Epidemiological.*

Gershon, A.A, (1995). Measles Virus. In: Mandell, G.L., Bennett, J.E. and Dolin, R. (Ed) *Mandell, Douglas and Bennett's Principles and Practices of Infectioous Diseases.* 4th ed., Churchill Livingstone, Philadelphia, PP. 1519-1522.

Gerber J.S and Offit P.A. (2009). Vaccines and autism: a tale of shifting hypotheses. *Clinical Infectious Diseases,* (48) 456 –61.

Greenberg B.L, Sack R.B, Salazar-Lindo E,(1991). Measles-associated diarrhea in hospitalized children in Lima, Peru: pathogenic agents and impact on growth. *Journal of Infectious Disease*, 163:495–502.

Griffin, D.E. and Bellini, W.J. (1996). Measles virus. In: Fields, B.N., Knipe, D.M., Howley, P.M. (Ed) *Fields' Virology.* Lippincott-Raven, Philadelphia. PP. 1267.

Griffin, D. E.(2001). Measles virus. In fields virology. 1401–1441. Edited by D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman and S. E. Straus Philadelphia, Lippincott Williams and Wilkins

Griffin, D. (2007). Measles Virus. In *Fields Virology,* 5th edn, pp. 1552-1585. Edited by D. M. Knipe, Howley, P.M.: Lippincott, Williams and Wilkins. Hajar, R. (2005). Al-Razi: Physician for all Seasons. *Heart Views,* 6, 39-43.

Hasley N.A, and Hyman S.L.(2001) Measles-mumps-rubella vaccine and autistic spectrum disorder: report from the New Challenges in Childhood Immunizations Conference convened in Oak Brook, Illinois, *Journal of Pediatrics,* 107:E84.

Hashiguchi, T., Kajikawa, M., Maita, N., Takeda, M., Kuroki, K., Sasaki, K., Kohda, D., Yanagi, Y. and Maenaka, K. (2007). Crystal structure of measles virus hemagglutinin provides insight into effective vaccines. *Proceedings of theNational Academy of Sciences of the United States of America,* 104, 19535-19540.

Henneman, P.L, Birnbaumer, D.M, Cairns, C.B.(1996). Measles pneumonitis, Pediatric hospital admissions for measles. Lessons from the 1990 epidemic. *Western Journal of Medicine,* 165:20–5.

Holmes, S.J Morrow, AL., and Piekering, L.K. (1996). *Childcare practices: Effects of social change on the epidemiology of infectious diseases and antibodies resistances. Epidemiologic Reviews,* 18 (1): 10 – 27.

Hosoya, M., Mori, S., Tomoda, A., Mori, K., Sawaishi, Y., Kimura, H., Shigeta, S., and Suzuki, H. (2004). Pharmacokinetics and effects of ribavirin following intraventricular administration for treatment of subacute sclerosing panencephalities. *Antimicrobial Agents and Chemotheraphy,* **48** (12) 4631-4635.

Hsu, E. C., Dorig, R. E., Sarangi, F., Marcil, A., Iorio, C. and Richardson, C. D. (1997). http://www.who.int/mediacentre/factsheets/fs286/en/, World Health Organization Fact sheet NÂ°286. Retrieved June 28, 2012.

Huber, M., Cattaneo, R., Spielhofer, P., Orvell, C., Norrby, E., Messerli, M., Perriard, J. C. and Billeter, M. A. (2009). Measles virus phosphoprotein retains the nucleocapsid protein in the cytoplasm. *Virology,* 185, 299-308.

Hussey G.D, and Clements C.J. (2014). Clinical problems in measles case management.. *International Journal of Advanced Research*, Volume 2, Issue 5, 607-613

James, E.D.,Joseph, B. S., and M. B. Oldstone(2011). Immunologic injury in measles virusinfection. II. Suppression of immune injury through antigenic modulation. *Journal of Experimental Medicine,* 142:864-76.

Jangu, E. Banatval, A. Jennifer, M. TrenT J. Bosma, Karen M. Corbett, Siobhan O'shea, (1995). PCR for Detection of Rubella Virus RNA in Clinical Samples. *Journal of Clinical Microbiology*, (6) 1075–1079

Jaye, A., A. F. Magnusen, and H. C. Whittle. (1998). Human leukocyte antigen class I- and class II-restricted cytotoxic T lymphocyte responses to measles antigens in immune adults. *Journal Infectious Disease*, 177:1282-1289.

Jefferson, T., Price, D., Demicheli, V., andd Bianco, E. (2005). Unintended events following immunization with MMR: a systematic review Vaccine, (26) 3954-3960.

Kobune, F., Sakata, H. and Sugiura, A. (1990). Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. *Journal of virology, (*64) 700-705.

Lagunju I.A, Orimadegun A.E, Odeyemi D.G (2005). Measles in Ibadan: a continuous scourge. *African Journal of Medical Science,* 34: 383 – 7

Lamb, R. A., and G. D. Parks. (2007). Paramyxoviridae: the viruses and their replication, p. 1449-1496. In D. M. Knipe and P. M. Howley (ed.), Fields Virology, Fifth ed. Lippincott, Williams and Wilkins, Philadelphia.

Leonard, V.H., Sinn, P.L., Hodge, G., Miest, T., Devaux, P., Oezguen, N., Braun, W., McCray, P.B., J.r., McChesney, M.B., Cattaneo, R. (2008) Measles virus blind to its epithelial cell receptor remains virulent in rhesus monkeys but cannot cross the airway epithelium and is not shed. *Journal of Clinical Investigation*, (118) 2448–2458

Lievano, F.A., M.J. Helfand, R.F. (2004). Lack of evidence of measles virus shedding in people with inapparent measles virus infections. *Journal of Infectious Diseases,* **198** (Suppl 1): S165-S170

Liszewski, M. K., Post, T. W. and Atkinson, J. P. (1991). Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. *Annual Review of Immunology,* 9, 431-455.

Longhi, S. (2009). Nucleocapsid structure and function. *Current Topics in Microbiology and Immunology* 329, 103-128.

Manirakiza A., Kipela J.M., Sosler, S., Daba R.M and Vasilache, I.G. (2011) Seropervalence of Measles and Natural rubella antibodies among children in Bangui, Central Africa Republic. BMC Public Health 11:1471 – 2458.

Manning, S.C, Ridenour, B, Brown, O.E, and Squires J.(1991) Measles: an epidemic of upper airway obstruction. *Otolaryngol Head Neck Surgery*. 105: 112-122

Macdonald, S. (2002). Aspirin use to be banned in under 16 year olds. *British Medical Journal,* 325: 988.

McCarthy A.J, and Goodman S.J. (2009). Reassessing conflicting evolutional histories of the Paramyxoviridae and the origins of respiroviruses with Bayesian multigene phylogenies. Infectious Genes. *Journal of Evolutional Research*. pp. 97–107.

McNeil, G.C. (2013). "Eradication Goal for Measles Is Unlikely, Report Says". New York Times. Retrieved May 3, 2013.

Michaela, Fu, Hanqing He; En-fu Chen; Qian Li; Zhifang Wang; Rui Yan; Jian Fu;Jinren Pan.(2005). Waning immunity to measles in young adults and booster effects of revaccination in secondary school students. *Journal of Vaccine,* (31) 533–537

Moll, M., Klenk, H. D., Herrler, G. and Maisner, A. (2001). A single amino acid change in the cytoplasmic domains of measles virus glycoproteins H and F alters targeting, endocytosis, and cell fusion in polarized Madin-Darby canine kidney cells. *Journal of biological chemistry,* 276, 17887-17894.

Moss, W.J., Ryon, J.J., Monze, M., Cutts, F., Quinn, T.C., nad Griffin, D.E. (2002). Suppression of human immunodeficiency virus replication during acute measles. *Journal of Infectious Diseases,* 00185: 1035-1042

Moss, W.J., Ota, M.O., and Griffin, D.E, (2004). Measles: immune suppression and immune responses. *The International Journal of Biochemistry and Cell Biology,* **36**(8): 1380-1385.

Muhammed Adeboye, Omotayo Adesiyun, Abdulrasheed Adegboye, (2011). Measles in a Tertiary Institution in Bida, Niger State, Nigeria: Prevalence, Immunization Status and Mortality Pattern. *Oman Medical Journal,* 26: 114- 117.

Muhammed, A. and Danish, O. (2014). Relationship between child immunization and household soico – demographic characteristics in Pakistan. *Research, Humanities and Social Sciences.* 4(7): 82 – 90.

Muhlebach, M.D., Mateo, M., Sinn, P.L., Prufer, S., Uhlig, K.M., Leonard, V.H.; Navaratnarajah, C.K., Frenzke, M., Wong, X.X., Sawatsky, B. (2011) Adherens junction protein nectin-4 is the epithelial receptor for measles virus. *Nature*, (480) 530–533.

Nakano T, Shimono Y, Sugiyama K,. (1996) Clinical features of measles in immunocompromised children. *Paediatrics Journal*, 38: 212–7.

Naniche, D., Wild, T. F., Rabourdin-Combe, C. and Gerlier, D. (1992). A monoclonal antibody recognizes a human cell surface glycoprotein involved in measles virus binding. *The Journal of General Virology,* (10), 2617-2624.

Naniche, D,, Wild, T. F,, Rabourdin-Combe, C. and Gerlier, D. (1993). Measles virus haemagglutinin induces down-regulation of gp57/67, a molecule involved in virus binding. *The Journal of General Virology,* 74: 1073-1079.

Navaratnarajah, C. K., Vongpunsawad, S., Oezguen, N., Stehle, T., Braun, W., Hashiguchi, T., Maenaka, K., Yanagi, Y. and Cattaneo, R. (2008). Dynamic interaction of the measles virus hemagglutinin with its receptor signaling lymphocytic activation molecule (SLAM, CD 150). *The Journal of Biological Chemistry* 283, 11763-11771.

Nester W., Dennise G., Nancy N., Martha T (2010): Microbiology a human perspective (4): 594 - 597 Mcgraw Hill, Usa.

Nguke O.A, Vargas P.A, Bernardi F.D, Alves V.A, (2012). Uncommon histopathologicalfindings in fatal measles infection: pancreatitis, sialoadenitis, and thyroiditis.*Journal of Histopathology*, **37**: 141–6.

Niedermeyer H.P, Arnold W, Neubert W.J, and Sedlmeier R. (2000). Persistent measles virus infection as a possible cause of otosclerosis state of the Ear Nose Throat. *Journal of medicine*, 79: 552–558.

Nishoe G.M., McAnerey J.M., Archer B.N., Smit S.B., Harris B.N., Stefano T., Mashele M., Singh B., Thomas J., Cengimbo A., Blumberg L.H., Puren A., Moyes J., Heever J., Schoub B.D and Cohen C. (2013). *Measles Outbreak in South Africa Epidemiology of*

Laboratory – Confirmed Measles Cases and Assessment of Intervention 2009 – 2011. Plos one, 8 (2): 1371.

Norby. E. and Oxman, M.N. (1990). Measles virus. In: Fields, B.N., Knipe, D. and Howley P.M. (Ed) *Fields' Virology,* Raven press, New York, PP. 103-108.

Odoemele C.F, Ukwandu N.C, Adu F.D, Nmorsi O.P, Anyanwu L.C, (2007) Odike of the nomenclature for describing the genetic characteristics of wild-type measles virus *Journal of Medicine,* 4: 125-132

Odega CC, Fatiregun AA, Osagbemi GK. (2010) Completeness of suspected measles reporting in a southern district of Nigeria. *Public Health Journal*, (124) 24-7.

Okada H, Kobune F, Sato T.A. Onyiruika A.N (2011). "Clinical profile of Children presenting extensive lymphopenia Practice,(4) 413-416 prevalence of maternal antibody to measles virus throughout rates to measles vaccine among children in Nigeria", *Report* 72, 349-353.

Onyiruka, O., Muhlebach, M.D.; Mateo, M.; Sinn, P.L.; Prufer, S.; Uhlig, K.M.; Leonard, V.H.; Navaratnarajah, C.K.; Frenzke, M.; Wong, X..X.; Sawatsky, B.(**2011**) Adherens junction protein nectin-4 is the epithelial receptor for measles virus. *Nature*,(480) 530–533.

Oyedele O.O., Odemuyiwa S.O Ammerlaan W., C.P.,and Adu F.D. (2005). Passive Immunity to measles in the breast milk and cord blood of some Nigerian subjects *Journals of Virology*, 77:7635 – 44.

Panum, P. L. (1940). Observations made during the epidemic of measles on the Faroe Islands in the year 1846. *American Publishing Association, New York.* 114-120

Parks C.L, Lerch R.A, Walpita P, Wang H.P, Sidhu M.S, Demsa (2009). Comparison of predicted amino acid sequences of measles virus strains in the edmonston vaccine lineage.*Journal of Virology*, 75 (2): 910 - 920.

Patel P.K., Al – Awaidy S.T., Bawikar S., and Al – Mahroqi S. (2009): Measles epidemiology and its implications for a vaccination programme in Oman. *Eastern Medit Health Journal*, 4 (3): 5 – 8.

Plaza J.A and Nuovo G.J. (2005) Histologic and molecular correlates o f fatal measles infection in children. *Diagnosis Molecular Pathology.* 14(2): 97-102.

Permar, S. R., Klumpp, S. A., Mansfield, K. G. (2003). Role of CD8 + lymphocytes in control and clearance of measles virus infection of rhesus monkeys. *Journal Virology,* 77, 396–4400.

Permar, S. R., S. A. Klumpp, K. G. Mansfield, A. A. Carville, D. A. Gorgone, M. A. Lifton, J. E. Schmitz, K. A. Reimann, F. P. Polack, D. E. Griffin, and N. L. Letvin. (2004). Limited contribution of humoral immunity to the clearance of measles viremia in rhesus monkeys. *Journal of Infectious Diseases,* 190: 998-1005.

Perry, R.T. and Halsey, N.A. (2004). The clinical significance of measles: a review. *The Journal of Infectious Disease,* **189**(S1): S4-16.

Premenko-Lanier, M., P. A. Rota, G. Rhodes, D. Verhoeven, D. H. Barouch, N. W. Lerche, N. L. Letvin, W. J. Bellini, and M. B. McChesney. (2003). DNA vaccination of infants in the presence of maternal antibody: a measles model in the primate. *Virology,* 307:67-75.provides an additional cysteine-rich protein. *Cell,* 56, 759-764.

Ravanel, K,, Castelle, C., Defrance, T., Wild, T. F., Charron, D,, Lotteau, V. and Rabourdin-Combe, C. (2008). Measles virus nucleocapsid protein binds to FcgammaRII and inhibits human B cell antibody production. *The Journal of Experimental Medicine,* 186, 269-278.

Richardson, C. D., Bondre, D., Noyce, R., Ha, M., Clattenburg, D. and Sisson, G. (2010). Wild type measles virus infects polarized adenocarcinoma cell lines using a unique receptor on the cellular apical surface. In Negative Strand Virus Meeting. Brugge, Belgium. *Journal of Immunology,* 4: 125-132

Richardson, C. D., Scheid, A. and Choppin, P. W. (1980). Specific inhibition of paramyxovirus and myxovirus replication by oligopeptides with amino acid sequences similar to those at the N-termini of the F1 or HA2 viral polypeptides. *Virology,* 105, 205-222.

Richardson, C. D., Welstead, G. G., Sisson, G., Sarangi, F. & Iorio, G. (2006). Evidence for a third receptor for measles on smooth airway epithelial cells. In Negative Strand Virus Meeting. Salamanca, Spain. *Virology,* 85: 213-219

Richardson, C. D,, Bondre, D., Noyce, R,, Ha, M., Clattenburg, D. and Sisson, G. (2009). A receptor for wild type measles virus is found on the apical surface of adenocarinoma cell lines of the lung, breast, colon, prostate, and liver. In *American Society of Virology,* (18) 122-129

Rima, B. K. & Duprex, W. P. (2009). The measles virus replication cycle. *Current Topics in Microbiology and Immunology,* 329, 77-102.

Rima, B. K., and W. P. Duprex. (2006). Morbilliviruses and human disease. *Journal of Pathology,* 208: 199-214

Rota, J. S., Wang, Z.-D., Rota, P. A. & Bellini, W. J. (2009). Seroepidemiology and decay rate of vaccine-induced measles virus. *Journal of Community Medicine,* 29: 214-226.

Rutter, M. (2005). Incidence ofautism spectrum disorders: changes over time and their meaning. *Acta Paediatrics,* **94**(1): 2-15.

Saleh, E. (2016**).** The paradigms of measles vaccinology. *CurrentTopics in Microbiology and Immunology,* **191**, 167-180.

Sarmukaddam S. B, and Gerad S.G, (2006): On validity of assumption while determining sample size. *Indian Journal of Community Medicine,* 29: 214-226.

Sawyer, L.A. (2000). Antibodies for the prevention and treatment of viral diseases. *Antiviral Research,* **47**(2): 57-77.

Schaffiner, W., Schluederberg, A.E., and Byrne, E.B. (1968). Clinical epidemiology of sporadic measles in a higly immunized population. *New England Journal,* 279:783.

Seki F., Takeda M., Minagawa H. and Yanagi Y(2006). Recombinant wild-type measles virus containing a single n481y substitution in its haemagglutinin cannot use receptor cd46 as efficiently as that having the haemagglutinin of the edmonston laboratory strain. *Journal of General Virology,* 87, 1643–1648.

Shaffer, J. A., Bellini, W. J. and Rota, P. A. (2003). The C protein of measles virus inhibits the type I interferon response. *Virology,* 315, 389-397.

Sheshberadaran H, Norrby E, McCullough KC, Carpenter WC, Orvell C. (1986).The antigenic relationship between measles, canine distemper and rinderpest viruses studied with monoclonal antibodies. *Journal of General Virology, 67: 1381–1392.*

Stein C.E, Birmingham M, Kurain M (2003). The global burden of measles in the year 2000, a model that uses country specific indicators. *Journal of Infectious Disease,* 187 Supplement: S8.

Stratton K, Gable A, Shetty P, and McCormick M.(2001). Immunization safety review: measles-mumps-rubella vaccine and autism.Washington, DC: National Academy Press, 144-150

Sugerman D.S, Barskey A.E, Delea M.G (2010). Measles outbreak in a highly vaccinated population,m Sandiego, 2008: role of the intentionally under vaccinated.*Pediatrics,* 125-747

Suryanarayana K., Baczko K., Termeulen V., and Wagner R(2008). Transcription inhibition and other properties of matrix proteins expressed by m genes cloned from measles viruses and diseased human brain tissue. *Journal of Virology,* 68, 1532– 1543

Takeuchi, K., Kadota, S. I., Takeda, M., Miyajima, N. and Nagata, K. (2003). Measles virus V protein blocks interferon (IFN)-alpha/beta but not IFN-gamma signaling by inhibiting STAT1 and STAT2 phosphorylation. *Federation of European Biochemical Societies Letters,* 545, 177-182.

Taylor B, Miller E, Lingam R, Andrews N, Simmons A, and Stowe J.(2002). Measles, mumps, and rubella vaccination and bowel problems or developmental regression in children with autism: *Population Study*, 324: 393–6.

Umeh C.A, and Ahaneku H.P. (2013). The impact of declining vaccination coverage on measles control: A case study of Abia state Nigeria. *Pan African Medical Journal,* (15):105.

Vitek C.R, Aduddel M., Brinton M.J (1999). Increased protection during a measles outbreak of children previously vaccinated with a second dose of measles-mumps-rubella vaccine. *Pediatric Infectious Diseases Journal,* 18: 620–623

Vieth M, Dirshmid K, Oehler U, Helpap B, Von Luckner A.G, and Stolte M. (2001). Acute measles gastric infection. *American Journal of Surgical Pathology,* (25) 259–62.

Von Messling, V., D. Milosevic, and R. Cattaneo. (2004). Tropism illuminated: lymphocyte-based pathways blazed by lethal morbillivirus through the host immune system. *Proceeding National Academic Science,* (101) 14216-14221.

WairagkarN.NS., Gandhi,B.V., Katrak S.M.,Shaiik, N.J., Parikh, P.R., Waia, N.H., F.R.C.P., and Gadkari, D.A. (2011), Acute renal failure with neurological involvement in adult associated with measles virus isolation. *Lancet, 354: 992-995*

Wang, N., Satoskar, A., Faubion, W., Howie, D., Okamoto, S., Feske, S., Gullo, C., Clarke, K,, Sosa, M. R., Sharpe, A. H. & Terhorst, C. (2004). The cell surface receptor SLAM controls T cell and macrophage functions. *The Journal of Experimental Medicine*, 199, 1255 - 1264.

Warren, J. and Gallian, M. J. (2010). Concentrated inactivated measlesvirus vaccine. Preparation and antigenic potency. *American Journal of Diseases of Children,* 103, 418-423.

Weldegebriel, G., Gasasira, A.,Harvy, P., Masresha, B., Goodson, J., Pate, M., Abanida, E., and Chevez, A. (2011). Measles Resurgence Following a Nationwide Measles Vaccination Campaign in Nigeria, 2005-2008. *Journal of Infectious Disease,* 204 (suppl 1): S226-S231.

Wichmann, O.D., Wechsler SL, W. H., Fields B.N.(2009). Immune response in SSPE: reduced antibody response to matrix protein of measles virus. *Journal of Immunology* 123: 884-889.

World Health Organization (2009). Measles vaccine: W.H.O Position paper. Weekly Epidemiological Records, 35 (84): 349 - 360

World Health Organization (2010) Global eradication of measles". Sixty-third world health assembly agenda provisional agenda item 11.15. http:www.who.int/mediacentre

World Health Organization (2012). Progress in Global control and regional elimination of measles. Weekly Epidemiological Records, 9(87): 73-80

World Health Organization. Measles fact sheet February (2013). Available Fact sheet United Nations. Retrieved 18 march. http;www.who.int/mediacentre/factsheet.8(68):73-82

World Health Organization /UNICEF(2013). Joint press release - Global plan for reducing measles mortality.

World Health Organization (2015). Manual for the laboratory diagnosis of measles virus infection. WHO/V and B/00.16. Geneva, Switzerland.9(87): 73-80

World Health Organization (2016) Measles surveillance data. Distribution of Measles genotypes in 2010.6(78): 37-40

Woo PC, Wong AY, Wong BH, Lam CS, Fan RY, Lau SK, Yuen KY (2016). Comparative genome and evolutionary analysis of naturally occurring Beilong virus in brown and black rats. *Infectious Genetic Evolution,* (16) 30399 - 9

Wong RD, Goetz MB.(1993) Clinical and laboratory features of measles in hospitalized adults. American *Journal of Medicine*, 95:377–83.

Yanagi, Y., Takeda, M., Ohno, S. & Hashiguchi, T. (2009). Measles virus receptors. *Current Topics in Microbiology and Immunology*, 329, 13-30.

Zhang C, Liu JW, Lin B, (2012). Effect of fluoride arsenic combination on the oxidative damange of brain of rat's offspring. *Journal of Health Toxicology*, (14): 156-158.

APPENDIX I: KADUNA STATE MINISTRY OF HEALTH RESEARCH ETHICAL CLEARANCE

MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

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

MOH/ADM/744/VOL.I/272

24[nd] April, 2015

Health Research Ethical Committee Kaduna State Ministry of Health.

To...

Ministry of Health Research Ethical Clearance.

RE: SEROPREVALENCE AND MOLECULAR CHARACTERIZATION OF MEASLES VIRUS AMONG CHILDREN 0-12 YEARS OF AGE IN SOME STATES IN NORTH WESTERN NIGERIA

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee.

Name of Investigator	Abdulfatai Khadijat
Address of Investigator	Microbiology Department, Ahmadu Bello University, Zaria
Date of Receipt of Application:	19[th] March, 2015
Date of Ethical Approval:	1[st] April, 2015
Research Period:	1 year

You are kindly requested to give researchers maximum cooperation during the period of research.
However researcher is, kindly requested to submit a copy of your findings to the state Ministry of Health please.

F. A. Kurah (Mrs.)

Secretary, Research Ethical Committee

APPENDIX II: SOKOTO STATE MINISTRY OF HEALTH RESEARCH ETHICAL CLEARANCE

MINISTRY OF HEALTH

Block 16, Shehu Kangiwa Secretariat
P.M.B. 2113, Sokoto State – Nigeria
Tel: 060-232856, 232425,2321

Ref: No. MH/1150/Vol.III **27th April, 2014**

AbdulFatai Khadijat,
Department of Microbiology,
Ahmadu Bello University, Zaria,
Kaduna State

ETHICAL CLEARANCE TO CONDUCT RESEARCH ON "SERO-PREVALLENCE AND MOLECULAR CHARACTERIZATION OF MEASLES VIRUS AMONG CHILDREN 0-12 YEARS OF AGE IN SOME STATE IN NORTH WESTERN NIGERIA" (SKHREC/015/015)

I am directed to refer to your application dated 19th March, which was received on 21/04/2015 on the above and to inform you that, the proposal submitted was reviewed by State Health Research Ethics Committee and found the protocol and other documents related to the survey satisfactory.

In the light of the above, I am further directed to convey the approval of the committee for the conduct of the above survey. It is however, expected that, the results of the survey will be sent to the committee for documentation as soon as it is concluded.

Accept the congratulations of the committee on behalf of the Honourable Commissioner, please.

(DADI ADARE) Dallatun Gobir
Director HPRS/Co-chairman HREC
For: Honourable Commissioner

E.O. I/CNO. kindly attend to the bearer & give the necessary support. Thank you PMO

The PMO WCWC

Pls PMO respond to the request of the bearer accordingly. 28/5/14

APPENDIX III: JIGAWA STATE MINISTRY OF HEALTH RESEARCH ETHICAL CLEARANCE

JIGAWA STATE HEALTH RESEARCH ETHICS COMMITTEE

STATE MINISTRY OF HEALTH

MOTTO: FOSTERING CHANGE THROUGH HEALTH RESEARCH

Registration number NHREC/10/11/2011

JHREC/A/R/18/2015

Monday, July 27, 2015
11 Shawwal 1436 AH

Abdulfatai Khadijat
Department of Microbiology
Ahmadu Bello University Zaria.

NOTICE OF APPROVAL TO CONDUCT A STUDY ON SERO-PREVALENCE AND MOLECULAR CHARACTERIZATION OF MEASLES VIRUS AMONG CHILDREN 0-12 YEARS OF AGE IN THE STATE

With reference to your research proposal submitted to the office of the Chairman of the Jigawa Health Research and Ethics Committee (JHREC) on the above topic, and pursuant to your request for ethical approval to conduct the study in the state, I wish to inform you that the research described in the submitted protocol has been reviewed appropriately.

Considering the fact that your research is basically a study of sero-prevalence and molecular characterization of measles virus in some states, obtain the prevalence of measles epidemic in our society, and determine the circulating measles virus genotype strain, I wish to convey clearance on behalf of the Ethics Committee for you to undertake the study in accordance with the specifications outlined in your protocol.

Please note that JHREC reserve the right to conduct monitoring visits to your research sites without prior notification and to have copy of the study findings at the end of the research. I wish you a successful and hitch free exercise in the state.

Thank you and Best Regards.

Pharm. Ibrahiim Hassan **MNIM, FCAI**
For: Chairman, Jigawa State Health Research Ethics Committee

APPENDIX IV: INFORMED CONSENTFORM

INFORMED CONSENT FORM (ICT)

Serial No ……………………………… *Hospital No* ……………………………… *Phone No*………………………………

This informed consent is basically for a Ph. D research title Sero – Prevalence and molecular characterization of measles virus among children 0 – 12years of age in some states in North Western Nigeria. You are being requested to participate in this study because we believe you may be able to contribute to our understanding of the disease. Besides reviewing the medical information of the child. Blood 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 strict 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 being subjected to any pressure.

Sign / right thumb print ……………………………………… ………………………………

Parent / Guardian *Date*

APPENDIX V: QUESTIONNAIRE

I am a post-graduate student of the Department of Microbiology, Ahmadu Bello University, Zaria. I am carrying out my research on "The Seroprevalence and Molecular Characterization of Measles Virus among Children 0-15 years of age in some State in North Western Nigeria."

I will appreciate your contribution to the success of this research by filling this questionnaire.

1. The educational level of parent:

   None [ ], Primary [ ], Secondary [ ], Tertiary [ ]
2. Socio-economic status: High [ ], Middle [ ], Low [ ],
3. Name of child____
4. Sex of child: Male [ ], Female [ ]
5. Age of your child:____
6. Place of birth____
7. Nutritional status of your child: Normal [ ], Average [ ], Poor [ ]
8. Did your child receive vitamin A supplement? YES [ ], NO [ ]
9. Any previous vaccination given to your child. YES [ ], NO [ ]
10. Any recent case of measles infection in your family? YES [ ], NO [ ]
11. Has your child ever been hospitalized for febrile illness involving rash? YES [ ], NO [ ]
12. Which of the following vaccines has your child received?

    Measles-mumps-rubella vaccine [ ], Polio vaccine [ ], Chickenpox vaccine [ ]

APPENDIX VI: ANALYSIS REPORT OF IgG ELISA

PLATE 1 IgG

MULTISKAN EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.050

BLANK WELL A1

ABSORBANCES
14. MAY 2016 15:01:25

	1	2	3
A	0.000	2.432	0.337
B	0.001	1.229	1.676
C	1.612	0.224	1.772
D	1.680	2.368	0.585
E	2.663	2.066	0.640
F	1.509	2.553	2.181
G	1.368	2.710	1.335
H	0.181	0.694	0.635

	4	5	6
A	0.822	0.122	0.288
B	2.237	0.883	2.664
C	0.548	2.002	2.062
D	2.759	0.646	0.996
E	2.169	0.214	0.372
F	0.611	0.160	1.964
G	1.677	0.379	0.860
H	2.690	1.066	0.288

	7	8	9
A	2.273	2.154	0.514
B	0.457	2.159	2.569
C	0.234	2.028	0.146
D	0.154	1.047	0.206
E	2.091	1.189	0.414
F	1.769	1.521	1.540
G	1.941	1.804	0.466
H	2.558	1.397	0.639

	10	11	12
A	0.575	0.622	2.426
B	0.178	0.209	2.701
C	0.294	0.555	0.225
D	0.310	0.554	0.370
E	0.092	0.984	1.179
F	1.048	0.652	2.695
G	0.960	0.504	0.730
H	0.430	0.896	0.633

PLATE 2 ISM

F -0.000-0.000 MULTISKAN
EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.086

BLANK WELL A1

ABSORBANCES
13. MAY 2016 14:00:10

	1	2	3
A	0.000	2.658	0.320
B	-0.004	0.646	0.000
C	1.893	2.206	0.394
D	1.876	0.515	0.330
E	2.624	1.246	0.465
F	1.106	0.422	2.476
G	0.850	0.685	1.248
H	0.442	2.819	0.184

	4	5	6
A	2.064	2.970	1.546
B	2.892	2.591	2.383
C	1.254	0.537	2.109
D	1.819	1.325	2.799
E	2.231	2.992	2.642
F	1.142	0.374	0.596
G	0.019	1.056	0.463
H	0.695	0.790	0.112

	7	8	9
A	1.586	1.056	0.909
B	0.381	0.380	1.282
C	0.582	1.009	2.545
D	0.704	0.996	0.483
E	0.016	0.516	1.156
F	0.237	0.203	1.011
G	0.520	1.083	1.917
H	-0.003	1.166	1.345

	10	11	12
A	0.064	0.645	0.252
B	0.118	1.335	0.484
C	0.215	0.644	1.347
D	0.335	0.779	0.731
E	0.528	0.210	0.275
F	0.428	0.432	0.194
G	1.438	0.569	1.615
H	0.593	0.298	2.406

MULTISKAN EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.050

BLANK WELL A1

ABSORBANCES
14. MAY 2016 15:05:07

	1	2	3
A	0.000	1.812	2.292
B	0.010	0.095	1.773
C	1.997	0.875	1.933
D	2.105	2.354	2.122
E	2.733	2.370	2.292
F	0.098	0.197	2.708
G	2.538	1.925	1.964
H	1.656	2.074	2.652

	4	5	6
A	1.498	2.308	0.253
B	2.007	0.277	2.235
C	1.765	2.613	2.432
D	1.333	2.789	1.461
E	2.121	2.601	1.658
F	2.403	1.176	2.617
G	2.263	2.724	2.162
H	2.275	0.320	2.636

	7	8	9
A	2.075	0.769	2.679
B	0.068	1.727	2.332
C	0.379	0.203	0.643
D	0.224	0.625	2.062
E	1.791	0.295	1.229
F	2.077	0.902	1.261
G	1.361	1.103	0.015
H	0.186	0.690	2.204

	10	11	12
A	1.963	0.880	2.219
B	2.677	1.782	1.551
C	2.348	0.094	2.579
D	0.351	0.628	0.591
E	0.182	0.819	0.704
F	0.472	0.451	0.546
G	0.425	0.607	0.869
H	0.054	2.668	0.398

PLATE 4 IgGs

MULTISKAN EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.055

BLANK WELL A1

ABSORBANCES
14. MAY 2016 15:21:49

		1	2	3
RB	A	0.000	0.526	0.641
—	B	0.007	0.469	0.102
C1	C	1.839	0.742	0.092
C2	D	1.861	2.052	0.013
+	E	2.660	0.524	0.423
	F	0.951	1.346	0.236
	G	1.995	0.215	0.333
	H	0.800	0.531	0.026

	4	5	6
A	0.587	0.404	0.039
B	0.024	0.388	0.175
C	0.247	0.007	0.328
D	0.008	0.176	0.094
E	2.794	0.491	0.195
F	0.087	0.032	0.014
G	0.438	0.230	0.222
H	0.052	0.513	0.062

	7	8	9
A	0.169	0.177	1.194
B	0.150	0.147	0.187
C	0.121	0.150	0.093
D	0.013	0.142	0.004
E	0.133	0.333	0.007
F	0.246	0.022	0.009
G	0.212	1.219	0.009
H	0.170	0.514	0.007

	10	11	12
A	0.005	1.056	0.382
B	0.005	2.619	0.479
C	0.002	0.047	1.992
D	0.005	0.120	0.286
E	0.012	0.261	0.074
F	0.372	0.147	0.220
G	0.332	0.395	0.180
H	0.364	0.158	0.045

PLATE 5 IgG

MULTISKAN EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.048

BLANK WELL A1

ABSORBANCES
14. MAY 2016 15:56:35

	1	2	3
A	0.000	0.605	0.115
B	0.001	0.388	0.057
C	1.621	0.131	0.705
D	1.484	0.016	0.189
E	2.111	0.170	0.193
F	2.015	1.700	0.179
G	0.633	0.103	0.111
H	0.275	0.342	0.410

	4	5	6
A	0.107	0.289	0.282
B	1.199	0.081	0.226
C	0.229	0.066	0.264
D	0.170	1.919	0.096
E	0.037	0.037	0.271
F	0.209	0.226	0.049
G	0.381	0.230	0.054
H	0.074	0.457	0.673

	7	8	9
A	0.084	0.390	0.050
B	1.518	0.557	0.298
C	0.182	0.098	0.089
D	2.143	0.112	0.164
E	0.407	0.134	0.786
F	0.021	0.050	0.053
G	0.158	0.166	0.948
H	0.243	0.436	0.136

	10	11	12
A	0.041	0.908	0.143
B	0.053	0.041	1.257
C	0.046	0.069	0.176
D	0.019	0.039	0.258
E	0.521	0.460	0.960
F	0.982	0.268	0.905
G	0.197	0.119	0.046
H	0.058	0.128	1.331

APPENDIX VII: ANALYSIS REPORT OF IgM ELISA

Plate 1 IgM

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
RC	Hole	B	S1	S2	S3	S4	S5
	OD	0.212	4.00*	4.00*	4.00*	2.937	1.406
	Quant		*	*	*	2.937	1.406
	Quali						
NC	Hole	S12	S13	S14	S15	S16	S17
	OD	0.000	0.573	0.963	0.407	0.017	0.885
	Quant	0.000	0.573	0.963	0.407	0.017	0.885
	Quali						
C1	Hole	S24	S25	S26	S27	S28	S29
	OD	1.302	0.044	1.976	0.166	0.858	0.927
	Quant	1.302	0.044	1.976	0.166	0.858	0.927
	Quali						
C2	Hole	S36	S37	S38	S39	S40	S41
	OD	1.305	0.856	1.792	2.136	0.128	0.175
	Quant	1.305	0.856	1.792	2.136	0.128	0.175
	Quali						
PC	Hole	S48	S49	S50	S51	S52	S53
	OD	2.834	1.563	1.520	2.468	0.243	0.807
	Quant	2.834	1.563	1.520	2.468	0.243	0.807
	Quali						
	Hole	S60	S61	S62	S63	S64	S65
	OD	2.815	2.665	2.494	2.486	0.206	0.715
	Quant	2.815	2.665	2.494	2.486	0.206	0.715
	Quali						
	Hole	S72	S73	S74	S75	S76	S77
	OD	2.650	2.525	2.869	1.237	0.610	0.730
	Quant	2.650	2.525	2.869	1.237	0.610	0.730
	Quali						
	Hole	S84	S85	S86	S87	S88	S89
	OD	2.682	2.854	2.711	2.668	0.156	0.733
	Quant	2.682	2.854	2.711	2.668	0.156	0.733
	Quali						

	7	8	9	10	11	12
Hole	S6	S7	S8	S9	S10	S11
OD	4.00*	0.962	0.673	0.871	0.482	0.385
Quant	*	0.962	0.673	0.871	0.482	0.385
Quali						
Hole	S18	S19	S20	S21	S22	S23
OD	0.917	0.088	0.000	0.000	2.692	0.552
Quant	0.917	0.088	0.000	0.000	2.692	0.552
Quali						
Hole	S30	S31	S32	S33	S34	S35
OD	2.218	0.355	0.322	0.144	2.271	2.252
Quant	2.218	0.355	0.322	0.144	2.271	2.252
Quali						
Hole	S42	S43	S44	S45	S46	S47
OD	2.727	0.289	2.117	0.115	2.700	2.898
Quant	2.727	0.289	2.117	0.115	2.700	2.898
Quali						
Hole	S54	S55	S56	S57	S58	S59
OD	1.361	0.272	0.736	0.454	1.360	2.801
Quant	1.361	0.272	0.736	0.454	1.360	2.801
Quali						
Hole	S66	S67	S68	S69	S70	S71
OD	1.363	0.285	1.203	1.186	1.886	2.874
Quant	1.363	0.285	1.203	1.186	1.886	2.874
Quali						
Hole	S78	S79	S80	S81	S82	S83
OD	1.845	0.435	0.844	0.541	0.471	1.163
Quant	1.845	0.435	0.844	0.541	0.471	1.163
Quali						
[illegible]	[illegible]	[illegible]	[illegible]	S93	S94	S95
OD	2.583	1.450	2.642	1.411	0.888	1.769
Quant	2.583	1.450	2.642	1.411	0.888	1.769
Quali						

Test date:2016-05-12　　Test time:23:(9:08

Print date:2016-05-12　　Print time:23:10:17

PLATE 2 ISM

F -0.000-0.000 MULTISKAN
EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.086
BLANK WELL A1

ABSORBANCES
13. MAY 2016 14:00:10

		1	2	3
	A	0.000	2.658	0.320
—	B	-0.004	0.646	0.000
C1	C	1.893	2.206	0.394
C2	D	1.876	0.515	0.330
+	E	2.624	1.246	0.465
	F	1.106	0.422	2.476
	G	0.850	0.685	1.248
	H	0.442	2.819	0.184

	4	5	6
A	2.064	2.970	1.546
B	2.892	2.591	2.383
C	1.254	0.537	2.109
D	1.819	1.325	2.799
E	2.231	2.992	2.642
F	1.142	0.374	0.596
G	0.019	1.056	0.463
H	0.695	0.790	0.112

	7	8	9
A	1.586	1.056	0.909
B	0.381	0.380	1.282
C	0.582	1.009	2.545
D	0.704	0.996	0.483
E	0.016	0.516	1.156
F	0.237	0.203	1.011
G	0.520	1.083	1.917
H	-0.003	1.166	1.345

	10	11	12
A	0.064	0.645	0.252
B	0.118	1.335	0.484
C	0.215	0.644	1.347
D	0.335	0.779	0.731
E	0.528	0.210	0.275
F	0.428	0.432	0.194
G	1.438	0.569	1.615
H	0.593	0.298	2.406

PLATE 3 IgM

F 0.217 0.194 MULTISKAN EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.126

BLANK WELL A1

ABSORBANCES
13. MAY 2016 14:53:00

	1	2	3
A	0.000	0.698	0.686
B	0.013	0.454	1.207
C	2.765	0.177	0.895
D	2.033	0.504	0.860
E	3.004	0.549	0.681
F	0.474	0.396	1.440
G	0.732	0.270	0.696
H	1.174	0.474	1.222

	4	5	6
A	3.199	0.873	-0.009
B	1.343	1.347	2.239
C	1.113	0.912	1.365
D	1.057	0.870	0.509
E	1.248	0.281	0.977
F	0.894	0.687	1.179
G	1.040	0.734	0.102
H	0.885	1.224	1.452

	7	8	9
A	0.170	3.064	2.129
B	0.515	3.072	3.130
C	2.527	2.902	0.642
D	0.225	2.875	0.152
E	3.004	0.665	1.637
F	0.586	2.910	0.310
G	3.400	2.701	0.132
H	3.064	2.630	0.762

	10	11	12
A	0.232	1.181	2.047
B	0.777	1.870	0.938
C	0.909	2.789	0.394
D	1.538	1.551	2.495
E	0.436	2.052	2.175
F	1.891	2.295	2.928
G	1.077	2.477	3.048
H	0.246	0.530	1.705

PLATE 4 IgM

E_{EX} 0.803 1.070 MULTISKAN
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.076

BLANK WELL A1

ABSORBANCES
13. MAY 2016 17:00:01

	1	2	3
A	0.000	0.008	0.005
B	0.022	0.010	0.080
C	1.340	-0.005	2.585
D	1.412	0.006	0.766
E	2.269	2.731	2.754
F	-0.006	2.678	0.572
G	0.002	2.599	0.039
H	0.030	0.047	0.035

	4	5	6
A	0.022	0.022	2.723
B	0.039	0.021	1.758
C	0.027	0.010	0.017
D	-0.002	2.926	0.028
E	0.030	1.877	0.021
F	0.001	0.004	0.010
G	0.006	0.632	0.488
H	0.023	1.223	2.519

	7	8	9
A	1.526	2.990	0.001
B	2.331	0.007	0.046
C	0.602	-0.003	-0.008
D	0.536	-0.002	-0.003
E	0.490	-0.007	0.003
F	0.187	-0.005	0.310
G	0.505	0.000	0.002
H	0.120	0.018	0.021

	10	11	12
A	0.014	0.021	0.026
B	0.010	0.029	0.057
C	0.000	0.050	0.045
D	-0.003	0.013	0.015
E	0.001	0.005	0.006
F	0.002	-0.005	0.005
G	0.015	-0.000	0.046
H	0.024	0.054	0.014

PLATE 5 I5M

MULTISKAN EX
PRIMARY EIA
V. 2.3
ABSORBANCE MODE
CONTINUOUS MOVEMENT
FILTER 450
SINGLE WELL BLANK 0.090

BLANK WELL A1

ABSORBANCES
13. MAY 2016 17:45:29

	1	2	3
A	0.000	1.318	0.052
B	0.058	0.217	0.032
C	1.783	0.033	2.047
D	1.364	0.012	2.589
E	2.411	0.025	2.837
F	0.144	1.255	0.105
G	1.886	0.016	0.103
H	0.048	0.339	0.500

	4	5	6
A	0.068	1.442	0.121
B	0.763	0.018	2.685
C	0.035	0.047	0.382
D	0.045	1.448	0.799
E	0.070	0.030	0.034
F	0.886	0.038	0.055
G	0.027	0.045	0.041
H	0.021	0.072	0.023

	7	8	9
A	2.971	0.346	0.034
B	0.039	0.047	0.024
C	2.219	0.043	0.019
D	0.032	0.040	0.054
E	2.482	0.025	0.038
F	0.165	0.035	0.668
G	0.025	0.003	0.045
H	0.057	0.042	0.077

	10	11	12
A	0.061	0.846	0.084
B	0.034	0.038	0.065
C	0.021	0.050	0.050
D	0.040	0.055	0.097
E	0.035	0.056	0.063
F	2.123	0.044	0.094
G	0.040	0.063	0.103
H	0.094	0.043	0.070

APPENDIX VIII

BIONEER
Innovation • Value • Discovery

100 bp Plus DNA Ladder
DNA Molecular Weight Markers

(V1/2016-02-15)

Bioneer Corporation
8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302
Republic of Korea
Tel: +82-42-930-8777 (Korea : 1588-9788)
Fax: +82-42-930-8688 E-mail: sales@bioneer.com

Bioneer Inc.
1301 Marina Village PKWY, Suite 110
Alameda, CA 94501, USA
Toll Free: +1-877-264-4300 Fax: +1-510-865-0350
E-mail: order.usa@bioneer.us.com

Bioneer R&D Center
Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro
Bundang-gu, Seongnam-si Gyeonggi-do, 13488
Republic of Korea
Tel: +82-31-628-0500 Fax: +82-31-628-0555

[Cat. No.]
D-1035

[Lot No.]
1601B

[Volume]
500 µl

[Concentration]
80.7 ng/µl

Consult Instruction For Use

RUO Research Use Only

REF Catalog Number

LOT Batch Code

Contains Sufficient for (n) tests

Caution, Consult Accompanying documents

USE BY

Temperature Limitation

Note

For research use only. Not for use in diagnostic or therapeutic procedures.

Bioneer shall not in any event be liable for incidental or special damage of any kind resulting from any use except on application(s).

• **Description :** 100 bp Plus DNA Ladder is specially designed for determining the size of double strand DNA from 100 to 10,200 base pairs. The DNA Ladder consists of 19 double strand DNA fragments ranging in sizes from 100 to 1,000 bp in 100 bp increments, and additional fragments of1,200, 1,600, 2,000, 2,961, 4,000, 5,007, 5,991, 8,000, 10,200 bp. The 500, 1,000 and 2,000 bp bands are two to three times brighter for easy identification.

• **Application :** Size confirmation of DNA fragments.

(bp)	(ng / 4µl)
10,200	7.2
8,000	7.2
5,991	8.4
5,007	8.4
4,000	9.6
2,961	12.0
2,000	56.6
1,600	13.4
1,200	10.0
1,000	40.0
900	18.0
800	16.0
700	14.0
600	12.0
500	40.0
400	12.0
300	12.0
200	12.0
100	14.0

• **Features**
- Ready-to-use
- Recommended Loading :
  3 ~ 4 µl / 5 mm lane width
- Typical Loading Number of Lanes :
  125 ~ 166 (5 mm lane width)
- Range (bp) : 100 ~ 10,200
- Number of Bands: 19

• **Storage Buffer**
- 10 mM Tris-HCl (pH 8.0)
- 1 mM EDTA
- 2.5% Ficoll
- 0.005% Bromphenol Blue
- 0.005% Xylene Cyanol

• **Recommend Electrophoresis Condition**
- 1% agarose gel.
- 0.5X TBE buffer.
- Agarose use for small DNA analysis.

• **Storage Condition**
- This product is shipped on dry ice and should be stored at -20°C until use.

• **Quality control assay**
- Agaros gel analysis shows that 19 bands are clearly distinguishable. The 500 bp, 1,000 bp and 2,000 bp band must be more intense than any other band.

• **Note**
- The DNA ladder can be applied directly onto an agarose gel.
- Do not heat before loading.
- Repeated freezing and thawing should be avoided.
- If TAE buffer is reused several times, DNA bands occasionally appear smeared in agarose gel electrophoresis. Because the buffering capacity of TBE buffer is higher, it can be used longer than TAE buffer.

• **Ordering Information**

Cat .No.	Product
D-1010	10 bp DNA Ladder, 100 µl (522 ng/µl)
D-1020	25/100 bp Mixed DNA Ladder, 250 µl (150 ng/µl)
D-1030	100 bp DNA Ladder, 250 µl (135 ng/µl)
D-1035	100 bp Plus DNA Ladder, 500 µl (80.7 ng/µl)
D-1040	1 kb DNA Ladder, 500 µl (130 ng/µl)
D-1050	Lambda DNA / *Hind* III Marker, 500 µl (200 ng/µl)
D-1060	Lambda DNA / *Eco*R I Marker, 500 µl (200 ng/µl)
D-1070	Lambda DNA / *Eco*R I + *Hind* III Marker, 500 µl (200 ng/µl)
D-2510	Lambda DNA, 500 µl (250 µg)
D-1030-1	*AccuLadder* 100 bp DNA Size Marker, 320 µl x 2 (54 ng/µl)
D-1040-1	*AccuLadder* 1 kb DNA Size Marker, 500 µl x 2 (65 ng/µl)

APPENDIX IX: Cycle Sequencing Products

Location	MV Genotype B3 Sequencing
SOK3	TAGGGGTGTCCGTGTCTGAGTCTTGTCTTAGATTCCTGCCATGGCTTGC AGCCTGAGCAGGGCATCGGCTGACCTTCGACTGTCCTGCGGATCTTGGC TGAACTCCGATGCAGTGTCAATGTCTAGGGGTGTGCCGGTTGGAGGAT GGGCAGCTCTCGCATCACTTGCTCTGCTGGGCTCGGTTTCTCTGTGGCT CTCCCCGGCTTCTCCTCGGTTCTGTTTGACCCTCCTGTCCTCCTTAACCC CCAATCTCGGCAGCTCATTCTCACTTTGATCACCGTGTAGAAATGACAC TTGGGCTTGTCTGGGTCCAACTGATCTGCTGATCCTGTCCTCAGTAGTA TGCATTGCAATCTCTGAAACAAGCCTTGCATCCTCGGCAGTTATACCGA GTTCAGATGCCAATGTGGAACTGACCTTTCCAGCTGACCTCCTTACCAT C
SOK4	CATTGTACACTCTAGGGGTGTCCGTGTCTGAGTCTTGTCTTCCGAGATT CCTGCCATGGCTTGCAGCCTAAGCAGGGCATCGGCTGACCTTCGACTGT CCTGCGGATCTTGGCTGAACTCCGATGCAGTGTCAATGTCTAGGGGTGT GCCGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGTGGGCTCGG TTTCTCTGTGGCTCTCCCGGGCTTCTCCTCGGTTCTGTTTGACCCTCCTG TCCTCCTTACCCCCCAATCTCGGTAGCTCATTCTCACTTTGATCACCGTG TAGAAATGACCTTGGGCTTGTCTGGGTCCAACTGCTCTGCTGATCCTGT CCTCAGAGTATGCATTGCAATCTCTGAAACAAGCCTTGCATCCTCGGCA GTATACCGAGTTTCAGATGCCAATGTGGAATGACCTTTCCAGCTGACCT
JG21	TTGTACACTCTAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTCC TGCCATGGCTTGCAGCCTAAGCAGGGCATCGGCTGACCTTCGACTGTCC TGCGGATCTTGGCTGAACTCCGATGCAGTGTCAATGTCTAGGGGTGTGC CGGTTGGAGGATGGGCAGCTCTCGCATCACTTGCTCTGCTGGGCTCGGT TTCTCTGTGGCTCTCCCGGGCTTCTCCTCGGTTCTGTTTGACCCTCCTGT CCTCCTTACCCCCCAATCTCGGCAGCTCATTCTCACTTTGATCACCGTGT AGAAATGACACTTGGGCTTGTCTGGGTCCAACTGATCTGCTGATCCTGT CCTCAGTAGTATGCATTGCAATCTCTGAAACAAGCCTTGCATCCTCGGC AGTTATACCGAGTTCAGATGCCAATGTGGAACTGACCTTTC
KD24	CTCTAGGGGTGTCCGTGTCTGAGTCTTGTTCTTCCGAGATTCCTGCCATG GCTTGCAGCCTAAGCAGGGCATCGGCTGACCTTCGACTGTC

KEY: Sok3= Sokoto sample number 3, Sokoto4 = Sokoto sample number 4, JG21 = Jigawa sample number 21, KD24 = Kaduna sample number 24