SEROPREVALENCE OF RUBELLA VIRUS ANTIBODIES AMONG SCHOOL CHILDREN 0-10 YEARS IN JOS, PLATEAU STATE

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE MASTER IN PUBLIC HEALTH, FIELD EPIDEMIOLOGY AND LABORATORY TRAINING PROGRAMME

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DECLARATION

I declare that the work in this dissertation entitled "SEROPREVALENCE OF RUBELLA VIRUS ANTIBODIES AMONG SCHOOL CHILDREN 0-10 YEARS IN JOS, PLATEAU STATE, NIGERIA " was done by me under the supervision of Dr. F. J. Giwa and Prof A.T. Olayinka . The information derived from literature has being duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any University.

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CERTIFICATION

This thesis entitled "SEROPREVALENCE OF RUBELLA VIRUS ANTIBODIES AMONG SCHOOL CHILDREN 0-10 YEARS IN JOS, PLATEAU STATE, NIGERIA" by Waziri Samuel Hyelshilni meets the regulations governing the ward of the degree of Master in Public Health Field Epidemiology of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Rubella disease occurs worldwide with seasonal variation and affects both the young and elderly and may present as an acute, mild or asymptomatic illness. Infection during early pregnancy may result in congenital rubella syndrome (CRS), stillbirth or miscarriage. CRS often results in multiple birth defects such as heart problems, deafness and blindness which makes rubella a leading cause of preventable congenital defects. This study aimed to determine the seroprevalence of rubella-specific antibodies in school children 0-10 years in Jos.

A cross-sectional descriptive study was done to determine the seroprevalence of rubella specific antibodies, prevalence of recent rubella infection and factors associated with its transmission in Jos. Children aged 0-10 in selected schools in Jos North and South were studied. Serum was obtained from children and tested for IgG and IgM using ELISA.

A total of 405 children were studied with a mean age of 6.3 years (SD ± 2.5), out of which 220 (54.3%) were female and the age group, 7-8 had the most participants (113 [27.9%]). 336 (83.0%) children tested positive for rubella IgG while only 9 (2.2%) of children tested were positive for IgM. Age ≥5 years was significantly associated with rubella seropositivity with CI 1.01-3.08 at 95% CI and p value 0.043. There was no significant association between sex of child and rubella seropositivity. Lack of Western education was significantly associated with rubella seropositivity among children at 95% CI (1.21-4.25) and p value 0.009. LGA of residence was also significantly associated with rubella seropositivity as residents of Jos North were more 10 times more likely to be have rubella specific antibodies than residents of Jos South. None of the study participants had ever taken rubella vaccine.

A large proportion of children had no antibodies and were still susceptible to rubella virus infection. There is a need to include rubella virus vaccine in the national routine immunization programme.

Key words : Children, ELISA, Jos, Seropositivity, Rubella

LIST OF ACRONYMS

CMV - - - - - -	Cytomegalovirus
CRS - - - - - -	Congenital rubella syndrome
EIA - - - - - -	Enzyme Immunoassay
ELISA - - - - - -	Enzyme linked immunosorbent assay
EPI - - - - - -	Expanded Programme on Immunisation
GVAP - - - - - -	Global Vaccine Action Plan
HRP - - - - - -	Horseradish peroxidase
H_2SO_4 - - - - - -	Tetraoxosulphate VI acid
IFU - - - - - -	Instructions for use
IgG - - - - - -	Immunoglobulin G
IgM - - - - - -	Immunoglobulin M
MMR - - - - - -	Mumps, measles, rubella vaccine
MOG - - - - - -	Myelin oligodendrocyte glycoprotein
MR - - - - - -	Mumps and rubella vaccine
OD - - - - - -	Optical density
RCV - - - - - -	Rubella-containing vaccine
RNA - - - - - -	Ribonucleic acid
RV - - - - - -	Rubella virus
SIA - - - - - -	Supplementary immunization activities
TMB - - - - - -	Tetramethyl benzidine
UNICEF - - - - -	United Nations Children's Fund
WHO - - - - - -	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Rubella disease occurs worldwide with seasonal variation and affects both the young and elderly. It may present as an acute, mild or asymptomatic illness thus serological tests for detection of specific rubella antibodies in suspected cases remain important. This infection, also known as German measles or three-day measles, is caused by the Rubella virus (RV). It is often mild especially in children with a lot of people not realizing that they are sick. Infection during early pregnancy may result in a child born with congenital rubella syndrome (CRS), stillbirth or miscarriage. Rubella is usually spread through contact with people who are infected via coughing or sneezing.[1] The incubation period is 14 to 21 days, with the majority of individuals developing a rash 14 to 17 days after exposure. Individuals with rubella are infectious from one week before symptoms appear to four days after the onset of the rash. Babies with CRS may spread the virus for more than a year.[2]

Rubella reinfection following natural infection is very rare.[3] Primary RV infections during the first trimester of pregnancy have high teratogenic potential leading to severe consequences- CRS which may occur in 80–85% of cases. It should be emphasized that more than 50% of RV infections in non-immunized persons in the general population (and in pregnant women) are subclinical.[4]

Naturally acquired rubella generally confers lifelong and usually high degree of immunity against the disease for the majority of individuals.[5,6] Rubella vaccination induces immunity that confers protection from viraemia in the vast majority of vaccinees, which usually persists for more than 16 years.[5,6] A small fraction of the vaccinees fail to respond or

develop low levels of detectable antibodies which may decline to undetectable levels within 5–8 years from vaccination.[7-10] Rubella can be prevented with a safe, effective and inexpensive vaccine. This can be delivered as a rubella vaccine alone, or combined with measles vaccine (MR) or with measles and mumps vaccines (MMR).

Assessment of primary rubella infection relies primarily on the detection of specific IgM antibodies in combination with either seroconversion or a >4-fold rise in rubella specific IgG antibody titer in paired serum samples (acute/convalescent).[10]

A seroprevalence study in 1989 showed a high rate of rubella susceptibility in school-age children, particularly in males.[11]

Rubella disease occurs worldwide with seasonal variation. The virus tends to peak during the spring in countries with temperate climates.[12,13] Before the vaccine to rubella was introduced in 1969, widespread outbreaks usually occurred every 6–9 years in the United States and 3–5 years in Europe, mostly affecting children in the 5-9 year old age group.[14] Since the introduction of vaccine, occurrences have become rare in those countries with high uptake rates.

Rubella can be prevented with a safe, effective and inexpensive vaccine. This can be delivered as a rubella vaccine alone, or combined with measles vaccine (MR) or with measles and mumps vaccines (MMR).[14]

In many developing countries, parents do not have access to immunization services that could protect their children from rubella. Factors such as poverty, poor health systems and a lack of information can make it difficult for families to secure preventative vaccinations for each of their children.

1.2 PROBLEM STATEMENT

Rubella can have serious consequences for pregnant women and their unborn children. If infected with rubella in the first trimester women have a very high risk of giving birth to a child with Congenital Rubella Syndrome (CRS).[4] Primary RV infections during the first trimester of pregnancy have high teratogenic potential leading to severe consequence which may occur in 80–85% of cases.[3,4,11] CRS often results in multiple birth defects including as heart problems, deafness and blindness. More than 100,000 children are born with CRS each year which makes rubella a leading cause of preventable congenital defects.[12] The lifelong complications and disabilities can have an immeasurable emotional, social and financial cost for families. The CRS burden is highest in South East Asia (approximately 48%) and African regions (approximately 38%).[11]

In the USA, before the introduction of the vaccine, a single epidemic resulted in 20,000 infants being born with permanent damage due to intrauterine infection with rubella virus.[15] In Saudi Arabia, the antibody prevalence among girls aged 5-25 years has been reported to be 92%.[16] In Japan, 15,000 cases of rubella and 43 cases of congenital rubella syndrome were reported to the National Epidemiological Surveillance of Infectious Diseases between October 15, 2012, and March 2, 2014 during the 2012–13 rubella outbreak in Japan. They mainly occurred in men of ages 31 to 51 and young adults aged 24-34.[17]In a study in Yemen among school girls, the prevalence of rubella IgG was as high as 91.64%[18]

In Eritrea, the prevalence of antibodies to rubella has been reported to be as high as 99% in female population.[19] Rubella antibody prevalence in Ghana has been found to be up to 92% among pregnant women, with susceptibility associate with a younger age[20] In Nigeria, rubella antibody prevalence in women of child bearing age has been reported to be 77% in Imo.[21] Another study showed the seroprevalence of Rubella IgG Antibody in Pregnant

Women in Osogbo, Nigeria was found to be 87.5%.[22] Seroprevalence of rubella antibodies among pregnant women was found to be 93.1% and 38.8% for IgG and IgM in a Teaching Hospital in Zaria, Nigeria.[23]

1.3 JUSTIFICATION

There are only a few studies done on rubella in children in Nigeria, however, a study done in Jos in 2010 revealed a prevalence of IgM antibodies of 45.2% out of a sample of 93 children.[24] This study was hospital based and all subjects were ill children who came to the hospital.

Even though fetuses are the worst hit by rubella in terms of CRS, and get infected from their mothers in-utero, children are usually the source of infection to their mothers due to close contact between them.[25]

Several seroprevalence studies showed a high rate of rubella susceptibility in school-age children.[13] This is the main reason for the choice of school age children in this research. An understanding of the epidemiology of this important virus in Jos will provide additional information for the design and institution of effective guidelines to reduce spread of infection with the virus and ultimately CRS.

Many studies have shown school-age children to be particularly susceptible to rubella infection. These children easily spread the disease among themselves especially in places where they aggregate for example schools. These children in turn serve as the source of infection to their caregivers who are mostly women who may be or get pregnant making their unborn babies susceptible to CRS. Nigeria does not currently have a programme that offers immunization services against rubella to either children or women of child bearing age.

The study will provide information about the current status of the immunity level of children and help to identify the proportion of children that are susceptible to rubella and thus the potential danger of an outbreak and consequently CRS. It will also help identify the proportions of affected individuals that pose serious risks to their mothers and what preventive practices should be put in place.

Information from this study will serve as a follow up to the earlier study carried out in Jos to help determine the trend of rubella infection among children and to institute control measures.

Outcome of the study will help in planning, strategizing and probably implementing surveillance systems for rubella infection to stem the tide of the infection at various level of prevention.

The study will help in considering the introduction of Rubella vaccine into the routine immunization programme since it is currently not part of the nation's routine immunization plan. This study will also provide additional data for further research in Jos

AIMS AND OBJECTIVES

1.4 AIM

To determine the prevalence of antibodies to rubella and factors associated with risk of rubella infection among children 10 years and below in Jos.

1.5 SPECIFIC OBJECTIVES

1. To determine the prevalence of IgG antibodies to rubella among children 10 years and below in Jos

2. To determine the prevalence of IgM antibodies to rubella among children 10 years and below in Jos

3. To identify factors associated with risk of rubella infection among the children.

CHAPTER TWO

LITERATURE REVIEW

2.1 BACKGROUND

The name rubella is derived from Latin, meaning “little red.” Rubella was initially considered to be a variant of measles or scarlet fever and was called “third disease”. It was not until 1814 that it was first described as a separate disease in the German medical literature, hence the common name “German measles”[26]. Rubella is an acute exanthematous viral infection of children and adults characterized clinically by fever, rash, lymphadenopathy and mimics a mild case of measles (rubeola).

2.2 MICROBIOLOGY OF THE VIRUS

Rubella virus was first isolated by Parkman and colleagues[27] and by Weller and Neva[28] in 1962. Rubella virus is the only member of the genus *Rubivirus* and belongs to the family of *Togavirideae*, whose members commonly have a genome of single-stranded RNA which is enclosed by an eicosahedral capsid.[29] It is closely related to the alphaviruses but in contrast to the alphaviruses, no vector is required for its transmission and it is serologically distinct from the alphaviruses. It is a spherical, 40- to 80-nm, positive-sense, single-stranded RNA virus with spike-like, hemagglutinin-containing surface projections. Its electron-dense 30 to 35 nm core is surrounded by a lipoprotein envelope.[30]It matures by budding from the cell membrane.[30] There are three structural proteins associated with rubella virus namely E1, E2 and C. E1 and E2 are transmembrane glycoproteins while C is the capsid protein that surrounds the RNA of the virion. Varying mixtures and proportions of the structural proteins make up the haemagglutinin and complement fixing antigen. Nonstructural proteins that are relate to replication and transcription probably exist also.[30-32]

There are 10 genotypes of the virus known worldwide viz 1A, 1B, 1C, 1D, 1E, 1F, 1G, 2A, 2B and 2C. However, there is little information available on genotypes of rubella viruses in Africa but 1E and 2B have been found to have a wide geographical distribution. There are few studies in Africa which have investigated on molecular epidemiology of rubella virus, therefore little is known regarding genotypes of rubella virus strains circulating in Africa.[33]

E1 and E2, are anchored to the external layer of the membrane. The E1 protein is responsible for receptor-mediated endocytosis and is the immunodominant antigen. Antibodies against the neutralizing domain of E1 when measured, can be used as a correlate of protection against rubella virus. The E2 protein is forms connections between rows of E1 proteins.[34]There is no known definitive cellular receptor for rubella virus however, the rubella E1 protein plays a role in in-vitro infection through binding with myelin oligodendrocyte glycoprotein (MOG).[34]

Rubella virus is relatively unstable and is inactivated by lipid solvents, trypsin, formalin, ultraviolet light, extremesf pH and heat, and inhibited by amantadine.[35]

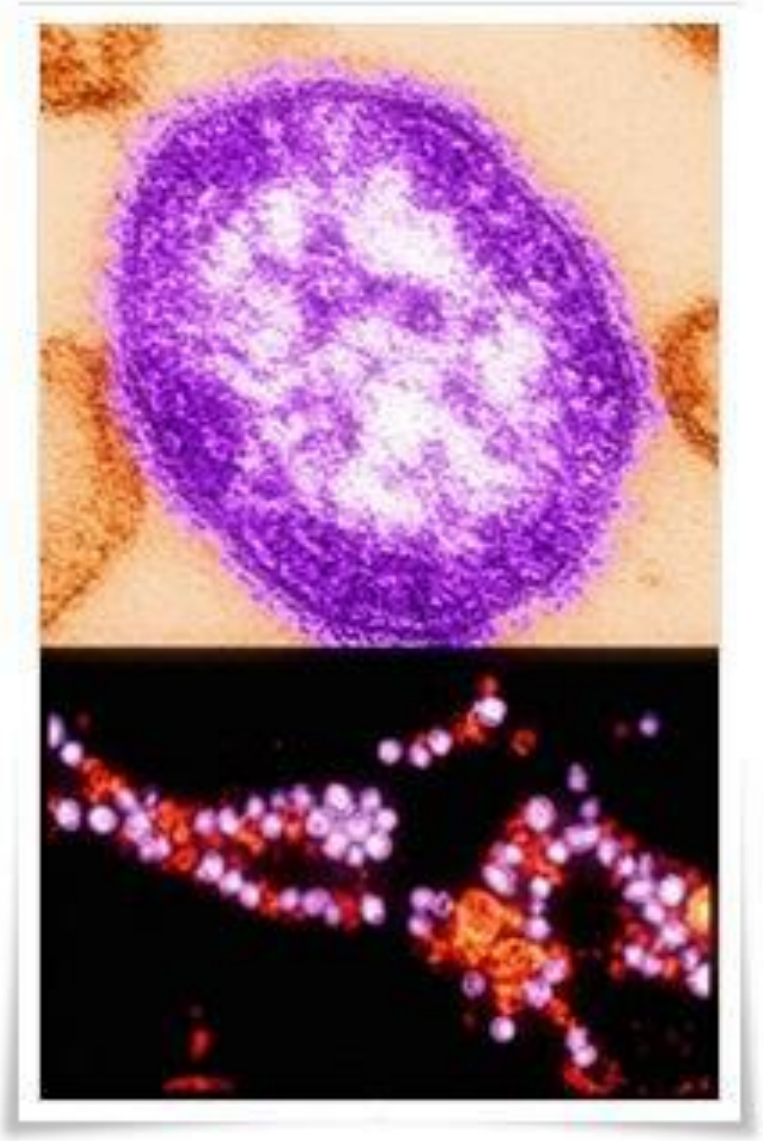

Fig. 1. Electron micrograph of the rubella virus

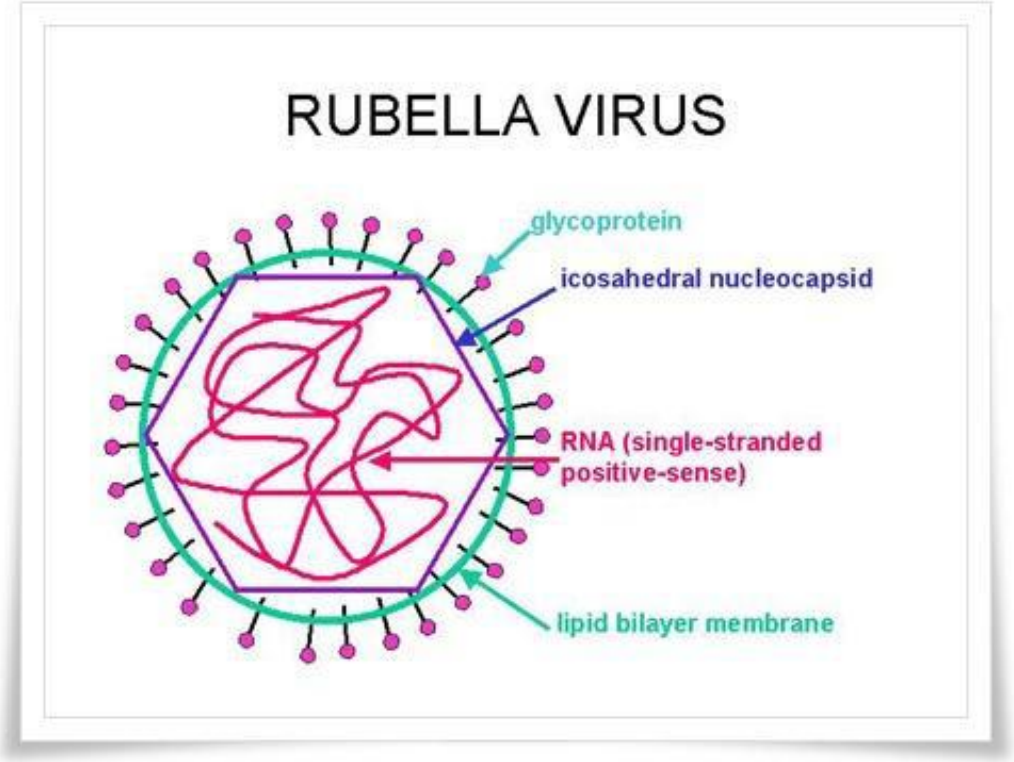

Fig. 2. Structure of rubella virus

2.3 TRANSMISSION

Rubella is transmitted in droplets spread shed from the respiratory secretions of infected persons. Individuals are most infectious when the rash is just erupting, however they may shed virus from the throat from 10days before the onset of rash to 15days after its onset.[35]Neonates with congenital rubella may shed large quantities of the virus from body secretions for prolonged periods through infancy, thereby putting their caregivers at great risk of contracting the virus. The mechanism through which these infants excrete the virus despite titres of neutralizing is not yet understood.[36]Individuals who have subclinical illness may also transmit the infection to others.[35] Individuals who have been vaccinated against rubella do not transmit the virus, although the virus may sometimes be isolated from their pharynx however, the quantity of the virus shed may be too small to be infectious.[37-39]

2.4 PATHOGENESIS

Rubella virus infects by invasion of the respiratory epithelium.[40] Following respiratory transmission of rubella virus, replication of the virus is thought to occur in the nasopharynx and then it extends to local lymph nodes where virus amplification, during the prodomal stage, gives rise to giant multinucleated lymphoid or reticuloendothelial cells (i.e., Warthin–Finkeldy cells). These syncytia, identified in the submucosal areas of tonsils and pharynx, are thought to be a major source of virus spread to other organs and tissues through the blood stream. Subsequent additional replication in selected target organs, such as the spleen and lymph nodes, leads to a secondary viremia with wide distribution of rubella virus. A secondary viraemia follows whereby the virus is further spread to involve the skin, the viscera, kidney and bladder. At this time the virus can be detected in the blood and respiratory secretions.[40-43]

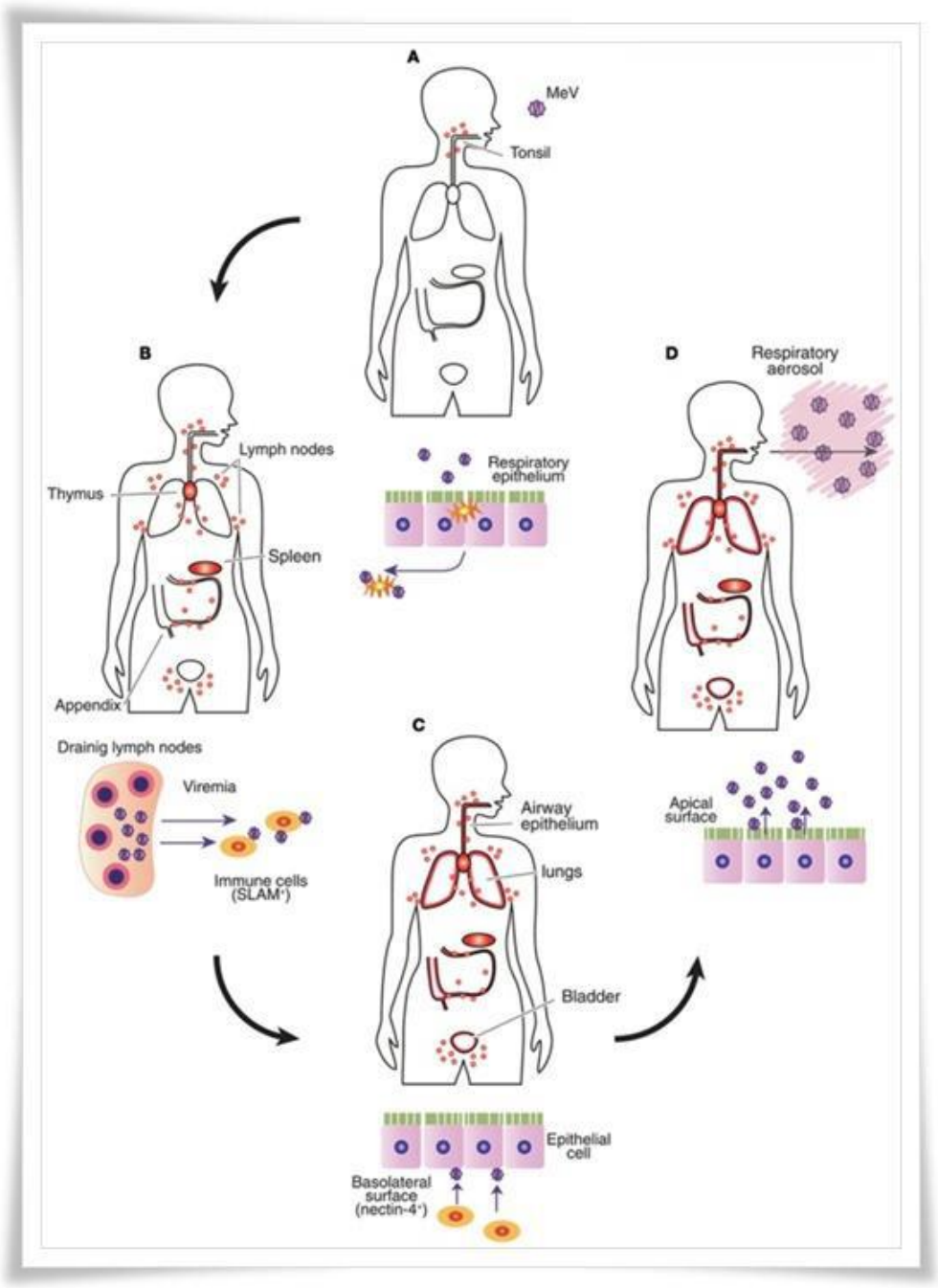

Fig. 3 Diagram showing the penetration and multiplication of rubella in the respiratory tract and lymph nodes.

2.5 EPIDEMIOLOGY OF RUBELLA

Rubella has a worldwide distribution the disease incidence is usually highest in spring and winter. Before the introduction of vaccination outbreaks tend to occur every 6 to 9 years in America and 3 to 5 years in Europe.[14] The last major epidemic in the United States occurred during 1964-1965, when there was an estimated 12.5 million rubella cases in the United States, resulting in 2,000 cases of encephalitis, 11,250 therapeutic or spontaneous abortions, 2,100 neonatal deaths, and 20,000 infants born with CRS.[26] During the 1990s, the incidence of rubella in the United States, among children younger than 15 years decreased (from 0.63 per 100,000 population in 1990 to 0.06 1999), whereas the incidence among adults aged 15 to 44 years increased (from 0.13 per 100,000 in 1990 to 0.24 in 1999).[26] However, since 2001, the incidence both among persons younger than age 15 years and those aged 15 to 44 years has been less than 1/10,000,000 population.[44,45] Since 2001, only three rubella outbreaks have been reported in the USA, each with five or fewer cases. The number of reported cases of rubella in the United States has declined dramatically to a median of 11 cases annually in 2005-2011.[46] Between 2000–2012, global reported rubella cases decreased 86% from 670,894 to 94,030;[47] however, rubella cases are substantially under-reported, particularly in countries not yet using rubella vaccine. The greatest decrease in reported rubella cases was a 95% decrease in the European Region, from 621,039 to 30,509, and a 99.9% decrease in the Americas, from 39,228 in 2000 to only 21 cases in 2012. In other regions, the number of cases increased during this period in parallel with the increase in the number of countries reporting rubella cases.[47] Compared to reporting of acquired rubella cases, fewer countries report CRS cases, though the number increased from 75 (39%) in 2000 to 129 (66%) countries in 2012. Compared to model estimates, the number of reported CRS cases is very low, with 300 reported CRS cases in 2012 versus a model-based estimate of 110,000 CRS cases in 1996. [47]

Immunity following natural infection or vaccination is life-long. The average age of rubella infection is 5–9 years of age in the absence of vaccination, with annual seasonal outbreaks usually occurring in the spring, and large outbreaks occur every 3–8 years. The cyclical nature of rubella is related to the accumulation of susceptible people in the population and contact rates. A review of the epidemiology of rubella in Africa between 2002–2009 found the median age of rubella IgM positive cases to be 7.3 years (interquartile range: 4.2–9.0 years).[48] The observed seasonal pattern was more marked in West Africa and Southern Africa, with Central and East Africa having a bimodal pattern with transmission throughout the year.[48] It is estimated more than 100,000 infants are born with CRS annually worldwide.[12] The burden of Congenital Rubella Syndrome (CRS) is typically underestimated in routine surveillance thus its magnitude is best estimated using seroprevalence data.[47] In 2010, 105,000 (95% CI: 54,000–158,000) CRS cases were estimated globally, compared to 119,000 (95% CI: 72,000–169,000) in 1996.[47] Approximately 110,000 children were born with CRS in 1996 in 78 developing countries which had not introduced rubella-containing vaccine (RCV) in their national programme.[49]In 2010, the incidence was <2 per 100,000 live births in the Americas and Europe, 25 (95% CI 4–61) per 100,000 live births in the Eastern Mediterranean, 90 (95% CI: 46–195) in the Western Pacific, excluding China, 116 (95% CI: 56–235) per 100,000 live births in Africa and 121 (95% CI: 31–238) per 100,000 live births in and South East Asia. [47]

The worldwide CRS burden is highest in South East Asia (approximately 48%) and African regions (approximately 38%).[41] In Saudi Arabia, the antibody prevalence among girls aged 5-25 years has been reported to be 92%.[16] In Japan, 15,000 cases of rubella and 43 cases of congenital rubella syndrome were reported to the National Epidemiological Surveillance of Infectious Diseases between October 15, 2012, and March 2, 2014 during the 2012–13

rubella outbreak in Japan. They mainly occurred in men of ages 31 to 51 and young adults aged 24-34.[17]In a study in Yemen among school girls, the prevalence of rubella IgG was as high as 91.64%.[18] In a study done in Bangui, Central African Republic, the overall prevalence of rubella-specific IgG was 55.4%. Presence of rubella-specific IgG antibodies increased with increasing age of study subjects with protective antibody in 38% of children 1-4 years of age, 71% of children 5-9 years of age, and 87% of children 10 years of age and older.[50] Another study done in Western Sudan showed a prevalence of 65.3% among pregnant women.[51] There is a wide variation of rubella susceptibility in Africa indicating that transmission rates differ among regions.[33] Generally, no significant difference was observed for IgG seroprevalence between urban and rural settings in Africa.[33] This study showed high transmission rates during childhood emphasizing importance of vaccination in this age group.[33] There are few studies in Africa which have investigated on molecular epidemiology of rubella virus, therefore little is known regarding genotypes of rubella virus strains circulating in Africa.[33] The seroprevalence of rubella specific IgG in Malta was found to be 97.7%.[52]

Seroprevalence of Rubella IgG Antibody in Pregnant Women in Osogbo, Nigeria was found to be 87.5%.[22] Another study done in a Teaching Hospital in Zaria, Nigeria found the seroprevalence of rubella antibodies among pregnant women to be 93.1% and 38.8% for IgG and IgM.[23] In Imo Nigeria, rubella antibody prevalence in women of child bearing age has been reported to be 77%.[21] Seroprevalence of IgM antibodies among children aged 0-10 years has been found to be 42.5% in Jos.[24]

According to a survey of the member countries in the World Health Organization (WHO), the number of countries that have incorporated rubella-containing vaccines into their routine national immunization programs increased from 83 (13% of the birth cohort) in 1996 to 130 countries (40% of the birth cohort) in 2010.[47] As of October 2010, the WHO

Region of the Americas and European Region have established rubella elimination goals for the year 2010 and 2015, respectively; the Western Pacific Region has established targets for accelerated rubella control and CRS prevention goal (<1 case per 100,000) by 2015; and the Eastern Mediterranean Region has established a goal of CRS prevention without a target date for countries that have introduced national rubella vaccination programs.[47] In 2010, the Pan American Health Organization (PAHO) announced that the Region of the Americas had achieved the rubella and CRS elimination goals set in 2003 based on surveillance data.[47] The World Health Organisation recommends that countries without rubella vaccination programmes to assess the burden of rubella infection and CRS using sero-epidemiological surveys because, there is limited data on epidemiology of rubella infection and CRS in many African countries. [33]

In 2011, WHO recommended for all countries that are providing two doses of measles vaccine and have not introduced rubella vaccine, to consider including rubella-containing vaccine in their immunization program.[23]

2.6 CLINICAL FEATURES

Rubella disease occurs worldwide with seasonal variation. The virus tends to peak during the spring in countries with temperate climates.[53] Age is an important determinant of the severity of rubella. Postnatal rubella is mostly asymptomatic as it is for most viral infections and children often have milder symptoms than adults while transplacentally acquired rubella during early pregnancy poses a great risk to the development of severe rubella with serious sequelae.[54,55]

Postnatal Rubella

The features vary from being asymptomatic to a mild prodromal illness involving a low-grade fever, malaise, coryza and mild conjunctivitis. Among patients who are symptomatic, children do not experience a prodromal phase, but adults may have a prodrome of, fever, an anorexia for several days. Lymphadenopathy involving posterior auricular, posterior cervical and sub-occipital glands may precede the rash lasting several weeks.[13,56] The rash is usually transitory, erythematous and maculopapular which appears first on the face and neck and quickly spreads to the trunk and upper extremities and then to the legs. It often fades on the face while progressing downwards. The lesions tend to be discrete at first, but rapidly coalesce to produce a flushed appearance.[28] An enanthem consisting of petechial lesions on the soft palate (Forscheimer spots) has been described for rubella, but unlike Koplik spots in measles, this enanthem is not diagnostic for rubella.[3] Clinical diagnosis is unreliable as the rash may be fleeting and is not specific to rubella.[13]

Postnatal rubella may be accompanied with complications, however these complications are by far less common compared with complications of measles. Arthritis / arthralgia has been reported in up to a third of women with rubella, this is however less common in men and children.[44] The pathogenesis of arthritis associated with rubella has not been clearly understood, however a serving as some form of explanation for chronic forms of rubella arthritis.[45,46,57]

Haemorrrhagic manifestations occur as complications of rubella in approximately 1 in 3000 cases.[37,43,57] This occurs more in children than in adults and may be secondary to thrombocytopaenia and vascular damage and probably immune mediated.[56]

Mild hepatitis has been described as an unusual complication of rubella.[57] Encephalitis is an even rarer complication occurring in about 1 in every 5000 and is more common in adults than in children with a mortality of 20-50%[44,58,59]

Congenital Rubella

Rubella in early pregnancy can be fatal leading to premature delivery, foetal death and a wide range of congenital defects. The effects of rubella virus on the foetus are to a large extent dependent on the time of infection, younger fetuses are more severely affected.[54,55,60] A foetus has a 65-85% chance of being affected during the first 2 months of gestation with either spontaneous abortion or multiple congenital defects.[31,61,62] Conversely, a foetus has a 30-35% chance of being affected in the third month of gestation with a single defect such as congenital heart disease or deafness. Furthermore, foetal infection during the fourth month of gestation is associated with a 10% risk of a single congenital birth defect while occasional foetal damage may be seen if it occurs up to 20 weeks gestation.[29,54,55,63]

The clinical features of congenital rubella can be classified as

- Temporary such as low birth weight
- Permanent such as deafness
- Developmental such as eye defects

A list of major clinical presentations of congenital rubella is shown in the table below

Table 1. Various presentations of congenital rubella

TEMPORARY	PERMANENT	DEVELOPMENTAL
Low birthweight	Hearing loss	Hearing loss
Thrombocytopaenia	Cataract (Microphthalmia)	Pulmonary stenosis
Hepatosplenomegaly	Retinopathy	Mental retardation
Bone lesions	Patent ductus arteriosus	Cloudy cornea
Large anterior fontanelle	Pulmonary stenosis	Severe myopia
Meningoencephalitis	Mental retardation	Diabetes mellitus
Jaundice	Behaviour disorders	Thyroid disorders
Cloudy cornea	Central language disorders	Seizure disorders
Hepatitis	Cryptorchidism	Precocious puberty
Generalised lymphadenopathy	Inguinal hernia	Degenerative disorders of the brain
Haemolytic anaemia	Spastic diplegia	
Rubella pneumonitis	Microcephaly	
	Dermatoglyphic abnormality	
	Glaucoma	
	Severe myopia	
	Myocardial abnormalities	

A few children whose mothers had rubella during pregnancy, and whose children were presumed to be normal were found to have features of congenital rubella on approaching school age thus it is thought to be a dynamic disease.[53,54,55] The pathologic basis for the manifestations of congenital rubella have been proposed in several models, it has been proposed that infection with rubella virus results in mitotic arrest of cells, which in turn leads to inhibition of growth and thus retarded organ growth.[63,64] Other theories propose that the growth retardation associated with congenital rubella is due to angiopathy with foetal and placental vasculitis which leads to cellular damage and tissue necrosis.[55,56,64] Fibroblasts infected with rubella in-vitro have been shown to a produce growth inhibitor which may be responsible for foetal growth retardation.[57,58,65] Furthermore, rubella infection interferes with the normal balance of growth and differentiation leading to defects in organogenesis.[31,59,65]

Approximately 50 developing countries have already conducted substantial studies to assess their CRS disease burden.[26] In 1995, the WHO Global Programme for Vaccines and Immunization carried out a global survey of rubella immunization policies.[12] Cases of CRS may be prevented as follows: by directly protecting women and/or schoolgirls (a selective vaccination strategy); by vaccinating boys and girls in childhood to provide indirect protection by reducing the transmission of rubella virus (a childhood vaccination strategy); or by a combination of these approaches (a combined strategy).[27]

2.7 DIAGNOSIS

It is often difficult to diagnose rubella clinically, because it is often mild and presents with non-specific symptoms. It has similar features with mild measles, scarlet fever, roseola, erythema infectiosum, infectious mononucleosis, toxoplasmosis e.t.c. Specific laboratory techniques are necessary diagnosis of rubella. Rubella may be diagnosed in the laboratory by virus isolation, identification of rubella antigen in infected tissues or by demonstration of serological response to rubella virus.

Laboratory diagnosis of postnatal rubella is most commonly done serologically. There are a number of methods available including latex agglutination test, radial haemolysis test, enzyme-linked immunosorbent assay (ELISA). In latex agglutination test, latex particles are coated with rubella antigens that then react to antibodies in the serum being tested. Agglutination shows the presence of antibodies. ELISA assay uses microplates coated with rubella antigen which upon presentation with antibodies combine to form antigen-antibody complexes which can be detected in various ways. These tests measure either immunoglobulin G or M (IgG or IgM respectively). Demonstration of specific IgG in one serum sample indicates some immunity to rubella. Acute rubella infection may be diagnosed by a demonstration of specific IgM in one serum sample or by a four-fold or more increase in rubella antibody titre in acute and convalescent specimens assayed in the same test.[60,61]

Isolation of the virus from throat swabs, synovial joint, urine or other body fluids can also be done except that this is time consuming and expensive so, it is usually reserved for special circumstances such as the diagnosis of congenital rubella or investigating arthritis and other conditions thought to be complications of postnatal rubella.[31,34]

When diagnosing congenital rubella serologically, antibodies from the maternal and infant sera should be measured several times. Rising antibody titre in a neonate may suggest rubella infection while a falling antibody titre may indicate passively acquired maternal antibody. Rubella IgM detection in neonates indicates transplacental infection. Placental biopsy, demonstration of rubella antigen with monoclonal antibody, cordocentesis and detection of RNA by polymerase chain reaction (PCR) and in situ hybridization are other methods that have been used to detect rubella as early as12 weeks gestation.[62]

2.8 RUBELLA VACCINE

Between 1965 and 1967, several live, attenuated rubella strains were developed, and most industrialized countries began rubella vaccination soon afterwards. The rationale for use of the vaccine was to prevent congenital rubella by controlling postnatal rubella. Immunization with live attenuated rubella virus vaccine has the demonstrated ability to prevent infection and its most feared complications. The live rubella vaccine currently distributed in most countries contains the RA 27/3 strain, which is prepared in human diploid cell culture.[63] The vaccine is produced in monovalent form (rubella only), and in the following combinations: measles-rubella (MR) vaccine and measles-mumps-rubella (MMR) vaccine. When properly administered, the vaccine produces a seroconversion rate of about 95% and this is not impaired in children with upper respiratory tract infection.[64,65] The vaccine may cause viraemia.[66,67] therefore the main complications are fever, lymphadenopathy, arthritis, and arthralgia. These complications are more common in adults than in children and most common in women who are more than 25 years old.[67-75] Immunization with live attenuated rubella virus vaccine has the demonstrated ability to prevent infection and its most feared complications.[76,-78] Despite the progress made, rubella remains an important pathogen and public health concern around the world.[47,79] The recent rubella epidemic in Japan, had more than 11,000 rubella cases occurring within the first

half of 2013 and at least 13 CRS cases, this points to the fact that a partial vaccination strategy leads to major outbreaks.[8] For this reason, a global commitment to rubella control, elimination, and eventual eradication must be in place.

Rubella virus is a candidate for global eradication because humans are the only known host, safe and highly efficacious vaccines (≥95% efficacy following a single dose) exist, accurate diagnostic and molecular assays exist, and there has been demonstration of sustained interruption of endemic transmission in the Americas since 2009.[47]

2.9 CURRENT USE OF RUBELLA VACCINE IN NATIONAL IMMUNIZATION PROGRAMMES

Worldwide, 78 countries (> one-third of all countries) reported a national policy of rubella vaccination as at 1997.[12] This did not include countries where rubella vaccine is only used in certain areas or the private sector.[12] Then, there were no countries in the African Region that included rubella vaccine in their national immunization schedules. Rubella vaccine was then used by approximately half the countries in the Region of the Americas and the Eastern Mediterranean Region, 64% in the European Region, and 31% in the Western Pacific Region. In the South-East Asia Region, Sri Lanka and Thailand introduced rubella vaccine in 1996. Of the 78 countries that used rubella vaccine, a combined strategy was reported by 47 (60%); 7 (9%) reported selective immunization of women and/or schoolgirls; and 24 (31%) reported only childhood immunization.[44]

Use of rubella vaccine is related to national economic status. Based on the United Nations country classification[27], rubella vaccine is used by 92% of industrialized countries, 36% of economies-in-transition, and 28% of developing countries.[47] A total of 38% of all countries currently include rubella vaccine in their national immunization programmes.[47]

Rubella vaccine had, however, not been recommended for inclusion in the Expanded Programme on Immunization (EPI) in developing countries[80] since when sustained high coverage could not be guaranteed its introduction could increase the susceptibility of adult women by slowing, but not interrupting, rubella transmission.[81]

The new Global Vaccine Action Plan (GVAP) has goals of establishing regional elimination of measles and rubella in at least five WHO regions by 2020.[47] This resolve was made at the World Health Assembly in May 2012. Furthermore, the core partners of the Measles and Rubella Initiative (American Red Cross, US Centers for Disease Control and Prevention, United Nations Foundation, UNICEF and WHO) launched the Global Measles and Rubella Strategic Plan, 2012–2020.[7] The plan was set to "achieve a world without measles, rubella and congenital rubella syndrome" with existing global control and regional elimination targets as milestones toward this. The plan includes a five-pronged strategy to: 1) achieve and maintain high levels of population immunity by achieving ≥95% vaccination coverage with two doses of measles- and rubella-containing vaccines; 2) monitor disease using effective surveillance, and evaluate programmatic efforts; 3) develop outbreak preparedness and respond rapidly to outbreaks; 4) communicate and engage to build public confidence and demand for immunization; and 5) perform the research and development needed to support cost-effective operations and improve vaccination and diagnostic tools. The outbreak in Japan in 2013 has shown that a partial vaccination strategy leads to major outbreaks.[7]

Rubella vaccines are highly cost-effective, because of their high efficacy and relatively low cost. Cost-benefit studies of rubella vaccination in industrialized and less industrialized countries in Latin America and the Caribbean with a coverage of >80% have demonstrated that the benefits outweigh the costs and that rubella vaccination is economically justified, particularly when combined with measles vaccine.[7]However, no such studies have been

conducted in low-income countries in Africa and Asia.[7] One of the major factors limiting universal use of rubella vaccine has been concern about a "paradoxical effect" – that sustained low rubella immunization coverage in infants and young children might decrease exposure to rubella during childhood, which may lead to increased susceptibility among women of childbearing age compared to the pre-vaccine era because such women are neither vaccinated nor exposed to virus. This has the theoretical potential to result in an increased risk of CRS above the pre-vaccine era level. Studies have shown that to avoid increasing the risk of CRS, countries should achieve and maintain immunization coverage of 80% or greater with at least one dose of RCV delivered through routine services and/or regular supplementary immunization activities (SIAs).[47]

There has been a gradual increase in the number of countries using rubella vaccine in their national immunization program over the last 15 years. The increase has been steady over the years of the countries providing at least one dose of RCV from 99 (52%) in 2000 to about 132 (68%) countries in 2012.[7] The proportion of countries that have introduced rubella vaccine by 2012 varied from 7% in the African Region to 100% of countries in the American and European Regions. Between 2000–2012, the estimated global coverage with one dose of RCV increased from 22% to 43% and by 2012, the American and European Regions of WHO had >93% estimated RCV coverage.[7] Rubella vaccination coverage through the routine immunization program is almost identical to that of measles, because most countries that give measles vaccines provide rubella vaccine combined with measles or measles and mumps vaccines.[7]

CHAPTER THREE

METHODOLOGY

3.1 STUDY AREA

The study sites were nursery and primary schools in Jos. Jos is the capital of Plateau State. Plateau state is located at latitude 9º55'N and longitude 8°53'E. Its altitude ranges from around 1,200 meters (about 4000 feet) to a peak of 1,829 metres above sea level. It is located approximately in the centre of the country. It is geographically unique in Nigeria because its boundaries surround the Jos Plateau. It is bordered by Bauchi State to the Northeast, Kaduna State to the Northwest, Nasarawa State to the Southwest and Taraba State to the Southeast. Plateau State is the 12th largest State in Nigeria with an area of 26,899 square kilometres. It has 17 LGAs with and an estimated population of 3,933,822 based on 2006 National Population Census. It has over 40 ethno-linguistic groups most of which are farmers with similar cultural and traditional ways of life.At the time of study, there were 101 registered nursery and primary schools in Jos North and about 246 registered nursery and primary schools in Jos South LGA.

3.2 STUDY POPULATION

The study population were pupils of nursery and primary schools 10 years and below in 22 schools across Jos North and Jos South LGAs.

3.3 INCLUSION CRITERIA

All children who were 10 years of age and below attending the selected schools.

3.4 EXCLUSION CRITERIA

Children 10 years and below who consented but not present on the days of sample collection.

3.5 STUDY DESIGN

The study was a cross-sectional descriptive study carried out from May to September 2016.

3.6 SAMPLE SIZE

A minimum sample size of 381 was arrived at using the formula [82]

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

Where : *n* = Sample size

Z = Standard normal deviate at 95% confidence interval that is, 1.96

P = Prevalence (using 0.452) Junaid et al[24]

d = 5% level of precision at 95% confidence interval

A total of 424 samples would have accounted for 10% non-response rate however, a total 405 samples were gotten and processed.

3.7 SAMPLING TECHNIQUE

Two- stage cluster (modified cluster) sampling was done. Proportional allocation was done to each LGA based on the number of registered schools in the LGAs. Jos North has 101 registered schools, Jos South has 346 registered schools. This made the ratio of schools in Jos North to Jos South to be 1:3.4. Thus with proportional allocation, 5 schools were

sampled from Jos North LGA and 17 schools were sampled from Jos South LGA. Random sampling was done from the list of nursery and primary schools in Jos North and Jos South to determine the schools that participated in each LGA. An average of 18 pupils were sampled from each of the schools chosen, to meet the minimum sample size of 381 however, 20 children were sampled from 4 schools and 19 children were sampled from 1 school arriving at a total 405 subjects for this study. The participants were recruited from the schools by simple random sampling until the sample size was reached. A ballot box was placed in each of the schools sampled with 2 possible outcomes 'R' and 'N'. Any pupil who picked the letter 'R' was sampled and the others were asked to go back to the class.

3.8 STUDY TOOLS

Questionnaires, samples collected, Rubella IgM and IgG test Kits, ELISA microplate washer and reader were used.

3.8.1 QUESTIONNAIRE

The questionnaire was interviewer administered with open ended questions, it had sections which include sociodemographic characteristics, social history, past medical history. The questionnaire was pretested using 10 women and 8 research assistants were trained to help with data collection.

3.8.2 SAMPLE COLLECTION AND PROCESSING

Informed consent from the care-givers was requested before sample collection Three milliliters (ml) of venous blood was collected by qualified personnel under strict aseptic conditions using sterile syringes and needles for the children into plain red top venepuncture tube without additives or anti-coagulants. The samples collected were stored in coolers containing ice packs and transported to the laboratory for storage. The blood was

allowed to clot and the specimens were centrifuged at 3000 revolutions per minute for 5 minutes to separate the serum from the cells. Serum samples were collected using Pasteur pipettes into plain cryovials. The samples were stored at temperatures of -20ºC for about 20 days. Repetitive freezing and thawing were avoided. Commercially available rubella antibody test kit (Prestige Diagnostics®) was used based on the manufacturer's instructions for detecting antibodies to rubella (both IgM and IgG).

3.9.1 EQUIPMENT NEEDED

Prestige Diagnostics® IgG/IgM kits, distilled water, timer, adjustable micropipettes, micropipette tips (10µl, 100µl) incubator, mixing troughs, microplate reader and microplate washer. Each Prestige Diagnostics® IgG/IgM kit contained the following:

1. Microwell Plate 1x96 wells[(12x8 well plate) One 96 well microplate coated with rubella antigen. Stable when stored at 2-8ºC till the expiry date].
2. Negative Control 1x1ml [Tris Buffer containing salts and preservatives. Stable when stored at 2-8ºC till the expiry date. Once opened to be used within 60 days].
3. Positive Control 1x1ml [Tris Buffer containing rubella IgG/IgM, salts and preservatives. Stable when stored at 2-8ºC till the expiry date. Once opened to be used within 60 days].
4. HRP Conjugate 1x6.5ml [1vial containing Anti-human IgG/IgM conjugated with HRP. Stored at 2-8ºC. Once opened to be used within 60 days].
5. Sample Diluent1x11ml [Calf Serum for diluting samples. Store at 2-8ºC. Once opened to be used within 60 days].
6. Wash Solution Concentrate 1x20ml [One vial containing a surfactant in buffered saline. A preservative had been added. Stored at 2-8ºC].
7. Substrate A 1x7ml [One bottle containing Hydrogen peroxide in buffer. Stored at 2-8ºC.

8. Substrate B 1x4ml [One bottle containing TMB in buffer. Stored at 2-8ºC].
9. Stop Solution 1x6ml [One bottle containing a strong acid 1N H_2SO_4. Stored at 2-30ºC].
10. Plastic Sealable bag, IFU and Cardboard plate covers.

3.9.2 PRESTIGE DIAGNOSTICS® RUBELLA IGM/IgG KIT

The Prestige Diagnostics® Rubella IgM/IgG Kit is a rapid test intended for the qualitative detection of IgM/IgG antibodies against rubella virus in human serum or plasma. It is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is the microplate well coated with rubella antigen.

3.9.3 PRINCIPLE

This ELISA assay uses microplates coated with rubella antigen. Upon presentation of the sample and if it contains Rubella IgG or Ig M antibodies, these antibodies combine with the Rubella antigen. Horseradish peroxidase (HRP) conjugated anti-human IgG or IgM is then added which forms a blue colour with a substrate. The reaction is stopped using a low pH acid solution that changes the colour to yellow. The use of a cut off control and a positive control provides the necessary absorbances to classify patient samples as being positive or negative.

In the storage and handling of the kit, storage of the kit was under normal refrigeration (2° – 8° C). The kit should was not frozen. Before conducting the test, all kit elements and specimen were brought to room temperature, for 60 – 120 minutes. Assays were performed at room temperature 20° – 25° C. Spillage and cross-contamination of solutions were avoided. Reagents were mixed by gently shaking the developing plate several times prior to use. The wells of the Prestige Diagnostics® IgG/IgM microwell plate were not touched.

The Prestige Diagnostics® kit contained inactivated biological material. The kit was handled and disposed of in accordance with accepted sanitary requirements.

3.9.4 REAGENT PREPARATION:

The wash buffer concentrate was diluted in the ratio of 1:40. (i.e., for every ml of the concentrate 40 ml of distilled water was added) Diluted buffer could be stored at 2-30ºC for up to 60 days.

3.9.5 TEST PROCEDURE:

All reagents and controls with samples were brought to room temperature (20-27ºC) and the following steps were carried out

1. The microplate wells for each blank well, control and patient specimen were formatted. Unused microwell strips were replaced back into the aluminum bag, sealed and stored at 2-8ºC.
2. The sample Diluent 100µl (2drops) was added into the appropriate wells except the blank well and the negative control well.
3. 10ul of the sample was added to the wells and mixed with the sample diluent by repeatedly pipetting in and out of the well until the liquid turned blue. 100µl of Negative and the positive control were dispensed to their respective wells. Nothing was dispensed into the blank well.
4. The microplate was swirled gently for 30 seconds to mix and the plate was covered with the plate cover and incubated for 20 minutes at 37ºC.
5. At the end of the incubation period, the plate cover was removed and discarded with the contents of the microwell. Each well was washed 5 times with diluted washing buffer of 350µl with a soak time of 20 seconds each time. After the final washing cycle,

the plate was turned down onto a blotting paper and tapped to remove any residual buffer.

6. 50µl of the HRP Conjugate was added to each well except the blank well. The contents of the well were gently mixed.
7. The plate was covered with the plate cover and incubated for 20 minutes at 37ºC. The incubation was done in the dark according to the manufacturer's guide.
8. At the end of the incubation period, the plate cover was removed and the contents of the microwell were discarded. Each well was washed 5 times with diluted washing buffer of 350µl with a soak time of 20 seconds each time. After the final washing cycle, the plate was turned down onto a blotting paper and tapped to remove any residual buffer.
9. 1 drop of Substrate A (50µl) and one drop of Substrate B (30µl) were added into all wells except the blank well and incubated at 37ºC for 10 minutes. The contents were mixed gently and incubated for a further 10 minutes at 37ºC.
10. Approximately 50ul of the Stop solution was added into each well and mixed gently. The plate was shaken gently for 15-20 seconds to mix, till the solution changed to yellow from blue.
11. The absorbance of the wells was read at 450/630nm using a microplate reader. The absorbances were noted down. The results were read within 30 minutes of addition of the stop solution.

Calculation of results:

- The absorbances obtained from the microplate reader were recorded and interpreted using the cut-off OD as 2.1x Negative control OD.

As a rule, if the od value of the negative control was lower than 0.09 the cut off would be calculated using 0.09 absorbance. however the od value of the negative controls were greater than 0.09 and the actual absorbance was used.

3.9.6 INTERPRETATION

Positive for Rubella IgG/IgM : sample OD is >/= Cut off OD

Negative for Rubella IgG/IgM : sample OD is < Cut off OD

Quality control measures:

1. Reagents from different batches of reagents were not mixed.
2. The reagents were gently mixed before use.
3. The wash buffer concentrate was ensured to be homogeneous before use.
4. Unused microwells were kept in the original bag.
5. Haemolytic, lipaemic, bacteria contaminate or heat inactivated samples were not used
6. Run validity was determined through the performance of the positive and negative controls as well as the blank
7. Positive and negative controls were intended to monitor reagent failure and not ensure precision at the assay cut off levels

3.10 DATA MANAGEMENT / STATISTICAL ANALYSIS

Data were collected using pretested interviewer administered questionnaires and kept in a password protected computer. The Epi info™ 7 Statistical Program (Centre for Disease Control and Prevention, Atlanta, GA) was used for data analysis. Data was analysed using descriptive statistics and chi-square. Association between positive samples for rubella IgM and IgG and other risk factors was performed using bivariate and multivariate analysis. Statistical significance was accepted at p value ≤ 0.05 and confidence interval of 95%.

3.11 ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the Research Ethics Committee of Jos University Teaching Hospital. Permission was sought from the Ministry of Health, Education and Plateau State Universal Basic Education Board. Participation by individuals was voluntary and opting out of the study or failure to give consent did not negatively affect them. The benefits of the study were explained to the caregivers as there may be no direct benefits to their children but, there are benefits ultimately to the State and the nation as a whole. The procedure was explained to the caregivers and children and that it was not harmful to either of them but were made to understand the children will feel needle prick pain which is transient. Consent was obtained from each care-giver and assent from participating children. Confidentiality was maintained throughout the study. Data collected was used for the sole purpose of research. Samples remaining may be used for further research.

CHAPTER FOUR

4.1 RESULTS

A total of 405 children participated in this research alongside their caregivers with 95.5% response rate. The mean age of the children was 6.3years (SD ±2.5), while that of the caregivers was 33.8years (SD ±5.3). More than half of the children studied were females (54.3%) and they were mostly in primary schools (70.0%).

Table 2. Sociodemographic characteristics of school children aged 0-10 years in Jos

Characteristic	Frequency	Percentage (%)
Age		
1-2	43	10.6
3-4	59	14.6
5-6	95	23.5
7-8	113	27.9
9-10	95	23.5
Sex		
Male	185	45.7
Female	220	54.3
School designation		
Nursery	119	29.4
Primary	286	70.6
Type of school		
Private	154	38.0
Public	251	62.0

The age group 7-8 years had the highest proportion of children, (27.9%) and majority of the children studied were from public schools (62.0%).

Table 3. Sociodemograhic characteristics of caregivers of school children aged 0-10 years in Jos

Characteristic	Frequency (n)	Percentage(%)
Age		
20-24	5	1.2
25-29	88	21.7
30-34	138	34.1
35-39	111	27.7
40-44	50	12.4
≥45	13	3.2
Sex		
Male	57	14.1
Female	348	85.9
Marital status		
Single	5	1.2
Married	391	96.5
Divorced	5	1.2
Widowed	4	1.0
Type of marriage		
Monogamy	313	78.0
Polygamy	85	22.0
Level of education		
None	126	31.1
Islamic	11	2.7
Primary	54	13.3
Secondary	94	23.2
Tertiary	120	29.6
Occupation		
Artisan	43	10.6
Banker	3	0.7
Civil servant	112	27.7
Farmer	11	2.7
Housewife	107	26.4
Security	3	0.7
Trader	126	31.1
Place of residence		
Jos North	111	27.4
Jos South	294	72.6

The ages of the caregivers ranged from 20 to 49 years with the age group 30- 34 having the highest representation (34.1%). Majority were females (85.9%) and married (96.5%) in a monogamous setting (78.0%). Most of the caregivers either had no form of education (31.1%) and trading was the most common occupation (31.1%). This is shown in table 2.

Table 4. Rubella IgG Antibody results of school children 0-10 years tested in Jos

	Rubella IgG Positive		Rubella IgG Negative		
Age group	**Frequency (n)**	**Percentage %**	**Frequency (n)**	**Percentage %**	**Prevalence of IgG %**
1-2	33	9.8	10	14.5	8.2
3-4	45	13.4	14	20.3	11.1
5-6	77	22.9	18	26.1	19.0
7-8	97	28.9	16	23.2	24.0
9-10	84	25.0	11	15.9	20.7
Total	**336**	**100**	**69**	**100**	**83.0**

A total of 336 (83.0%) children tested positive for rubella IgG antibodies. 28.9% of this were between the age group 7-8, accounting for 24.0 % of all children tested. 9.8% of those that tested positive were between the age group 1-2 accounting for 8.2% of children tested. Of all the children that tested negative, 26.1% were between 5 and 6 years old.

Table 5. Rubella IgM antibody results of school children 0-10 years tested in Jos

	Rubella Ig M positive		Rubella IgM negative		
Age group	**Frequency (n)**	**Percentage %**	**Frequency (n)**	**Percentage %**	**Prevalence of IgM %**
1-2	0	0.00	43	10.9	0
3-4	1	11.1	58	14.7	0.3
5-6	1	11.1	94	23.7	0.3
7-8	4	44.4	109	27.5	1.0
9-10	3	33.3	92	23.2	0.7
Total	**9**	**100**	**396**	**100**	**2.2**

Only about 2.2% (9) of children tested were positive for IgM showing recent infection. Of the children that tested positive for Ig M, 4(<1%) were between 7 and 8 years of age.

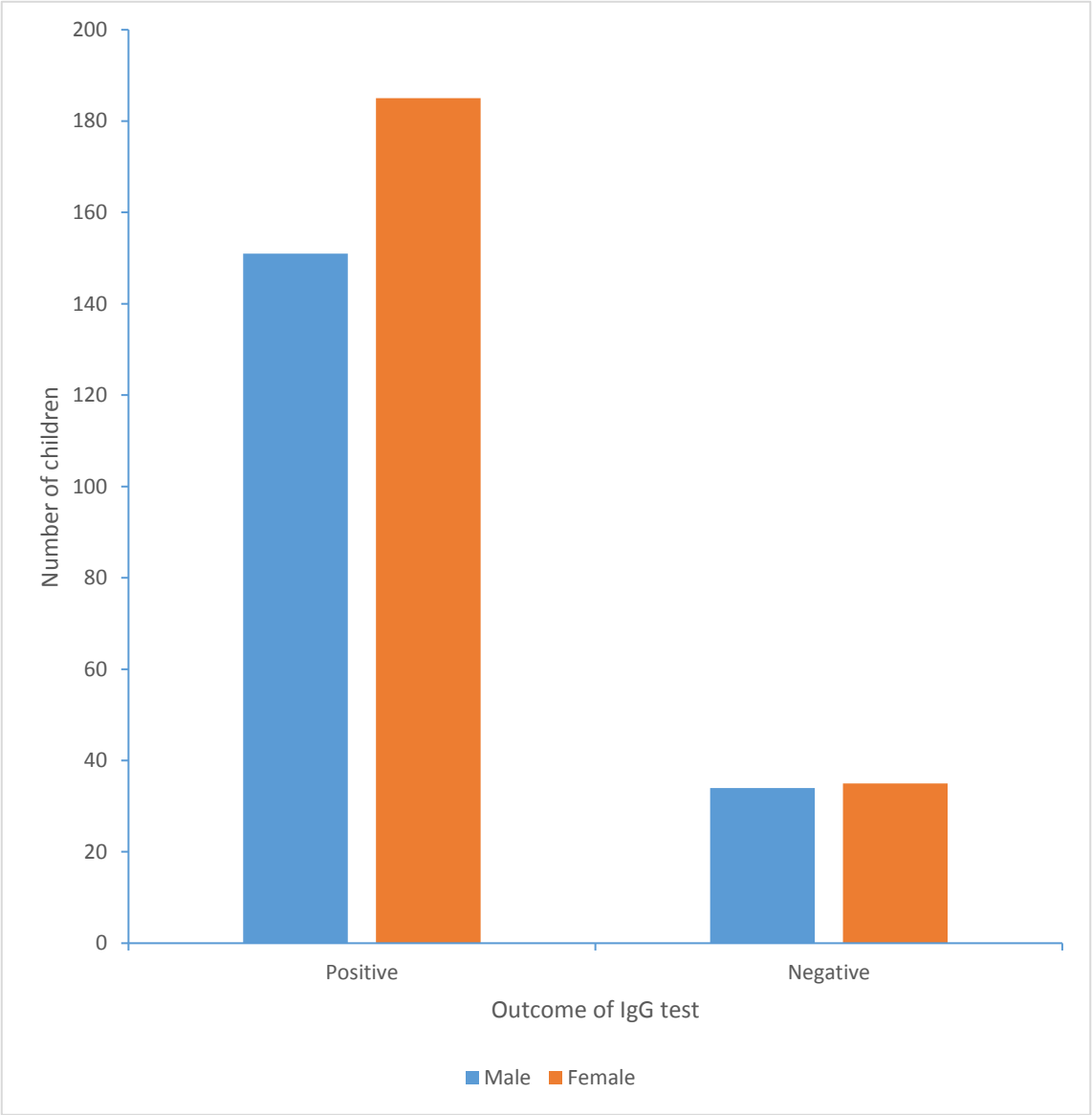

Fig 4. Distribution of IgG results among male and female children

More females tested positive to rubella IgG, however both sexes had about equal number of children who tested negative

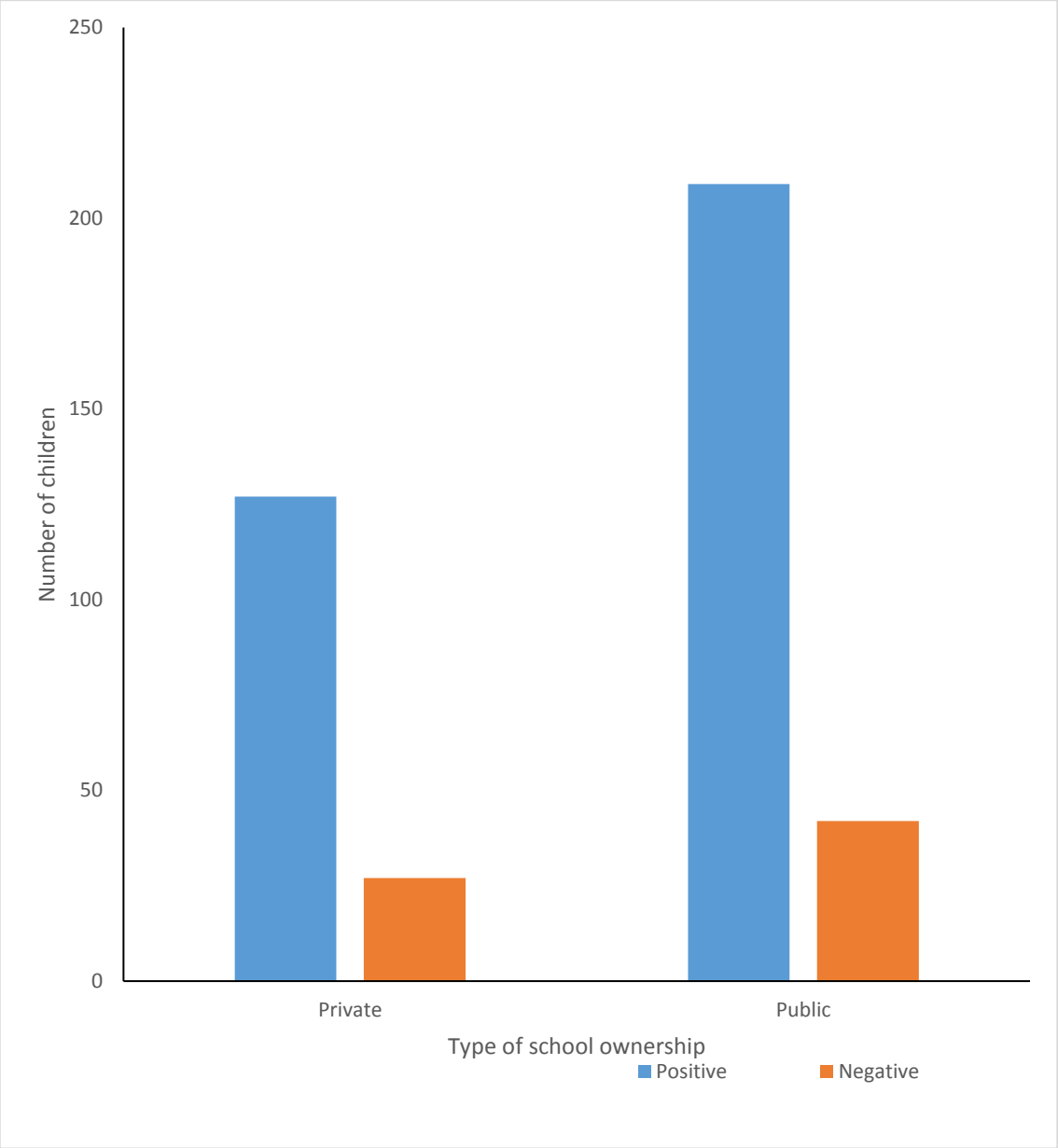

Fig 5. Distribution of IgG results among the types of school

Majority of the children that tested positive were from public schools.

Table 6. Factors associated with risk of rubella infection in school children aged 0-10 years in Jos (n=405)

Characteristic	Frequency	Percentage (%)
Number of rooms in the house		
1-2	184	45.4
3-4	196	48.4
5-6	23	5.7
7-8	2	0.5
Number of people sleeping in the room the child sleeps in		
1-2	99	24.4
3-4	199	40.1
5-6	82	20.3
≥7	25	6.2
Number of windows in the room the child sleeps in		
0	1	0.3
1	33	32.8
2	260	64.2
3	9	2.2
4	2	0.5
Cross ventilation in the room child sleeps		
Yes	242	59.8
No	163	40.2
Hand washing after wiping child's nose		
Yes	228	56.3
No	177	43.7
Sucking mucus from child's nose		
Yes	236	58.3
No	169	41.7
History of fever in the last 3 weeks		
Yes	128	31.6
No	277	68.4
History of rashes in the last 3 weeks		
Yes	43	10.6
No	362	89.4
History of flu-like illness in the last 3 weeks		
Yes	107	26.4
No	298	73.6
History of flu-like illness and rashes present at same time		
Yes	27	6.7
No	378	93.3
History of flu-like illness in household member in the last 3 weeks		
Yes	88	21.7
No	317	78.3
History of blood transfusion in the child		
Yes	34	8.4
No	371	91.6

The households had varying numbers of rooms ranging from 1 to 8 with about 40% having 3 rooms for their families. Some families had as high as 9(%) children sleeping in a room

but most (28.9%) had 4 people sleeping in a room. About 64.2% of the rooms in which the children slept had 2 windows while 59.8% claimed to have cross ventilation in their rooms.

About 31.6% of the children had a history fever in the last 3 weeks preceding the interview while 10.6% of them had a history of rashes. About 26.4% had a history of flu-like illness with only 6.7% having but symptoms and rashes present at the same time.

Table 7. Risk factors associated with rubella IgG antibodies among study Participants

Characteristic	IgG Positive	IgG Negative	Odds ratio (95% CI)	p value
Age				
≥5 years	258	45	1.76(1.01-3.08)	0.043
<5 years	78	24		
Educational level of caregiver				
No western education	123	14	2.27(1.21-4.25)	0.009
Western education	213	55		
Marital status				
Married	332	68	1.22(0.13-11.09)	0.859
Unmarried	4	1		
Occupation of caregivers				
Not employed	89	18	1.02(0.57-2.59)	0.945
Employed	247	51		
Number of people in a room				
>3	192	32	1.54(0.92-2.60)	0.101
≤3	144	37		
LGA of residence				
Jos North	108	3	10.42(3.20-33.90)	<0.00
Jos South	228	66		1
Type of school ownership				
Private	127	27	0.95(0.56-1.61)	0.835
Public	209	42		
Sex of child				
Male	151	34	0.84(0.51-1.41)	0.510
Female	185	35		
Sex of caregiver				
Male	48	9	1.11(0.52-2.39)	0.073
Female	288	60		
School designation				
Nursery	94	25	0.68(0.40-1.18)	0.170
Primary	242	44		
Number of windows in room where child sleeps				
<2	117	17	1.63(0.90-2.95)	0.102
≥2	219	52		
Crossventilation in the room child sleeps				
Yes	194	48	0.60(0.34-1.04)	0.068
No	142	21		
Washing hands after wiping child's nose				
Yes	187	41	0.86(0.51-1.45)	0.566
No	149	28		
Sucking mucus from child's nose				
Yes	196	40	1.02(0.60-1.72)	0.956
No	140	29		
History of fever in the last 3 weeks				
Yes	104	24	0.84(0.49-1.45)	0.533
No	232	45		
History of rashes in the last 3 weeks				
Yes	32	11	0.56(0.27-1.16)	0.115
No	304	58		

History of flu-like illness in past 3 weeks				
Yes	86	21	0.79(0.45-1.39)	0.406
No	250	48		
The rashes and flu-like illness present at the same time				
Yes	20	7	0.56(0.23-1.38)	0.204
No	316	62		
Household member having flu-like illness in the past 3 weeks				
Yes	72	16	0.91(0.49-1.68)	0.756
No	263	53		
History of blood transfusion				
Yes	25	9	0.54(0.24-1.21)	0.126
No	311	60		

Age ≥ 5 years, lack of Western education and residence in Jos North were significantly associated with Ig G seropositivity with OR(95% CI) 1.76(1.01-3.08), p value 0.043, 2.27(1.21-4.248), p value 0.009 and 10.42(3.20-33.90) p value <0.001 respectively at 95% CI.

Table 8. Multivariate analysis of factors associated with rubella IgG antibodies seropositivity

Factors	Odds ratio	95% CI	P-Value
Age group	0.58	0.33-1.04	0.069
Education	0.60	0.31-1.18	0.140
Cross ventilation	0.81	0.45-1.47	0.483
LGA of Residence	0.10	0.03-0.34	<0.001

In the logistic regression model, only LGA of residence was significantly associated with rubella IgG seropositivity.

CHAPTER FIVE

5.1 DISCUSSION

The presence of rubella specific IgG antibodies in an unvaccinated population is a long-term marker of previous infection, which helps to assess the immune status of that population while presence of IgM indicates recent infection. A seroprevalence of 83.0% for rubella IgG was found in this study which is in keeping with a study in Egypt which found a similar prevalence of 88.2%.[83]Similar studies done in the United States and South Africa found a prevalence of 35% and 96.3% respectively.[84]

Our study found a prevalence of 8.2% and 11.1% among 1-2 year olds and 3-4 year olds respectively similar to a study in England which found a prevalence of 20% and 27% among children in these age groups,[85] however, a similar study in India showed a seropositivity rate among children 1-5 years of 17.8% [86] A possible explanation is that these countries routinely offer rubella vaccine and understandably, a higher proportion of children would have IgG antibodies against rubella since they have received vaccines against it.

Out of 405 children tested for IgM antibodies to rubella, only 9 (about 2.2%) were positive for IgM showing recent infection. This is in contrast to a serosurvey conducted in 2010 in Jos where they found IgM antibodies prevalence in children 10 years and below to be 45.2%.[24] However, their research was hospital based and only ill children were sampled probably explaining the reason for the higher prevalence of IgM in their study.

The rubella IgG positivity of the children assayed was highest among caregivers within the age group 30-34 and 35-39 and were found to be 34.1% and 27.4% respectively. This is in contrast to a study done in Jos which showed that rubella seropositivity decreased with increase in age of caregivers.[24] This could be attributed to the fact that caregivers at these

ages are more active and tend to visit congregate environment frequently with their children and thereby exposing their children to several risk of contracting rubella via contact or respiratory route.[24]

Children whose caregivers were full time house wives had the highest prevalence of 26.4%. Bankers, civil servants, traders, and artisans had seroprevalence rates of 0.9%, 19.9%, 25.3% 9.8% respectively. This is similar to a study in Jos on the seropravlence of rubella antibodies where children whose mothers were housewives also had the highest prevalence of 21.5%.[24] This is probably due to the fact that full time housewives are less likely to go out and seek knowledge on preventive measures in tackling diseases and general health promotion, in addition to the fact that they have more contact time with the children.

Children whose caregivers had no form of education were the ones with the most seropositivity (33.9%). This contrasts the findings in a similar study done in Jos in which children with the least seropositivity were those whose mothers had no education (1.1%).[24]However the findings in our study were in keeping with findings in a study among German children in which seropositivity was also highest among children whose caregivers were uneducated.[87] Education generally leads to greater access to information on health promotion and preventive practices and thus may explain why our study showed that those with no education had children with the highest seropositivity. Lack of Western education among caregivers was statistically significant at 95%CI (1.21-4.25) p value 0.009 and those without Western education were 2.25 times more likely to be seropositive than those who had Western education.

Children whose caregivers were married were the ones with highest seropositivity 323 (96.1%). This is in consonance with a study done previously in Jos.[24] The possible reason may be due to the fact that most of the caregivers of the children studied were married

391(96.5%) and also probably as a result of high level of contact/interactions at the family level which promotes rubella transmission.

Sex distribution of the seropositive children in our study showed that there was no significant association between sex and seropositivity. The same was observed in a similar study in Jos in 2010[24] as well as another study conducted in 2002 in Bolivia,[88] This however contrasts with findings in a German study where females (girls) were found to be more likely to be seropositive to rubella antibodies.[87] The probable reason for this was because parents often decide to get their daughters vaccinated against rubella more often than their sons because of the possibility of the daughters growing into women and the risk of giving birth to babies with CRS if not vaccinated.[79]

Age ≥5 years was significantly associated with rubella seropositivity with CI 1.01-3.08 at 95% CI and p value 0.043. This is in keeping with fact that acquisition of rubella antibodies occurs more in school age children.[85]

LGA of residence was also significantly associated with rubella seropositivity as residents of Jos North were more 10 times more likely to be have rubella specific antibodies than residents of Jos South. A study in Tanzania showed that living in rural areas was found to be associated with rubella seropositivity.[89] Even though both LGAs are urban in nature, the probable reason accounting for this could be due to the crowded nature of most schools visited in Jos North as against Jos South. Also, Jos North has fewer schools and greater general population than Jos South fostering the fast and easy spread of rubella.

Multivariate regression analysis was done on the significant variables found on bivariate analysis which included age group ≥ 5years, acquiring Western education, and LGA of residence and only LGA of residence was found to still be significantly associated with

rubella IgG positivity further buttressing the significant association found in the bivariate analysis 0.03-0.34 at 95% CI and p value of <0.001.

Marital status of caregivers had no significant association with rubella seropositivity at 95% CI and p value of 0.859. A similar outcome was found in the study in Jos in 2010.[24] This was possibly due to the fact that almost all (96.5%) the women studied were married women. Occupation of caregivers also had no significant association with rubella serprevalence at 95% CI, p value of 0.945. This is in keeping with the study done in 2010 in Jos possibly due to the fact that most of the women would have a basic knowledge of health promotion and disease prevention practices no matter their occupation since the whole areas studied were urban in nature.

Number of windows in room where child sleeps, crossventilation in the room child sleeps Washing hands after wiping child's nose and sucking mucus from child's nose all did not have any significant association with rubella seropositivity at 95% CI and p values of 0.102 0.068, 0.566 and 0.956 respectively

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Rubella is recognised as a public health challenge in developing countries like Nigeria and its control is essential in eliminating congenital rubella syndrome. It has been seen that, even though, a large proportion of individuals are seropositive to rubella IgG antibodies (83%) from this study about 17% may still be susceptible to rubella. This may be due to the rubella vaccine not being part of the routine immunization schedule in Nigeria.

The study showed that only 2.2% of the children had IgM antibodies and age of children ≥ 5 years, place of residence and lack of Western education were significantly associated with rubella seropositivity however, only place of residence was really significantly associated with rubella seropositivity on multivariate analysis.

6.2 RECOMMENDATIONS

1 A significant number of individuals are not immune to rubella, because of the enormity of the proportion of individuals with samples negative for the rubella IgG antibody in them. It is imperative that the government of Nigeria should ensure that rubella vaccine is made available and routine to avert the menace of congenital rubella syndrome.

2 Awareness campaigns should be carried out to ensure the public gets enlightened about rubella and the dangers of CRS by the Federal government of Nigeria.

3 Information, education and communication materials should be made available for rubella and CRS from the Federal Government down to the primary health centres and not restricting to measles only.

4 Information should be shared between major stakeholders such as epidemiologists and health care providers and policy makers.

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APPENDIX 1

CONSENT FORM

Ahmadu Bello University Zaria and Nigeria Field Epidemiology and Laboratory Training Program, No 50 Haile Selassie Crescent, Asokoro Abuja.

Part 1: Parental consent

Title of the research: Seroprevalence of Rubella virus children 10 years and below in Jos.

Name(s) and affiliation(s) of researcher(s) of applicant(s): This study is being conducted by Waziri S. Hyelshilni of the Ahmadu Bello University Zaria and the Nigeria Field Epidemiology and Laboratory Training Program.

Purpose(s) of research: Rubella, also known as German measles or three-day measles, is an infection caused by the Rubella virus. It is often mild especially in children with a lot of people not realizing that they are sick. Infection during early pregnancy may result in a child born with congenital rubella syndrome (CRS), stillbirth or miscarriage. This study will help to identify the children that pose serious risks to their mothers.

The study may help in considering the re-introduction of Rubella vaccine into the routine immunization programme.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

We are recruiting 424 participants into this study from Jos North and South. We will use nursery and primary schools within the two LGAs. Your child's school has been randomly selected to be one of 22 schools to participate in this study. Each participant will be asked a series of questions to be filled in a questionnaire. We will collect 5mls of blood from the child.

Expected duration of research and of participant(s)' involvement:

We expect the whole procedure to take not more than about 20 minutes for each participant.

Risk(s):

The blood sample collection may produce minimal pain which will ease off within a very short while.

Costs to the participants, if any, of joining the research:

Your child's participation in this research will not cost you financially .

Benefit(s):

Information from this study will serve as a follow up to the earlier study carried out to help determine the trend of rubella infection among children and to institute control measures.

Outcome of the study will help in planning, strategizing and probably implementing surveillance systems for rubella infection to stem the tide of the infection at various level of prevention.

Confidentiality: All information collected in this study will be given code numbers and no name will be recorded. This cannot be linked to you in anyway and your name or any identifier will not be used in any publication or reports from this study. We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study. As part of our responsibility to conduct this research properly, the ethics committee may have access to these records.

Voluntariness: The participation of your child in this research is entirely voluntary.

Alternatives to participation: If you choose not to allow your child to participate, your child will not be penalized in anyway.

Due inducement: Your child will not be paid any fees for participating in this research.

Right to Refuse or Withdraw: Can I choose not to be in the research? Can I change my mind?

Your child does not have to be in this research. No one will be mad or disappointed with your child if you say no. It's your choice. You can think about it and tell us later if you want. You can say "yes" now and change your mind later and it will still be okay.

Sharing the Findings:

When we finish the research, we will not tell you personally about our findings. However, we will report the outcome of the research in form of publications so that other scientists and the public would know about the research and what we found. We will also make recommendations to the Nigerian government about the outcome of the study.

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE: _____ SIGNATURE: _____

NAME: _____

Statement of person giving consent:

I have read the description of the research and have had it explained to me in language I understand. I have also talked it over with the researcher to my satisfaction. I understand that the participation of my child is voluntary. I know enough about the purpose, methods, risks and I have read the description of the research and have had it explained to me in language I understand. I have also talked it over with the researcher to my satisfaction. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want my child/ward to take part in it. I understand that I may freely stop my child from being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself. I therefore, consent to the use of my child's blood for the seroprevalence of rubella virus among children 10 years and below. I also give permission for the leftover specimen of my child/ward to be kept for future research.

DATE: _____ SIGNATURE: _____

NAME: _____

Detailed contact information including contact address, telephone, fax, e-mail and any other contact information of researcher(s), institutional HREC and head of the institution:

This research has been approved by the Jos University Teaching Hospital (JUTH), Institutional Health Research Ethical Committee and the Chairman of this Committee can be contacted at JUTH Permanent site Lamingo, Jos. The phone number and email address are +234 7034502269 and juth@infoweb.abs.net respectively. In addition, if you have any question about your participation in this research, you can contact the researcher, Dr Waziri S.Hyelshilni at Department Medical Microbiology Jos University Teaching Hospital, Lamingo, Jos.

Ahmadu Bello University Zaria and Nigeria Field Epidemiology and Laboratory Training Program.

Parental assent form

Name of Investigator: Waziri S. Hyelshilni

Name of Organization: Ahmadu Bello University Zaria and Nigeria Field Epidemiology and Laboratory training Program

Title of Project: Seroprevalence of Rubella virus in children 10 years and below in Jos.

This Informed Assent Form has two parts:

- **Information Sheet (gives you information about the study)**
- **Certificate of Assent (this is where you sign if you agree for your child to participate)**

You will be given a copy of the full Informed Assent Form

Part I: Information Sheet

Introduction

My name is Waziri S, Hyelshilni and I want to research about Seroprevalence of Rubella virus among children 10years and below in Jos. I am going to give you information and invite you to be part of a research study. You can choose whether or not you want to participate. We have discussed this research with your parent(s)/guardian and they know that we are also asking you for your agreement. If you are going to participate in the research, your parent(s)/guardian also have to agree. But if you do not wish to take part in the research, you do not have to, even if your parents have agreed. You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. You do not have to decide immediately. There may be some words you don't understand or things that you want me to explain more about because you are interested or concerned. Please call me at any time and I will take time to explain to you.

Purpose:

Rubella, also known as German measles or three-day measles, is an infection caused by the Rubella virus. . It is often mild especially in children with a lot of people not realizing that they are sick. Infection during early pregnancy may result in a child born with congenital rubella syndrome (CRS), stillbirth or miscarriage. This study will help to identify the children that pose serious risks to their mothers.

The study may help in considering the re-introduction of Rubella vaccine into the routine immunization programme.

Choice of participants:

We are collecting blood sample from children 10 years and below to test for Rubella antibodies.

Participation is voluntary: Your child does not have to be in this research if you don't want him/ her to. It's up to you. If you decide not to let your child be in the research, it's okay and nothing changes. Even if you say "yes" now, you can change your mind later and it's still okay.

I have checked with the child and he/she understands that participation is voluntary _____

Procedures: We are recruiting 424 participants into this study from Jos North and South. I will be asking you a series of questions to be filled in a questionnaire. We will collect 5mls of blood from him/her.

Discomforts: The process of collecting the blood sample might hurt for just a few seconds. That should go away shortly after collection.

I have checked with the child and they understand the risks and discomforts _____ (initial)

Benefits: Nothing might directly happen to your child. But this research might help us to put in place effective ways to prevent rubella infection.

I have checked with the child and they understand the benefits_____ (initial)

Confidentiality: We will not tell other people that your child is in this research and we won't share information about you to anyone who does not work in the research study. Information about you that will be collected from the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is.

Sharing the Finding: When we finish the research, we will not tell you personally about our findings. However, we will report the outcome of the research in form of publications so that other scientists and the public would know about the research and what we found. We will also make recommendations to the Nigerian government about the outcome of the study.

Further research: We would keep the leftover of your sample for further research.

Right to Refuse or Withdraw: Your child does not have to be in this research. No one will be mad or disappointed with her if you say no. It's your choice. You can think about it and tell us later if you want. You can say "yes" now and change your mind later and it will still be okay.

Who to Contact: You can ask me questions now or later on 08034526494.

If you choose that your child/ward be part of this research I will also give you a copy of this paper to keep for yourself your child. You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

Nigeria Field Epidemiology and Laboratory Training Program, No 50 Haile Selassie Crescent, Asokoro Abuja.

Certificate of Assent

I understand the research is about testing the sample my child will provide for antibodies to rubella virus. I understand that my child will give her blood sample for the tests. I also understand that the left over specimen of my child would be kept for future research.

I have read this information (or had the information read to me) I have had my questions answered and know that I can ask questions later if I have them.

I agree for my child to take part in the research.

Name of child _____

Signature of Parent: _____ Date: _____

I have given the potential participant the assent form to take home to parent read and provided them with contact number for the opportunity to ask questions. I confirm that the individual has given assent freely.

Name of researcher_____

Signature of researcher_____

Date_____

Statement by the researcher/person taking consent

I have given out the information sheet to the potential participant to take home to his/her parent, and to the best of my ability made sure that I presented to them that blood sample will be collected

I confirm that the child has brought back the form and was given an opportunity to ask questions about the study, and all the questions asked by her have been answered correctly to the best of my ability. I confirm that the parent has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this assent form has been provided to the participant.

Print Name of Researcher/person taking the assent_____

Signature of Researcher /person taking the assent _____

Date _____

Copy provided to the participant _____ (initialed by researcher/assistant)

Parent/Guardian has signed an informed consent and assent form ___Yes ___No _____(initialed by researcher/assistant)

SEROPREVALENCE OF RUBELLA ANTIBODIES AND THE DETERMINANTS AMONG CHILDREN 10 YEARS AND BELOW IN JOS, PLATEAU STATE

Questionnaire for Care givers

Date of Interview _____

Name of interviewer _____

Questionnaire ID _____

Name of School _____

Private ☐ Public ☐

Primary ☐ Primary ☐

Nursery ☐ Nursery ☐

Sociodemographic characteristics_____

1. Age of child at last birthday _____
2. Age of caregiver at last birthday (Years) _____
3. Sex of child (a) Male (b) Female
4. Sex of caregiver (a) Male (b) Female
5. Marital status (a) Married (b) Singe (c) Divorced (d) Widowed
6. Type of marriage (a) Monogamy (b) Polygamy
7. Occupation _____
8. What is your highest educational qualification? _____
9. What year did you obtain your highest qualification? _____

Social history

10. How many rooms do you have in your house? _____
11. How many people sleep in a room? _____
12. How many windows are in the bedroom where the child sleeps in?

_____

13. Is there cross ventilation in the room? (a) Yes (b) No
14. Do you wash your hands after wiping the child's nose? (a) Yes (b) No
15. Do you suck mucus from your child's nose? (a) Yes (b) No

Past Medical History

16. Is there a history of fever in the last 3 weeks? (a) Yes (b) No
17. Has the child had 'flu-like' illness in the past 3 weeks? (a) Yes (b) No
18. Were there rashes in the last 3 weeks?
19. Were the rashes and fever/ 'flu-like' illness present at the same time?
20. Is there anybody in the household who has had 'flu-like' symptoms in the past 3 weeks? (a) Yes(b) No
21. Is there any history of blood transfusion in the child?

Thanks for your participation.

JOS UNIVERSITY TEACHING HOSPITAL
JOS, NIGERIA

Phone: 073-450226-9
E-mail: juth@infoweb.abs.net

Cables & Telegram: JUTH
P.M.B. 2076
JOS

JUTH/DCS/ADM/127/XIX/6533
Ref:.....

11th April, 2016.
Date:.....

Dr. Waziri Samuel Hyelshilni,
Nigeria Field Epidemiology and
Laboratory Training Program,
No. 50 Haile Selassie Crescent
Asokoro, Abuja.

RE: ETHICAL CLEARANCE/APPROVAL

I am directed to refer to your application dated 6th April, 2016 on the research proposal titled:

"Seroprevalence of Rubella Virus Infection among Children 10 years and below in Jos"

Following recommendation from the Institutional Health Research Ethics Committee, I am to inform you that Management has given approval for you to proceed on your research topic as indicated.

You are however required to obtain a separate approval for use of patients and facilities from the department(s) you intend to use for your research.

The Principal Investigator is required to send a progress report to the Ethical Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Submission of final research work should be made to the Institutional Health Research Ethical Committee through the **Secretary, Administration Department,** please.

On behalf of the Management of this Hospital, I wish you a successful research outing.

Hajia R. Danfillo
For: Chairman, MAC

INSTITUTIONAL HEALTH RESEARCH ETHICAL COMMITTEE
DESPATCHED
11 APR 2016
JOS UNIVERSITY TEACHING HOSPITAL, P.M.B 2076 JOS

PLATEAU STATE UNIVERSAL BASIC EDUCATION BOARD

Telephone: 073 - 464260.

Ref: _____
Plateau State Universal Basic Education Board,
Private Mail Bag 2063,
Jos,
Plateau State,
Nigeria.

All correspondence should be directed to the Executive Chairman

Date: 24th June, 2016 20____

Dr. Waziri Hyelshilni Samuel
Nigeria Field Edidemiology Training Program
No. 50 Haille selassie Crescent Asokoro,
Abuja.

RE – INTRODUCING DR. WAZIRI GYELSHILNI SAMUEL, RESIDENT, NIGERIA FIELD EPIDEMIOLOGY AND LABORATORY TRAINING PROGRAM NFELTP

The Board is in receipt of your letter dated 20th June, 2016 intending to carry out a research on "seroprevaleance of Rubella among children aged ten (10) years and bellow" in the Public Primary Schools in Jos North and South.

I have been directed to write and convey the Board's approval to you to enable you carry out the research but the Board want to know the number of the schools to enable it communicate the education Secretaries.

Mrs. Sarauniya Mallum
Director, Primary Education
For: Executive Chairman
Plateau SUBEB, Jos

GOVERNMENT OF PLATEAU STATE

MINISTRY OF HEALTH HEADQUARTERS

P.M.B. 2014, JOS, PLATEAU STATE

MOH/MIS/202/VOL.T/X

10th June, 2016

Dr. Waziri Hyelshilni S.
Nigeria Field Epidemiology and Laboratory Training Programme
No. 50, Haile Selassie Crescent,
Asokoro Abuja

RE: REQUEST FOR PERMISSION TO CARRY OUT RESEARCH

I have been directed to refer to your communication on the above subject.

The Ministry has granted you permission to conduct the study on the proposed topic "Seroprevalence of Rubella among children aged 10 years and below in Jos, a cross sectional descriptive study targeting Nursery and Primary school children in Jos North and Jos South.

Please note that the participation of any individual or group in this study is optional.

You are requested to send a copy of your research findings to the Ministry, please.

Thank you.

Paul D. Dwagas
For: Hon. Commissioner

Prestige Diagnostics

RUBELLA IgM Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIARUB2	RUBELLA IgM Elisa	96 Tests

Intended Use:

The RUBELLA IgM Elisa is intended to be used for the detection of IgM antibodies to Rubella in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

The Rubella virus is the causative virus of the congenital rubella syndrome when infection occurs during the first weeks of pregnancy. Congenital rubella syndrome is a disease in which children suffer from hearing impairments, eye and heart defects and other lifelong disabilities, including autism, diabetes mellitus and thyroid dysfunction. This Elisa assay uses microplates coated with monoclonal IgM antibodies. Upon presentation of the sample and if it contains Rubella IgM antibodies, these antibodies combine with the monoclonal IgM antibodies coated onto the microplate. HRP Conjugated Rubella antigen is then added which forms a blue colour with a substrate. The reaction is stopped using a low pH acid solution that changes the colour to yellow. The use of a cut off control and a positive control provides the necessary absorbances to classify patient samples as being positive or negative.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	One 96 well microplate coated with monoclonal IgM antibodies. Stable when stored at 2-8°C till the expiry date.
Negative Control	1x1ml	Tris Buffer containing salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
Positive Control	1x1ml	Tris Buffer containing Rubella IgM, salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
HRP Conjugate	1x6.5ml	1vial containing Rubella antigen conjugated with HRP. Store at 2-8°C. Once opened use within 60 days.
Sample Diluent	1x11ml	Calf Serum for diluting samples. Store at 2-8°C. Once opened use within 60 days.
Wash Solution Concentrate	1x20ml	One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
Substrate A	1x7ml	One bottle containing Hydrogen peroxide in buffer. Store at 2-8°C.
Substrate B	1x4ml	One bottle containing TMB in buffer. Store at 2-8°C.
Stop Solution	1x6ml	One bottle containing H2SO4. Store at 2-30°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

NOTE 1: Do not use reagents beyond the kit expiration date.

NOTE 2: Avoid extended exposure to heat and light. Opened reagents are stable for 60 days when stored at 2-8°C. Kit and component stability are identified on the label.

NOTE 3: Above reagents are for single 96 well microplate.

Materials required but not provided:

Distilled water, Timer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection, Storage and Stability:

Serum is the sample of choice. Collect serum samples in accordance with correct medical practices. Ensure that the samples are clear and do not have suspended particles or sediments. Blood should be collected in plain red top venepuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, the samples may be stored in temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml of the specimen is required.

Procedure:

Reagent preparation:

1. **Wash Buffer:** Dilute wash buffer concentrate in the ratio of 1:40. (i.e., for every ml of the concentrate add 40 ml of distilled water) Store diluted buffer at 2-30°C for up to 60 days.

TEST PROCEDURE:

Before proceeding with the assay, being all reagents and controls to room temperature (20-27°C)

STEP 1

Preparation: Format the microplate wells for each blank well, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminium bag, seal and store at 2-8°C.

STEP 2

Addition of Sample Diluent: Add Sample Diluent 100ul (2drops) into the appropriate wells except the blank well, positive control well and the negative control well.

STEP 3

Addition of Sample: Add 10ul of the sample to the wells mixing it with the sample diluent by repeatedly pipetting in and out of the well until the liquid turns blue. Dispense 100ul of Negative and the Positive control to their respective wells. Do not dispense anything into the blank well.

STEP 4

Incubation: Swirl the microplate gently for 30 seconds to mix and cover the plate with the plate cover and incubate for 20 minutes at 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 6

Addition of HRP Conjugate: Add 1 drop (50ul) of the HRP Conjugate to each well except the blank well. Gently mix the contents of the well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at 37°C. Ensure that the incubation is done in the dark.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 9

Addition of Substrate: Add 1 drop of Substrate A (50ul) and one drop of Substrate B (30ul) into all wells except the blank well. Incubate at 37°C for 10 minutes

STEP 10

Stopping the Reaction: Add 1 drop (50ul) of the Stop solution into each well and mix gently. Shake the plate gently for 15-20 seconds to mix, till the solution changes to yellow from blue.

STEP 11

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances. Read the results within 30 minutes of addition of the stop solution. (when using a reader without the facility of a secondary wavelength use the blank well absorbance and subtract the absorbance from the negative, positive and the sample absorbances).

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.

CUT OFF OD: 2.1 x Negative Control OD.

IF THE OD VALUE OF THE NEGATIVE CONTROL IS LOWER THAN 0.05 CALCULATE CUT OFF USING 0.05 ABSORBANCE. IF THE OD VALUE OF THE NEGATIVE CONTROL IS GREATER THAN 0.05 USE THE ACTUAL ABSORBANCE.

Interpretation:

Positive for Rubella IgM: sample OD is >/= Cut off OD

Negative for Rubella IgM: sample OD is < Cut off OD

Validation:

Negative OD/Cut-off OD (Nc/CO) should be between 0.1 and 0.8

Positive OD/Cut-off OD (Pc/CO) should be between 4.5 and 18.0

Performance Characteristics:

Specificity: 100% and Sensitivity: 90%

Notes:

1. Do not mix reagents from different batches of reagents.
2. Gently mix the reagents before use.
3. Wash Buffer concentrate may have crystals under different temperature conditions. Ensure that the wash buffer concentrate is homogeneous before use.
4. Keep unused microwells in the original bag.
5. Do not use haemolytic, lipaemic, bacteria contaminated or heat inactivated samples.
6. Assay performance characteristics have not been established for visual estimations.
7. Caution should be used when evaluating samples obtained from immunosuppressed patients.
8. Run validity is determined through the performance of the positive and negative controls as well as the blank.
9. Positive and negative controls are intended to monitor reagent failure and not ensure precision at the assay cut off levels.
10. NCCLS document C24-A3: Statistical Quality Control for Quantitative measurements can be used as a guidance document for QC practices.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

Prestige Diagnostics U.K. Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

V2: rev Apr 2015

Prestige

RUBELLA IgG Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIARUB1	RUBELLA IgG Elisa	96 Tests

Intended Use:

The RUBELLA IgG Elisa is intended to be used for the detection of IgG antibodies to Rubella in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

The Rubella virus is the causative virus of the congenital rubella syndrome when infection occurs during the first weeks of pregnancy. Congenital rubella syndrome is a disease in which children suffer from hearing impairments, eye and heart defects and other lifelong disabilities, including autism, diabetes mellitus and thyroid dysfunction. This Elisa assay uses microplates coated with Rubella Antigen. Upon presentation of the sample and if it contains Rubella IgG antibodies, these antibodies combine with the Rubella Antigen. HRP Conjugated Anti Human IgG is then added which forms a blue colour with a substrate. The reaction is stopped using a low pH acid solution that changes the colour to yellow. The use of a cut off control and a positive control provides the necessary absorbances to classify patient samples as being positive or negative.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	One 96 well microplate coated with Rubella Antigen. Stable when stored at 2-8°C till the expiry date.
Negative Control	1x1ml	Tris Buffer containing salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
Positive Control	1x1ml	Tris Buffer containing Rubella IgG, salts and preservatives. Stable when stored at 2-8°C till the expiry date. Once opened use within 60 days.
HRP Conjugate	1x6.5ml	1vial containing Anti Human IgG conjugated with HRP. Store at 2-8°C. Once opened use within 60 days.
Sample Diluent	1x11ml	Calf Serum for diluting samples. Store at 2-8°C. Once opened use within 60 days.
Wash Solution Concentrate	1x20ml	One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
Substrate A	1x7ml	One bottle containing Hydrogen peroxide in buffer. Store at 2-8°C.
Substrate B	1x4ml	One bottle containing TMB in buffer. Store at 2-8°C.
Stop Solution	1x6ml	One bottle containing H2SO4. Store at 2-30°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

NOTE 1: Do not use reagents beyond the kit expiration date.

NOTE 2: Avoid extended exposure to heat and light. Opened reagents are stable for 60 days when stored at 2-8°C. Kit and component stability are identified on the label.

NOTE 3: Above reagents are for single 96 well microplate.

Materials required but not provided:

Distilled water, Timer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection, Storage and Stability:

Serum is the sample of choice. Collect serum samples in accordance with correct medical practices. Ensure that the samples are clear and do not have suspended particles or sediments. Blood should be collected in plain red top venepuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, the samples may be stored in temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml of the specimen is required.

Procedure:

Reagent preparation:

1. **Wash Buffer**: Dilute wash buffer concentrate in the ratio of 1:40. (i.e., for every ml of the concentrate add 40 ml of distilled water) Store diluted buffer at 2-30°C for up to 60 days.

TEST PROCEDURE:

Before proceeding with the assay, being all reagents and controls to room temperature (20-27°C)

STEP 1

Preparation: Format the microplate wells for each blank well, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminium bag, seal and store at 2-8°C.

STEP 2

Addition of Sample Diluent: Add Sample Diluent 100ul (2drops) into the appropriate wells except the blank well, positive and the negative control well (blank and positive well set 1 well, negative well set 2 wells).

STEP 3

Addition of Sample: Add 10ul of the sample to the wells mixing it with the sample diluent by repeatedly pipetting in and out of the well until the liquid turns blue.

Dispense 100ul of Negative and the positive control to their respective wells. Do not dispense anything into the blank well.

STEP 4

Incubation: Swirl the microplate gently for 30 seconds to mix and cover the plate with the plate cover and incubate for 20 minutes at 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 6

Addition of HRP Conjugate: Add 1 drop (50ul) of the HRP Conjugate to each well except the blank well. Gently mix the contents of the well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at 37°C. Ensure that the incubation is done in the dark.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350ul with a soak time of 20 seconds each time. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer. If a squeeze bottle is used (instead of an automatic microplate washer) then fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

STEP 9

Addition of Substrate: Add 1 drop of Substrate A (50ul) and one drop of Substrate B (30ul) into all wells except the blank well. Incubate at 37°C for 10 minutes.

STEP 10

Stopping the Reaction: Add 1 drop (50ul) of the Stop solution into each well and mix gently. Shake the plate gently for 15-20 seconds to mix, till the solution changes to yellow from blue.

STEP 11

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances. Read the results within 30 minutes of addition of the stop solution. (when using a reader without the facility of a secondary wavelength use the blank well absorbance and subtract the absorbance from the negative, positive and the sample absorbances).

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.

CUT OFF OD: 2.1 x Negative Control OD.

IF THE OD VALUE OF THE NEGATIVE CONTROL IS LOWER THAN 0.09 CALCULATE CUT OFF USING 0.09 ABSORBANCE. IF THE OD VALUE OF THE NEGATIVE CONTROL IS GREATER THAN 0.09 USE THE ACTUAL ABSORBANCE.

Interpretation:

Positive for Rubella IgG: sample OD is >/= Cut off OD

Negative for Rubella IgG: sample OD is < Cut off OD

Performance Characteristics:

Specificity: 100% and Sensitivity: 90%

Notes:

1. Do not mix reagents from different batches of reagents.
2. Gently mix the reagents before use.
3. Wash Buffer concentrate may have crystals under different temperature conditions. Ensure that the wash buffer concentrate is homogeneous before use.
4. Keep unused microwells in the original bag.
5. Do not use haemolytic, lipaemic, bacteria contaminated or heat inactivated samples.
6. Assay performance characteristics have not been established for visual estimations.
7. Caution should be used when evaluating samples obtained from immunosuppressed patients.
8. Run validity is determined through the performance of the positive and negative controls as well as the blank.
9. Positive and negative controls are intended to monitor reagent failure and not ensure precision at the assay cut off levels.
10. NCCLS document C24-A3: Statistical Quality Control for Quantitative measurements can be used as a guidance document for QC practices.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

Prestige Diagnostics U.K. Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

V2: rev Apr 2015