A SURVEY FOR RUBELLA VIRUS AND ANTIBODIES AMONG PREGNANT WOMEN IN KADUNA STATE NIGERIA

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA

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TITLE PAGE

A SURVEY FOR RUBELLA VIRUS AND ANTIBODIES AMONG PREGNANT WOMEN IN KADUNA STATE NIGERIA

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NOVEMBER, 2016

DECLARATION

I declare that the work reported in this thesis entitled a survey for rubella virus antibodies among pregnant women in Kaduna State Nigeria has been carried out by me in the Department of Microbiology, Faculty of Life Science, Ahmadu Bello University, Zaria.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project thesis was previously presented for another degree or diploma at this or any other institution.

.....	.....	.....
Aishatu Bintu Gubio	Signature	Date

CERTIFICATION

This thesis, entitled "A SURVEY FOR RUBELLA VIRUS ANTIBODIES AMONG PREGNANT WOMEN IN KADUNA STATE NIGERIA", by AISHATU BINTU GUBIO, meets the regulations governing the award of Doctorate degree (PhD Microbiology) Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedication to the mothers who were infected with rubella virus and babies who had congenital anomalies.

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ABSTRACT

Rubella Virus (RV) causes a mild disease, but maternal infection early in pregnancy often leads to birth defects known as congenital rubella syndrome (CRS). Rubella remains poorly controlled in Africa despite being a vaccine preventable disease. The aim of this study were to determine the survey for rubella virus and antibodies among pregnant women in Kaduna State Nigeria. The study was carried out on pregnant women attending ante-natal clinic in three different senatorial district in Kaduna State from the year 2013-2014. Blood serum samples from 900 pregnant women were screened for rubella IgM and IgG antibody using enzyme linked immunosorbent assay (ELISA). The risk factors associated with rubella virus transmission among these pregnant women were identified. Reverse Transcription Polymerase Chain Reaction (RT-PCR), and gene sequencing were used to confirm the presence of rubella virus in the serum sample. Of the 900 pregnant women screened, 572(63.1%) were positive for rubella IgG and 39(4.3%) were positive for rubella IgM. The prevalence of rubella IgG was highest among the age group 41-45 years old (83.3%) and IgM was highest among the age group 21-25 years old (6.1%).The IgG test results shows that out of the 497 pregnant women enrolled in their first trimester, 273 (54.9%) pregnant women tested positive, while the IgM positive results shows 24(4.8%) in their first trimester. Although the southern senatorial district had the highest sero-prevalence 14(35.9%) among the three centres, the differences were not statistically significant (p=0.05). Only three people claimed to have been vaccinated against rubella virus and these people were negative for rubella. Acquisition of secondary education and being a house wife were insignificantly associated (p=0.05).The serological evidence of rubella virus found in pregnant women in this study is an indication that rubella is prevalent in Kaduna State, Nigeria. It is however still necessary to immunize seronegative women against rubella before they get pregnant.

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LIST OFABBREVIATIONS

Abbreviation	Word
ANC	Ante Natal Clinic
ATP	Adenosine Triphosphates
APO-1 (CD 95)	Mutagenesis of fas
AUG	Adenine Uracil Guanine
AVE	RNase-Free water
AVL	A Viral Lysis Buffer
AW1	Wash Buffer 1
AW2	Wash Buffer 2
Bax	Pro-Apoptotic Proteins
Bcl-xL	Heterodimeric Partner
Bcl-xS	Pro-Apoptotic Proteins
Bcl-2	Inner Mitochondrial protein
BHK-21 cells	Baby Hamster Kidney
BLAST	Basic Local Alignment Search Tool
Bp	Base Pair
C	Cytosine
CA	California
CC	Confronting Cisternae
CDC	Centre for Disease Control
CDD	Conserved Domain Database
cDNA	Complementary Deoxyribonucleic Acid
CM	Confronting Membranes
CM-1	Confronting membranes type 1
CM-2	Confronting membranes type 2
CNS	Central Nerves System
CPE	Cytopathic Effect
CPV	Cytopathic Vacuoles type 1
CRI	Congenital Rubella Infection
CRS	Congenital Rubella Syndrome
CSD	Central Senatorial District

CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
dsRNA	Double Stranded Ribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endoplasmic Reticulum
G	Guanine
HAI	Haemagglutination Inhibition
HNIG	Human Normal Immunoglobulin
HPV	Human Papillomavirus
HPV77.DE5	First Rubella Vaccines Strain
IDDM	Insulin-Dependent Diabetes Mellitus
IF	Immunofluorescence
IgA	Immunoglobulin Antibody
IgG	Immunoglobulin Gamma
IgM	Immunoglobulin Miu
Iμ/ml	Micro litre per millimeter
IUGR	Intrauterine Growth Retardation
KAHP	Key Actions for Health Promotion
kDa	Adipocyte complement-related protein
LGA	Local Government Area
MMR	Measles Mums Rubella Vaccines
mRNA	Messenger Ribonucleic Acid
NCBI	National Center for Biotechnology Information
Ns	Non Structure
NSD	Northern Senatorial District
Nt	Nucleotide
OD	Optical Density
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PHC	Primary Health Care
p value	Prevalence value
RA27/3	Rubella Vaccine Strain
RB	Retinoblastoma
RER	Rough Endoplasmic Reticulum

RF	Replicative Forms
RI	Replicative Intermediates
RIA	Radio Immuno Assay
RK	Rabbit Kidney
RLP	Rubella Virus like Particles
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription- Polymerase chain reaction
RV	Rubella Virus
SFV	Semliki Forest Virus SIRC
SRH	Single Radial Hemolysis
SSD	Southern Senatorial District
ssRNA	Single Stranded Ribonucleic Acid
T Cells	T lymphocytes
TM	Transmembrane
TP53	Tumor Protein
TSEM	Transmission Scanning Electron Microscopy
USA	United States of America
UK	United Kingdom
UV	Ultra Violet Rays
WHO	World Health Organization
$\chi 2$	Chi Square
Yrs	Years

CHAPTER ONE

1.0 INTRODUCTION

1.1Rubella Virus

Rubella virus (RV) causes a benign systemic rash illness when it infects humans as a result of acute infection and causes severe birth defects if acute maternal infection occurs during the first trimester of pregnancy (Willey *et al*., 2011). This virus is a positive-strand RNA virus that replicates in the cytoplasm of the infected cell. Rubella virus is taxonomically unique, being the sole member of the genus *Rubivirus* of the family *Togaviridae* (Brooks *et al*., 2007).

The rubella virus causes "German measles," also known as "three-day measles"(Bukbuk *et al.,* 2002), this is usually a milder disease than red measles. Infections occur most commonly in children and the resulting natural immunity is most probably lifelong (Matthews *et al.,* 2011). However, in pregnant women the risk of intrauterine transmission is up to 90% if infection occurs in early pregnancy (8-10 weeks gestation). As the viraemia leads to placental infection and spread of the virus, it causes a chronic infection of the foetus leading to the development of congenital rubella syndrome (CRS) (Matthews *et al*., 2011). Red/Hard measles or just measles is caused by Rubeola virus.

A pregnant woman who is infected with rubella virus can transmit the virus to the foetus (Bukbuk *et al.,* 2002), to cause congenital rubella infection (CRI). Several organ systems can be effected when CRI occurs and these includes the eyes, ears, heart, brain, and endocrine system (Chantler *et al*., 2001). A CRS encompasses all outcomes associated with intrauterine rubella infection, including miscarriage, stillbirth, abortion, combinations of birth defects, or asymptomatic infection in the infant (Reef and Cordero, 2000a).The pathogenesis of CRS is not understood and CRS patients are persistently infected with RV at birth and can suffer

progressive squeal in addition to those apparent at birth (Brooks *et al*., 2007; Adewumi *et al.,* 2013).

The two clinical symptoms observed in rubella virus infection are CRI which is usually mild with most cases passing as subclinical or unrecognized events and CRS which is the outcome of infection of rubella virus from the mother to the foetus after transplacental transmission of rubella disease. The clinical manifestations of congenitally acquired rubella are usually severe (Chantler *et al.,* 2001; Kimberlin, 2002). The classical sign of malformations in congenital rubella includes the eyes (cataract, microphthalmia, glaucoma and chorioretinitis), the heart (most often patent ductus arteriosus) and the ear (unilateral or bilateral sensoryneural deafness) (Haukenes, 2002).

The virus is transmitted from person to person via respiratory aerosols (Lee and Bowden, 2000), and replicates in the nasopharynx and regional lymph nodes. Rubella virus infects only humans and is spread from person to person through contact or from a cough or sneeze as the rubella virus lives in the mucus of an infected person (Agbede *et al*., 2011). Symptoms for postnatal Rubella virus include a rash spreading down from the face to the extremities and in some cases a runny nose, fever or joint pain. It is possible for these symptoms to be asymptomatic (Lee and Bowden, 2000).

Rubella occurs worldwide with a seasonal distribution. The peak incidence of infection is in late winter or early spring (Adesina *et al.,* 2008). The mechanism by which rubella virus causes foetal damage is not well understood till now. The possible mechanism is direct viral damage of infected cells (Margaret, 2011). Worldwide, Rubella is known to affect children,

and being a childhood disease it is predominantly endemic throughout the world (Kimberlin, 2002; Haukenes, 2002; Brooks *et al*., 2007; Willey *et al*., 2011).

In 1998, standard case definitions for surveillance of CRS and rubella were developed by W.H.O. In 2001, 123 countries/territories reported a total of about eight hundred and thirty five thousand rubella cases. More countries are expected to report on rubella as a global measles/rubella laboratory network is further developed under the coordination of W.H.O (Frey, 2008).

Rubella epidemic and pandemics are controlled in the United States (Brooks *et al*., 2007; Centers for Disease Control and prevention, 2008; NewYork Times, 2015). During the 1962–1965 global pandemic, an estimated 12.5 million rubella cases occurred in the United States, resulting in 2000 cases of encephalitis, 11,250 spontaneous abortions, 2100 neonatal deaths, and 20,000 infants born with congenital rubella syndrome (Mayo Clinic, 2011).The rubella-induced congenital anomalies were observed and collectively referred to as the expanded congenital rubella syndrome (Best and Banatvala, 2000; Kimberlin, 2002). The economic impact of this epidemic in the United States was estimated to be $1.5 billion (Kimberlin, 2002).

In a study in Karachi, the prevalence for rubella IgM and IgG rubella were 13% and 29% respectively among 355 pregnant women screened. Out of this number, total of 212 pregnant women had abortion (Bamboye *et al.,* 2004). It was recommended that to reduce morbidity and mortality related to rubella virus, premarital screening and vaccination of sero-negative girls should be done (Bamboye *et al.,* 2004). There is at least a 20% chance of damage to the foetus if a woman is infected with rubella early in her pregnancy (Adesina *et al*., 2008).

Immunizing children on schedule is recommended to protect children and others, including pregnant women and their unborn babies from rubella infection (Bukbuk *et al.,* 2002).

In Africa there is general lack of awareness of Rubella and it is not often diagnosed clinically, which may be due to the dark skin colour (Christelle *et al.,* 2010). Sero-epidemiological survey conducted throughout Africa has shown the presence of the virus throughout the continent (Christelle *et al.,* 2010). World Health Organization (2015) has reported 466 rubella cases in 2007 in Nigeria. Studies in Ghana, Togo, Ivory Coast, Burkina Faso and Mali all showed a high prevalence of rubella antibody in the adult population (Christelle *et al.,* 2010).In Lagos State Nigeria, a study on rubella-IgG antibody in women of childbearing age was conducted and 77% of all the female subjects were positive for Rubella-IgG antibody. This means that the remaining 23% did not have immunity to this rubella virus (Dontigny *et al.,* 2008).

The prevalence of IgG antibody specific to the rubella virus was assessed in 207 consenting pregnant women in Maiduguri Borno State (Bukbuk *et al.,* 2002). The pregnancy outcome was normal in 27% of the women studied (Bukbuk *et al.,* 2002). Miscarriage, premature delivery and stillbirth occurred with the others. There was no clinically detectable malformations in 25 (44.6%) of all the deliveries from the pregnant women (Bukbuk *et al.,* 2002). A study conducted at the Ahmadu Bello University Teaching Hospital, Zaria, showed a rubella IgG sero-prevalence of 97.9% among pregnant women and 2.1% sero negative for rubella IgG antibody this was out of 430 serum samples tested (Muhammad *et al.,* 2010). Differences in socio demographic factors were of little significance between the groups but awareness of the infection was low (Bamboye*et al.,* 2004). Another study on rubella conducted at the Ahmadu Bello University Zaria showed a prevalence of 1.9% for rubella

IgM antibodies (Pennap *et al*., 2009). This was determined from 160 pregnant women (Olajide *et al*., 2012). A study in Benue state showed that 4.2% of the pregnant women tested were seropositive to rubella IgM (Pennap *et al*., 2009; WHO, 2015).

The advent of molecular techniques such as the polymerase chain reaction and sequencing has made it possible to examine the genetic makeup of the virus itself and perform molecular epidemiology studies (Lee and Bowden, 2000). Genotypes 1A, 1E, 1F, 2A and 2B have been isolated in China. Genotype 1j has only been isolated from Japan and the Philippines. Genotype 1E is found in Africa, the Americas, Asia and Europe. Genotype 1G has been isolated in Belarus, Cote d'Ivoire and Uganda. Genotype 1C is endemic only in Central and South America. Genotype 2B has been isolated in South Africa. Genotype 2C has been isolated in Russia (Dominguez *et al*., 1990).

1.2 Statement of the Research Problem

The research Problem of this study include:

1. The lack of routine immunization against the infection
2. Cases of misdiagnosis which include a rash spreading down from the face to the extremities and in some cases a runny nose, fever or joint pain.
3. The sequalae of outcome which includes CRS and the irreversibility of the outcome
4. Lack of awareness of the disease and control measures

Worldwide, it is estimated that there are more than 100.000 infants born with CRS each year (Frey, 2008). Cases of rubella infection still occur in Nigeria among pregnant women (Bamboye *et al.,* 2004, Olajide *et al*., 2012). In 2006, a case of confirmed CRS was reported in Port Harcourt in a three month old male with heart failure (Otaigbe *et al.,* 2009). Other

studies conducted in Maiduguri, Ibadan, Lagos and Zaria have shown the presence of rubella virus in pregnant women with prevalence of rubella IgG antibodies of76%, 54%, 68.5%, 95.9% and 97% respectively (Onyenekwe *et al.,* 2000;Bukbuk *et al.,* 2002; Bamboye *et al.,*2004;Muhammad *et al.,* 2010; Olajide *et al*., 2012).

1.3 Justification

Rubella virus only has a large risk of infection if the disease is contracted by the mother in the first trimester, after which birth defects are less likely (Lee and Bowden, 2000; Sallam *et al*., 2003). Even if a woman who develops rubella has no rubella symptoms, her baby can still develop serious congenital rubella syndrome symptoms. Therefore, it is important to determine the rubella immunity status for all women, either before conception or very early in the first trimester of pregnancy.

Worldwide, it is estimated that there are more than 100.000 infants born with CRS each year (Frey, 2008). Cases of rubella infection still occur in Nigeria among pregnant women (Bamboye *et al.,* 2004, Olajide *et al*., 2012). In 2006, a case of confirmed CRS was reported in Port Harcourt in a three month old male with heart failure (Otaigbe *et al.,* 2009). Other studies conducted in Maiduguri, Ibadan, Lagos and Zaria have shown the presence of rubella virus in pregnant women with prevalence of rubella IgG antibodies of 76%, 54%, 68.5%, 95.9% and 97% respectively (Onyenekwe *et al.,* 2000; Bukbuk *et al.,* 2002; Bamboye *et al.,*2004; Muhammad *et al.,* 2010; Olajide *et al*., 2012). In Markurdi, Benue state, a serological survey of specific rubella IgM in the sera of pregnant women showed that only 4.2% of the women were susceptible (Pennap *et al*., 2009).

A study in Kaduna State by Muhammad *et al.* (2010) reported a rubella IgG prevalence of 97.9% among pregnant women attending ante natal clinic in Ahmadu Bello University Teaching Hospital Shika. Olajide *et al.* (2012) also conducted a study between the months of June and August 2012 on rubella IgM among pregnant women and reported a prevalence of 38.8%. This makes it necessary to conduct a survey across the senatorial districts of Kaduna State.

This research will give information on the current prevalence of rubella virus infection in pregnant women in Kaduna State. The result would provide data that the Ministry of Health could use this information to guide its routine immunization programs in health facilities in Nigeria. Prenatal diagnosis of infectious diseases has been shown to be indispensable to confirm or exclude due to rubella. Consequently, the central challenge and the most important public health priorities at this time are to determine if there are other infected persons and if necessary, implement measures to prevent additional human infections (WHO, 2015).

1.4 Research Question

1. What is the prevalence of rubella virus among pregnant women in Kaduna State Nigeria?
2. What are the socio-demographic characteristics of women with rubella IgM or IgG antibodies?
3. What are the risk factors for rubella virus among pregnant women in Kaduna State?

1.5 Aim

"Aimed at monitoring the disease by way of surveillance study in Kaduna Nigeria"

1.6 Specific Objectives

The specific objectives of this study were to:

1. Identify the risk factors associated with rubella virus infection among pregnant women in Kaduna State and Follow up the babies of these pregnant women whose serum were positive for IgM by checking their case note, calling or tracing their homes.
2. Determine rubella viruse IgG and IgM antibodies among pregnant women in Kaduna State Nigeria using Enzyme Linked Immuno- sorbent Assays.
3. Confirm rubella virus in the serum of pregnant women attending ante-natal clinic in Kaduna State using conventional Reverse Transcription Polymerase Chain Reaction (RT-PCR).
4. Sequence the Rubella virus isolated from these pregnant women attending ante-natal clinic in Kaduna State.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Rubella Virus

Rubella was first described in the mid-eighteenth century. Friedrich Hoffmann made the first clinical description of rubella in 1740, which was confirmed by de Bergen in 1752 and Orlow in 1758. In 1814, George de Maton first suggested that it should be considered a disease distinct from both measles and scarlet fever. All these physicians were German, and the disease was known as Rötheln (contemporary German *Röteln*), hence the common name of "German measles"(Best *et al.,* 2007). Henry Veale, an English Royal Artillery surgeon, described an outbreak in India. He coined the name "rubella" (from the Latin word meaning "little red") in 1866(Wellington 2006; Atkinson *et al*., 2007).

It was formally recognized as an individual entity in 1881, at the International Congress of Medicine in London. In 1914, Alfred Fabian Hess theorized that rubella was caused by a virus, based on work with monkeys. In 1938, Hiro and Tosaka confirmed this by passing the disease to children using filtered nasal washings from acute cases (Atkinson *et al*., 2007). In 1940, there was a widespread epidemic of rubella in Australia. Subsequently, ophthalmologist Norman McAllister Gregg found 78 cases of congenital cataracts in infants and 68 of them were born to mothers who had caught rubella in early pregnancy (Atkinson *et al*., 2007). Gregg published an account, *Congenital Cataract Following German Measles in the Mother*, in 1941. He described a variety of problems now known as congenital rubella syndrome (CRS) and noticed that the earlier the mother was infected, the worse the damage was. The virus was isolated in tissue culture in 1962 by two separate groups led by physicians Parkman and Weller (Lee *et al.,* 2000; Wellington 2006).

There was a pandemic of rubella between 1962 and 1965, starting in Europe and spreading to the United States. In the years 1964-65, the United States had an estimated 12.5 million rubella cases. This led to 11,000 miscarriages or therapeutic abortions and 20,000 cases of congenital rubella syndrome. Of these, 2,100 died as neonates, 12,000 were deaf, 3,580 went blind and 1,800 mentally retarded. In New York alone, CRS affected 1% of all births (Pan America Health Organisation, 2007).

In 1969 a live attenuated virus vaccine was licensed (Atkinson *et al*., 2007). In the early 1970s, a triple vaccine containing attenuated measles, mumps and rubella (MMR) viruses was introduced (Wellington, 2006; CDC, 2011).

2.2 Genome and Cell Structure of Rubella Virus

2.2.1 Genome Organization

The full-length RV genome (fig 1) measures 9,762 nucleotide (nt) and contains two long open reading frames (ORFs) (Dominguez*et al*., 1990). The RV 5′-proximal ORF of 6,345 nt encodes the viral nonstructural (ns) proteins, p150 and p90, while the 3′ ORF of 3,189 nt encodes the structural proteins, capsid (C), E2, and E1 (Yao*et al.,* 1998). Thus, the gene order for the RV 40S RNA is 5′-p150-p90-C-E2-E1-3′. The complete nucleotide sequence of RV has been determined for three strains, Therien, M33, and RA27/3. Nucleotide and deduced amino acid sequencing analysis reveals a high degree of homology between strains, ranging from 97.2 to 99% and 97.6 to 98.9% at the nucleotide and amino acid levels, respectively. The RV genome has an extraordinarily high guanine cytosine (G, C) content of 69.5%, the highest of any known RNA virus to date, and this has undoubtedly contributed to some discrepancies in the reported RV genomic sequences (Pugachev*et al.,* 1998).

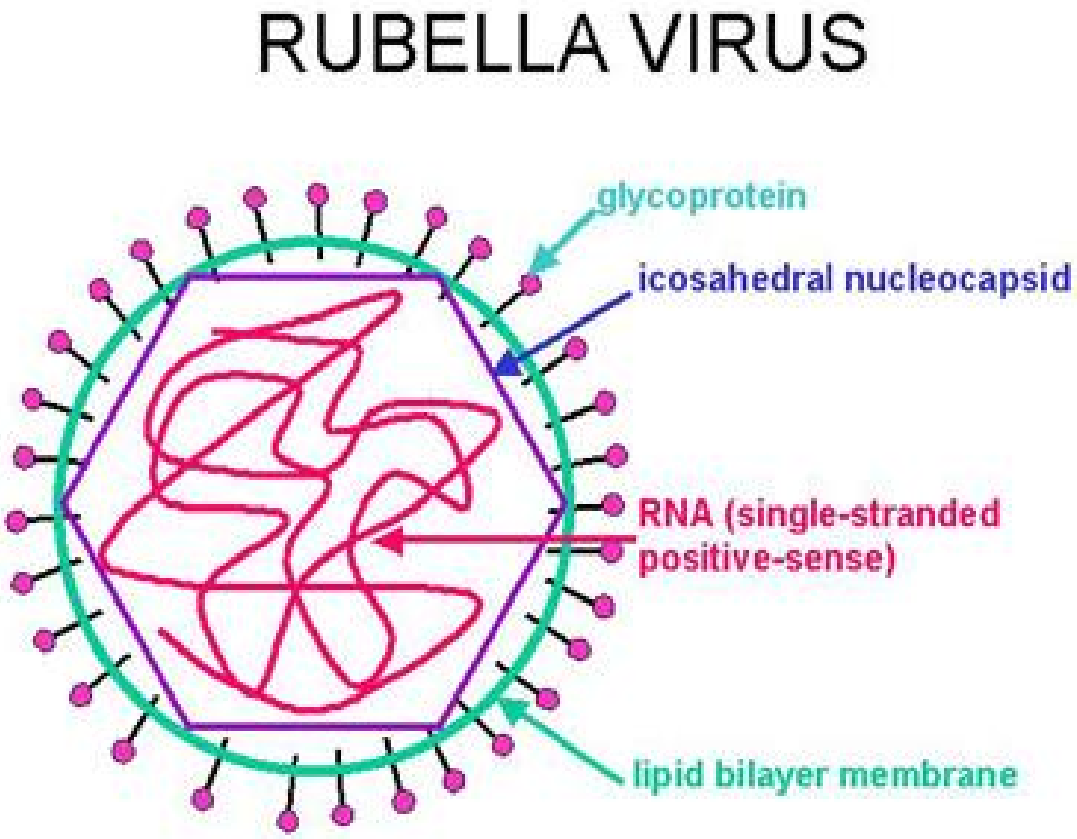

Fig 2.1: Genome Cell Structure of Rubella Virus

Rubella grows in a wide range of cell lines. It induces a cytopathic effect (CPE) only in continuous cell lines such as RK13 (rabbit kidney) and Vero cells. Immunofluorescence is used to identify the presence of the virus in culture. Rubella nucleotides and has similar genomic structure to other Toga viruses. The Rubella Virus has the highest concentration of G, C nucleotides of any RNA virus with 69.5% of the genome consisting of those nucleotides. Rubella has been sequenced completely for three strains showing greater than 95% homology between the three strains (Lee *et al*., 2000).

It is a non-arthropod-borne Togavirus and only member of the genus Rubivirus. It is an enveloped ssRNA circular or oval shaped virus of 60nm in diameter and nucleocapsid of 33nm symmetry thought to be icosahedral but virus particle has a pleomorphic appearance (Lee *et al*., 2000). Rubella is a positive 40S RNA virus consisting of 3 structural proteins. The outer membrane is a lipid bilayer containing specialized glycoproteins (E1 and E2: believed to be responsible for attachment). Only one serotype of Rubella virus E1 has 6 distinct antigenic determinants; 4 associated with hemagglutination, and 2 with neutralization. It is also believed that a pH of 6.0 or less induces conformational changes in the glycoproteins making attachment of the viral envelope to host cells more likely. Rubella virus likely enters cells via endocytosis. Once in the cell a conformational change occurs in the capsid shell releasing the genetic information into the cell. Replication is slow with a latency period of 8-12 hours, with structural proteins appearing at 12-16 hours and peak viral 36-48 hours after infection. In volunteer subjects infected via aerosol the characteristic rash typically appears 16-20 days from the time of exposure (Lee *et al*., 2000).

The virion contains an electron-lucent spherical core composed of multiple copies of the RV capsid protein and a single copy of the viral RNA genome. The RV core is surrounded by a

host-derived lipid bilayer containing 5- to 6-nm-long spikes which project from the virion surface; the spikes are composed of the E2 and E1 glycoproteins (Ilkow *et al*., 2010).

2.2.2The Capsid Protein

It is a non-glycosylated, phosphorylated, disulfide-linked homodimer with a reported molecular mass of 33 to 38 kDa. The capsid protein contains clusters of proline and arginine residues, which have been postulated to be involved in binding to the RV genomic RNA to form the viral nucleocapsids. In particular, a 28-amino-acid domain containing a large number of basic residues appears to be directly involved in binding to the RNA genome (Zhou *et al*., 2007). However, the interaction of the capsid protein with the viral RNA may not be solely dependent on the density of basic residues because other basic regions within the protein were found to bind poorly. It remains to be determined whether other domains of the protein are involved in nucleocapsid formation. On the RV genome, a 29 nt stretch nt 347 to 375 interacts with the capsid protein, although it is not clear whether this is sufficient for packaging of the genome (Zhan*et al.,* 2012).

2.2.3 The E1 and E2 Glycoproteins

The virion envelope proteins, E1 and E2, are type 1 membrane glycoproteins observed as spikes in the form of E1-E2 heterodimers on the virion surface. The E1 and E2 proteins each contain a putative transmembrane (TM) domain, which are 22 and 39 residues in length, respectively. For E2, the putative TM domain is followed by a positively charged 7-residue sequence, RRACRRR, and a 20-residue region which acts as a signal sequence for E1; the positively charged 7-residue region is believed to interact with the negatively charged

phospholipid head groups of the lipid bilayer. For E1, the TM domain is followed by a 13-residue cytoplasmic domain (Hobman*,et al.,* 1989a and Lee *et al.,* 2000).

The RV E1 glycoprotein migrates as discrete band with a molecular mass of 58 kDa, while the E2 glycoprotein migrates as broad heterogeneous band of 42 to 47 kDa. Amino acid sequence analysis of the E1 protein has since revealed that it contains three N-linked glycosylation sites for all strains so far sequenced. In contrast, the number of N-linked glycosylation sites of the E2 protein appears to vary depending on the strain. The E2 protein of the M33 and HPV-77 strains possesses four N-linked glycosylation sites, while the E2 protein of the Therien and RA27/3 strains possesses three. Studies using RV-infected cells and full-length cDNA clones of E1 and E2 have shown that all the N-linked glycosylation sites are utilized, with N-linked sugars representing approximately 6 kDa and 15 to 20 kDa of the molecular mass of the mature E1 and E2, respectively (Pugachev, 1998).

Studies in which recombinant E1 was expressed in *Escherichia coli* have indicated that glycosylation may be required for correct folding of E1 for the expression of important antigenic and immunogenic epitopes (Rebertson *et al.,* 2003a). For E2, mutagenesis studies have shown that removal of any of the N-linked sites results in slower glycan processing and lower stability, with the severity of the defect increasing with the number of N-linked glycosylation sites removed (Pennap *et al.,* 2009). In addition to N-linked sugars, the RV E2 protein contains O-linked carbohydrates. The presence of these O-linked sugars most probably contributes to the heterogeneous nature of the viral form of E2. Pulse-chase labeling of RV-infected cells has revealed the presence of intracellular forms of E2 (39 kDa), which migrate more rapidly than the viral form of E2 (42 to 47 kDa) (Pugachev, 1998).

The functions of the RV E1 and E2 glycoproteins have been studied extensively. Using monoclonal antibodies, it has been shown that the E1 protein contains at least six non

overlapping epitopes, some of which are associated with hemagglutination and neutralization. E1 appears to be the main surface protein, with domains involved in the attachment of the virus to the cell (Theodoro, 1997). More recent studies have revealed that a 28-residue internal hydrophobic domain of E1 is responsible for the fusogenic activity of RV. In addition, this region is involved in the binding to E2 for heterodimer formation. The function of E2 has been more difficult to determine. E2 is disulfide-linked to E1 in the mature virion and is poorly exposed. Therefore, the antigenic sites of E2 are less accessible to characterization by monoclonal antibodies (Yang, 1998; Bellini *et al.,* 2000). However, E2 does contain partial hemagglutination and neutralizing epitopes and may also carry strain-specific epitopes.

2.3 Post-Natal and Congenital Rubella Viral Infections

Postnatal infections occur after delivery and congenital rubella syndrome (CRS) is an illness resulting from rubella virus infection during pregnancy:

2.3.1 Postnatally Acquired Rubella

Infection is acquired via inhalation of aerosol or transmitted by droplet spread or direct contact with infectious patients. The virus infects cells in the upper-respiratory tract, after which cell penetration occurs by receptor-mediated endocytosis (Wolinsky, 1996). Rubella spreads and replicates in the lymphoid tissue of the nasopharynx and upper-respiratory tract, after which a viraemia leads to systemic infection, involving many organs, including the placenta. Infected people excrete high concentrations of rubella in nasopharyngeal secretions (Wolinsky, 1996). Thus, vaccines may excrete more than 10^5 TCID50 per 0·1mL, although diurnal variations as large as 1000-fold have been reported. Individuals acquiring natural

infection probably excrete even higher concentrations of infectious virus (Rebertson *et al.,* 2003a).

2.3.1.1 Clinical Features of Postnatally Acquired Rubella Virus

Among children, constitutional features are mild or absent but adults might develop fever and malaise associated with viraemia before the development of rash. The rash disappears as humoral immune responses develop, and at this stage viraemia is terminated (Burns *et al.,* 2010). Since rubella-like illnesses can be induced by other viruses that have no teratogenic potential, serological investigation is important in women who might be pregnant and who have been exposed to close contacts who might have rubella or who have rubella-like illness. Rubella virus may continue to be excreted for 1–2 weeks, sometimes even longer, and might also be recovered from the nasopharynx during the week preceding the onset of rash. Therefore, the date of first exposure could precede the onset of rash in the contact by 7–10 days (Lee and Bowden 2000).

2.3.1.2 Complications from Postnatally Acquired Rubella Virus

Postnatally acquired rubella is seldom associated with complications, apart from joint symptoms. Arthralgia or a frank arthritis, although uncommon among boys and prepubertal girls, occurs in up to 60% of postpubertal women. Symptoms generally persist for 3–4 days, although they occasionally last for 1 month or even longer, sometimes with a fluctuating course. Since rubella virus can be detected in the synovial fluid and the synovium in arthritis related to naturally acquired infection and vaccination, and since symptoms appear as rash subsides and humoral antibody develops, immune complexes might be involved in the pathogenesis (Brooks*et al*., 2007).

Although some findings suggest that rubella is involved in the pathogenesis of some forms of chronic arthritis, this association has not been confirmed in other studies (Brook*et al*., 2007). .

2.3.2. Congenital Rubella

Rubella virus enters the foetus during the maternal viraemic phase through the placenta. The virus first infects the mother and then passes to the baby during pregnancy. The virus interrupts the development of the baby. The damage to the foetus seems to involve all germ layers and results from rapid death of some cells and persistent viral infection in others. This results to the congenital rubella syndrome, associated with chromosomal aberrations and reduced cell division. The foetus is almost invariably infected if the mother is infected during the first trimester. After the first trimester, the virus is isolated infrequently from the neonates, probably because foetal immune mechanisms can be activated and infection can be terminated (Bellini *et al*., 2007). Following intrauterine infection in early pregnancy the virus persists throughout the gestation and can be isolated from most organs at autopsy. The virus can also be recovered from nasopharyngeal secretions, urine, stools and CSF from survivors. However by the age of 3 months the proportion of virus excreted, will declined to 50-60% and by 1 year, 10%. The mechanism of virus persistence is not known but may be due to defects in cell-mediated immunity (Bellini *et al*., 2007; Morice *et al.,* 2009).

2.3.2.1 Risk to the Fetus

The risk of major malformations following infection in the first trimester varies from 10% to 54%, the risk being greatest in the first 8 weeks of pregnancy (Hanshaw *et al*., 1985). However this data was compiled from prospective studies which were carried out before laboratory diagnosis of rubella infection became available. More recent studies suggest that

the actual risk of major fetal damage is much higher than realized. Cardiac and eye defects are more likely to result when maternal infection is acquired during the first 8 weeks of pregnancy that is during the critical phase of organogenesis. Whereas, retinopathy and hearing defects are more distributed throughout the first 16 to 20 weeks of gestation (Ehrlich, 2010; Kolawole *et al*., 2014). Rubella virus is seldom isolated from infants whose mothers acquired rubella after the first trimester. However rubella - specific IgM can be detected in a high proportion of these infants which means that they were infected. Major abnormalities are very rare because organogenesis is complete by 12 weeks and the immune response may be more developed (Lawn *et al*., 2000). Deafness and retinopathy (which does not affect vision), are likely to be the only abnormalities associated with post first trimester rubella. Deafness is usually the sole clinical manifestation of foetal infection occurring between 13 and 16 weeks (Derek, 2013).

2.3.2.2 Clinical Features of Rubella Virus

The clinical features of the congenital rubella syndrome (CRS) may be categorized as follows:

a. **Transient features:** Intrauterine Growth Retardation **(**IUGR), thrombocytopenic purpura, hepatosplenomegaly and haemolytic anaemia. These abnormalities are present during the first few weeks of life and are not associated with permanent sequelae. Transient bone lesions occur in 20% of congenitally infected infants. About 25% have meningoencephalitis which may or not leave neurological sequelae. Jaundice is commonly present (National Health Service, 2013).

b. **Developmental abnormalities:** Sensorineural deafness, mental retardation, insulin-dependent diabetes. Developmental defects may take months before they become apparent but persists permanently (Zhu, 2007). Congenital rubella remains the

commonest cause of congenital deafness in developed countries. Rubella deafness may be unilateral or bilateral and varies considerably in severity. Insulin Dependent Diabetes Mellitus (IDDM) is actually a common manifestation of CRS (up to 20%). However onset may be delayed till adolescence or adulthood. Autoimmune mechanisms may be involved. Between 3 - 12 months some infants develop a rubbelliform rash (Lombardo, 2011), persistent diarrhoea and pneumonitis which are referred to as "late onset disease". This carries a high mortality (Pugachev *et al.,* 1998). .

c. **Permanent damage:** Heart defects, eye defects (retinopathy, cataract, micro-opthalmia, glaucoma, severe myopia), CNS defects (microcephaly, psychomotor retardation). (National Health Service, 2013).

In the early sixties before the advent of vaccination, a large outbreak of CRS occurred in America. A follow-up study was conducted 25 years later and it was found that one-third of those affected were leading normal independent lives, one-third had to live with their parents, and one-third were institutionalized. Late sequelae, especially those affecting the heart were commonly seen (National Health Service, 2013).

2.4 Rubella Virus link to Teratogenicity

For RV, the explicit pathway leading to teratogenicity remains to be elucidated. Many of the steps leading to CRS and the consequences of CRS are well documented, but exactly how the virus causes this dramatic effect has been the subject of much speculation (Pugachev *et al.,* 1998). Cellular damage seen during early gestation of RV-infected foetuses is unlikely to involve the immune system, since no foetal immune response can be detected at this early

stage (Tahita *et al.,* 2013). Although the presence of immunoglobulins such as immunoglobulin M (IgM), IgG, and IgA, T cells, natural killer cells, and interferon can be detected by mid-gestation in infected foetuses, the extent to which they limit or contribute to further foetal damage has yet to be determined (Leopardi, 1996; Ezike *et al*., 2002). A closer examination of some of the unusual features of RV replication and virus-host cell interactions may provide important clues. Works to investigate the ability of RV to induce apoptosis, and a mechanism for the teratogenic effect of RV has been proposed (Pugachev *et al.,* 1998 and Megyeri *et al.,* 1999).

2.4.1 Mitochondrial Changes

A role for mitochondria in the budding and replication of RV has been suggested based on early studies of viral lipid content and metabolic changes in RV-infected cells. Cardiolipin, a phospholipid that is relatively specific to the inner mitochondrial membrane, was reported to be present in virions (Ilkow *et al*., 2010). These findings led the investigators to speculate that RV may bud from mitochondria. There were further suggestions of mitochondrial involvement in RV infection when the same group reported a decrease in the level of ATP in RV-infected BHK-21 cells within the first hour of infection coincidental with the period of viral adsorption and penetration. In addition, there appeared to be an increase in respiration, glycolysis, and alanine synthesis during the same period (Ilkow *et al*., 2010).

A direct link between mitochondria and RV infection was confirmed more than a decade later, when mitochondrial changes were detected in RV-infected Vero cells by Transmission Scanning Electron Microscopy (TSEM). Electron-dense zones associated with mitochondria were identified in three different configurations: between the outer membrane of a mitochondrion and one membrane of the Rough Endoplasmic Reticulum (RER), between the outer membranes of two adjacent mitochondria, and between two opposing membranes of the

RER. These ultra-structural changes have been designated confronting membranes type 1 (CM-1), confronting membranes type 2 (CM-2), and confronting cisternae (CC), respectively. Furthermore, it was noted that during the course of RV infection, the mitochondria appeared to become club shaped (Lee, 2000). It remains to be determined whether mitochondrial function is impaired as a result of such deformities during RV infection. These findings were seen only in RV-infected cells and were not present in the mock-infected preparations or in semiliki Forest Virus (SFV-infected cells). Structures similar to CM-2 have been detected in cells infected with Nodamura virus, while CC have been found in a variety of cells including cells infected with herpes viruses (Atreya *et al.,* 1995a). However, CM-1 appears unique to RV (Atreya *et al.,* 1995a) .

The composition of the electron-dense zones within these structures is unknown, although analysis using RV-specific antibodies should show whether viral proteins are localized within these zones. The cellular changes observed in these studies do not correspond to those described by Kistler, who performed an ultrastructural examination of cells from RV-infected human embryos and foetuse (Kistler, 1975). Another intriguing feature of RV infection is the association of RV core particles with mitochondria; no such association has been reported for the alphaviruses. This association is perplexing, since there appears to be no mitochondrial targeting signal on the RV capsid protein. It may be that the capsid protein has a specific affinity for mitochondrial membranes or an affinity for cell membranes in general. The RV capsid protein is a phosphorylated protein with a unique feature in possessing the E2 signal peptide. The precise role of the peptide has not been determined, although in vitro and in vivo studies have revealed that it has an affinity for membranes. Further studies are necessary to see whether this signal peptide or phosphorylation plays a role in targeting the RV capsid proteins for assembly at mitochondria or whether assembled core particles localize to the mitochondria (Atreya *et al*., 1998b).

In addition to these observations, pronounced clustering of mitochondria around RV replication complexes was reported; this was similarly observed with the SFV replication complexes. In light of these observations, it has been suggested that togavirus replication complexes are sites of a high-energy requirement that induces mitochondria to migrate to their vicinity. This phenomenon does not appear to be confined to the togaviruses. Mitochondria in Vero cells infected with African swine fever virus, a dsDNA virus, have also been reported to migrate in large numbers to viral assembly sites (Baron *et al.,* 1992). The migration was accompanied by a dramatic change in mitochondrial ultrastructure, characteristic of active respiration. In addition, a fourfold increase in the levels of mitochondrial stress proteins and mitochondrial chaperone proteins was observed (Rojo *et al*., 1998). For RV, cell metabolic and viral kinetic studies need to be performed in parallel with ultrastructural studies to gain a better understanding of RV-induced mitochondrial changes.

2.4.2 Cytoskeletal Changes

The effects of RV infection on the cytoskeletal components of cells have been investigated by immunofluorescence studies using antibodies to actin (Rojo *et al*., 1998). A significant alteration in the arrangement of actin filaments following RV infection was evident. Instead of the filamentous actin cables observed in uninfected cells, amorphous clumps of fluorescent foci, representing depolymerized actin filaments, were detected in RV-infected cells (Kujala *et al*., 1999). Little depolymerization was evident until 16hr after infection, suggesting that the synthesis of some viral product may have been necessary to achieve this effect. As infection progressed, fewer actin filaments were stained, until almost all the actin was found to be disaggregated into large, intensely stained foci; this effect was noted in both RV-infected Vero and BHK-21 cells. Studies by Kujala *et al*. (1999) also reported similar

findings. Interestingly, no changes were observed in the microtubules, another cytoskeletal component, during RV infection (Kujala *et al*., 1999).

2.4.3 Apoptosis

Apoptosis is a form of cell death that involves a genetically programmed series of events culminating in the destruction and disposal of unwanted cells (Reed, 1998). It is now established that apoptosis is characterized by specific morphological and biochemical features that can vary with tissue and cell type. Morphologically, changes within the cell, such as nuclear chromatin condensation, plasma membrane blebbing, and cellular fragmentation into membrane apoptotic bodies, are typical features of apoptosis. Biochemically, degradation of chromatin, as determined by DNA fragmentation assays, is generally characteristic of this form of cell death. (Schwartzman, 1993) More specifically, apoptotic events can be characterized by the over expression of certain regulatory proteins that function to trigger or regulate apoptosis. The proteins within the Bcl-2 family best exemplify the diverse and complex biochemical pathways involved in the regulation of apoptosis. Within the Bcl-2 family are anti-apoptotic proteins such as Bcl-2 and Bcl-xL and pro-apoptotic proteins such as Bax and Bcl-xS, which act directly or indirectly to activate a family of proteases called caspases, the major effector proteins for apoptosis (Reed, 1998). The Bcl-2 family of proteins can indirectly promote apoptosis through the regulation of a variety of signal transduction stimuli, e.g., through ceramide, collapse of mitochondrial transmembrane potential, p53 gene activation, and activation of cytokine receptors such as fas/APO-1 (CD 95) (Reed, 1998).

It is becoming increasingly evident that apoptosis plays an important role in the pathogenesis of many viruses. Alphaviruses, adenoviruses, Epstein-Barr virus, human papilloma virus, hepatitis B virus, and human immunodeficiency virus are some of the viruses known to

induce or interfere with the apoptotic pathway. Some of these viruses possess genes that encode either homologues of the Bcl-2 family of proteins or inhibitors of caspases. Alternatively, some of these viruses have evolved proteins that block apoptosis and, in so doing, prolong the life of the infected cell for virus dissemination (Leopardi *et al*., 1998). The versatility of the virus to control the cellular apoptotic machinery can result in a range of pathogenic outcomes such as persistence, latency, and tissue tropism. The increase in the volume of literature on RV-induced apoptosis have revealed some understanding of the mechanisms of RV persistence and teratogenicity (Höfmann *et al*., 1999).

While RV can establish persistent non-cytocidal infection in many cell lines, it can also cause cytopathic effects (CPE) in cell lines such as Vero, BHK-21, and RK13 (Junaid*et al.,* 2011). It is now clear that the RV-induced CPE, seen as cell rounding followed by detachment from the monolayer, is due to apoptotic cell death. Characteristic markers of apoptosis such as DNA fragmentation, nuclear chromatin condensation, and annexin V staining have been reported during acute infection with RV. The ability of RV to induce apoptosis varies considerably with cell type and appears to be associated with cells that cause CPE during infection. Active replication is required for RV-induced apoptosis during acute infection, although transfection studies of cells expressing only RV structural proteins indicate that the RV E1, E2, and capsid proteins may not be required for this process. The apparent lack of RV structural protein involvement with apoptosis indicates that the trigger for virus-induced apoptosis may be different between RV and the alphaviruses. For Sindbis virus, the prototype alphavirus, the E1 and E2 proteins, and in particular their respective TM domain, can induce apoptosis as shown in cDNA transfection studies (Junaid *et al*., 2011; Kolawole*et al*., 2014).

Since RV replication requires the formation of replication complexes, it is likely that viral components such as dsRNA and non-structural proteins found within these structures play a role in the apoptotic process. Studies of Vaccinia virus infection showed that dsRNA caused apoptosis via the activation of dsRNA-dependent protein kinase, a key player in interferon-mediated host defense against viral infection. While this has not been shown for RV, it is possible that the RV RI and RF RNAs may elicit similar responses; interestingly, alpha interferon is present in 90% of RV-infected fetuses and is readily detectable in the sera of fetuses at mid-gestation (21 to 29 weeks) (Junaid *et al.,* 2011). The RV non-structural proteins may also play a role in RV-induced apoptosis, since determinants of cytopathogenicity have been mapped to these proteins. However, the presumed interplay between the non-structural proteins and cellular proteins involved with the apoptotic pathway remains to be elucidated. (Junaid *et al.,* 2011). The cellular proteins and biochemical pathways involved in RV-induced apoptosis are also not well characterized. Studies using chemically defined caspase inhibitors have shown indirectly that apoptosis during RV infection is mediated by caspases, and it is likely that the Bcl-2 family of protein are involved. There are conflicting reports on the role of p53 in inducing apoptosis during RV infection. Megyeri *et al.*(1999) demonstrated the involvement of the p53-dependent pathway in RV-induced apoptosis, while Höfmann *et al*.(1999) using a similar cell line, reported that cell death during RV replication is mediated by a p53-independent pathway.

Apoptosis in RV-infected cell cultures occurs asynchronously and is confined mainly to RV-infected cells that have detached from the monolayer as a consequence of CPE (Junaid *et al.,* 2011). Ultrastructural studies of Vero cell monolayers infected with RV confirm these findings, since no evidence of chromatin condensation within the nuclei of cells displaying RV replication complexes and mitochondrial changes were found, even at

late stages of infection. This asynchronous induction of apoptosis is intriguing, particularly when the majority of the cells are RV-infected ((Junaid *et al.,* 2011). The extent to which viral or host cellular proteins are involved in controlling or limiting apoptosis in these infected cells is not known. The inhibition of apoptosis in the RV-infected monolayer has clear advantages for the virus, since persistence develops as a consequence (Junaid *et al.,* 2011). To date, there has been no report on the role of apoptosis in RV teratogenicity. It would be of interest to determine whether the non-inflammatory necrosis observed in organs of RV-infected fetuses is caused by RV-induced apoptosis. Further analyses using more sophisticated biochemical and molecular tools in apoptosis are required (Lee *et al.,* 2000).

2.5 Life Cycle of Rubella Virus

2.5.1 Pathogenesis of Rubella Virus

a. **Attachment and Entry**: Preliminary viral attachment and penetration studies by thin-section electron microscopy (TSEM) indicate that at physiological pH of 7.4, RV enters predominantly via the endocytic route. RV virions were observed to attach to cell surface projections which were either adjacent to or in coated pits, these events were observed as early as 3 min after the addition of virus to the cell monolayer. Similarly, pre-embedding immunogold-labeling studies using antibodies to RV revealed that during the viral latent period, gold-labeled RV virions were seen attached to the plasma membrane adjacent to coated pits; unlabeled virions were observed in endosome-like vacuoles (Lee *et al.,* 2000). Molecular characterization of RV has been largely performed in Vero and BHK-21 cells because of their ability to produce high titers of virus, perhaps due to the absence of an interferon system. However, RV can establish infections in a variety of cell lines (Pertruzziello *et al*., 1996), indicating that the host cell receptor is likely to be an ubiquitous molecule.

Although the host cell receptor has not been identified, it appears that membrane phospholipids and glycolipids may be involved in viral attachment. There is some evidence suggesting that RV enters cells via the endocytic pathway, similar to that reported for the Alphaviruses (Lanzrein *et al.,* 1994). Early biochemical studies by Katow and Sugiura, (1997) showed that exposure of the RV E1 and E2 glycoproteins to pH 6.0 or less induced a conformational change within the glycoproteins that favoured the fusion of the viral envelope to the endosomal membrane. This hypothesis was further supported by a study which demonstrated the inhibition of viral replication following the use of lysosomotropic agents (Petruzziello *et al.,* 1996).

b. **The uncoating:** The uncoating event for RV is also not well defined. It has been shown that between pH 5.0 and 5.5, the RV capsid protein undergoes a structural change from having hydrophilic to hydrophobic properties. This conformational change in the capsid protein presumably allows uncoating to occur within the endosome, allowing the release of viral genomic RNA into the cytoplasm. Thus, it seems that the low-pH environment of the endosome serves not only to induce virion envelope fusion to the endosomal membrane but also to trigger uncoating of the capsid protein. This is in contrast to the mechanisms of Alphavirus uncoating while for SFV, the viral nucleocapsid is uncoated by the binding of the capsid proteins to ribosomes (Singh *et al*., 1992).

c. **Replication:** RV is characterized by slow replication, which is reflected in the long viral latent period of 8 to 12hr (Frey, 1994). During RV infection, four distinct viral RNA species can be detected. A single-stranded 40S RV genomic RNA (3.8×10^3 kDa) and a 24S subgenomic RNA (1.2×10^3 kDa) that corresponds to the 3′ one-third

of the genomic RNA present in infected cells. Both contain a methyl guanosine cap at the 5′ terminus and a polyadenylate tail at the 3′ terminus. In addition, viral replicative intermediates (RI) of 21S, representing partial double-stranded RNA (dsRNA), and viral replicative forms (RF) of 19 to 20S, representing full dsRNA, have been detected in RV-infected cells (Oker-Blom *et al*., 1984).During viral replication, the 40S RV genomic RNA serves as a messenger for the nonstructural (ns) proteins and as a template for the synthesis of a 40S negative-polarity RNA strand. The minus strand in turns acts as a template for the transcription of both the 40S RNA and the 24S RNA. Nascent 40S RNA is packaged with the RV capsid protein to form nucleocapsids. In terms of viral kinetics, both the RV 40S RNA and 24S RNA were detected at the end of the viral latent period, with viral structural proteins appearing 4hr later. Peak virus production occurs during the period from 36 to 48hr post infection. One-step multiplication studies have shown that RV is unable to infect every cell at any specific time, irrespective of the titer of the virus. Moreover, the proportion of cells infected by RV at any one time is cell type dependent. However, as infection proceeds, the entire culture eventually becomes infected (Hemphill *et al*., 1988).

d. **Structural Proteins** (Translation, Processing, and Assembly): The RV structural proteins are translated as a polyprotein precursor, p110, in the order NH2-C-E2-E1-COOH from the 24S subgenomic mRNA; two possible AUG initiation codons are present within the RV 24S RNA, and it has been suggest that both AUG codons are used indiscriminately. The p110 protein is translocated into the ER by two separate signal peptides, 23 and 20 amino acids in length, located at the amino termini of E2 and E1, respectively. Within the ER, the RV capsid protein is cleaved from E2 and E2

is cleaved from E1. Unlike the Alphaviruses, where an autoprotease cleaves the Alphavirus capsid protein from the polyprotein precursor, cleavage of RV capsid protein is mediated by a cellular signalase found within the lumen of the ER. A unique feature of the RV capsid protein is the retention of the E2 signal peptide on the carboxy terminus of the capsid protein. Similarly, the E1 signal peptide of 20 amino acids is retained on the carboxy terminus of the E2 protein after cleavage from E1 by host signalase. The respective signal peptides of E2 and E1 direct the insertion of the proteins into the ER (Hobman *et al*., 1989). The assembly pathway of RV has not been fully elucidated. Nevertheless, significant progress has been made in understanding RV assembly with the use of cDNA constructs containing the RV structural genes. Of particular significance is the development of cDNA constructs express RV structural proteins and assemble into rubella virus-like particles (RLP). These RLPs have similar morphology and sites of budding similar to their wild-type counterpart, and they serve as a convenient tool in dissecting the RV assembly pathway (Qiu *et al*., 1994). For RV, the assembly of structural proteins begins immediately following translation of these proteins. Following proteolytic cleavage in the ER, the E1 and E2 proteins form disulfide-linked heterodimers while the capsid proteins form disulfide-linked homodimers. E1-E2 heterodimer formation is necessary for the transport of E1 from the ER to the Golgi complex and the cell surface; in the absence of E2, E1 is arrested in the post-ER, pre-Golgi complex compartment (Baron *et al*., 1991).It has been suggested that E1 and E2 dimerization facilitates the proper folding of E1. An ER retention signal on E1 of 22 amino acids, spanning both the cytoplasmic and transmembrane (TM) domains of the protein, functions to retain unassembled E1 subunits and immature E2-E1 dimers in the ER until folding and heterodimer formation are complete. In the process, E1 undergoes a

conformational change that masks the ER retention signal, thereby allowing the transport of the heterodimers to the Golgi complex. It was later found out in another study that an internal hydrophobic domain in E1 is involved in E1-E2 interaction leading to the formation of the heterodimer (Yang *et al*., 1998). The E2-E1 heterodimers are retained in the Golgi complex by a retention signal of 18 amino acids located in the TM domain of E2. Studies using RLPs have indicated that the TM and cytoplasmic domain of E2 are required for the targeting of the heterodimers to the sites of budding such as the Golgi complex (Garbutt *et al.,* 1999). In contrast, the TM and cytoplasmic domains of E1 were shown not to be required for this process but were necessary for the secretion of RLPs into the medium. A better-defined mutagenesis study on the E1 TM and cytoplasmic domain within a RV infectious cDNA has revealed similar findings. Collectively, the data suggest that the E1 TM and cytoplasmic domains play a critical role in the very late stages of virus budding (Yao *et al.,* 1998). The role of the capsid protein in RV assembly is less well defined. It has been proposed that maintenance of the E2 signal sequence as part of the capsid protein after cleavage allows the capsid protein to be transported along with the glycoproteins to the Golgi complex. Earlier studies postulated that the interaction of the E1 cytoplasmic domain and the RV capsid protein triggered virus budding (Beatch *et al*., 2005). However, another study revealed that this interaction is not the driving force for virus budding. The mechanisms involving the interaction between the nucleocapsid and E2-E1 heterodimers have yet to be elucidated. While studies with RLPs have provided important insights into RV assembly with respect to virus protein-protein interactions, the precise mechanisms for budding where the virions acquire the host membrane are far from clear. Moreover, recent observations that RV particles and RLPs can be secreted into the apical and basolateral surfaces of

polarized cells have raised questions whether the assembly and budding events are similar for both compartments (Lee *et al*., 2000).

e. **Nonstructural Proteins:** Translation and Processing the characterization of RV nonstructural (ns) proteins has largely been hampered by the limited production of ns proteins in infected cells. The task was further complicated by the presence of host cell proteins that obscured the detection of the viral ns proteins because RV does not inhibit host protein synthesis. Early studies reported the detection of several ns proteins, of 200, 150, 87, 75, and 27 Adipocyte complement-related protein (kDa) in RV-infected cells. Pulse-chase studies during hypertonic salt treatment of RV-infected cells demonstrated that the (non-structural) ns 200 protein was cleaved to (non-structural) ns 150. More recent work employing cDNA constructs containing the RV 5′ ORF of the Therien and M33 strains demonstrated that the 200-kDa protein is ns-polyprotein precursor that is cleaved to produce two products of 150 and 90 kDa. Thus, the gene product order for the RV 5′ ORF-encoded protein is NH2-p150-p90-COOH. The 87-kDa protein of Bowden and West away in RV-infected cells most probably represents the 90 kDa protein observed in transfection studies (Ilkow, 2008).

The cleavage of the polyprotein precursor, p200, into two fragments is mediated by a protease residing in p150 domain or region with catalytic residues of Cis-1151 and His-1272; the protease cleavage site is found within Gly-1300–Gly-1301. Based on comparative amino acid sequence analysis, the RV protease was proposed to be a Main protease with similarities to cellular proteases. Transfection studies have since confirmed that the RV protease is indeed a main protease because it can function in cis and trans cleavage. The p150 protein was shown to be localized to RV replication complexes. The only other functional activity demonstrated within the RV 5′ ORF is that of the RV helicase (Kujala *et al*., 1999)

2.6 Role of Cellular Proteins in RV Replication

Studies using RV-infected cells showed that the addition of actinomycin D (an inhibitor of DNA-dependent RNA polymerase) in the early stages of RV infection inhibited virus replication, suggesting that cellular or host proteins may also be involved in viral RNA replication. Moreover, there are several sequences at the 5′ and 3′ termini of the RV genome that can potentially form stable stem-loop structures representing possible sites for interactions between RV RNA and host proteins (Beatch *et al.,* 2005). A synthetic RNA modeled from the 3′ stem-loop structure has been shown to interact specifically with three phosphorylated cytoplasmic proteins. One such protein has been identified as calreticulin, a host protein that is involved in the modulation of genes and that binds specifically to regions located at the 3′ end of the RV RNA (Athreya *et al*., 1995a). Although the 3′ untranslated region contains cis-acting elements necessary for RV replication, the role of calreticulin binding to this region is unclear. It would be of interest to determine whether calreticulin associates with RV replication complexes where active viral RNA synthesis occurs. In addition to host proteins interacting with the RV genome, the cellular retinoblastoma (RB) protein interact with a known RB-binding motif located within the carboxy-terminal half of the RV p90 protein. Because the RB protein is involved in the regulation of cell growth, it has been proposed that this interaction may induce RV teratogenesis (Athreya *et al*., 1998b).

a. **Morphogenesis:** Numerous investigators have employed TSEM to study RV morphogenesis in a variety of cell lines including RK13, BHK-21, and Vero cells. Early TSEM studies on RV-infected cells reported predominantly on the morphology and maturation of RV (Athreya *et al*., 1995b). RV maturation is a budding process in which the viral core acquires an envelope membrane after passing through modified host cell

membranes. The Golgi apparatus, rough ER (RER), cytoplasmic vacuoles, and plasma membrane have been identified as sites of RV maturation. This is in contrast to the Alphaviruses, where the plasma membrane is the only site of virus budding (Wollinsky, 1996). The maturation of RV at cytoplasmic sites is presumably due to the presence of an ER and a Golgi retention signal on the E1 and E2 glycoproteins, respectively, that facilitates the accumulation and assembly of the proteins in the respective organelles. Mechanisms responsible for triggering virion assembly are poorly defined, and it is not known whether similar mechanisms are involved at the different maturation sites (Athreya *et al*., 1995b and Lee *et al.,* 2000) early morphological studies revealed several cellular changes associated with RV infection. However, discrepancies with these findings exist. Cytoplasmic inclusions have been reported in RV-infected RK13 cells but were never detected in a similar cell line studied by other investigators. Moreover, the significance of these cytoplasmic inclusions is not known. Annulate lamellae were observed in LLC-MK2 and RK13 cells infected with RV, but measurements and descriptions of virus-like particles in these studies did not correlate with the characteristic morphology of RV virions. Thus, it appears unlikely that these structures were associated with RV replication as has been previously reported. In addition, annulate lamellae have been found in other virus-infected cells, indicating that the occurrence of these structures is probably due to a nonspecific cellular response to viral infection (Kessel, 1992).

2.7 Replication Complexes

b. Although early TSEM studies have produced several important findings on the morphology and maturation of RV, interest in RV morphogenesis waned with the advent of molecular biology applications. Consequently, in the past two decades, RV research has focused mainly on the characterization of RV replication at the

molecular and biochemical level (Athreya *et al*., 1995b). Nevertheless, interest in RV morphogenesis has continued, leading to the discovery of "replication complexes" in RV-infected cells (Lee, 2000). It is now recognized that the results of these studies complement existing molecular information on RV replication. Furthermore, these morphological studies revealed that RV replication complexes are similar in morphology, function, and biogenesis to Alphavirus cytopathic vacuoles type 1 (CPV-1) (Kessel, 1992).

It must be noted that RV replication complexes were so named in reference to their functional role while their Alphavirus counterparts were termed CPV-1 according to their morphology (Magliano *et al.,* 1998).

Lee and Bowden (2000) first described RV replication complexes as membrane-bound cytoplasmic vacuoles lined internally with vesicles measuring approximately 60 nm in diameter. These vesicles contained thread-like inclusions and were found either free in the vacuole or attached to the inner membrane of the vacuole via a membranous neck. Although these vesicles have similar dimensions to those of RV virions, they do not constitute immature or aberrant virion forms. Close examination reveals that the vesicles contain irregular internal structures which are distinct from the spherical electron-lucent cores observed in mature RV virions. In addition, aberrant forms of RV virions were reported as multicore structures surrounded by a single membrane. Importantly, RV virions have not been detected in the vacuoles of the replication complex where the vesicles were found (Lee and Bowden 2000).

2.8 Cellular Changes in RV-Infected Cells

A typical replication complex is observed with the characteristic vesicles and the close association of the RER. RV core particles can be seen at the cytoplasmic side of the vesicles of the replication complex. Core particles can also be detected in association with the outer membrane of mitochondria. Electron-dense zones are frequently observed between the outer membranes of adjacent mitochondria. A distinctive feature of RV replication complexes is the close association of the RER with the vacuole complex. During RV infection, RER was found to associate only with the side of the replication complex vacuole where the vesicles were located, but as the infection progressed, the RER surrounded the whole replication complex. RV replication complexes were detected as early as 8hour post infection, coincident with the end of the viral latent period, and peak numbers of these structures were reported at 24hour post infection, coinciding with peak production of the virus (Lee *et al*., 1991).

The presence of RV replication complexes in infected cells was first postulated by Bowden *et al*., (1985) who observed discrete cytoplasmic fluorescent foci in RV-infected cells in an immunofluorescence assay using antibodies to dsRNA, a marker for viral replicative Intermediates (RI) and replicative forms (RF). When studies employing immunogold-labeling EM and anti-dsRNA were performed on RV-infected cells, dsRNA was observed to localize within the vacuole of the replication complex, indicating that these virus-induced structures are associated with viral RNA synthesis. The precise site of viral replication appears to be the vesicles within the replication complex. The localization of RV p150 within these complexes further confirms their role in viral replication (Bowden and Westaway, 1985).

RV replication complex-associated core particles were often detected between the cytoplasmic side of the replication complex and the adjacent RER membrane; these particles were frequently found at the base of the vesicles lining the vacuole complex. Immunogold-labeling studies using monoclonal antibodies to the capsid protein confirmed the identity of the particles. Hence, RV replication complexes appear to serve as sites for nucleocapsid assembly. This is not surprising, since the processing of the capsid, E2, and E1 proteins occurs in the RER with the host signal peptidase involved in cleaving E2 from the capsid and E2 from E1. The attachment of the E2 signal peptide at the carboxy terminus of the capsid protein tends to render the protein hydrophobic; thus, the capsid protein is likely to remain attached to the RER membrane. Given the close proximity of the RER to the replication complex, it is conceivable that the assembled capsid protein then associates with newly synthesized genomic RNA as the viral RNA is expelled from the vesicles of the replication complex (Figure2.1). Interestingly, another group has reported the co-localization of RV capsid proteins with p150, thereby indicating the association of capsid proteins with RV replication complexes (Kujala *et al.,* 1999).

"The vesicles of the replication complex are postulated to provide a protective environment for the synthesis of nascent viral genomic RNA. Newly synthesized viral RNA is then rapidly encapsidated by RV capsid proteins, which are synthesized from the adjacent RER. The mechanisms involved in the translocation of the resulting nucleocapsids for interaction with RV E2-E1 heterodimers have not been defined (Figure 2.1).

While it is well recognized that endosomes and lysosomes play an important role in viral entry via the endocytic pathway, the use of these organelles as sites of viral replication appears to be unique to the togaviruses. A model for the biogenesis of RV replication

complexes is proposed based on the collective findings from RV studies (Figure2.1). In this model, the RV virion binds preferentially to a cell surface projection and then translocates into an electron-dense coated pit adjacent to the surface projection. The virion is then transported into the cell when the coated pit invaginates to form a coated vesicle. The virus is subsequently delivered through a series of endosomes with progressively acidic compartments until it reaches an endosome where the environment is sufficiently acidic (pH 5.3) to induce conformational changes within the E1 and capsid protein, resulting in the release of the genomic RNA into the cytoplasm. The events from virus uncoating to early formation of the replication complex are unknown. Presumably, the virus nonstructural proteins are synthesized immediately from the newly released viral RNA and remain associated with the endosome where uncoating has occurred (Mauracher *et al*., 1991).

The production of non-structural proteins, replicating viral RNA, and/or host proteins contributes to the formation of vesicles within the endosome, which provides a protected environment for the transcription of the genomic and possibly subgenomic RNAs. Concomitantly, the RER migrates to the vicinity of the endosome, allowing the translation and processing of the viral structural proteins from the 24S subgenomic RNA to occur. As infection progresses, the vesicles within the endosome increase in number as more non-structural proteins are synthesized either from newly synthesized RV genomic RNA or from the original viral genomic RNA. Late in infection, the RER is found surrounding the entire vacuole, which has been lined internally with vesicles. While these viral events are occurring, the endosome, which now represents a replication complex, continues in its life cycle and fuses to a lysosome. It is important to bear in mind that the life cycle of both the virus and the endosome/lysosome are intertwined. The decrease in the number of replication complexes late in infection and the detection of vesicles containing thread-like inclusions on the cell

surface indicate that the virus-modified lysosome has fused to the plasma membrane, thereby expelling the vesicles and other lysosomal by-products extracellularly (Lee and Bowden, 2000).

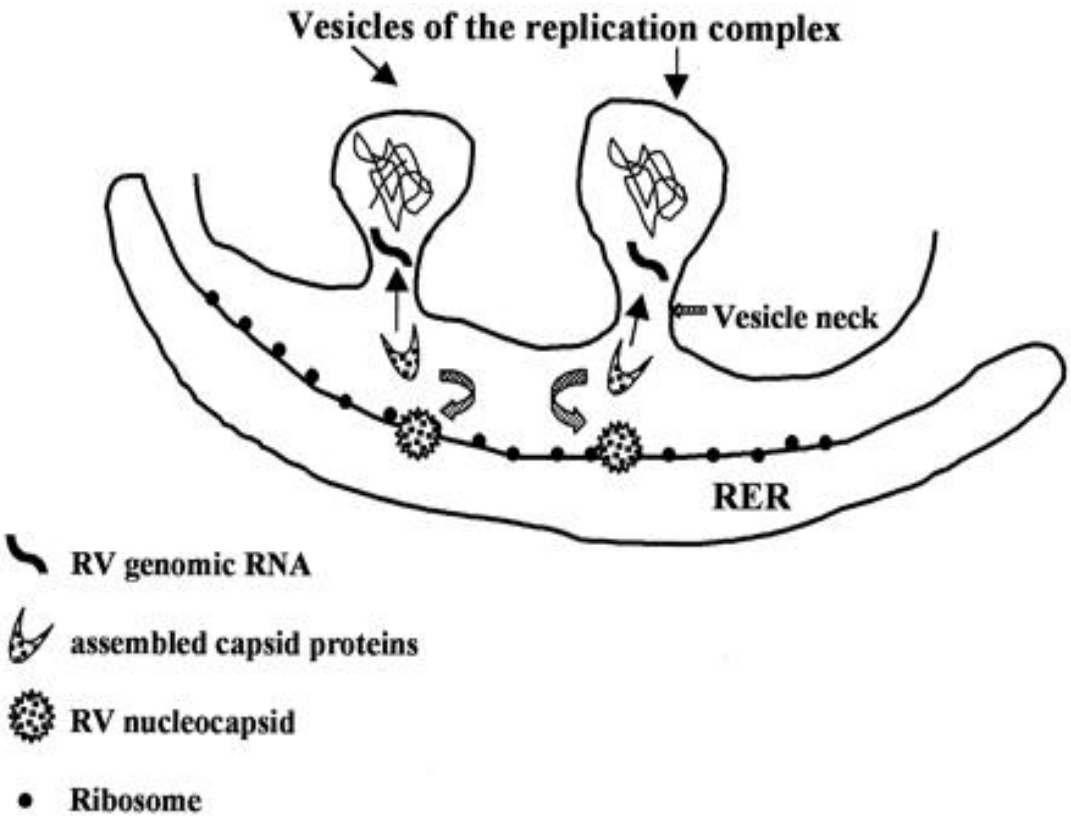

Figure 2.2:Role of Vesicles within the RV Replication Complex at Precise Sites of Viral RNA synthesis (Lee and Bowden, 2000).

2.9 Summary of Rubella Virus Life Cycle

"Step 1, the RV virion attaches to the cell surface and is translocated to the coated pit. Step 2, the coated pit then pinches off to form a coated vesicle that contains the virion. Step 3, the virion passes through a series of endosome with progressively acidic pH until it arrives at an endosome where the environment is sufficiently acidic to trigger the uncoating process. The E1 and capsid proteins undergo conformational changes that result in the release of the viral genomic RNA into the cytoplasm. Step 4, release of the viral RNA triggers the transformation of the endosome, and vesicles are induced to form within the endosome. Step 5, as infection progresses, the RER surrounds the entire vacuole, which is lined internally with vesicles. While these events are occurring, the virus-modified endosome fuses to a lysosome as part of its life cycle. Step 6, the replication complex continues in its life cycle as a virus-modified lysosome and eventually expels its lysosomal contents, including the vesicles, after fusion of the lysosomal vacuole membrane to the plasma membrane (Figure 2.2).

Vesicles play a vital role in the replication of many single-stranded RNA viruses such as the picornaviruses and flaviviruses. The vesicles induced by these viruses are postulated to represent replication complexes that are derived from or accumulate in the ER rather than in endosomes and lysosomes. It is unclear, however, why the togaviruses, flaviviruses, and picornaviruses have utilized vesicles for viral replication. It has been postulated that the membranous structure of the replication complex acts as scaffolding, providing an architectural framework for the assembly of all the components of replication, as well as providing a large surface area for viral replication and hence a more rapid synthesis of progeny RNA. In addition, the double membrane vesicles may serve to protect the nascent

single-stranded viral genomic RNA from degradation by cellular RNases (Lee and Bowden, 2000).

The mechanisms involved in vesicle formation within RV replication complexes are not well understood. However, transfection studies have provided some important clues by showing that SFV genomic RNA, rather than infectious virions, was able to induce the formation of replication complexes. Later, confocal microscopy studies by the same group found that the alpha virus non-structural P1 (nsP1) was localized to endosomes and lysosomes (Pattison, 2000). It is not known whether nsP1 alone was sufficient to induce formation of these vesicles, since no EM procedures were performed. Vesicle formation is not a characteristic confined to toga virus infection.

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2.10 Rubella Re-Infection

Natural infection is followed by a high level of protection from re-infection. However re-infection can occur which is generally asymptomatic. Re-infection in pregnancy is thought to pose minimal risk to the fetus. Reports of fetal infection are exceedingly rare. In re-infection, the IgG is highly elevated whilst IgM may be demonstrable, giving equivocal results. It may

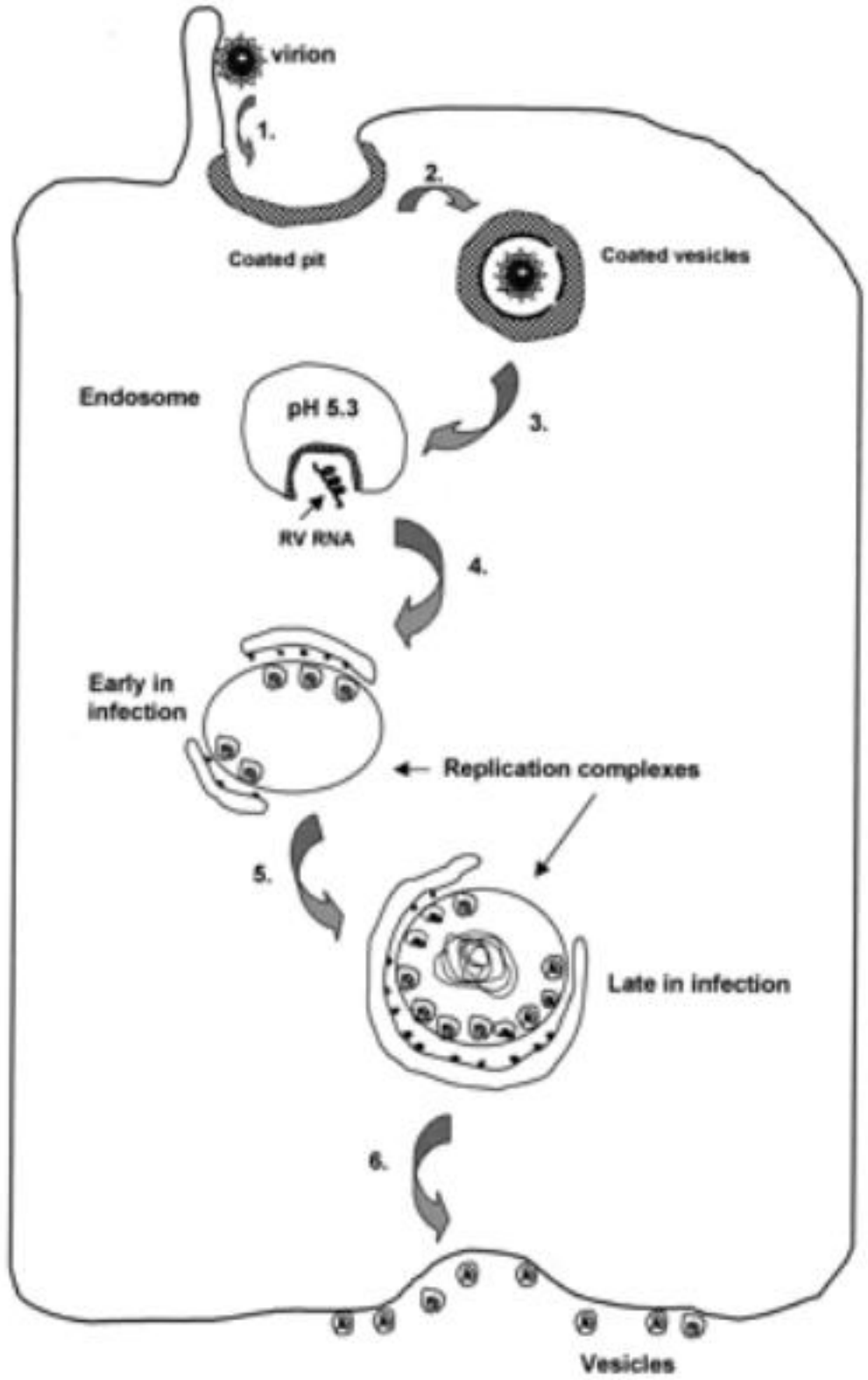

Figure 2.3: Biogenesis of Rubella Virus Replication Complexes (Lee and Bowden**,** 2000)**.**

now be possible to distinguish re-infection from primary infection by examining the avidity of specific IgG (IgG from patients with re-infection have a higher avidity) (Derek, 2013).

2.11 Epidemiology

Rubella has a worldwide distribution. The peak incidence occurs during spring months in countries with a temperate climate, although the disease is present throughout the year (Hobman *et al*., 2007). In the pre-vaccine era, rubella epidemics occurred every 6 to 9 years in the United States and at shorter intervals of 3 to 5 years in the United Kingdom. In developed countries before vaccine development, infection was most common in the 5- to 9-year-old group (Reef *et al*., 2002), corresponding to the early school years. With the advent of childhood vaccination in the United States, there was a shift in disease incidence to young adults. In countries practicing the selective vaccination policy, there were much higher notification rates in males, as expected. During the epidemic in the US from 1962 to 1965, Rubella virus infections during pregnancy were estimated to have caused 30,000 still births and 20,000 children to be born impaired or disabled as a result of CRS. (Plotkin, 2001). Universal immunization aim at producing a high level of herd immunity is important in the control of epidemics of rubella (Danovaro-Holliday, 2000).

On January 22, 2014, the World Health Organization and the Pan American Health Organization declared and certified Colombia free of the rubella and it became the first Latin American country to abolish the disease within its borders (WHO, 2014).

From studies with monoclonal antibodies, it was widely agreed that there is essentially only one serotype of RV. With the advent of molecular techniques such as PCR, it is now also possible to look at the genetic makeup of the virus itself and perform molecular epidemiological studies. A number of groups have examined the nucleotide sequence of the

RV envelope E1 gene and performed phylogenetic analyses. Geographical isolates were derived from three continents and included wild-type, laboratory, and vaccine strains. The virus strains analyzed were derived from initial rubella isolates collected in the 1960s through to the 1990s, allowing some evolutionary comparisons to be made. Overall, RV could be divided into two genotypes, which differ from each other by 8 to 10% at the nucleotide level. Genotype I was a large intercontinental group containing 60 of the 63 isolates derived from North America, Europe, and Japan, and genotype II represented only 3 Asian isolates from China and India. The diversity seen at the nucleotide level was not evident at the deduced amino acid level, where the two genotypes differed by only 1 to 3%, indicating that they were antigenically very similar. The isolates included strains isolated from patients with CRS; no apparent signature mutations or other changes could be identified to distinguish CRS strains from other strains (Frey *et al*., 1998).

2.12 Laboratory Diagnosis

a. **Serological techniques for diagnosis of rubella infection**: Serology is the mainstay of diagnosis of rubella infection. A recent rubella infection can be diagnosed by Single Radial Haemolysis (SRH), Latex agglutination (LA) and ELISA are used for screening for immunity against rubella. SRH is reckoned to be slightly less sensitive than LA or ELISA. False negative results may occur with SRH due to the interference with red cell lyses by a "blocking factor" which may be removed by absorbing the sera with erythrocytes from the same species used in SRH tests. ELISA is now the choice of test in many laboratories but it is considerably more expensive than the SRH. An antibody titre of equal or greater than 15 IU/ml is regarded indicative of being immunity to rubella. However, there is some controversy as to the 15 IU/ml cutoff since it was arrived at empirically in the first place. It is quite clear that lower

levels of antibody, such as 10 IU/ml would probably be protective as well. HAI is not used for rubella antibody screening because it is not sensitive enough. IgM antiglobulins such as Rheumatoid Factor can seldom cause false positive results as can heterophil antibodies. Indirect ELISA and RIA have also been developed but these are not as sensitive as direct ELISA (Derek, 2013).

It is essential to obtain accurate information relating to the date and time of exposure, the date of onset of illness, history of previous rubella vaccination as well as previous results of rubella screening tests. Blood should be collected from pregnant women with features of rubella-like illness as soon as possible after onset of symptoms. A significant rise in antibodies can often be demonstrated. However rubella-specific IgM is the test of choice for demonstrating current infection. It has been shown though that low and transient levels of IgM can be detected in cases of re-infection. Furthermore, low levels of rubella IgM may persist for a few months to 4 years following rubella vaccination (Derek,2013).

a. **Virus isolation and identification**: Virus isolation is now seldom used for diagnosing postnatally acquired rubella infection. It may still be useful in diagnosing congenital acquired disease and in determining the duration of virus excretion in these infants since they may transfer infection to susceptible adults. The specimen should be minced or mixed with culture medium and the supernatant is then inoculated into culture medium. RK13 cell cultures are used exclusively in the UK for virus isolation, cells are used in Scandinavian countries. Rubella virus is fastidious and produces cytopathic effect (CPE) in RK13 cells under carefully controlled conditions. The virus is more

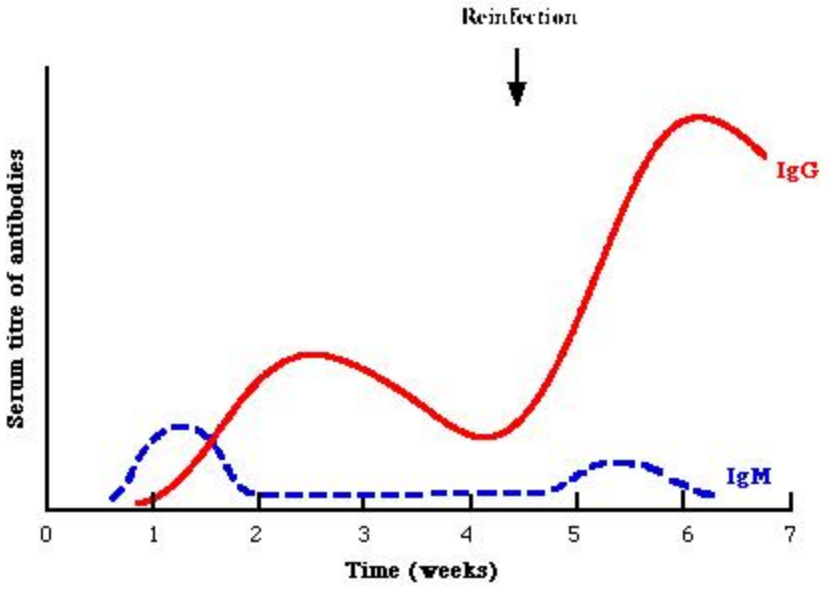

Figure 2.4: Typical Serological Events Following Acute Rubella Infection.

Note that in Re-Infection, Rubella-Specific IgM is Usually Absent or Present at a Low Level Transiently (Derek, 2013).

rapidly identified using immunofluorescence (IF) techniques. Inoculation of specimens into Vero cells followed by passage into RK13 or SIRC cells, in which the virus can be identified by its characteristic CPE or by IF represents the most sensitive method for rubella isolation available (Derek, 2013).

2.13 Diagnosis of Rubella Congenital Acquired Infection

This is usually made by the presence of rubella IgM in cord blood or serum samples taken in infancy, detection of rubella antibodies at a time when maternal antibodies should have disappeared (approx. 6 months of age) and isolation of rubella virus from infected infants in the first few months of life (Ilkow, 2008). The detection of rubella-specific IgM in cord blood or infant sera is the method of choice for the diagnosis of congenital rubella. Specific IgM has been demonstrated in all confirmed cases to the age of 3 months, in 86% 3 to 6 months, 62% 6 months to 1 year and 42% 12 to 18 months and rarely over 18 months. If the IgM result is negative or equivocal and where there has been a history of rubella in pregnancy, a serum can be taken at 9-12 months to look for the presence of specific IgG (Kesson, 2012).

The detection of specific IgG may be of value where tests for IgM have not been conducted in early infancy. Since rubella is uncommon under the age of 2, IgG detected between 1 and 2 may be indicative of congenital infection. However each case must be assessed individually, taking into account factors such as age, maternal history and presence of clinical findings. Rubella virus may be recovered from nasopharyngeal secretions of most neonates with severe CRS. But by 3 months the proportion has declined to 50 - 60% (Derek, 2013).

2.14 Prenatal Diagnosis of Congenital Rubella Infection

Prenatal diagnosis of congenital infection is of value when maternal infection occurred after the first trimester, in cases of maternal re-infection and in cases where equivocal serology results from the mother were obtained (Ilkow, 2008). Possible methods include the testing of foetal blood samples obtained by fetoscopy for rubella specific IgM. However the fetus does not produce sufficient IgM for detection before 22 weeks, Virus may be isolated from amniotic fluid but the reliability of this technique has not been demonstrated and lastly detection of rubella RNA or viral proteins in chorionic villus biopsies and amniotic is currently being evaluated (Derek, 2013).

2.15 Prevention

The first vaccines were developed in the early 60's (HPV77.DE5 and Cendehill strain) and were licensed for use in 1969. In 1979 the HPV77.DE5 strain was replaced with RA27/3 and Cendehill is no longer available. RA 27/3 is now the most widely used vaccine strain one of whom is made by Merck and Co., Inc (manufacturer). All vaccines are administered subcutaneously and are well tolerated and produced a response in 95% of recipients. Although the virus is excreted by the vaccines, it is not transmitted to susceptible twist contacts because it is life attenuated (Kesson, 2012; Derek, 2013).

2.15.1 Vaccination Policies

2.15.1.1 Two main policies were initially used

a. Universal childhood immunization; the aim was to eradicate rubella infection by vaccinating all preschool children. At least 85% of these children are vaccinated with this policy

b. Selective vaccination; the aim is to protect the population at risk. All prepubertal schoolgirls in the UK were selectively vaccinated. This policy is more suitable for countries which are unlikely to attain the necessary 85% uptake.

Universal childhood vaccination was adopted by the USA in 1969 with great success. It was offered as part of the MMR (Mumps, Measles, and Rubella) vaccine. The uptake was very high as proof of immunization of measles is mandatory before school entry. Since 1978 there has been a steady decline in CRS as well as postnatally acquired rubella and CRS is on verge of being eliminated in the USA. The USA policy results in financial saving: cost-benefit analysis shows that the cost of rubella in an unvaccinated population is approximately 11 times more than the cost of vaccination policy (Plotkin, 2001).

In the UK selective vaccination of 11 - 14 yrs. old girls was introduced in 1970. The vaccine was also offered to susceptible people e.g. Nurses, doctors and schoolteachers and to women who were found to be seronegative when they attended antenatal clinics. Seronegative women attending antenatal clinics were offered vaccination in the immediate postpartum period. It appears that overall 90% of schoolgirls have been vaccinated. However there are districts where the uptake rates are lower (Ilkow, 2008). The proportion of susceptible women attending antenatal clinics between 1984 and 1986 varied between 2.3% and 5.8%. Although there has been some decline in cases of reported CRS, maternal rubella is still relatively common (Ilkow, 2008). In 1986, 173 cases of laboratory confirmed rubella were reported during the first 16 weeks of pregnancy. A high proportion of these pregnancies were terminated. (Kesson, 2012: Derek, 2013).

Since it became apparent that complete vaccination of the target population was an unrealistic goal and because despite high uptake rates, rubella still infects pregnant women,

the rubella vaccination program in the UK was augmented in 1988 by offering rubella vaccination to preschool children of both sexes. Rubella vaccine is given as part of the MMR vaccine (Market Research Report, 2015). As part of the “catch up" program MMR is also given to children age 4 - 5 years. The vaccination of 10 to 14 years. olds and seronegative women is to continue until it can be demonstrated that rubella is no longer circulating in the community and that serological surveillance shows that 90-95% of adolescents are already immune (Plotkin, 2001). The augmented program is designed to eradicate rubella. However in the USA this has been harder to achieve than expected. Mathematical models show that poor uptake amongst preschool children may actually increase the proportion of rubella susceptible in older age groups. Therefore high vaccine uptake is totally essential (Derek, 2013).

The rubella vaccination policy in Nigeria is to ensure that every Nigerian child receives life-saving vaccination by finding ways to simplify vaccination procedures in the field; improving vaccination delivery to reach every last child, especially those living in remote and inaccessible areas; ensuring vaccine affordability and strengthening vaccine supply chains; training more health workers; improve the quality of data collected by countries and using this to improve immunization operations; overcoming challenges posed by conflict, natural disasters and other crises (Chukwuma, 2015).

2.16 Immune Response

Rubella vaccination induces an immune response in 95% of recipients, but antibody concentrations are generally lower after vaccination than after naturally acquired infection. Testing for antibodies should commence 8 weeks after immunization (NHSC, 2012). Of the 5% who fail to seroconvert, the majority will respond if revaccinated. A few may fail to

respond or respond poorly due to concurrent infection or a low level of preexisting antibodies, which may be undetectable by HAI or SRH. Antibodies persists at levels greater than15 IU/ml in the majority of vaccinees for at least 21 years. In approximately 10%, the antibody levels fall below 15 IU/ml within 5 to 8 years and a small number may become completely seronegative. IgM may persist in 73% of vaccines after 6 months and occasionally been shown to persist up to 4 years (Parkman, 1999). Virus excretion can be detected in the majority of vaccinees between 6 to 28 days after vaccination. However transmission of the virus to susceptible host rarely occur. Vaccine strain of the virus may also be shed in the breast milk of women who were vaccinated postpartum. However even though some infants are infected, they develop no clinical features. Rubella vaccines are well tolerated. Lymphadenopathy, rash may occur between 10 days and 4 weeks after vaccination. These reactions are less severe than the natural infection. Post pubertal females are more likely to develop symptoms than children. Like other live attenuated vaccines, rubella vaccine should not be given to immunocompromised patients, as a result of disease or treatment (Derek 2013).

Pregnancy is an absolute contraindication and pregnancy should be avoided for 1 month after vaccination. Where inadvertent rubella vaccination had occurred just before or during pregnancy, there had not been a single case of foetal damage reported (Plotkin, 2001). Even though it has been shown that vaccine strain virus does cross the placenta and establishes a persistent foetal infection. In a series of 486 babies delivered by women who were inadvertently vaccinated during the first trimester, no congenital abnormalities consistent with CRS were reported. However, there was serological evidence of infection in 8 babies (Derek, 2013).

2.17 Reinfection

Re-infection with rubella may occur. It is more likely to occur in those whose immunity is induced by vaccination rather than natural infection. The IgG response is highly elevated. A slight and transient IgM response may be present. It has been suggested that IgG avidity assays may be useful in distinguishing between primary and re-infection (Derek, 2013).

2.18 Passive Immunization

Post-exposure prophylaxis with immunoglobulins does not prevent infection in non-immune contacts and is therefore not recommended for protection of women exposed to rubella. However it may reduce the likelihood of clinical symptoms which may reduce the level of maternal viraemia and the risk to the fetus. Women who contract rubella during the first trimester of pregnancy but are determined to proceed with the pregnancy may be offered human normal immunoglobulin (HNIG) or rubella immunoglobulin. There is evidence to suggest that infants of women who experienced subclinical rubella in early pregnancy following administration of HNIG is less likely to be infected in *utero*, or if infected, likely to be severely affected. Possible mode of action seems to be decreased maternal viraemia in the presence of HNIG. Dudgeon advocates the administration of HNIG to women who are determined to proceed to term. He suggested a dose of 1500 mg i.m. as soon as possible after exposure and 3 to 4 days later (Kesson 2012; Derek, 2013).

2.19 WHO recommendation

World Health Organization (WHO) recommends that all countries that have not yet introduced rubella vaccine should consider doing so using existing well-established measles immunization program (WHO, 2014).

In April 2012, the Measles Initiative – now known as the Measles and Rubella Initiative – launched a new Global Measles and Rubella Strategic Plan which covers the period 2012-2020. The Plan includes new global goals for 2020.

The strategy focuses on the implementation of five core components:

a. Achieve and maintain high vaccination coverage with two doses of measles- and rubella-containing vaccines;
b. Monitor the disease using effective surveillance and evaluate programmatic efforts to ensure progress and the positive impact of vaccination activities;
c. Develop and maintain outbreak preparedness, rapid response to outbreaks and the effective treatment of cases;
d. Communicate and engage initiate programmes to build public confidence and demand for immunization;
e. Perform the research and development needed to support cost-effective action and improve vaccination and diagnostic tools (WHO, 2014).

Implementation of the Strategic Plan can protect and improve the lives of children and their mothers throughout the world, rapidly and sustainably. The Plan provides clear strategies for country immunization managers, working with domestic and international partners, to achieve the 2015 and 2020 measles and rubella control and elimination goals. It builds on years of experience in implementing immunization programmes and incorporates lessons from accelerated measles control and polio eradication initiatives. As one of the founding members of the Measles & Rubella Initiative, WHO provides technical support to governments and communities to improve routine immunization programmes and hold targeted vaccination campaigns. In addition, the WHO Global Measles and Rubella

Laboratory Network support the diagnosis of rubella and CRS cases and tracking of the spread of rubella viruses (WHO, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Kaduna State is a state in central northern Nigeria. It has political significance as the former administrative headquarters of the north during the colonial era. It is located on the southern end of the high plains of Northern Nigeria. One of the richest states in the region is generally regarded as the gateway to the north and has roads leading from all directions. The state has a total land area of 46,053sqkm this rank the 3rd from the 36 states in Nigeria. Population Census 2006: a total of 6,066,562 also rank the 3rd of the 36 states in Nigeria. It has a density of 131.7/km2 (34.2/sq mi).The state is bordered to the north by Katsina, Zamfara and Kano State, to the east by Plateau and Bauchi States to the south by Nasarawa State and Abuja and, to the west by Niger State (Kaduna State of Nigeria. Nigeria Information Guide 2013).

Kaduna State forms a part of the country's cultural melting point. Apart from the six major ethnic groups found in the state, there are over twenty other ethnic minority groups, each with its language and arts or religion different from each other. Among the major ethnic groups are Kamuku, Gwari, and Kadara in the west, Hausa and Kurama to the north and northwest. "Nerzit" is now used to describe the Jaba, Kaje, Koro, Kamanton, Kataf, Morwa and Chawai instead of the derogatory term "southern Zaria people." Also the term "Hausawa" is used to describe the people of Igabi, Ikara, Giwa and Markafi LGAs which include a large portion of rural dwellers that are strictly "Maguzawas". There is the central market where trading of goods and food stuffs from the different ethnic groups from within kaduna state and outside the state are sold. The presence of one of the most populous universities in Nigeria, makes Kaduna state an area where the presence of rubella virus could be investigated due to the in flocks of traders and students in the state.

Figure 3.1: Map of Kaduna State and its Three Senatorial Districts (Map of Kaduna State 2015).

Kaduna State is divided into three senatorial district. These comprise of the north senatorial district, south senatorial district, and central senatorial district (Figure 3.1). The state comprise of nine hundred and ninety (990) primary and secondary health facilities spread across the senatorial districts of Kaduna state (Primary Health Care Agency Kaduna State, 2012).

3.2 Study Design

This is a cross sectional study. The area of study was selected using representative sampling technique (stratification into three senatorial districts). Random sampling was done to select three local government areas (LGA) from each senatorial districts. These include Zaria for northern senatorial district, Jama'a for southern senatorial district and Igabi for central senatorial district. Three health care facilities each wereselected from each senatorial district as follows.

A- Northern Senatorial District (Zaria LGA).

a) Major Ibrahim Abdullahi Memorial Hospital Sabon Gari.

b) Primary Health Care Tudun wada.

c) Babbandodo Primary Health Care.

B- Southern Senatorial District (Jama'a LGA).

a) Primary Health Care Kafanchan

b) Primary Health Care Godogodo.

c) Primary Health Care kofarsarki

C- Central Senatorial District (Kaduna North LGA)

a) Primary health Care Badarawa.

b) Primary Health Care Ungwar Shanu.

c) Primary Health Care Malali.

3.3 Study Population

The study population included randomly selected pregnant women in their different trimesters attending ante-natal clinics at the selected health care facility in Kaduna State. The clients were recruited irrespective of age, educational/ethnic group.

3.4 Inclusion Criteria

Pregnant women attending ante- natal clinic within the study period that expressed interest in participating in the study at the selected health care facility were included in this study.

Consenting pregnant women were also included.

3.5 Exclusion Criteria

Women who are pregnant but not attending ante-natal clinic at the selected health care facility.

Non-Consenting pregnant women

3.6 Ethical Approval

Ethical permit was obtained from the Department of Health and Human Resources at the State Ministry of Health. In order to have access to patients and/or data, samples (Appendix 1).

Viberal permit was also obtained from Local Government Council. In order to have access to patients and/or data, samples.

3.7 Sample Size Determination

The sample size was calculated using the formula below

$$n=\frac{z^2pq}{i^2}$$ (Muhammad *et al*., 2010).

where z = 1.96 for 95% confidence interval

p = 0.979(Muhammad *et al.,* 2010). Prevalence rate statistics recommends a p value of 97.9)

q = 1 – p

i = allowable error (5%)

n = number of samples

$$= \frac{(1.96)^2 \times 0.979 \times 0.021}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.979 \times 0.021}{0.0025}$$

$$= \frac{0.0790 \times 10}{0.0025}$$

= 316.01(A sample size of at least 346.01was used)

The calculated sample size was346.01 which was the least number of women to be enrolled in the study. However 900 women were enrolled in the study for significant representation.

3.8 Data Collection Using Questionnaire

Questionnaires (Appendix III) and consent forms (Appendix IV) in English were administered to participants and was translated into languages other than English when participants preferred and then participants were asked about demographic information,

clinical symptoms and some possible information regarding possible risk factors associated with rubella virus.

3.9 Collection of Samples

Blood samples were collected as the women came into the clinic and gave consent (randomly) from women attending ante-natal clinics at the selected health care facilities in Kaduna State senatorial districts. The sample collection was: non-probability sampling - Convenience sampling. Equal ratio of sample size 1:1:1 distribution was shared among each senatorial district.

A total of 5ml of blood was collected from each participants with the assistance of trained research assistants. The trained research assistants examined the patients and prepared the site by conventional venepuncture using 5cc syringes fixed with 21 gauge needles. The samples were transferred into non-anticoagulated blood containers and allowed to clot. After which the sera were separated and dispensed into two aliquots labelled and stored. The separation was done by centrifugation at 2500 revolutions per minute (rpm) for 15 minutes. The samples were stored at -20^{o}C at the Yusuf Dantsoho Memorial Hospital, Tudun Wada Kaduna, until analyzed. Samples collected from other facilities were transported using cold chain to the Yusuf Dantsoho Memorial Hospital Tudun-Wada Kaduna and stored at -20^{o}C until analysis required. The analysis were carried out at the Yusuf Dantsoho Memorial Hospital, Tudun Wada Kaduna and DNA labs at Danja road off Katuru Road Unguwar Sarki, Kaduna State.

3.10 Laboratory Analysis of Rubella Virus

Enzyme Linked Immunosorbent Assay (ELISA kits, Axiom Diagnostics Made in Germany) was carried out according to the manufacturer's instruction.

3.10.1 Procedure for Elisa Serum Analysis.

The ELISA kit which contained Carbonate-bicarbonate buffer, Antigens, Test sera and positive control sera were placed at room temperature one hour before the test was performed.Microtiter plates were cleaned and numbered carefully. Using a micro dropper antigen were coated with 2.5mg/ml of protein concentrations in carbonate-bicarbonate buffer (pH 9.6). 5µl of the test sera was placed in well 1F-12H with an Eppendorf pipette (Using new tip for each serum sample, 5µl positive sera diluted was placed on well 1D-1E).The serum was diluted 1:100 and reacted for 2 hours (hrs) at 37^{o}C. Peroxidase conjugated anti-human IgG were diluted at 1:1,000 and further reacted for 2hrs at 37^{o}C. The reaction developed using O-phenylene diamine chromogen (Sigma, st Locus, MO, USA). The absorbance (abs) were read at 490nm using micro-titre plate (M3, 550, Bio-Rad Laboratories, Hercules, CA, USA). Cut off absorbance were set at 0.18 for Rubella (Zeus Scientific, 2009).

Sera which give optical density (OD) 490 value of more than the cut off (mean of 50 negative serum samples + 2SD) wasconsidered as positive (Zeus Scientific, 2009). Various controls was used for validity of the assay such as antigen blank, antibody blank, negative and positive control serum the same procedures was used as above for the detection of IgM antibodies to rubella (Appendix VII-XXVII). The Peroxidase conjugated anti- human IgM was used (Zhonghua, 1996).

3.11 Reverse Transcription Polymerase Chain Reaction (rtPCR).

PCR (polymerase chain reaction) is a method to analyze a short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA. PCR is used to reproduce (amplify) selected sections of DNA or RNA.

3.12 RNA Extraction of Rubella Virus

The serum samples were equilibrated to room temperature (15-25^{o}C). Buffer AVE (viral elution buffer is RNase –free water containing 0.04% sodium azide to prevent microbial growth and subsequent contamination with RNase) was also equilibrated at room temperature. All wash buffers were prepared according to manufacturer's instruction. The prepared buffer AVL containing carrier RNA was pipetted into a micro centrifuge tubes. Then 140µl of the sample was added to buffer AVL carrier RNA in the micro centrifuge tubes mixed and vortexed (Fisher scientific vortex mixer analog count) for 15min.The same procedures were repeated for all the samples and nuclease free water (negative control), known RNA a(positive control). Samples were incubated at room temperature (15-25 ^{o}C) for 10mins then briefly centrifuged to remove drops from lid. Ethanol (100%) was added to the samples and mixed by pulse vortexing for 15secs (Pattison, 2000).

After mixing, the tubes were briefly centrifuged to remove drops from inside the lid. Ethanol 5ul was added to the QIAamp mini spin column without wetting the rim Cap was closed and centrifuged (Eppendorf centrifuge 5415D) at 8000 revolution per minute (rpm) for 1min. QIAamp spin column was placed into a clean 2ml collection tube. The tube containing filtrate was discarded. This process was repeated again. AW1 buffer 5ul was added into the QIAamp mini spin column closed cap and centrifuged at 8000rpm for 1min. QIAamp was placed in a 2ml collection tube and tube containing filtrate was discarded. The process was

repeated with buffer AW2 and then centrifuged at 14000rpm for 3min and the filtrate was discarded. QIAamp spin column was centrifuged for 1 min to remove residual buffer. QIAamp spin column was again placed in a clean 2ml tube. Tube containing filtrate was discarded. Buffer AVE was equilibrated to room temperature and incubated for 1min was added to the spin column and centrifuged at 8000rpm for 1 min. QIA spin column was discarded and elution buffer was transferred to a sterile RNase – free tube for storage at -20^{o}C at the DNA lab independence way Kaduna (DNA Lab, 2014, Mosquera *et al*., 2002).

3.13 Reverse Transcription (RT) of Rubella Virus

Volumes of prepared samples above were aliquoted appropriately into PCR tubes according to manufacturer's procedures using a micro pipette. This was inserted into the Mx3005P Strategene machine for amplification and detection by PCR.

Two pairs of specific primers were chosen for use in the nested-set RT-PCR to amplify an 876-nt region of the RV E1-coding region, which encompassed the 739-nt region recommended by WHO for use for RV Sequencing.

3.14 Amplification and detection by RT-Polymerase Chain Reaction (RT-PCR)

Three major steps were involved in PCR (Denaturation, Annealing, and Extension). These three steps were repeated for 45 cycles. The cycles were done on an automated thermocycler (type Mx3005P Strategene machine), a device which rapidly heats and cools the test tubes containing the reaction mixture. Each step denatauration (alteration of structure) was placed at 50^{o}C for 30 mins, annealing (joining) was placed at 95^{o}C for 2 mins, and extension was placed at 95^{o}C for 15 secs and 45 cycles 55^{o}C for 30 secs.

During denaturation, the double-stranded DNA melts and opens into two pieces of single-stranded DNA. While during annealing, the primers pair up (anneal) with the single-stranded "template" (The template is the sequence of DNA to be copied.) On the small length of double-stranded DNA (the joined primer and template), the polymerase attaches and starts copying the template. During the extension the polymerase works best, and DNA building blocks complementary to the template are coupled to the primer, making a double stranded DNA molecule (Chey, 2010).

3.15 Sequencing of Rubella Virus

3.15.1 Nested-set RT-PCR for Sequencing.

For nested PCR- in the first round has amplified a large region while in the second round we amplified region of interest using another different set of primers. Two pairs of specific primers were chosen for use in the nested-set RT-PCR to amplify an 876-nt region of the RV E1-coding region, which encompassed the 739-nt region recommended by WHO for use for RV Sequencing.

(a)The first-round primers were 8656F (5′-CACCGACACCGTGATGAG) and Rub3′ (5′-TTTCTATACAGCAACAGGTGC).

(b)The second-round primers were 8669F (5′-AGCGTGTTCGCCCTT) and 9549R (5′-TGTGTGCCATAC). RT-PCR with an Access RT-PCR system kit (Promega, Madison, WI) was performed. For the first-round RT-PCR, 5 µl of RNA was added to 45-µl reaction mixtures containing reaction buffer, a 0.2 mM deoxynucleoside triphosphate mixture, 1 mM $MgSO_4$, 1 µM each of the first-round primers, 0.1 U avian myeloblastosis virus reverse transcriptase, 0.1 U *Tfl* DNA polymerase, and 1 M betaine. Betaine to the kit components to facilitate amplification of the RV G, C-rich RNA. Thermal cycling as follows: 45°C for 45

min, followed by 94°C for 2 min and then 40 cycles of 94°C for 30 s, 60°C for 1 min, and 68°C for 2 min. For the second round, 3µl of the first-round product was used as the template in the PCR mixture, as described above, except that the avian myeloblastosis virus reverse transcriptase were eliminated and the 45°C segment of cycling was removed (Yumei, 2007).

(This protocol usually allows the genotypes of the rubella viruses present to be determined from specimens which are positive by conventional RT-PCR).

The sequences obtained were aligned with the reference sequences representing the different genotypes described by WHO (2001) by using the Clustal-W function of the BioEdit program (T. Hall, Department of Microbiology, North Carolina State University, USA). Distance matrices were calculated using maximum likelihood function of the phylip package (Felsenstein, 1985) and the phylogenetic relation was then inferred using the neighbor joining method of the phylip package (Felsenstein, 1985) in combination with bootstrap analysis (100 replications).

3.16 Statistical Analysis of the data

Results were subjected to statistical analysis using Epi Info 3.5.1 software to generate frequencies and proportions of the data collected. The pearson chi square test at 95% confidence interval and 0.05 level of statistical significance using this software. Sequence analysis was done with the SeqScape program, version 2.5 (Applied Biosystems) to confirm the presence of the rubella virus.

CHAPTER FOUR

4.0 RESULTS

Sero-prevalence of the pregnant women enrolled in this present study were presented in Figure 4.1. The response rate was 100%. Up to 63.5% of the pregnant women were rubella IgG positive, 4.3% were positive for rubella IgM while 1.7% were positive for both rubella IgG and IgM. While the overall rubella antibody positivity was 69.5%.

The highest sero-positivity for rubella IgG was among the age group 41-45 years (83.3%). The lowest 0% sero-positivity for rubella IgG was among ages greater than 45 years. The highest sero-positivity for rubella IgM was among age group 21-25 years (6.1%). This was followed by the pregnant women within the age group 36-40 years (4.9%) and 16-20 years (4.4%).The prevalence of both Rubella IgG and IgM antibody among pregnant women was highest among the age group 41-45years with (6.7%). The lowest was among ages less than 45 years(Table 4.1). There was a statistical significant difference (IgG p=0.05, IgM p=0.05, IgG $\chi 2$= 1.42, IgM $\chi 2$=1.96) in the rate of occurrence of the rubella antibodies among the age groups.

In the total of 388 who were screened at the primary educational level, 61.6% were sero-positive for rubella IgG. The secondary educational status had the highest (68.4%) sero-positivity for rubella IgG. Both then one educational status and primary educational status (61.2% and 61.6%) had the lowest sero-positivity for rubella IgG. The secondary educational status had the highest sero-positivity for rubella IgM. The tertiary educational status had (1.2%) the lowest IgM sero-positivity. This was followed by those who never had any form of education (15.4%). Women who were sero-positive for rubella IgG and IgM were highest with (2.3%) in the primary educational status and lowest (0%) in the tertiary educational

status (Table 4.2). There was no statistical significant difference in the rate of occurrence of the rubella antibodies with respect to educational status (IgG p= 0.06, IgM p=0.08. IgGχ^2 = 1.52, IgM χ^2=2.51).

Rubella IgG was highest in pregnant women (76.0%) who were farmers and was lowest in pregnant women who were students (53.6%). Rubella IgM was highest in pregnant women (12.0%) who were farmers and lowest in pregnant women who were students (0%) (Table 4.3). There was no statistical significant difference in the rate of occurrence of the rubella antibodies with respect to type of occupation (IgG p =0.7, IgM p=0.6. IgG χ^2= 2.92, IgM χ2 =2.42).

Rubella IgG was highest in pregnant women who were married (64.1%) and lowest (30.8) in divorced women. Rubella IgM was highest in pregnant women who were Divorced (7.7%) and lowest in the single and widowed women (0%). Rubella antibodies were generally low in pregnant women who were single, divorced and widowed (Table 4.4). There was no statistical significant difference (IgG p =0.05, IgM p=0.05. IgGχ^2= 3.04, IgM χ2=3.86) in the rate of occurrence of the rubella antibodies with respect to marital status.

Rubella IgG was highest (100.0%) in pregnant women who said they had received rubella vaccination. Out of the 39 pregnant women who were positive to rubella IgM, 4.2% said they did not receive any rubella vaccination. Those who did not know if they had received the rubella vaccination before had the highest (6.0%) sero-positivity for rubella IgM (Table 4.5). There was a statistical significant difference (IgG p =0.01, IgM p=0.04. IgG χ^2=0.53,IgM χ2=0.45) in the rate of occurrence of the rubella antibodies with respect to vaccination status.

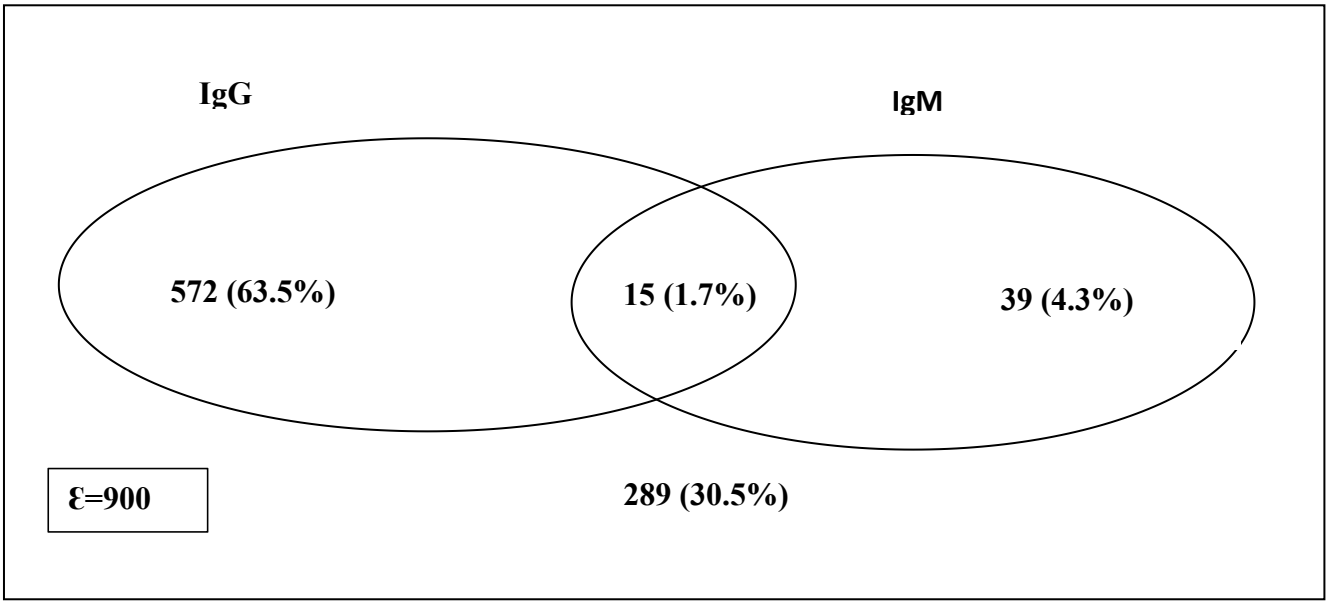

Figure 4.1: Venn diagram showing Sero-Prevalence of Rubella Antibodies IgG, IgM, and IgG and IgM among Pregnant Women Attending Ante- Natal Clinic in Kaduna State.

Table 4.1:Sero-prevalence of Rubella Antibodies Among Pregnant Women Attending Antenatal clinic in Kaduna State Based on Age

Age grp (Years)	Screened	IgG Positive (%)	IgM Positive (%)	IgG and IgM Positive (%)
<15	8	5 (62.5)	0 (0)	0 (0)
16-20	160	99 (61.5)	7 (4.4)	3 (1.9)
21-25	328	198 (60.4)	20 (6.1)	7 (2.1)
26-30	224	155 (69.2)	7 (3.1)	3 (1.3)
31-35	132	82 (62.1)	3 (2.3)	0 (0)
36-40	41	28 (68.3)	2 (4.9)	1 (2.4)
41-45	6	5 (83.3)	0 (0)	1 (6.7)
>45	1	0 (0)	0 0)	0 (0)
Total	900	572(63.5)	39(4.3)	15(1.7)

(IgG) χ^2 =1.42, p =0.05 (IgM) χ^2 =1.96, p=0.05

Table 4.2: Sero-Prevalence of Rubella Antibodies Among Pregnant Women Attending Ante-Natal Clinic inKaduna State Based on Educational Status.

Educational Status	Screened	IgG Positive (%)	IgM Positive (%)	IgG and IgM Positive (%)
Primary	388	239(61.6)	19 (4.9)	9(2.3)
Secondary	253	173 (68.4)	13(5.1)	5(2.0)
Tertiary	81	51(63.0)	1(1.2)	0(0)
None	178	109 (61.2)	6 (3.4)	1(0.6)
Total	900	572(63.5)	39(4.3)	15(1.7)
(IgG) χ^2=1.52, p =0.06			(IgM) χ^2=2.51, p =0.08	

Table 4.3: Sero-Prevalence of Rubella Antibodies among Pregnant Women Attending Ante-Natal Clinic in Kaduna State Based on Occupation

Occupation	Screened	IgG Positive (%)	IgM Positive (%)	IgG and IgM Positive (%)
House wife	515	320(62.1)	23(4.5)	10(1.9)
Civil Servants	69	42 (60.9)	1(1.4)	1(1.4)
Business	263	176(66.9)	12(4.6)	3(1.1)
Farmer	25	19 (76.0)	3(12.0)	1(4.0)
Student	28	15 (53.6)	0(0)	0(0)
Total	900	572(63.5)	39(4.3)	15(1.7)
(IgG) χ^2 =2.92, p=0.7			(IgM) χ^2=2.42, p =0.6	

Table 4.4: Sero-prevalence of Rubella Antibodies among Pregnant Women Attending Ante-Natal Clinic in Kaduna State Based on Marital Status.

Marital Status	Screened	IgG Positive (%)	IgM Positive (%)	IgG and IgM Positive (%)
Married	870	558(64.1)	38(3.9)	14(1.6)
Single	7	4(57.1)	0(0)	0(0)
Divorced	13	4(30.8)	1(7.7)	1(7.7)
widowed	10	6(60.0)	0(0)	0(0)
Total	900	572(63.5)	39(4.3)	15(1.7)

(IgG) $\chi^2 = 3.04$, $p = 0.05$ (IgM) $\chi^2 = 3.86$, $p = 0.05$

Table 4.5: Sero-prevalence of Rubella Antibodies among Pregnant Women Attending Ante-Natal Clinic in Kaduna State Based on Vaccination Status.

Previous Vaccination	Screened	IgG Positive (%)	IgM Positive (%)	IgG and IgM Positive (%)
Do not Know	84	52(61.9)	5(6.0)	2(2.4)
Yes	3	3(100.0)	0(0)	0(0)
No	813	517(6.30)	34(4.2)	13(1.6)
Total	900	572(63.3)	39(4.3)	15(1.7)
(IgG) χ^2=0.53 p=0.01			(IgM) χ^2=0.45, p =0.04	

Each senatorial district had three PHCs that screened 100 samples each making a total of 900 samples over all. The results indicated that in the southern senatorial district Kafachan PHC had the highest sero-positivity for rubella IgG (72%) followed by Godogodo (71%). Godogodo had the highest sero-positivity for rubella IgM (6%) Godogodo followed by kofarsarki (5%). In the northern senatorial district, babandodo had the highest positivity for rubella IgG (77%) with MIBA having the least rubella sero-positivity IgG (58%). Rubella sero-positivity IgM (4%) was equal in both Babandodo and MIBA while Tudun Wada had the highest sero-positivity IgM (5%). In the central senatorial district, Unguwar Shanu had the highest rubella positive IgG (62%) and Badarawa had the least sero-positivity for rubella IgG (49%) and rubella IgM (5%) was highest in Badarawa and lowest in (3%) Unguwar Shanu (Table 4.6).

In the southern senatorial district, the third trimester had the highest sero-positivity for rubella IgG (133.3%: 40/30) and the lowest (52.5%: 96/183) was in women in their first trimester. In the northern senatorial district, the second trimester had the highest sero-positivity for rubella IgG(90.4%: 66/73) and the lowest (50.6%: 85/168) was in women in their first trimesters. In the central senatorial district, the women in their second trimester also had the highest sero-positivity for rubella IgG, (70.5%: 62/88) and the lowest was in women in their first trimester. (Table 4.7).In the southern senatorial district, the third trimester had the highest sero-positivity for rubella IgM (6.7%: 2/30) and the lowest (3.8%: 7/183) was in women in their first trimester. In the northern senatorial district, the first trimester had the highest sero-positivity for rubella IgM (4.8%: 8/168) and the lowest (3.4%:2/59) was in women in their third trimesters. In the central senatorial district, the women in their first trimester also had the highest sero-positivity for rubella IgM, (6.2%: 9/146) and the lowest was in women in their third trimester (0%) (Table 4.7).

Table 4.6: Sero-prevalence of rubella antibodies among pregnant women attending ante-natal clinic in Kaduna state based on the three senatorial districts and their phcs.

Senatorial District	PHCs	Screened	IgGPos	IgM Pos	IgG+IgM Pos	**IgM Rate /1000000**
SSD*	KFC*	100	72	3	2	56
	GOGO*	100	71	6	3	112
	KS*	100	64	5	2	94
NSD*	TW*	100	63	5	1	33
	MIBA*	100	58	4	1`	45
	BABAND*	100	77	4	1	45
CSD*	MALA*	100	56	4	1	50
	U/SHANU*	100	62	3	2	38
	BADARA*	100	49	5	2	63
TOTAL		900	572	39	15	491

SSD-KFC-Southern Senatorial District Kafanchan
SSD-GOGO-Southern Senatorial District Godogodo
SSD-KS-Southern Senatorial District Sarki
NSD-TW-Northern SenatorialDistrict Wada
NSD-MIBA-Northern Senatorial District Major Abdullahi Memorial Hospital
NSDZ-BABAND-Northern Senatorial Distrcit Babandodo
CSDZ-MALA-Central Senatorial District Malali
CSDZ-U/SHANU-Central Senatorial District Unguwar Shanu

Table 4.7: Sero-prevalence of Rubella Antibodies among Pregnant Women Attending Ante-Natal Clinic in Kaduna State Based on Trimesters

Trimester	Senatorial District	Screened	IgG Positive %	IgM Positive %	IgG and IgM Positive %
First	SSD*	183	96(52.5)	7(3.8)	6(3.3)
Second		87	71(81.6)	5(5.7)	1(1.1)
Third		30	40(133.3)	2(6.7)	0(0)
First	NSD*	168	85(50.6)	8(4.8)	3(1.8)
Second		73	66(90.4)	3(4.1)	0(0)
Third		59	47(79.7)	2(3.4)	0(0)
First	CSD*	146	92(6.2)	9(6.2)	5(3.4)
Second		88	62(70.5)	3(3.4)	0(0)
Third		66	13(19.7)	0(0)	0(0)
Total		900	572(63.6)	39(43.3)	15(1.7)

SSD-Southern SenatorialDistrict

NSD-Northern Senatorial District

CSD-Central Senatorial District

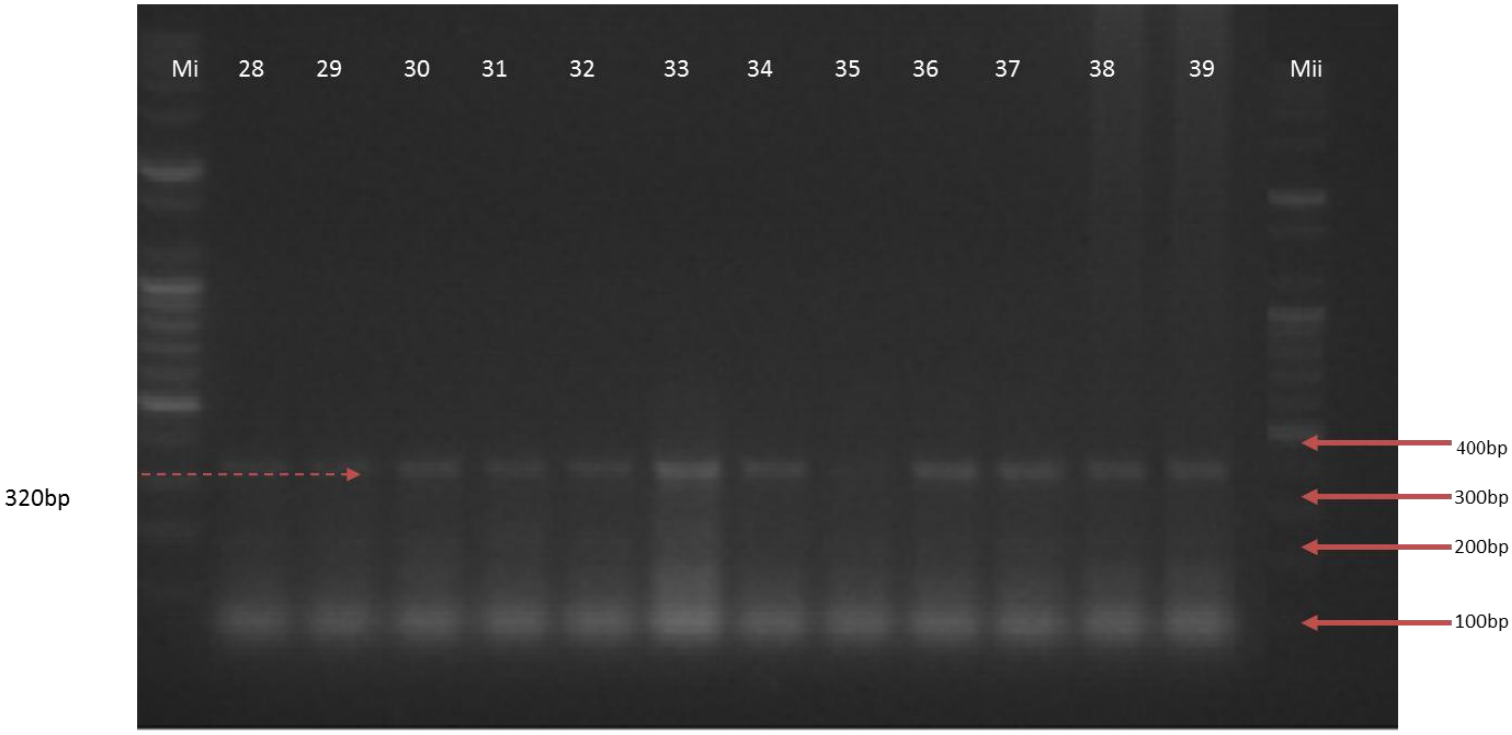

Plate I: Rubella Virus Amplicon at 320bp.

Rubella virus expression under UV transilluminator. (Expression of rubella virus protein on gel electrophoresis viewed under UV transilluminator (Plate III). The bands shown are at 320bp on comparison with standard molecular weight markers of 1kb DNA plus ladder of 0.9µg/lane.)

M = Molecular weight marker of 100bp intervals

Mi = Molecular weight marker 1 (positive control)

Mii = Molecular weight marker 2 (negative control)

Lane 28-39= Rubella samples from subject 28 to

This figure 4.2 shows the distribution of rubella virus-specific IgG and IgM in the sera of the pregnant women that participated in this study. The IgG test results shows that 198 (66.0%) pregnant women tested positive for the northern senatorial district, 207(68.8%) tested positive for the southern senatorial district and 167(55.9%) for central senatorial zone, while the IgM positive results shows 13(4.33%) for the northern senatorial district, 14 (4.67%) southern senatorial district, and 12 (4.00%) tested positive for the central senatorial district. A total of 36.4% was negative for rubella IgG and a total of 95.7% was negative for rubella IgM.

The outcome of the total number of pregnant women who tested positive for rubella IgM in all of the PHC facilities chosen from the three senatorial districts of Kaduna State was 39(4.3%).The northern senatorial district had a total of 13 cases, while the southern senatorial district had a total of 14 cases and the central senatorial district had a total of 12 cases. A total of 11 sero-positive women did not give birth at the health facility, and 9 of the total sero-positive mothers were transferred to a higher health facility. The case record for 2 of the 39 sero- positive pregnant women who attended anta natal clinic in some of the PHCs was not found. A total of 9 babies whose mothers were sero-positive for rubella IgM had no clinical symptoms although 2 babies died at birth and 3 mothers and babies died during delivery. The northern and southern senatorial district had 1 case of miscarriage each and also 1 out of the 39 babies who had eye infections at birth came from the northern senatorial district PHCs (Table 4.8).

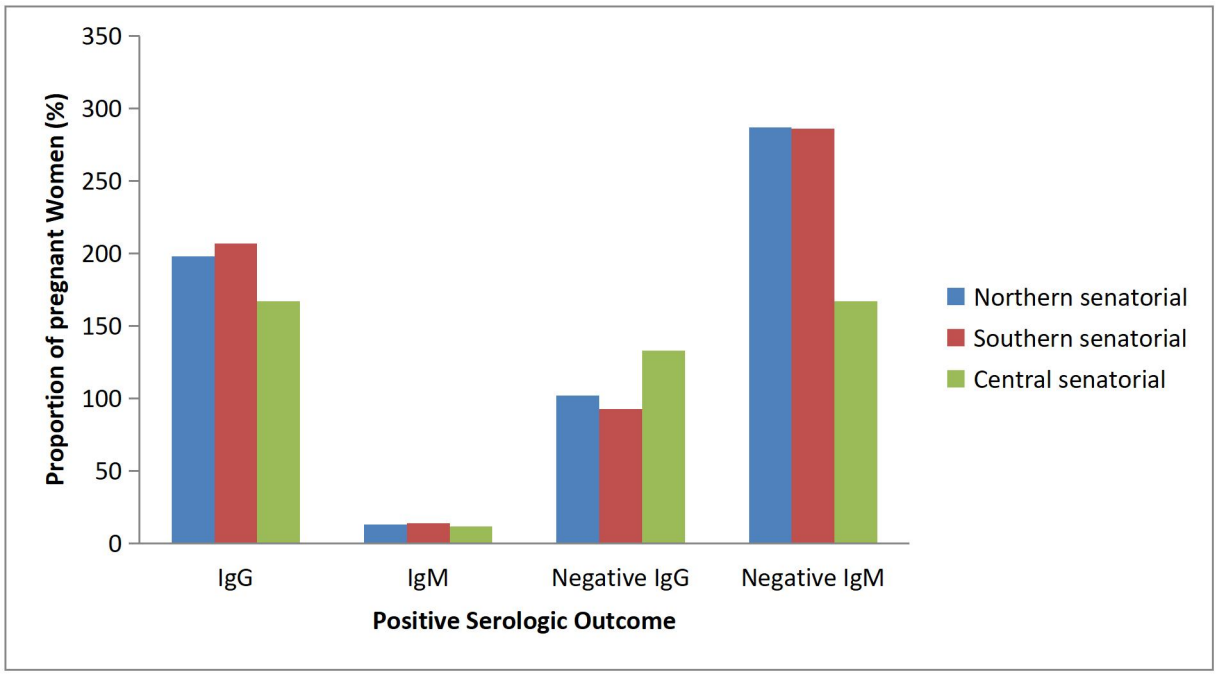

Figure 4.2: Prevalence of Rubella-Virus-Specific Immunoglobulin G and M Among Pregnant Women Attending ANC in the Three Senatorial Districts of Kaduna State

Table 4.8:Follow up cases of Rubella Virus in the Three Senatorial District in Kaduna State.

Cases	Total No. Positive	NSZKD* (%)	SSZKD* (%)	CSZKD* (%)
Did not give birth in health facility	11	5(45.5)	4(36.4)	2(18.2)
Transfer to a higher health facility	9	3(33.3)	2(22.2)	4(44.4)
Miscarriages	2	1(50.0)	1(50.0)	0(0)
Eyes Infection	1	1(100.0)	0(0)	0(0)
Case record not found	2	0(0)	2(100)	0(0)
Mother and baby Died	3	0(0)	2(66.7)	1(33.3)
Baby died	2	2(100.0)	0(0)	(0)
Normal	9	1(11.1)	3(33.3)	5(55.6)
Total	39	13(33.3)	14 (35.8)	12(30.7)

*NSD- Northern Senatorial District Kaduna State

*SSD- Southern Senatorial District Kaduna State

*CSD- Central Senatorial District Kaduna State

The outcome of the total number of pregnant women who tested positive for rubella IgM in the northern senatorial PHC facilities chosen from the three senatorial district of Kaduna State was 13(%). A total of 5 sero-positive women did not give birth at the health facility, and 3 of the total sero-positive mothers were transferred to a higher health facility. All the case record for the pregnant women who attended anta natal clinic in the PHCs was available. Only 1 baby whose mother was sero-positive for rubella IgM had no clinical symptoms although 2 babies died at birth and no mother and baby died during delivery. The MIBA PHC had 1 prevalence of miscarriage and 1 out of the 21 babies who had eye infections at birth came from Babandodo PHC Table (4.9).

The outcome of the total number of pregnant women who tested positive for rubella IgM in one of the PHC facilities chosen from the three senatorial district of Kaduna State was 10(25.6%). A total of 4 sero-positive women did not give birth at the health facility, and 1 of the total sero-positive mothers were transferred to a higher health facility. The case record for 2 of the 10 sero- positive pregnant women who attended anta natal clinic in some of the PHCs was not found. A total of 0 babies whose mothers were sero-positive for rubella IgM had no clinical symptoms no babies died at birth and 2 mothers and babies died during delivery. The southern senatorial district had 1 case of miscarriage and no baby had eye infection at birth (Table 4.10).

Table 4.9: Follow up Cases for Rubella Virus in Northern Senatorial District in Kaduna State.

Cases	Total No. Positive	TW PHC* (%)	MIBA PHC* (%)	BABANDODO PHC* (%)
Did not give birth in Hospital	5	2(40.0)	0(0)	3(60.0)
Transfer to another health facility	3	3(100.0))	0(0)	0(0)
Miscarriages	1	0(0)	0(0)	1(50.0)
Eyes Infection	1	0(0)	0(0)	1(50.0)
Case record not found	0	0(0)	0(0)	0(0)
Mother and Baby died	0	0(0)	0(0)	0(0)
Baby died	2	1(50.0)	1(50.0)	0(0)
Normal	1	1(100.0)	0(0)	0(0)
Total	13	7(53.9)	1(7.7)	5(38.5

TW PHC- Primary Health Care Tudun wada.

MIBA PHC- Major Abdullahi Memorial Hospital Sabon Gari.

BABANDODO PHC- Babandodo Primary Health Care.

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Table 4.10: Follow up Cases of Rubella Virus in SouthernSenatorial District in Kaduna State.

Cases	Total No. Positive	PHC Kafanchan (%)	PHC Godogodo(%)	PHC Kofar sarki (%)
Did not give birth in Hospital	40(0.0)		2(50.0)	2(50.0)
Transfer to another health facility	2	1(50.0)1(14.3)		1(14.3)
Miscarriages	1	0(0.0)	1(100.0)	0(0.0)
Eyes Infection	0	0(0.0)	0(0)	0(0.0)
Case record not found	2	0(0.0)	1(50.0)	1(50.0)
Mother and Baby died	2	1(50)	0(0.0)	1(50.0)
Baby died	0	0(0.0)	0(0.0)	0(0.0)
Normal	3	0(0.0)	2(66.7)	1(33.3)
Total	14	2(14.3)	7(50.0)	5 (35.7)

PHC Kafanchan-Primary Health Care Kafanchan

PHC Godogodo-Primary Health Care Godogodo.

PHC Kofar sarki-Primary Health Care kofar sarki

The outcome of the total number of pregnant women who tested positive for rubella IgM in the central senatorial PHC facilities chosen from the three senatorial districts of Kaduna State was12 (20.5%). A total of 2 sero-positive women did not give birth at the health facility, and 4 of the total sero-positive mothers were transferred to a higher health facility. All the case record for all the 12sero-positive pregnant women who attended anta natal clinic in the PHCs was available. A total of5babies whose mother was sero-positive for rubella IgM had no clinical symptoms and no baby died at birth although 1mother and baby died during delivery (Table 4.11). The central senatorial district had no cases of miscarriages and also no cases of eyes infections.

Table 4.11: Follow Up Cases of Rubella Virus in Central Senatorial District in Kaduna State.

Cases	Total No. Positive	PHC Badarawa* (%)	PHC Ungwar Shanu* (%)	PHC Malali* (%)
Did not give birth in Hospital	2	0(0)	0(0)	2(100)
Transfer to another health facility	4	2(50)	2(50)	0(0)
Miscarriages	0	0(0)	0(0)	0(0)
Eyes Infection	0	0(0)	0(0)	0(0)
Case record not found	0	0(0)	0(0)	0(0)
Mother and Baby died	1	1(100.0)	0(0)	0(0)
Baby died	0	0(0)	0(0)	0(0)
Normal	5	1(20.0)	2(40)	2(40)
Total	12	4(33.3)	4(33.3)	4(33.3)

PHC Badarawa-Primary health Care Badarawa.

PHC Ungwar-Primary Health Care Ungwar Shanu.

PHC Malali-Primary Health Care Malali.

CHAPTER FIVE

4.0 DISCUSSION

This study showed the prevalence of rubella antibodies (IgG and IgM) among pregnant women attending anta-natal clinics in the three senatorial districts of Kaduna State to be 63.3% and 4.3% respectively out of the 900 women screened. This is similar with the finding of Muhammad et al, (2010) whose study showed a 97% IgG sero-positivity among pregnant women attending clinic at the Ahmadu Bello University Teaching Hospital Zaria. This could be as a result of previous exposure to the rubella virus which shows the prevalence. It may not be as a result of vaccination because over 90% of the women screened said they were not vaccinated against this virus probably because it is not part of the routine immunization exercise in Nigeria.

The prevalence of low history of vaccination 3(0.3%) IgG rate for rubella is suggestive of the low priority attention being given to rubella which is not included in the routine immunization program in Nigeria. The routine immunization program have a low patronage has less than 30% of the children between the ages 0-24months receive complete vaccination. The primary purpose for rubella vaccination is the prevention of congenital rubella infection (CRI) including congenital rubella syndrome (CRS). In studies conducted on rubella epidemiology in the African region, susceptibility among women of reproductive age in West Africa ranges from 10%- 38% for rubella IgM and 97% for rubella IgG (Chimhuya *et al*., 2015). The measles, mumps, rubella vaccine is called MMR for short and this is one of the recommended child hood vaccines.

Sero-positivity rate for IgG and IgM among the southern senatorial districts was highest with 133.3% and6.7%in their first trimesters respectively. These patients would have come in

contact with the virus sometime during their life time. Also the mode of transmission of the infection and asymptomatic nature of rubella infection could be a contributing factor.

Married women accounted for majority 96% and the sero-prevalence rate IgG was highest in married women with 64.1% and pregnant women who were divorced had the highest (7.7%) rubella IgM sero-positivity. This is because in most northern Nigeria setting the age at marriage is 15years. Islam the predominant religion allows remarriage even after divorced or death of spouse consequently the proportion of married women remains high. Infection may differ from one particular location to the other.

Out of the 55% pregnant women who tested for rubella IgG IgM in their first trimester, 17% of this figure was positive for rubella IgG & 48% for rubella IgM. Infection with rubella virus during pregnancy, especially during the first trimester, can result in congenital rubella syndrome (CRS). The prevalence of rubella IgG antibody in all the different trimester was however found not to be statistically significant. The pregnant women that were IgG sero-positive only are considered to be immune. The immunity acquired by these women is due previous exposure to rubella virus since they were not vaccinated against this virus previously from the questionnaire survey.

The IgM sero-prevalence of 4.3% is an indication of active rubella infection. Primary infection of rubella depending on the gestational period is known to affect the pregnancy. It is important to note that the primary public health concern of rubella acquired in the first 12 weeks of pregnancy is associated with 90% risk of congenital malformation. Infection with rubella virus during pregnancy, especially during the first trimester, can result in congenital

rubella syndrome (CRS). Serious manifestations of CRS include deafness, cataracts, cardiac defects, mental retardation, and death.

The age distribution of participants with the modal 328(36%) age group is 21-25. This group had a sero-positivity rate for rubella IgG 60.4% the highest positivity among the age group. The highest age related sero-positivity rate for Rubella IgG was ages 21-25. The high prevalence which was statistically significant in this group may be indicative of a re-infection. This is corroborated by a study Hamkar (2009) that showed 85.3% high-avidity anti rubella virus which was regarded as a case of re-infection and another Study by Eilard (1974) which showed evidence of congenital malformation of a foetus from a mother who was re-infected with rubella IgM antibodies in the umbilical cord blood. Rubella re-infection can occur in both natural or vaccine induced immunity, once the IgG level is low (Tetsuya *et al*., 2007). If the infection is primary the infants whose mother acquire rubella infection during the first trimester presents congenital rubella. Cataracts results when infection occurs between third and eighth weeks, Deafness between third and eightieth weeks end heart abnormalities between the third and tenth weeks of gestation. The risk declined by 10% to 20% in the 16 weeks gestation. Foetal damage is rare by 20 weeks gestation (Australian National Immunization Handbook, 2008; Junaid *et al*., 2011).

The presence of the IgG antibodies indicates a past infection and the antibody levels is linked to B-memory cell activity. The lowest sero-positivity rate fell at ages less than 45years for rubella IgG and IgM this explains that women between this age group have been long exposed to the rubella virus.

Low level of education together with unemployment provides a fatal land for the proliferation of vaccine preventable illnesses. This is because the lack of education limits the adaption of those key actions for health promotion (KAHP). Paradoxically, the highest sero-positivity rate rubella IgG was in the farmer group being (76.0%). While those who were also farmers had the highest rubella IgM (12.0%) The socioeconomic status indicated that 100% of the pregnant women who were farmers were rubella IgG and IgM sero-positive. The health facilities showed similar positivity rate. These results showed no statistical significance.

The sero-prevalence of these antibodies did not show any significant correlation in terms of educational status of the pregnant women. Though the highest sero-prevalence of IgG, IgM and IgG and IgM was in pregnant women who had secondary education and the knowledge of rubella is low among the study participants. Thus, the level of education could have effect on the knowledge about rubella infection and its prevention by vaccination program which can help to reduce the burden of the disease.

Rubella virus continues to circulate elsewhere in the world, especially in regions where rubella vaccination programs have not been established (e.g., the African Region). In Nigeria only few surveys have so far been conducted (Agbede *et al*., 2011). This study like other previous studies has shown that a high proportion of our population has rubella immunity suggesting exposures to previous immune attacks. It has been reported that all immune Nigerians acquire their immunity by natural infection. This study reinforces the few existing information on the distribution of rubella immunity in Nigeria. An ELISA Rubella G index of 1.0 or greater or IU value greater than 15were sero-positive. It indicates prior exposure to the

rubella virus (greater than15 IU/ml). While Samples absorbance over cut off value higher than or equals to 1.00 were considered positive, which indicates that antibodies to the rubella virus have been detected with this kits.

The 63.6% sero-prevalence obtained in this study is similar to the 54.1%, 68.5% and 76.0% reported by Bukbuk *et al*. (2002) in Maiduguri, Bamgboye *et al.* (2004) in Ibadan and Onyenekwe *et al*. (2000) in Lagos respectively but contrast the sero-prevalence of 97.7% reported in Zaria, Nigeria (Muhammed *et al*., 2010), 92.9% reported in Eldoret, Kenya and 88.6% reported in Yaounde, Cameroun (Fokunang *et al*., 2010).

The same technique (ELISA) was employed for the detection of the antibody but the studies were not conducted at the same time, the recent studies have higher prevalence. Also comparing the marked increased in the sero-prevalence rate in Nigeria and some African countries is suggesting of an increased in circulation of rubella virus as reported by WHO that there is an increased in rubella cases in areas where there is no MMR vaccines (WHO 2000).

Occupation and rubella sero-positivity was not significant forIgG, the highest prevalence of 76.0% was observed among women that are farmers. This is similar with the findings conducted in Ibadan by Bamgboye *et al*. (2004). Which shows highest prevalence of 80% in unemployed women. For IgM, the prevalence of 12.0% was the same in unemployed women. This is similar with a study done by Eleazu *et al*. (2012) in Jos, highest prevalence of 3.68% in housewife. Occupation might be consider a risk factor for acquiring the infection, once the person involved is not immune against this virus.), highest in unemployed women 76.0% as compared to the 2.6% in employed women. Working in places such as pediatric department,

day care and schools especially primary school involves children and can expose susceptible individuals to rubella infection because is a disease of school age children. Occupation might be considered a risk factor for acquiring the infection, once the person involved is not immune against this virus.

The initial consequences of rubella infection early in pregnancy is either miscarriage or still birth when the foetus survives the infection, multiple congenital defects maybe present at birth. The previous miscarriages and stillbirth observed in this study could be due to rubella infection, other TORCH (Toxoplasma, Rubella, Cytomegalovirus, Herpes simplex virus) complexes or iatrogenic causes.Bukbuk *et al*. (2002) reported a 60% stillbirth from the immune women. Bamgboye *et al.* (2004) and Fokunang *et al*. (2010) reported 22.0% and 40.3% previous history of abortion. Hamdan *et al*. (2011) also report multiple congenital defects.

The knowledge and awareness of rubella among the pregnant women was low, from the questionnaire survey none of the respondent was aware of the infection (Appendix xxviii). This is because rubella is not incorporated in their routine ante-natal health talk the awareness and knowledge of other vaccine- preventable disease such as polio, measles, hepatitis among others was high among the pregnant women. Due to the minimal awareness and knowledge of the above vaccine- preventable disease as well as the availability and cost, the women do not usually participate fully in the national immunization programme.

The sero prevalence of rubella virus antibodies amongst pregnant women attending some ante-natal clinics in the three senatorial distribution in Kaduna State was studied. The sero-prevalence of 63.6 % for IgG antibody and 4.3% for Ig M antibody obtained from this study

could be attributed to the infection and asymptomatic nature of rubella infection. Close-contact is known to enhance transmission of infections that can be acquired through air. Also asymptomatic nature of the infection, allows asymptomatic carriers to transmit the infection to susceptible individuals through their nasopharyngeal secretion and infants born with CRS excretes the virus through body secretion as well.

IgM sero-prevalence obtained in this study is similar to 3.9% and 6.8% obtained by Pennap *et al.* (2009 in Benue and Eleazu *et al*. (2012) in Jos respectively. Also from the present study the sero-prevalence rate of IgG and IgM was 1.7%, this rate was lower than the prevalence of 10.0% from Benin by Onakewhor *et al*. (2011) andlower to the prevalence of 80.8% in Iraq by Bushra *et al*. (2010) butsimilar to 1.3% from Turkey by Yavuz*et al*. (2008). Generally there is scanty published information on sero-prevalence of antibodies to rubella among pregnant women in Africa especially on IgM antibodies. The sampling of this present study involved three different zones in Kaduna state while the previous studies mainly involved singled location. However, endemicity of specific infectious agent may vary with geographical location and population density.

The highest sero-positivity of IgG observed among age group 41-45yrs, is similar with the finding of Adewumi *et al*. (2013) in Ibadan who reported highest sero-positivity among age greater than 40yr. The sero-prevalence of IgM was highest in the age group 21-25yrs and none (0.0%) in the age group greater than 45yrs and above. This finding agrees with the report of Eleazu *et al*. (2012) where the older age group had a lower prevalence of IgM antibody than the younger age group. In addition, the sero-prevalence of IgG + IgM was highest in the age group 21-25yrs and lower in the age group greater than 45yr old. This results differ from reportby Bushra *et al.* (2010) in Iraq where they observed highest sero-

prevalence in the age group 25 to29 yrs. old lower among women aged greater than 40 years for IgG and IgM. Generally the virus can infect all age, but the infection occurs most often in children. The highest immunity observed in the older women could be probably due to higher frequency of childbearing and nursing experienced in such age, which predisposes them to a higher risk of infection and thus increased immunity. However, the sero-prevalence of these antibodies did not show any significant correlation in terms of educational status of the pregnant women. Though the highest sero-prevalence of IgG, IgM and IgG + IgM combination respectively was in pregnant women who had primary education, secondary education and non formal education. The knowledge of rubella is low among the study participants. Thus the level of education could have an effect on the knowledge about rubella infection and its prevention by vaccination program which can help to reduce the burden of the disease (Obijimi *et al.,* 2013).

Not all the women who were IgM positive and followed delivered in the hospitals. A total of 9 babies whose mothers were sero-positive for rubella IgM had no clinical symptoms although 2 babies died at birth and 3 mothers and babies died during delivery. This may be due to the presence of the rubella IgM virus infection during pregnancy or complications during labour.

Despite the fact that rubella virus has a similar genomic organization to the alpha viruses, the phylogenetic relationship between the Rubivirus genus and the Alpha viruses' genus are distant and the evolutionary events connecting rubella virus are apparently complicated (Lee*et al*., 1991).The advent of molecular techniques such as the polymerase chain reaction and sequence has made it possible to examine the genetic makeup of the virus itself and perform molecular epidemiology studies (Lee and Bowden, 2000).

The E1 genotype sequenced in this study was similar with Domingueez *et al*., 1990 study who stated that genotype E1 is found in Africa, the Americas, Asia and Europe. The E1 has been shown to be a type 1 membrane protein that is rich in cysteine residues with extensive intramolecular disulfide bonds. This is in correlation with marchler-bauer *et al*. (2011) of Conserved Domain Database (CDD) for the functional annotation of proteins. Studies have described phylogenetic analysis of a collection of 103 E1 gene sequences from rubella viruses isolated from 17 countries from 1961 to 2000 (Katow *et al.,* 1997;Zhu*et al*., 2007).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study has shown that the sero-prevalence of Rubella IgG among the pregnant women was 63.6 %. This is to say that there are still 45.5% of the population screened who might be at risk of rubella infection and can transmit the virus to their unborn babies. Risk factors such as maternal, age, trimester in pregnancy, number of deliveries, and previous history of congenital abnormalities were not statistically significant in the assessment of their association with sero-positivity for rubella infection. It is therefore necessary to immunize those women that are sero negative for rubella before they get pregnant to provide protection against the rubella virus.

6.2 RECOMMENDATIONS

The sero-prevalence 63% and 4% for rubella IgG and IgM antibodies to rubella indicates that the virus is in circulation in Kaduna State. We recommend to the Federal Ministry of Health that due to the congenital abnormalities associated with rubella virus, pregnant women should be screened against rubella virus and given health talk on rubella virus during ANC, young girls should be vaccinated against rubella before getting married.

This could be achieved if the government, community rulers and partners provide screening and vaccination program. Early detection of maternal rubella infection, has been documented to prevent congenital rubella by screening. The Knowledge on rubella and studies conducted in the communities is very low. This research was hospital based. In the feature, a community based study should be conducted to give an accurate description of a community based study.

Rubella virus transmission would be reduced if we avoid overcrowding in hospitals and homes. The Federal Ministry of Health, the State Ministry of Health, and stake holders & community rulers should participate in creating awareness on rubella virus infection and also make the vaccine available and accessible in all health facilities. Further research work on sero-epidemiology and molecular characterization of rubella virus should be carried out in tertiary health facilities to compare the prevalence.

REFERENCES

Adesina, O., Adeniji J.and Adeoti, M. (2008). Rubella IgG Antibody in Women of Child Bearing Age in Oyo State.*African Journal of Clinical and Experimental Microbiology,* 9(2):78–81.

Adewumi, O.M., Olasanya, R. B., Oladunjoye, B. A. and Adeniji, J.A.(2013). Rubella IgG Antibody Among Nigerian Pregnant Women Without Vaccination History. *African Journal of Clinical and Experimental Microbiology*, 14(1): 40-44

Agbede, O.O., Adeyemi, O.O., Olatinwo, A.W.O., Salisu, T.J. and Kolawole, O.M.(2011). Sero-Prevalence of Antenatal Rubella in University of Ibadan Teaching Hospital. *The Open Public Health Journal,* 4, 10–16.

Atkinson, W., Hamborsky, J., McIntyre, L. and Wolfe, S. (2007). Epidemiology and Prevention of Vaccine-Preventable Diseases. 10th ed. *Centers for Disease Control and Prevention.* 56(01):10

Atreya, C. D., Singh, N. K. and Nakhasi, H. L. (1995a). The Rubella Virus Binding Activity of Human Calreticulin is Localized to the N-Terminal Domain. *Journal of Virology*. 69(6):3848–3851.

Atreya, C.D.,Mohan, K.V.K.and Kulkarni, S. (1998b).Rubella Virus and Birth Defects: Molecular Insights into the Viral Teratogenesis at the Cellular Level.*Clinical and Molecular Teratology*. 70: 431-437.

Atreya, C. D., Lee, N. S., Forng, R.Y., Höfmann, J., Washington, G., Marti, G. and Nakhasi, H.L. (1998b). The Rubella Virus Putative Replicase Interacts with the Retinoblastoma Tumor Suppressor Protein. *Virus Genes,* 16: 177–183.

Australian National Immunization Handbook. (2008). Rubella. 9th edition. [Online] Available at:http://www.health.gov.au/internet/immunise/publishing./Handbook-home. Accessed 3 June 2012.

Bamboye, A.E., Afolabi, K.A., Esumeh, F. I. and Enweani, I.B. (2004).Prevalence ofRubella Antibody in Pregnant Women in Ibadan, Nigeria.*West Africa Journal of Medicine,*23 (3):245-248.

Baron, M. D. and Forsell, K. (1991). Oligomerization of The Structural Proteins of Rubella Virus. *Virology*. 185;811–819.

Baron, M. D., Ebel, T. and Suomalainen, M. (1992). Intracellular Transport of Rubella Virus Structural Proteins Expressed from Cloned cDNA. *Journal of General Virology.* 73: 1073–1086.

Beatch, M.D., Everitt, J.C., Law, L.J. and Hobman, T.C. (2005). Interactions Between Rubella Virus Capsid and Host Protein p32 are Important for Virus Replication. *Journal of Virology.* 79; 10807–10820.

Bellini, J. W. and Icenogle, J. P. (2007). Measles and Rubella Viruses. *In: Manual of Clinical Microbiology.* American Society for Microbiology (ASM), 2(9):1389-1403.

Bellini, J. W. and Sever, L. J. Rubella Virus. In: Specter, S., Hodinka, L. R. and Young, A. S. eds. (2000). *Clinical Virology Manual*. 3rd ed. Washington, DC: American Society of Microbiology (ASM) Press. Ch. 36.

Best, J. M. and Banatvala, J.E. Rubella. (2000) In:Zuckerman, A.J., Banatvala, J.E. and Pattison, J.R., eds. Principles and practice of clinical Virology, 4th ed. England: John Wiley and Sons Ltd. Ch.12.

Best, J.M. (2007). Rubella Seminar: Fetal Neonatal Medicine. *Public Medicine*. 12; 182–192.

Bowden, D. S. and Westaway, E. G. (1985). Changes in Glycosylation of Rubella Virus Envelope Proteins During Maturation. *Journal of General Virology*. 66; 201–206.

Brooks, F. G., Carroll, C.K., Butel, S.J. and Morse, A.S. (2007).*Jawetz, Melnick, & Adelberg's Medical Microbiology.*24th ed.U.S.A.:McGraw-Hill Companies. Pp562-564.

Bukbuk, D.N., El Nafaty A.U. and Obed, J.Y.(2002). Prevalence of Rubella – Specific IgG Antibody in Non – Immunised Pregnant Women in Maiduguri, North-Eastern Nigeria.*CentralEurope Journal Public Health,* 10(1-2): 21 – 23.

Bushra,A., Muhammed, A.,and Wisam H. (2010) "Evaluation of Anti- Rubella Antibodies AmongChildbearing Age Women in Babylon Governoratein Iraq" *Medical Journal of Babylon.* (7):1-2.

Burns,T., Breathnach,S., Cox,N. and Griffiths,C., eds.(2010).Rubella. *In: Rook'sTextbook of Dermatology.*[Online]Available at: http://onlinelibrary.wiley.com/book/10.1002/9781444317633. Accessed 15 Feb, 2013.

Centers for Disease Control and Prevention.(2008). Progress Towards Elimination of Rubella and Congenital Rubella Syndrome—The Americas. *Morbidity and Mortality Report weekly report.*[online] at file:///C:/contents/congenital-rubella- Accessed 1May 2011.

Centers for Disease Control and Prevention.(2011). National Center for Immunization and Respiratory Disease. *Division of viral diseases.*[Online]Available at: http://www.cdc.gov/Features/Rubella/ Accessed 1 May 2011.

Centre for Disease Control and prevention. (2012). Pinkbook Course – Epidemiology of Vaccine – Preventable Disease. [Online]Availableat:http://www.cdc.gov/vaccines/pubs/pinkbook/rubella.html Accessed 3 June 2013.

Chantler, J., Wolinsky, J. S. and Tingle, A. (2001). Rubella Virus.In: Knipe, D.M. and Howley, P.M., eds.*Field's Virologv.* Vol. 1, 4th ed. Lippincott Williams and Wilkins.Ch. 31.

Chey, S., Claus, C., Liebert, U.G.(2010).Validation and Application of Normalization Factors for Gene Expression Studies in Rubella Virus-Infected Cell lines with Quantitative Real-Time PCR.*Journal of Cellular Biochemistry.*110(1):118-128.

Chimhuya, S., Manangazira, P., Mukaratirwa, A., Nziramasanga P., Berejena, C., Shonhai, A., Kamupota, M., Gerede, R., Munyoro, M., Mangwanya, D., Tapfumaneyi, C., Byabamazima, C., Shibeshi, M.E. and Nathoo, K. J. (2015). Trends of Rubella incidence during a 5-year period of case based surveillance in Zimbabwe, *Bio Med Central Public Health* 15:292- 294.

Chukwuma M. (2015) Ensuring every Nigerian Child Receives Life-Saving Vaccination, The Guardian 01 may 2015.

Christelle, V.F. and Picone, O.(2010). Cytomegalovirus and the National Health and Nutrition Examination Surveys.*Clinical Infectious Diseases.*[Online] Available at:http://scholar.qsensei.com/content/1pnjvwAccessed December 2013.

Danovaro-Holliday,M.C., Lebaron, C.W., Allenswoth, C. R.,Bordan, I.G. Murray, A. B., Icenogle, J.P. and Reef, S.E. (2000). A large Rubella Outbreak with Spread fromthe Workplace to the Community. *Journal of America Medical Association*, 284 (21):2733-2739.

Derek, W. (2013). Rubella: Congenital viral infections slide set. *Wong's virology*. 14.

Dominguez, G., Wang, C.Y., Frey, T.K. (1990). "Sequence of The Genome RNA of Rubella Virus: Evidence for Genetic Rearrangement During Togavirus Evolution". *Virology,* 177 (1): 225– 238.

Dontigny, L., Arsenault, M. And Martel, M. (2008).Rubella in Pregnancy, *Journal of Obstetrics and Gynaecology of Canada*, 30(2):152-158.

EhrlichS.D. (2010).Rubella.*VeriMed Healthcare Network.* [Online] Available at:http://www.umm.edu/altmed/articles/rubella-000145.htm. Accessed 17 March 2013.

Eilard, T. and Strannegard, O. (1974). Rubella Infection in Pregnancy Followed by Transmission to the Fetus. *Journal of Infectious Disease*, 129(5):594-596

Eleazu, C.O., Chinedum, E.K., John, A. and Esther, A. (2012). "Survey of the Sero-Prevalence of IgM Antibodies in Pregnant Women Infected With Rubella Virus Plateau State SpecialistHospital in Jos" E3.*Journal of Biotechnology and Pharmaceutical Research* 3 (1): 10-14.

Ezike, E., Ang, J.Y., and Asmar, B. (2002).Rubella.*eMedicine Journal*, (3): 20-21

Felsenstein, J. (1985). MEGA, Molecular Evolutionary Genetics[Online]http://www.megasoftware.net/mega4/WebHelp/references/felsenstein_1985.htm Accessed 3 May 2015.

Fokunang, C.N., Chia, J., Ndumbe, P., Mbu, P. and Atashilli, J. (2010). Clinical Studies on Sero-Prevalence of Rubella Virus in Pregnant Women of Cameroon Regions. *African Journal of Clinical and Experimental Microbiology,* 11(2): 79-94.

Frey, T. K. (1994). Molecular Biology of Rubella Virus. *Advance Virus Research*. 44: 69–160.

Frey, T.K., Abernathy, E.S., Bosma, T.J., Starkey, W.G., Corbett, K.M., Best, J.M., Katow, S. And Weaver, S. C. (1998). Molecular Analysis of Rubella Virus Epidemiology AcrossThree Continents, North America, Europe, and Asia, 1961–1997. *Journal Infectious Disease*. 178; 642–650.

Frey, T.K. (2008).Rubella virus. *Encyclopaedia of Virology* (3):514-522

Garbutt, M., Law, M. J., Chan, H. and Hobman, T. C. (1999). Role of rubella virus glycoprotein domains in assembly of virus-like particles. *Journal of Virology*. 73; 3524–3533.

Hamdan, Z. H., Ismail, E.A., Nasser, M.N. and Ishag, A. (2011). Sero-prevalence of Cytomegalovirus and Rubella among Pregnant Women in Western Sudan. *Virology Journal.* 8: 217.

Hamkar, R., Jalilvand, S., Abdolbagh, M. H., Jelyani, K. N., Esteghamati, A., Hagh-goo, A., Mohktari-Azad, T., Nategh, R. (2009). Distinguish Between Primary Infection and Reinfection with Rubella Vaccine Virus by IgG Avidity Assay in Pregnant Women. *East Mediterrian Health Journal,*15(1):94-103.

Hanshaw, J.B., Dudgeon, J.A., and Marshall, W.C. (1985). Viral Diseases of The Fetus and Newborn. *WB Saunders Coporation, Philadelphia*.

Haukenes, G. (2002). In:Haaheim, L.R., Pattison, J.R. and Whitley, R.J.,eds. (2002). A Practical Guide to Clinical Virology. 2nded. England*:* John Wiley and Sons Ltd.

Hemphill, M. L., Forng, R.Y., Abernathy, E. S. and Frey, T. K. (1988). Time Course of Rubella Virus-Specific Macromolecular Synthesis DuringRubella Virus Infection in Vero cells. *Virology*. 162; 65–75.

Hobman, T.C. and Gillam S. (1989). Invitro and Invivo Expression of Rubella Virus Glycoprotein E2: The Signal Peptide is contained in The C-terminal Region of Capsid Protein. *Virology*, 173: 241–250.

Hobman, T., Chantler, J., Knipe, D.M., Howley, P.M., Griffin, D.E., Martin, M.A., Lamb, R.A., Roizman, B. and Straus, S.E. (2007). Rubella Virus. *Virology*, 5thed. PA, USA: Lippincott Williams & Wilkins, 1069-1100.

Höfmann, J., Pletz, M.W. and Liebert, U.G. (1999). Rubella Virus-Induced Cytopathic Effect Invitro is caused by Apoptosis. *Journal of General Virology*.80: 1657–1664.

Ilkow, C.S., Willows, S.D. and Hobman, T.C. (2008). Rubella Virus Capsid Protein: A Small Protein with Big Functions. *Future Microbiology*. 5; 571–584.

Junaid, S.A., Akpan, K.J. and Olabode, A. O. (2011). Sero-Survey of Rubella IgM Antibodies among Children in Jos, Nigeria. *Virology Journal*. 8:244.

Katow, S.and A. Sugiura (1997). Low PH- Induced Conformational Change of Rubella Virus Envelope Proteins. *Journal of General Virology*, 69:2797 – 2807.

Kaduna State of Nigeria. Nigeria Information Guide (2013). [Online] Available at: http://www.nigeriagalleria.com/Nigeria/States_Nigeria/Kaduna_State.html Accessed 13 October 2014

Kessel, R.G. (1992). Annulate Lamellae: A last Frontier in Cellular Organelles. International *Review of Cytology*. 133: 43–120.

Kesson, A. M. (2012).Rubella.*Netter's Infectious Disease*. 51-54

Kimberlin, W.D. In: Richman, D.D., Whitley, J.R. and Hayden, G. F., eds. (2002). Clinical Virology, 2nd ed. Washington D.C.: *American Society for Microbiology Press.*Ch 54.

Kistler, G. S. (1975). Cytoplasmic Tubuloreticular Complexes and Nuclear Bodies in Cells of Rubella-Infected Human Embryos and fetuses. *Beitr Pathology*. 155:101–138.

Kolawole, O.M., Anjorin, E.O., Adekanle, D.A., Kolawole, C.F. and Durowade, K. A. (2014). Sero-Prevalence of Rubella IgG Antibody in Pregnant Women in Osogbo, Nigeria. *International Journal of Preventive Medicine*. 5; 287-92

Kujala, P., Ahola, T., Ehsani, N., Auvinen, P., Vihinen, H. and Kääriäinen, L. (1999). Intra-Cellular Distribution of Rubella Virus Nonstructural Proteins P150. *Journal of Virology*. 73:7805–7811.

Lanzrein, M., Schlegel, A., Kempf, C. (1994). Entry and Coating of Enveloped Viruses. *Journal of Biochemistry*, 302:313 – 20.

Lawn, J.E., Reef, S., Adadevoh, S., Caul, E. O. and Griffin, G. E. (2000). Unseen Blindness, Unheard Deafness, and Unrecorded Death and Disability:Congenital rubella in Kumasi, Ghana. *American Journal of Public Health,* 90; 1555–61.

Lee, H. J., Shieh, C. K., Gorbalenya, A. E., Koonin, E. V., Monica, N. La. and Tuler, J. (1991). Conservation of the Putative Methl Transferase Domain: Hallmark of the Sindbis- Like' Supergroup of positive- Strand RNA Viruses. *Virology*, 180(2): 567-582.

Lee, J. and Bowden, S. (2000). Rubella Virus Replication and Links to Teratogenicity.*Clinical Microbiology Reviews*, 13(4):571-587.

Leopardi, R. and Roizman, K. (1996). The Herpes Simplex Virus Major Regulatory Protein ICP4 Blocks Apoptosis Induced by Virus or by Hyperthermia, *Proceeding of National Academy of Science. USA*, 93:9583 – 9587.

Lombardo, P.C. (2011). Dermatological Manifestations of Rubella.*Medscape Drugs, Diseases and Procedures*,[Online] Available at: http://emedicine.m*edscape.*com/article/1133108-overviewAccessed 5April 2014

Magliano, D., Marshall, J.A., Bowden, D.S., Vardaxis, N., Meanger,J.and Lee J.Y. (1998). Rubella Virus Replication Complexes are Virus-Modified lysosomes. *Virology* 240: 57–63.

Map of Kaduna State. (2015). [Online] Available at:http://www.bing.com/images/search?q=map Accessed 19 March 2016.

Market Research Reports, Inc.(2015). Mumps-and-Rubella-Combined-Vaccine-live-Industry-2015-Market-Report-Launched-via-Marketresearchreportscom-637662.htm [Online] Available at:http://www.sbwire.com/press-releases/global-measles-

Mauracher, C. A., Gillam, S., Shukin, R. and Tingle, A. J. (1991). PH-Dependent Solubility Shift of Rubella Virus Capsid Protein. *Virology,.*181: 773–777.

Matthew, L.A., Lawrence, L.M., Gray, D., Gray S. (2011). An Audit of Rubella IgG Antibody Status in Antenatal Women in a NHS Trust Over 5 years. *Epidemiology and Infection*. 319(11):1720-1726.

Mayo Clinic (2011).Mayo Clinic Guide to a Healthy Pregnancy. *Mayo foundation for Medical Education and Research,* [online] Availableat:http//www.mayoclinic.com/health/rubella/DS00332/DSECTION=prevention Accessed 2 April 2014.

Megyeri, K., Berencsi, K., Halazonetis, T.D., Prendergast, G.C., Gri, G., Plotkin, S.A., Rovera, G., Gönczöl, E. (1999).Involvement of a p53-Dependent Pathway in Rubella Virus-Induced Apoptosis.*Virology,* 259 (1): 74–84.

Morice, A., Ulloa-Gutierrez, R. and Avila-Aguero, M.L. (2009). Congenital Rubella Syndrome: Progress and Future Challenges. *Expert Review Vaccines*. 811-7.

Mosquera, M. M., Ory F., Moreno, M. and Echevarria J. E. (2002). Simultaneous Detection of Measles Virus, Rubella Virus, and Parvovirus B19 by Using Multiplex PCR. *Journal of clinical microbiology*. 40(1): 111–116.

Muhammad, D.A.,Shittu, O., Sadauki, H., Olayinka, A.,Kolawole, B. and Adejo, D. (2010). Prevalence of Rubella IgG Antibodies Amongst Pregnant Women in Zaria, Nigeria. *International Health,* 2, 156-159

National Center for Immunization and Respiratory Diseases.(2012). Rubella. In: Epidemiology and Prevention of Vaccine- Preventable Diseaeses. In: *The Pink Book*: Course Textbook. 12th ed. [Online] Available at: www.cdc.gov/vaccines/pubs/pinkbook/rubella.html Accessed 2 January 2012

National Health Services Choices. (2012). Rubella. National Health Services Choices.[Online]Available at: http://www.nhs.uk.Conditions /Rubella/Pages/Introduction.aspxAccessed 6 April 2014.

National Health Service. (2013). Rubella. [Online] Available at: www.nhs.uk/conditions/Rubella/pages/introduction.aspx Accessed 3 March 2015

NewYork Times. (2015).[Online]Available at:http://www.nytimes.com/2015/04/30/health/rubella-has-been-eliminated-from-the-americas-health-officials-say.html?_r=0 Accessed 30 October 2015.

Obijimi, T. O., Ajetomobi, A. B., Sule, W. F. and Oluwayelu, D. O. (2013). Prevalence of Rubella Virus-Specific Immunoglobulin-G and Immunoglobulin-M in Pregnant Women Attending Two Tertiary Hospitals in Southwestern Nigeria. *African Journal of Clinical and ExperimentalMicrobiology*,14: 3.

Oker-Blom, C., Ulmanen, I., Kääriäinen, L. and Pettersson, R. F. (1984). Rubella virus 40S genome RNA specifies a 24S subgenomic mRNA that encodes for a precursor to structural proteins. *Journal of Virology*. 49; 403–408.

Olajide O.M, Aminu M and Randawa A. (2012) Sero-Prevalence of Rubella-Specific IgM and IgG Antibodies in Pregnant Women Attending Ahmadu Bello University Teaching Hospital, Zaria.*Society for Occupational Safety and Environmental Health*.

Onakewhor, J. and Chiwuzie, J. (2011). Sero-Prevalence Survey of Rubella Infection in NigeriaPregnancy at the University of Benin Teaching Hospital, Benin City,.*Nigerian Journal of Clinical Practice.* 14(2). 140-145

Onyenekwe, C.C., Kehinde, T.A., Ofor, U.S. and Arinola, O.G. (2000). Prevalence of Rubella Antibody in Women of Child bearing age in Lagos Nigeria.*West African Journal of Medicine,*19 (1): 23-26.

Otaigbe, B.E., Brown, T., Esu, R. (2006). Confirmed Congenital Rubella Syndrome -A case report. *Nigeria Journal of Medicine*, 15(4):448-450.

Pan American Health Organization.(2007). "EPI Newsletter Volume XX, Number 4". *Retrieved* 2007-07-03.

Parkman, P. D. (1999). Making Vaccination Policy. The Experience with Rubella. *Clinical Infectious Diseases,*28: 5140–5146.

Primary Health Care Agency Kaduna State(2012). [Online] Available at: http://mohd.kd.gov.ng/primary-health-care-agencyAccessed 20 Feb 2014.

Pennap, G., Amauche, G., Ajoge, H., gabadi, s Agwale, S. and Forbi, J (2009).Serologic Survey of Speciric Rubella Virus IgM in the Sera of Pregnant Women in Markudi, Benue State, Nigeria.*African Journal of Reproductive Health.* 13(2):69-73.

Pertruzziello, R., Orsi, N., Maccia, S., Frey, T.K. and Mastromarino, P. (1996). Pathway of Rubella Virus Infections Entry into Vero Cells, *Journal of General Virology*, 77:303 – 308.

Pattison, J.R., (2000). *Principles and Practice of Clinical Virology*. 4th ed. England: John Wiley and Sons Ltd. Ch.12.

Plotkin, S.A. (2001). Rubella Eradication. *Vaccine,* 19 (25-26): 3311–3319.

Pugachev, K.V. and Frey, T. K. (1998). Rubella Virus Induces Apoptosis in Culture Cells. *Virology*. 250: 359–370.

Qiu, Z., Dawei, O. U., He, W. U., Tom, C. Honman, E. and Shirley G. (1994). Containing Rubella Virus Structural Protein. [Online] Available at:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC236923/pdf/jviro100015-0644.pdf Accessed 2 May 2013.

Rebertson, S.E., Cutts, F.T, Samuel, R, and Diaz –Ortega, J. (2003a). Control of Rubella and Congenital Rubella Syndrome (CRS) in Developing Countries, part 2: Vaccination Against Rubella. *WHO Bulletin* Vol 75.

Rebertson, S.E., Featherstone, D.A., Gacic-Dobo, M. and Hersh B.S. (2003b).Rubella and congenital rubella syndrome.*African Journal of Public Health 2003*; (5)306-15.

Reed, J. C. (1998) Bcl – 2 Family proteins, *Oncogene* 17: 3225 – 3236.

Reef, S. E., Frey, T. k., Theall, k., Abernathy, E., Burnett, C. L. and Wharton, M. (2002). The changing Epidemiologyof Rubella in The 1900s on The Verse of Elimination and New Challenges. For Controland Prevention. *Journal of America Medical Association*, 287 (4): 464–72.

Reef, S.E., Plotkin, S. and Cordero, J.F. (2000a). Preparing for Congenital Rubella Syndrome Elimination: Summary of the Workshop on Congenital Rubella Syndrome Elimination in the United States. *Clinical Infectious Diseases*, 31:85-95

Reef, S.E. and Cochi S.L. (2000b). The Evidence for the Elimination of Rubella and Congenital Rubella Syndrome in the United States: A Public Health Achievement: Clinical Infectious disease. (43)123-125.

Sallam T,A., Raja'a, Y,A., Benbrake, M.S., Alshaibani, K,S. and Al-Habani, A,A. (2003). Prevalence of Rubella Antibodies among School Girls in Sana'a, Republic of Yemen. *Eastern Mediterranean Health Journal,* 12; 1–4.

Schwartzman, R.A. and Cidlowski, J.A.(1993). Apoptosis: the biochemistry and molecular biology of programmed cell death, *Endocrinology Review,* 14:133-151.

Singh, I. and Helenius, A. (1992). Role of ribosomes in Semliki Forest virus nucleocapsid uncoating. *Journal of Virology,* 66; 7049–7058.

Tahita, M.C., Judith H. M., Zekiba Tarnagda, Da Ernest, Emilie, C., Jacques, K. R., Claude M. P. and Jean, O. B. (2013). *Boston Medical Center Infectious Diseases*.13:164

Theodoro, J.G. and Branton, P. E. (1997). Regulation of Apoptosis by Viral Gene Products. *Journal of Virology*, 71; 1739–1746.

Tetsuya M., Daiji E., Michiko O., Kazuya S., Hiroyuki S., Minetaro A., Shuetsu F., Masayuki S., Kouji S., Chang K., Mikako I., reiko N., Tomohiko T., Koji I., Tetsuro S., Ichiro K., Shigeru M., and Hidekazu N. (2007). Rapid Genome Sequencing of RNAViruses.*Emerging Infectious Diseases* 13: 322-324

Vijayalakshmi, P., Anuradha, R., Prakash, K., Narendran, K. and Ravindran, M. (2004). Rubella Sero-surveys at three Aravid Eye Hospitals in Tamil Nadu, India. *Bull World Health Organization*. 82; 259–264.

Wellington, N.Z. (2006). Ministry of Health. Immunisation Handbook 2006. Chapter 11 - Rubella Retrieved 2007-07-03. ISBN 0-478-29926-5.Wolinsky, J.S. and Knipe, D.M. (1996). Rubella in Fields '*Virology edition by Fields, Philadelphia, Lippen Cott-Raven*. pp. 899–929

Willey, M.J., Sherwood, M. L. and Woolverton, J.c. (2011). Human Diseases caused by Viruses and Prisons. In: Willey, M. J., Sherwood, M.L. and Woolverton, J. C. Eds. (2011). *Prescott's Microbiology the. New York: Mc Graw-Hill.* Pp 905-906

Wolinsky, J.S. and Knipe, D.M. (1996). Rubella in Fields 'Virology ed by Fields, Philadelphia, Lippen Cott-Raven. pp. 899–929.

World Health Organization. (1999). World Health Organization report on Infectious Disease: Removing Obstacles to Healthy Development. WHO Report on Infectious Diseases?

World Health Organization. (2000). Rubella vaccines. WHO position paper; *Weekly Epidemiological Record* 75:161-975:161-9.

World Health Organization. (2015a). WHO Rubella Fact sheet N°367 **www.who.int**/mediacentre/**factsheets**/fs367AccessedDecember 2015.

World Health Organization. (2015b). WHORubella Repoted cases[online] Available online at:http://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencerubella.html

Yang, D., Hwang, D., Qiu, Z. and Gillam, S. (1998). Effects in the mutation in the rubella virus E1 glycoproteins on E1-E2 interaction and membrane fusion activity. *Journal of Virology*. 72:8747–8755.

Yao, J., Yang, D., Chong, P., Hwang, D., Liang, Y. and Gillam, S. (1998). Proteolytic processing of rubella virus nonstructural proteins. *Virology,*246;74–82.

Yavuz U., Alaaddin B., Alper A., Cevat C.(2008) Prevalence of rubella and cytomegalovirus antibodies among pregnant women in northern Turkey. *New Microbiology* 31, 451-455.

Yumei, Z., Hiroshi, U., Teryl, k., Frey T.K. (2007). Animal RNA viruses.Genomic analysis of diverse rubella virus genotypes.*Journal of General Virology,* 88:932-941.

ZeusScientific.(2009). Rubella IgM ELISA test system: Available online www.Zeusscientific.com/fileadmin/media/pdfs/inserts/elisa/infectious/R2305E.pdf 2009.

Zhan, J., Chen, H., Gu, S., Tian, X., Liu, J., Chen, Y., Fu, H., Yang, X., Zheng, H., Liu, L., Zheng, L., Gao, H., He, J., Sun, L., Xu, W. (2012). Emergence and Continuous Evolution of Genotype 1E Rubella Viruses in China. *Journal of Clinical Microbiology*.50(2):353-63.

Zhonghua, M., Guo, W., Sheng, Wu Ji, M., Yi, X., Za Z. (1996). Laboratory Evaluation of Automated Enzyme Linked Fluorescent Assay for Detecting Serum Specific IgG Antibodies Against Rubella Virus. [Online]Available at:http://www.ncbi.nlm.nih.gov/pubmed/10592786-56 Accessed 4 July 2013

Zhou, Y., Ushijima, H., Frey, T. K. (2007). Genomic Analysis of Diverse Rubella Virus Genotypes. *The Journal of General Virology*. 88(3):932–941. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17325367

Zhu, Z., W,Xu., E. S., Abernathy, M., Chen, Q., Zheng, T., Wang, Z., Zhang, C., Li, C., Wang, W., He, S. Zhou, Y. and Icenogle, J. (2007). Comparison of four methods for confirming rubella virus infection using throat swabs. *Journal Clinical Microbiology.* 45:2847-2852.

APPENDIX I

APPENDIX II: Consent Form

SERO-PREVALENCE ANDMOLECULAR DETECTION OFRUBELLA VIRUSES AMONG PREGNANT WOMEN IN KADUNA STATES. 2013

Introduction

Good day ma, my name is AISHATU BINTU GUBIO I am a post-graduate student of Ahmadu Bello University, Zaria. I am conducting a study sero-prevalence and molecular detection of rubella viruses among pregnant women in Kaduna States. This study would help to give information on the current prevalence of rubella IgG and IgM antibodies in pregnant women. This information could be used to advise the government of Nigeria to include rubella vaccination in routine immunization to reduce rubella infection.

I seek your permission to ask you few questions about your knowledge, attitude and practices regarding your health. The interview will last for about 20 minutes. I assure you that all your responses will be treated with utmost confidentiality. Your participation in the study is voluntary and you may decide not to participate if you wish. However, your participation in the study will be highly appreciated.

Do you consent to participate in the study? Yes ☐ No ☐

Respondent's signature or right thumb-print if consented…….

If consented, proceed with the interview; if not, do not proceed with the interview

Interviewer's name…….

Date (dd/mm/yyyy)……Questionnaire number……..

APPENDIX III

SERO-PREVALENCE ANDMOLECULAR DETECTIONOF RUBELLA VURUSES AMONG PREGNANT WOMEN IN KADUNA STATES(Questionnaire) 2013.

DEMOGRAPHIC INFORMATION

Sample No…… LGA/PHC……..

Tel. Phone No.....Address.....

Age.....Age in last Pregnancy.....

Age in present pregnancy.....

No. of previous pregnancy..... No. Of previous abortion if any.....

Degree of schooling.....Occupation.....

Primary language spoken.....Country of origin.....

State of origin..... Tribe.....

CLINICAL DETAILS

Past history of skin rash in any of the pregnancies i) No ii) Yes

Past delivery of congenital malformed baby i) No ii) Yes

Gestational age of pregnancy i) 1st trimester ii) 2nd trimester iii) 3rd trimester

Past history of still births including abortion i) No ii) Yes

Any history of skin rash and fever in this pregnancy i) No ii) Yes

Do you have any Medical condition if yes specify..... i) No

HIV Status.....

VACCINE STATUS

Have you heard of Rubella vaccine .....

Have you been vaccinated against Rubella.....

No. of doses of rubella vaccination.....

If not vaccinated, reason for non-vaccination.....

RISK FACTORS

Any contact with a probable or confirmed case of rubella/Measles.....

APPENDIX IV

Sero-Prevalenceand Molecular Detection of Rubella Virus Antibody Among Pregnant Women in Zaria, Nigeria –Hausa Consent Form

Lambar Fom ɗin tambaya

(QuestionnaireNo).....

Assalamu alaykum.

Suna na Aishatu Bintu Gubio. Ni daliba ce a jami'ar Ahmadu Bello inda nake nazarin bincike don karatun digiri na biyu (M.Sc). Ina binciken tsananin ƙwayar cutar rubella a tsakanin mata masu juna biyu a Zaria dake Arewacin Nijeriya. Wannan nazarin binciken zai bayyana ƙiddiddigan sinadaran kariyar garkuwar jiki masu jinsin IgG and IgM (Ig G and IgM antibodies). Wannan nazarin binciken zai bayyana dangantar dake tsakanin kamuwa da ƙwayar cutar rubella da haihuwan jira-jirai masu raunin kama ko halitta. Bayanan da aka samu daga binciken zasu zama turbar shawartar gwamnatin tarayyar Nijeriya ga shigar da alluran rigakafin rubella a tsarin alluran rigakafin da ake yi.

Ina mai neman izininki don tambayarki bayanan da suka shafi zurfin sani, ra'ayi, da ɗaukan matakai da suka shafi ƙwayar cutar rubella da allurar rigakafin rubella.

TuntuƁar da zan yi maki na tambayyoyi zai ɗauki minti 20. Ina tabbattar maki zan sirranta amsoshin da kika bani. Kina da 'yancin fita daga wannan nazrin binciken bada ya saƁa da ingancin kulan da ya kamata ki samu.

Ina mai godiyan amincewa da shiga wannan binciken.

Kin amince da shiga wannan binciken?

Ki zaƁi akwatin wanda ya dace ra'ayin ki

Na'am / Ee na amince

A'a ban amince

Sa hannun mace mai juna biyu data amince da shiga nazarin bincike

.....

Sunan mai mai juna biyu data amince da shiga nazarin bincike

.....

Sunan Shaida.....

Sunan mai tambaya .....

Ranar wata.....

APPENDIX V

Budget for Research

NAME OF REAGENT	QTY	COST
Laboratory assistant	9	$100 X 9 =$900
Questionnaire	1000	$70
2ml syringe	1000pcs	$70
Cotton Wool	2pck	$20
Spirit	1btl	$20
Sterile Non-anticoagulant	1000	$50
Pipette	1pck	$100
Transportation cold box	3pcs	$50
Gloves	3pcks	$70
Tunicate	4pcs	$20
Dust Bins	4pcs	$50
Bio Harzard Bags	50	$20
Disposable Laboratory Coats	20pcs	$200
Pipette Tips	1000ul	$100
Face Mask	200	$50
Ice pcks	30	$20
Enzygnost Kit	24pcks	$3000
Total		$4810

APPENDIX VI

Content of the Diagnostic Automation ELISA IgG kit

I. Microtitre plate containing absorbed purified rubella antigen coated 96 U-shaped wells

II. Enzyme conjugate (alkaline phosphatase – labeled rabbit antibody to human gamma-globulin). Red colour solution. 12ml

III. Negative Calibrator (negative control serum containing zero IU/ml rubella IgG antibody) colorless. Natural cap. 150ml.

IV. Cut-off calibration (calibratrated positive serum containing 15iu/ml rubella IgG antibody) colorless. Yellow cap. 150ml.

V. Position calibrator (positive serum containing 30iu/ml rubella IgG antibody) colorless. Red cap.150ml.

VI. Positive calibrator (positive serum containing 10iu/ml rubella IgG antibody) green cap. 150ml

VII. Negative control. Colorless. Blue cap. 150ml & Positive control. Colorless. Brown cap. 150ml

VIII. Washing concentrate (Saline- Tween 20-BSA (Bovine serum albumin) white color solution. 100ml.

IX. Sample diluents (Saline – Tween20 – BSA). Blue color solution 22ml.

X. Chromogenic substrate & Stop solution (2N HCL) 12ml

XI. Sera from pregnant women attending antenatal clinics in Zaria

XII. Vacutainer tubes (BD vacutainer systems PL6 &BP UK) & Bijou bottles

XIII. Multi dispensermicropipette (Eppendorf Product. Germany)

XIV. Multiwell ELISA reader II (Sigma diagnostic EIA multiwall reader II Missouri U.S.A).

APPENDIX VII

ELISA RESULTS IGM: PHC-Babandodo
Printed at 13.03 on 08.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 08.04.04
TEST FILTER: 450nm, PLATE: 001, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.003	0.050	0.023	0.014	0.034	0.088	0.032	0.025	0.062	0.022	0.075	0.091
B	0.031	0.047	0.063	0.026	0.039	0.051	0.816	0.066	0.059	0.094	0.060	0.058
C	0.035	0.745	0.032	0.023	0.020	0.049	0.034	0.032	0.024	0.032	0.033	0.082
D	0.027	0.065	0.013	0.099	0.092	0.017	0.080	0.091	0.055	0.015	0.036	0.019
E	0.780	0.096	0.045	0.085	1.196	0.090	0.067	0.034	0.045	0.056	0.034	0.026
F	0.791	0.082	0.026	0.034	0.079	0.042	0.045	0.047	0.710	0.064	0.054	0.082
G	0.017	0.026	0.011	0.044	0.019	0.047	0.049	0.038	0.045	0.050	0.031	0.040
H	0.002	0.012	0.038	0.029	0.054	0.043	0.041	0.021	0.072	0.075	0.021	0.063

APPENDIX VIII

ELISA RESULTS IGM: PHC-Major Abdullahi Memorial Hospital

Printed at 13.17 on 08.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 08.04.04
TEST FILTER: 450nm, PLATE: 002, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.005	0.020	0.050	0.041	0.041	0.060	0.073	0.038	0.096	0.069	0.088	0.014
B	0.021	0.090	0.070	0.018	0.037	0.018	0.085	0.047	0.051	0.978	0.045	0.099
C	0.019	0.012	0.024	0.022	0.037	0.930	0.032	1.023	0.066	0.086	0.027	0.040
D	0.030	0.010	1.263	0.083	0.070	0.068	0.026	0.059	0.027	0.074	0.015	0.042
E	1.380	0.084	0.049	0.031	0.089	0.084	0.078	0.034	0.010	0.096	0.019	0.038
F	1.402	0.052	0.014	0.097	0.056	0.085	1.890	0.037	0.017	0.053	0.013	0.089
G	0.910	0.077	0.094	0.084	0.081	0.084	0.047	0.078	0.065	0.063	0.065	0.012
H	0.031	0.012	1.109	0.095	0.034	0.067	0.029	0.002	0.060	0.098	0.043	0.081

APPENDIX IX

ELISA RESULTS IGM: PHC-Tudun Wada

Printed at 13.32 on 08.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 08.04.04
TEST FILTER: 450nm, PLATE: 003, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.003	0.083	0.059	0.064	0.073	0.059	0.078	0.045	0.044	0.058	0.048	0.049
B	0.072	0.056	0.010	0.061	0.076	0.18	0.081	0.074	0.058	0.041	0.043	0.041
C	0.060	0.058	0.060	0.057	0.059	0.620	0.079	0.062	0.045	0.037	0.040	0.042
D	0.057	0.077	0.061	0.052	0.057	0.015	0.091	0.044	0.046	0.044	0.045	0.016
E	0.806	0.060	0.066	0.055	0.581	0.077	0.067	0.043	0.045	0.041	0.041	0.021
F	0.790	0.068	0.080	0.056	0.060	0.080	0.075	0.045	0.046	0.038	0.040	0.014
G	0.051	0.064	0.073	0.058	0.017	0.024	0.086	0.051	0.046	0.046	0.037	0.027
H	0.057	0.061	0.067	0.067	0.084	0.006	0.041	0.046	0.042	0.039	0.042	0.982

APPENDIX X

ELISA RESULTS IGM: PHC-Godogodo

Printed at 12.13 on 09.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 09.04.04
TEST FILTER: 450nm, PLATE: 001, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.008	0.060	0.082	0.090	0.099	0.027	0.043	0.031	0.019	0.957	0.015	0.031
B	0.064	0.068	0.081	1.236	0.032	0.031	0.065	0.097	0.062	0.046	0.097	0.029
C	0.012	0.056	0.036	0.096	0.007	0.026	0.036	0.022	0.091	0.033	0.093	0.012
D	0.069	0.099	0.092	0.072	0.010	0.092	1.178	0.096	0.034	0.053	0.025	0.022
E	1.062	0.035	0.082	0.080	0.263	0.045	0.062	0.035	0.045	1.042	0.033	0.096
F	1.001	0.095	0.005	0.061	0.011	0.031	0.051	0.071	0.031	0.022	0.014	0.035
G	0.063	0.047	0.001	0.062	0.061	0.065	0.089	0.021	0.019	0.058	0.028	0.071
H	0.064	0.893	0.015	0.058	0.070	0.058	0.012	0.093	0.025	0.097	0.032	0.062

APPENDIX XI

ELISA RESULTS IGM: PHC-Kafanchan

Printed at 12.38 on 09.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 09.04.04
TEST FILTER: 450nm, PLATE: 002, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.010	0.021	0.092	0.007	0.015	0.052	0.478	0.015	0.099	0.039	0.021	0.097
B	0.044	0.039	0.021	0.068	0.063	0.059	0.082	0.009	0.063	0.054	0.016	0.019
C	0.048	1.551	0.062	0.028	0.064	0.082	0.081	0.096	0.032	0.062	0.070	0.012
D	0.050	0.049	0.046	0.023	0.056	0.005	0.036	0.072	0.040	0.015	0.085	0.022
E	0.909	0.071	0.093	0.099	0.328	0.080	0.092	0.080	0.007	0.033	0.062	0.096
F	0.810	0.030	0.054	0.019	0.064	0.035	0.082	0.061	0.061	0.045	0.015	0.035
G	0.034	0.017	0.013	0.033	0.056	0.044	0.001	0.058	0.011	0.065	0.089	0.021
H	0.014	0.068	0.010	0.011	0.056	0.047	0.041	0.029	0.066	0.036	0.012	0.045

APPENDIX XII

ELISA RESULTS IGM: PHC-Kofan Sarki

Printed at 13.18 on 09.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 09.04.04
TEST FILTER: 450nm, PLATE: 003, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.002	0.025	1.032	0.031	0.008	0.081	0.019	0.021	0.015	0.055	0.015	0.088
B	0.035	0.018	0.097	0.040	0.045	1.071	0.086	0.090	0.031	0.048	0.048	0.099
C	0.040	0.024	0.150	0.002	0.024	0.094	0.017	0.860	0.024	0.002	0.050	0.057
D	0.025	0.033	0.017	0.043	0.095	0.079	0.080	0.096	0.079	0.089	0.080	0.096
E	1.078	0.021	0.068	0.033	0.039	0.043	0.013	0.047	0.094	0.021	0.043	0.087
F	1.650	0.030	0.079	0.045	0.005	0.044	0.086	0.004	0.023	0.091	0.659	0.065
G	0.019	0.027	0.040	0.974	0.033	0.022	0.015	0.011	0.084	0.104	0.038	0.044
H	0.007	0.055	0.061	0.046	0.073	0.069	0.088	0.038	0.040	0.021	0.043	0.024

APPENDIX XIII

ELISA RESULTS IGM: PHC-Badarawa

Printed at 12.45 on 10.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 10.04.04
TEST FILTER: 450nm, PLATE: 001, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.041	0.045	0.150	0.027	0.097	0.020	0.027	0.066	0.036	0.089	0.099	0.025
B	0.023	0.083	0.017	0.010	0.017	0.078	0.071	0.025	0.028	0.008	0.051	0.056
C	0.034	0.031	0.044	0.036	0.079	0.060	0.083	1.659	0.050	0.050	0.032	0.048
D	0.028	0.088	0.073	0.066	0.086	0.039	0.027	0.010	0.075	0.028	0.045	0.086
E	0.999	0.073	1.856	0.038	0.019	0.053	1.619	0.150	0.080	0.021	0.060	0.027
F	0.899	0.019	0.049	0.028	0.066	0.005	0.017	0.015	0.025	0.017	0.065	0.018
G	0.082	0.030	0.054	0.007	0.036	0.083	0.015	1.814	0.019	0.029	0.021	0.042
H	0.058	0.024	0.086	0.022	0.018	0.044	0.021	0.036	0.051	0.021	0.012	0.022

APPENDIX XIV

ELISA RESULTS IGM: PHC-Unguwa Shanu

Printed at 13.03 on 10.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 10.04.04
TEST FILTER: 450nm, PLATE: 002, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.063	0.037	0.095	0.071	0.071	0.022	0.056	0.046	0.033	0.023	0.057
B	0.040	0.062	0.013	0.025	0.019	0.013	0.035	0.010	1.638	0.046	0.055	0.076
C	0.043	0.058	0.019	0.058	0.025	0.095	0.055	0.023	0.047	0.063	0.051	0.065
D	0.033	0.023	0.025	0.048	0.032	0.071	0.075	0.031	0.079	0.032	0.075	0.791
E	0.893	0.150	0.032	0.062	0.033	0.047	0.093	0.037	0.027	0.023	0.093	0.056
F	0.991	0.018	0.080	0.063	0.010	0.046	0.058	0.029	0.047	0.058	0.079	0.076
G	0.025	0.027	0.033	0.070	0.031	0.033	0.093	0.023	0.077	0.040	0.084	0.084
H	0.048	0.079	0.037	0.032	0.037	0.093	0.084	0.093	1.728	0.022	0.093	0.015

APPENDIX XV

ELISA RESULTS IGM: PHC-Malali

Printed at 13.33 on 10.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 10.04.04
TEST FILTER: 450nm, PLATE: 003, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.039	0.017	0.016	1.493	0.028	0.021	0.079	0.039	0.054	0.094	0.021
B	0.050	0.074	0.075	0.027	0.061	0.028	0.015	0.095	0.094	0.068	0.090	0.080
C	0.019	0.040	0.097	0.042	0.091	0.041	0.078	0.061	0.038	0.013	0.041	0.018
D	0.031	0.007	0.020	0.028	0.022	0.023	0.063	1.174	0.046	0.050	0.010	0.013
E	0.784	0.045	1.878	0.047	0.093	0.011	0.012	0.150	0.052	0.094	0.084	0.054
F	0.792	0.014	0.003	0.067	0.032	0.051	0.062	0.042	0.076	0.038	0.078	0.047
G	0.084	0.022	0.091	0.096	0.060	0.001	0.025	0.061	0.083	0.041	0.068	0.094
H	0.039	0.056	0.089	0.019	1.938	0.088	0.047	0.083	0.042	0.084	0.014	0.043

APPENDIX XVI

ELISA RESULTS IGM: PHC- Babandodo, MIBA, T/ Wada, Godogodo, Kafanchan, K/ Sarki,Badarawa, U/ Shanu, Malali (last 10 samples respectively)

Printed at 14.20 on 10.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 10.04.04
TEST FILTER: 450nm, PLATE: 004, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.031	0.071	0.061	0.011	0.012	0.061	0.072	0.036	0.021	0.022	0.048
B	0.050	0.046	0.037	0.051	0.014	0.037	0.050	0.049	0.023	0.051	0.060	0.065
C	0.050	0.025	0.076	0.011	0.052	0.099	0.037	0.038	0.066	0.064	0.036	0.045
D	0.080	0.047	0.013	0.020	0.029	0.082	0.037	0.027	0.052	0.007	0.051	0.055
E	0.809	0.025	0.007	0.052	0.084	0.022	0.016	0.016	0.063	0.035	0.060	0.029
F	0.798	0.075	0.023	0.092	0.036	0.034	0.010	0.053	0.011	0.072	0.030	0.036
G	0.905	0.078	0.091	0.039	0.069	0.046	0.039	0.036	0.051	0.050	0.014	0.078
H	0.037	0.031	0.017	0.089	0.048	0.021	0.037	0.087	0.039	0.013	0.047	0.006

APPENDIX XVII

ELISA RESULTS IGG: PHC-Babandodo

Printed at 11.33 on 11.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 11.04.04
TEST FILTER: 450nm, PLATE: 001, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	2.203	0.026	0.099	0.378	1.896	1.688	1.968	2.658	0.024	2.458	1.653
B	0.015	1.704	0.041	1.831	1.377	1.568	0.684	2.515	1.601	1.865	2.207	1.433
C	0.067	2.251	0.021	1.319	0.017	1.818	0.850	1.766	0.016	0.740	1.240	0.028
D	0.013	0.491	0.041	1.975	1.707	2.348	1.844	1.666	2.658	0.961	0.091	1.408
E	1.041	0.145	0.023	1.847	0.005	0.046	1.672	2.270	1.601	1.538	1.627	0.009
F	0.981	1.943	0.039	1.953	0.035	2.603	0.735	1.177	2.697	1.636	1.150	1.042
G	2.474	2.041	0.014	0.059	0.021	2.180	0.782	0.010	0.058	0.984.	2.192	2.038
H	2.031	1.181	2.222	1.415	0.019	0.032	2.026	0.043	2.865	2.884	2.133	1.897

APPENDIX XVIII

ELISA RESULTS IGG: PHC-Major Abdullahi Memorial Hospital

Printed at 13.23 on 11.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 11.04.04
TEST FILTER: 450nm, PLATE: 002, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	1.012	0.013	1.060	0.044	1.505	0.051	0.075	0.080	2.263	0.032	1.998
B	0.062	1.818	1.047	1.087	2.047	2.161	2.059	1.490	1.888	0.008	0.078	1.398
C	0.011	0.021	0.090	1.707	2.066	1.450	0.041	0.789	2.432	0.084	0.048	0.073
D	0.001	1.609	0.510	1.550	0.012	0.018	0.029	0.027	1.457	1.193	2.447	2.051
E	1.004	1.389	1.045	1.047	0.098	0.092	1.076	0.067	0.017	1.312	0.012	0.099
F	1.491	2.189	0.045	0.023	1.590	0.341	2.968	0.415	2.212	0.073	1.202	0.968
G	2.038	1.053	1.302	1.520	1.802	1.556	0.092	1.115	0.056	0.033	0.047	0.020
H	1.897	0.032	1.062	0.045	2.057	1.940	1.051	2.143	0.020	0.031	1.100	1.672

APPENDIX XIX

ELISA RESULTS IGG: PHC-T/Wada

Printed at 12.43 on 14.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 12.04.04
TEST FILTER: 450nm, PLATE: 001, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	1.224	0.055	1.888	0.019	1.676	1.415	1.672	1.383	2.408	1.123	1.211
B	0.007	1.183	1.924	1.519	1.390	1.451	1.259	1.638	1.914	0.064	1.321	1.622
C	0.040	1.226	0.013	0.054	1.427	0.013	1.187	1.604	1.586	1.008	1.558	0.064
D	0.098	2.211	0.016	0.021	1.475	0.041	1.248	0.022	0.061	1.203	0.032	0.013
E	1.540	0.045	1.790	1.493	0.045	1.099	1.219	1.331	1.082	1.004	0.085	1.059
F	1.342	1.200	2.195	0.071	2.131	1.492	1.567	0.028	0.013	0.061	1.451	0.065
G	0.079	0.072	1.546	0.012	0.032	0.016	1.964	1.155	1.269	0.023	1.720	1.242
H	0.008	1.635	1.321	1.177	1.805	0.019	2.930	0.035	1.347	1.218	1.224	1.558

APPENDIX XX

ELISA RESULTS IGG: PHC-Godogodo

Printed at 13.00 on 12.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 12.04.04
TEST FILTER: 450nm, PLATE: 002, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	1.973	1.103	1.508	1.209	0.021	0.612	0.034	0.051	0.056	0.039	0.032
B	0.006	1.083	1.219	1.750	1.218	0.042	1.256	1.499	1.232	0.019	0.041	0.083
C	0.021	0.067	0.039	1.680	1.344	1.051	1.489	0.037	0.041	0.078	0.027	0.024
D	0.050	0.014	1.289	0.051	0.081	1.215	0.092	1.051	1.260	0.021	0.051	0.056
E	0.890	1.207	1.215	1.928	1.437	1.654	0.072	0.064	0.021	1.324	0.017	0.086
F	1.045	1.178	1.363	1.611	1.405	1.232	0.016	1.215	2.062	0.035	0.083	0.035
G	1.453	1.066	0.018	0.034	1.351	1.260	1.258	0.041	1.214	1.221	0.016	1.489
H	1.244	1.250	1.289	1.317	1.499	0.042	1.486	1.654	1.124	1.256	0.057	0.020

APPENDIX XXI

ELISA RESULTS IGG: PHC-Kafanchan

Printed at 15.33 on 14.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 14.04.04
TEST FILTER: 450nm, PLATE: 003, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.021	0.0	1.393	1.930	2.579	1.636	1.907	1.510	1.018	1.831	1.997	1.977
B	0.056	1.222	1.740	1.203	1.271	0.064	2.932	1.388	1.180	1.011	1.856	1.079
C	0.035	2.355	1.452	0.018	2.142	2.027	1.600	0.022	1.133	0.023	1.183	1.018
D	0.052	1.550	2.142	0.023	0.061	1.284	0.001	0.011	0.031	1.115	0.010	0.041
E	1.301	1.755	0.049	1.579	0.093	0.048	1.284	1.215	0.014	1.524	1.444	0.029
F	1.203	0.029	0.051	1.765	1.563	1.047	1.428	1.322	0.944	0.042	1.173	0.819
G	0.023	1.203	1.560	1.845	1.759	1.367	0.022	1.977	1.524.	1.895	1.132	0.013
H	1.400	1.891	1.755	1.891	1.203	1.679	1.413	1.079	0.028	1.872	0.807	1.215

APPENDIX XXII

ELISA RESULTS IGG: PHC-K/Sarki

Printed at 10.15 on 15.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 15.04.04
TEST FILTER: 450nm, PLATE: 001, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	1.045	0.071	0.075	1.039	1.039	0.071	1.003	0.0	1.407	1.217	1.611
B	0.091	0.042	0.964	1.240	1.094	0.576	0.944	1.363	1.378	1.224	0.051	1.476
C	0.003	0.048	0.053	1.044	1.041	0.752	1.066	1.312	1.231	1.232	0.024	1.456
D	0.050	0.380	1.066	0.842	0.022	1.136	1.021	0.071	1.371	1.397	1.529	0.030
E	1.706	1.601	0.038	1.746	0.031	0.938	0.083	0.056	0.033	0.041	1.360	0.041
F	1.094	1.620	1.970	0.049	1.050	0.020	0.092	0.016	0.047	0.030	0.842	1.363
G	1.351	0.037	0.031	1.110	0.019	0.063	0.743	1.462	1.230	0.051	1.220	1.078
H	1.051	0.081	0.019	0.843	0.946	1.014	0.740	1.276	1.516	1.511	1.369	1.127

APPENDIX XXIII

ELISA RESULTS IGG: PHC-Badarawa

Printed at 12.13 on 15.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 15.04.04
TEST FILTER: 450nm, PLATE: 002, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.012	0.018	0.015	2.041	1.258	2.473	1.558	2.325	1.508	1.340	1.312
B	0.001	0.019	2.525	2.251	1.181	0.056	1.138	0.020	2.325	0.075	1.540	1.211
C	0.002	2.031	1.776	0.021	0.072	1.254	1.211	1.809	2.151	0.753	1.312	1.806
D	0.080	2.203	0.050	0.776	2.222	1.662	0.036	1.451	0.080	1.604	0.068	0.012
E	1.340	1.906	2.128	0.491	1.831	0.040	0.075	0.720	0.041	0.036	1.211	1.602
F	1.056	1.128	1.501	1.145	1.319	1.321	1.622	0.019	1.569	1.331	1.383	1.384
G	1.836	2.102	1.704	0.048	0.031	1.910	1.059	2.224	1.648	1.155	1.914	0.031
H	2.474	1.843	0.003	1.943	1.975	2.349	1.242	1.949	1.383	1.363	0.586	2.408

APPENDIX XXIV

ELISA RESULTS IGG: PHC-Unguwan Shanu

Printed at 14.21 on 15.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 15.04.04
TEST FILTER: 450nm, PLATE: 003, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.002	1.060	1.595	1.590	1.609	1.258	1.611	1.454	1.270	1.311	0.0	1.336
B	0.004	1.064	0.043	1.082	1.396	1.299	1.270	1.231	0.030	1.266	1.433	1.321
C	0.021	2.168	0.072	1.205	0.036	1.632	0.067	1.261	0.067	1.392	1.365	0.041
D	0.003	0.031	1.447	1.305	1.472	0.048	0.030	0.093	1.611	0.091	1.581	0.478
E	1.450	0.016	1.782	0.018	1.080	0.092	1.311	0.012	1.107	0.013	0.023	1.316
F	1.041	1.656	0.019	0.003	1.261	0.015	1.266	1.162	1.407	1.454	0.014	1.170
G	1.063	1.199	1.281	1.301	0.043	1.407	1.392	1.258	0.014	1.231	1.433	0.023
H	1.064	1.235	1.336	1.415	1.162	1.107	0.016	1.632	0.924	0.013	1.365	1.385

APPENDIX XXV

ELISA RESULTS IGG: PHC-Malali

Printed at 11.06 on 16.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 16.04.04
TEST FILTER: 450nm, PLATE: 001, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.060	0.023	0.012	1.364	1.076	0.638	1.581	1.485	1.569	1.569	1.543
B	0.002	0.037	1.364	0.590	1.724	1.799	1.392	1.392	1.364	0.061	0.035	0.047
C	0.021	1.098	1.021	1.037	1.384	0.062	0.013	1.368	0.028	1.320	0.048	1.701
D	0.011	1.272	1.064	1.483	0.015	1.343	0.032	0.063	1.425	1.358	1.320	1.216
E	1.024	0.071	1.056	1.085	0.021	1.798	1.798	0.091	1.571	0.053	0.092	0.092
F	1.036	1.037	1.884	1.032	1.519	1.518	1.518	1.389	1.544	0.017	0.038	1.226
G	1.320	1.993	0.028	0.017	0.053	1.800	1.343	0.044	0.081	0.023	0.014	1.303
H	1.119	1.099	0.031	1.041	1.423	0.042	0.014	1.521	1.552	1.534	0.028	0.032

APPENDIX XXVI

ELISA RESULTS IGG: PHC-Babandodo, MIBA, T/ Wada, Godogodo, Kafanchan, K/ Sarki,Badarawa, U/ Shanu, Malali (last 10 samples respectively)

Printed at 13.05 on 16.04.04
TEST NAME: MULTISKAN EX PRIMARY EIA V.2.3
TEST NO. : A w/l MODE: DUAL DATE: 16.04.04
TEST FILTER: 450nm, PLATE: 002, C.O = 0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	1.906	0.032	1.408	2.131	0.046	1.293	1.310	0.016	0.018	0.018	0.050
B	0.004	1.128	1.176	1.038	0.093	0.082	1.465	1.237	1.580	0.014	0.026	0.021
C	0.012	2.102	1.193	1.521	1.032	0.013	1.479	1.293	1.453	0.053	0.052	0.015
D	0.038	1.843	1.099	0.028	2.091	0.072	0.033	0.029	0.053	0.019	0.097	0.053
E	1.043	2.525	1.122	0.015	1.327	0.091	1.477	1.479	0.030	0.020	0.049	0.040
F	1.076	1.776	1.233	2.143	2.133	0.012	1.464	1.271	0.044	0.024	0.023	0.051
G	2.251	2.128	1.334	0.017	1.310	0.067	0.048	1.477	0.034	1.207	0.017	0.082
H	1.704	1.501	0.718	1.442	1.237	1.891	1.241	1.464	0.083	0.070	0.019	0.095

APPENDIX XXVII

Sociodemographic and Risk Factors of Rubella Antibodies among Pregnant Women Attending Ante-Natal Clinics in Kaduna State

APPENDIX XXVIII

Table 10b: Sociodemographic and Risk Factors of Rubella Antibodies among Pregnant Women Attending Ante-Natal Clinics in Kaduna State

APPENDIX XXIX

Rubella virus isolate 6431-ITA97 E1 protein (E1) gene, partial cds

GenBank: AY161375.1

FASTA Graphics PopSet

Go to:

```
LOCUS       AY161375               1443 bp    RNA     linear   VRL 13-MAY-
2003
DEFINITION  Rubella virus isolate 6431-ITA97 E1 protein (E1) gene, partial
cds.
ACCESSION   AY161375
VERSION     AY161375.1  GI:30691388
KEYWORDS    .
SOURCE      Rubella virus
  ORGANISM  Rubella virus
            Viruses; ssRNA positive-strand viruses, no DNA stage;
Togaviridae;
            Rubivirus.
REFERENCE   1  (bases 1 to 1443)
  AUTHORS   Zheng,D.P., Zhu,H., Revello,M.G., Gerna,G. and Frey,T.K.
  TITLE     Phylogenetic analysis of rubella virus isolated during a period
of
            epidemic transmission in Italy, 1991-1997
  JOURNAL   J. Infect. Dis. 187 (10), 1587-1597 (2003)
   PUBMED   12721939
REFERENCE   2  (bases 1 to 1443)
  AUTHORS   Zheng,D.-P., Zhu,H. and Frey,T.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-OCT-2002) Biology, Georgia State University, 24
            Peachtree Center Ave., Atlanta, GA 30303, USA
FEATURES             Location/Qualifiers
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                     /db_xref="GI:30691389"

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YHPTACEVEPAFGHSDAACWGFPTDTVMSVFALASYVQHPHKTVRVKFHTETRTVWQL
```

APPENDIX XXX

Sequence Results of the 320 Length of the plates:

gcacggacaactcgaggtccaggtcccgccc
gaccccggggacctggttgagtacattat
gaattacaccggcaatcagcagtcccggtgg
ggcctcgggagcccgaattgccacggccccg
attgggcctccccggtttgccaacgccattc
ccctgactgctcgcggcttgtggggccacg
ccagagcgcccccggctgcgcctggtcgacg
ccgacgacccccctgctgcgcactgcccctgg
acccggcgaggtgtgggtcacgcctgtcata
ggctctcaggcgcgcaagtgcggactccaca
tacgcgctggac

APPENDIX XXXI

Rubella virus isolate 6431-ITA97 E1 protein (E1) gene, partial cds
Sequence ID: gb|AY161375.1|Length: 1443Number of Matches: 1
Related Information.
Range 1: 537 to 856GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
592 bits(320)	1e-165	320/320(100%)	0/320(0%)	Plus/Plus

```
Query  1    GCACGGACAACTCGAGGTCCAGGTCCCGCCCGACCCCGGGGACCTGGTTGAGTACATTAT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  537  GCACGGACAACTCGAGGTCCAGGTCCCGCCCGACCCCGGGGACCTGGTTGAGTACATTAT  596

Query  61   GAATTACACCGGCAATCAGCAGTCCCGGTGGGGCCTCGGGAGCCCGAATTGCCACGGCCC  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  597  GAATTACACCGGCAATCAGCAGTCCCGGTGGGGCCTCGGGAGCCCGAATTGCCACGGCCC  656

Query  121  CGATTGGGCCTCCCCGGTTTGCCAACGCCATTCCCCTGACTGCTCGCGGCTTGTGGGGGC  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  657  CGATTGGGCCTCCCCGGTTTGCCAACGCCATTCCCCTGACTGCTCGCGGCTTGTGGGGGC  716

Query  181  CACGCCAGAGCGCCCCCGGCTGCGCCTGGTCGACGCCGACGACCCCCTGCTGCGCACTGC  240
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Sbjct  717  CACGCCAGAGCGCCCCCGGCTGCGCCTGGTCGACGCCGACGACCCCCTGCTGCGCACTGC  776

Query  241  CCCTGGACCCGGCGAGGTGTGGGTCACGCCTGTCATAGGCTCTCAGGCGCGCAAGTGCGG  300
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Sbjct  777  CCCTGGACCCGGCGAGGTGTGGGTCACGCCTGTCATAGGCTCTCAGGCGCGCAAGTGCGG  836

Query  301  ACTCCACATACGCGCTGGAC  320
           ||||||||||||||||||||
Sbjct  837  ACTCCACATACGCGCTGGAC  856
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APPENDIX XXXII

APPENDIX XXXIII

APPENDIX XXXIV

APPENDIX XXXV