

CHAPTER 1

1.1 Introduction

Rubella is an infection transmitted by respiratory droplets that cause a generally mild disease with a rash and fever, primarily in children (WHO, 2000; David et al., 2001). Rubella virus causes a disease commonly known as German measles or Rubella (Wesselhoeft, 1947; Bellini et al., 2007). Rubella virus infection in women during pregnancy may cause foetal death or congenital rubella syndrome (CRS) in the infant (WHO, 2000; Grillner et al., 1993). In neonates and infants, CRS is a cause of deafness, blindness, congenital heart disease and mental retardation (WHO, 2000; Sadighi et al., 2005). The actual burden of Rubella virus is unknown for developing countries, but it is estimated that 110 000 CRS cases occur each year world-wide (Robertson et al., 1997; WHO, 2000). If infection occurs during the first trimester in pregnant women, Rubella becomes a concern in public health because it poses a significant risk of abortion and teratogenicity effects (Gregg, 1941; Lee et al., 2000). In Zimbabwe under reporting of milder rubella cases among women of child bearing age is a potential problematic bias because the measles-rubella surveillance system is primarily focused on detection and reporting of suspected measles cases.

The prevalence of rubella has been estimated in several African countries, ranging between 70-90% (Gomwalk, 1989; Onyenekwe et al., 2000; Pennap et al., 2009). In Zimbabwe confirmed rubella infections resulting in 18 clinically diagnosed CRS cases were described in the 1970s (Axton et al., 1979). Although in 2011 the World Health Organisation (WHO) and Ministry of Health and Child Welfare launched a CRS surveillance sentinel site at Harare Hospital. As of the end of 2012 the sentinel site had reported 45 clinically diagnosed cases

with 13 laboratory confirmed Rubella cases according to the Zimbabwe Measles- Rubella Laboratory records.

As other studies in Zimbabwe reveal that Rubella antibodies were highly prevalent in early childhood among the Korekore tribe, they stated that by the age of 5 years 90% of the children had rubella antibodies in 1974 (Illman et al., 1971). In 1984 Tswana and Mbengeranwa did another study in children and pregnant women in Harare using hemagglutinating inhibition test, they found out that Rubella antibodies to be higher (99%) in high density areas of the city than in low density areas (84%) (Tswana et al., 1984).

No serological study on women of child-bearing age and pregnant women has been published since Tswana and Mbengeranwa in 1984. Gomwalk *et al.*, provided an overview of published seroprevalence surveys, analysis of surveillance and laboratory data as a baseline of rubella epidemiology in Africa (Gomwalk et al., 1989). Incidence rates of rubella were reportedly high (96%) in children 5-9 years of age in 1969 (CDC, 1989). While in 1994 the Centres for Disease Control and Prevention (CDC) documented a shift in Rubella incidence to young adults and adolescents (CDC, 1994). Because of its mild nature, rubella surveillance based on clinical reports is not specific as it is related to other viral infections presenting with similar symptoms hence the need to include surveillance based on both clinical symptoms and laboratory confirmation (Ramsay et al., 1998).

Rubella virus infection is by contact with nasopharyngeal secretions of infected persons and humans are the only known host. Gregg and co-workers discovered the teratogenicity nature and the possibility of vertical transmission of rubella (Gregg et al., 1941).

Rubella is a self-limiting, mild illness that occurs during the second week after exposure to the virus (Forrest et al., 2002; Gregg, 1941). A prodromal illness consisting of fever ($< 39^{\circ}\text{C}$), malaise and mild conjunctivitis, is more common in adults (Dyne, 2008; El-Mekki, 1998). The rash is preceded by post auricular, occipital and posterior cervical lymphadenopathy by 5-10 days. Rubella infected persons have a maculopapular, erythematous and often pruritic rash which occurs in 50-80% affected individuals. The rash lasting 1-3 days starts on the face and neck before progressing down the body (David et al., 2001; Clarke, 1980). In pregnant women the virus infects the placenta and developing foetus. Infants with congenital rubella may excrete the virus for a year or more in pharyngeal secretions and urine (Bellini et al., 2007; CDC, 2001).

The virus is transmitted by the respiratory route, it initially replicates in the nasopharyngeal mucosa and then local lymph nodes (Horstmann et al., 1976; Plotkin et al., 2004). The incubation period ranges from 12 to 23 days, with an average of 18 days. Viraemia occurs 5-7 days after exposure and results in viral spread to different organs of the body. Rubella virus can be detected in nasopharyngeal samples from 1 week before the onset of the rash to 2 weeks after, with maximum shedding of the virus occurring 1-5 days after rash onset (Reef et al., 2002; Wesselhoeft, 1947; White et al., 1994).

Detection of Rubella ribonucleic acid (RNA) directly from clinical samples (throat swabs) is a critical factor in early laboratory diagnosis of recent congenital infection, in addition to the detection of Rubella specific immunoglobulin M (IgM) in blood samples (CDC, 1989; El-Mekki et al., 1998). The immunocolorimetric assay (ICA) is a technique whereby Vero/hSLAM (human signalling lymphocyte activation marker) cells are stained to identify

the presence of the Rubella virus using an ordinary light microscope, since the cytopathic effect (CPE) of Rubella can be difficult to detect in routine virus isolation techniques (Frey et al. 1998; Jin et al. 2007).

The cross sectional analytical study determined the immune status against Rubella infection in pregnant women 18-40 years of age using an enzyme linked immunoabsorbent assay (ELISA) of Rubella virus immunoglobulin (Ig) G. Whilst the retrospective analytical study determined the incidence of Rubella virus during the 2009-11 Measles outbreak. This study determined the proportion of pregnant women in Harare who show previous exposure to Rubella virus infection (92.2%) and factors that influence immunity to Rubella virus infection among pregnant women attending antenatal clinics. So far there are no recent Rubella seroprevalence studies that have been done in Zimbabwe. The cross sectional study characterised the pregnant women that were immune to Rubella virus in Harare and the retrospective study analysed the incidence of rubella (22%) during the Measles/Rubella outbreak of 2009-11 in Zimbabwe.

The WHO carried out a survey in 2009 for the prevaccine period on how to reduce the risk of Rubella infection and Congenital Rubella Syndrome in Zimbabwe using effective vaccination coverages of above 80% not considering that Zimbabwe has not included the Rubella vaccine in the immunisation programme (Goodson et al., 2011; CDC, 2010).

Gomwalk and Ahmad, stated that the prevalence of Rubella in the Zimbabwe among children was >80% in 1989 (Gomwalk et al., 1989). In their survey they were using hemagglutinating inhibition test to detect Rubella antibodies in the 11-18 years age group and came up with 93% antibody prevalence. They then came up with an average of 80%

antibody prevalence for Gambia, Egypt, Zimbabwe, Mali and Kenya. This study intended to determine seroprevalence of Rubella infection in pregnant women which is the first of its kind in Harare. In this study laboratory surveillance data of Rubella was analysed to determine the trends of Rubella infection if it is still a childhood disease and to determine whether it has changed since 1989.

1.2 Literature Review

1.2.1 Classification

Rubella virus is an RNA virus that is the of the *Rubivirus* genus, within the *Togaviridae* family which is a non-segmented positive sense, single stranded RNA virus (Frey, 1994; Frey et al., 1998). The Rubella virus genome has 10 000 nucleotides and encodes five protein products, including three virion proteins. The capsid protein (C) and two envelope glycoproteins E1 and E2 (Frey et al., 1993; Frey et al., 1998). Some authors have used the E1 gene sequence for genotyping and phylogenetic analysis of Rubella virus isolates (Frey et al., 1993; Katow et al., 1997; Reef et al., 2002). According to Frey et al., Rubella genotype 1 (RG1) has maximum diversity at the nucleotide level of 5% and is mostly found in Europe, Asia and North America (Frey et al., 1993; WHO, 2004; Zheng et al., 2003). Recently Italy had a distant phylogenetic branch differing from RG1 virus by 8-10% which now is referred to as RG2 (Bosma et al., 1996; Frey et al., 1998; Katow et al., 1997; Zheng et al., 2003). Both the CDC and WHO has a gene bank with the above mentioned sequences and nomenclature (WHO, 2004). They stated that due to limited sampling, the geographic range of RG2 has not been determined and in Zimbabwe there is no current or existing database for the Rubella virus genotype and phylogenetic analysis.

1.2.2 Virus structure

The Rubella virion is 60nm with a nucleocapsid 30-35nm in diameter and polymorphic in shape with often elongated multi or aberrant core (David et al., 2001; Jin et al., 2007). Rubella nucleocapsid has a single positive-strand 40S RNA molecule surrounded by 32 capsomers arranged in T=3 icosahedral symmetry (Frey et al., 1993; Katow et al., 1997). A lipoprotein envelope with two membrane-spanning glycoproteins E1 and E2 surrounds the nucleocapsid (Figure 1.1) (Jin et al., 2007; Lee et al., 2000).

The Rubella virus genome contains 30% guanosine (G) residues and 39% cytidine (C) residues (Frey, 1994). The genome contains two long, non-overlapping open reading frames (ORFs) and a 5' proximal ORF encompassing roughly two-thirds of the genome, which encodes non-structural proteins involved in viral RNA replicating, and a 3' proximal ORF encompassing the other one-third of the genome, which encodes the virion proteins (Frey et al., 1998; Katow et al., 1997; Lee et al., 2000). The order of the coding sequences for the virion proteins within this ORF is 5'-C-E2-E1-3', and the proteins are cleaved co-translationally by a cellular protease, signal endopeptidase (Figure 1.2) (Lee et al., 2000; Zuckerman et al., 2000).

According to Lee and Bowden, the Rubella virion has an electron lucent spherical core with multiple copies of the capsid protein and a single copy of the viral RNA genome. The spherical core is surrounded by host-derived lipid bilayer of 6 nm spikes projecting from the virion envelope (E1 and E2 glycoproteins) (Lee et al., 2000). The capsid which is nonglycosylated, phosphorylated and disulphide linked homodimer is found inside the lipid envelope, (Figure 1.1). The E1 glycoprotein is immunodominant in the humoral response

induced against the structural proteins than E2 which more difficult to determine. E1 glycoprotein has both neutralizing and haemagglutinating determinants (Bardeletti et al., 1979).

The diagram below shows the core section of Rubella virus with genomic detail, while on the right is the surface structure.

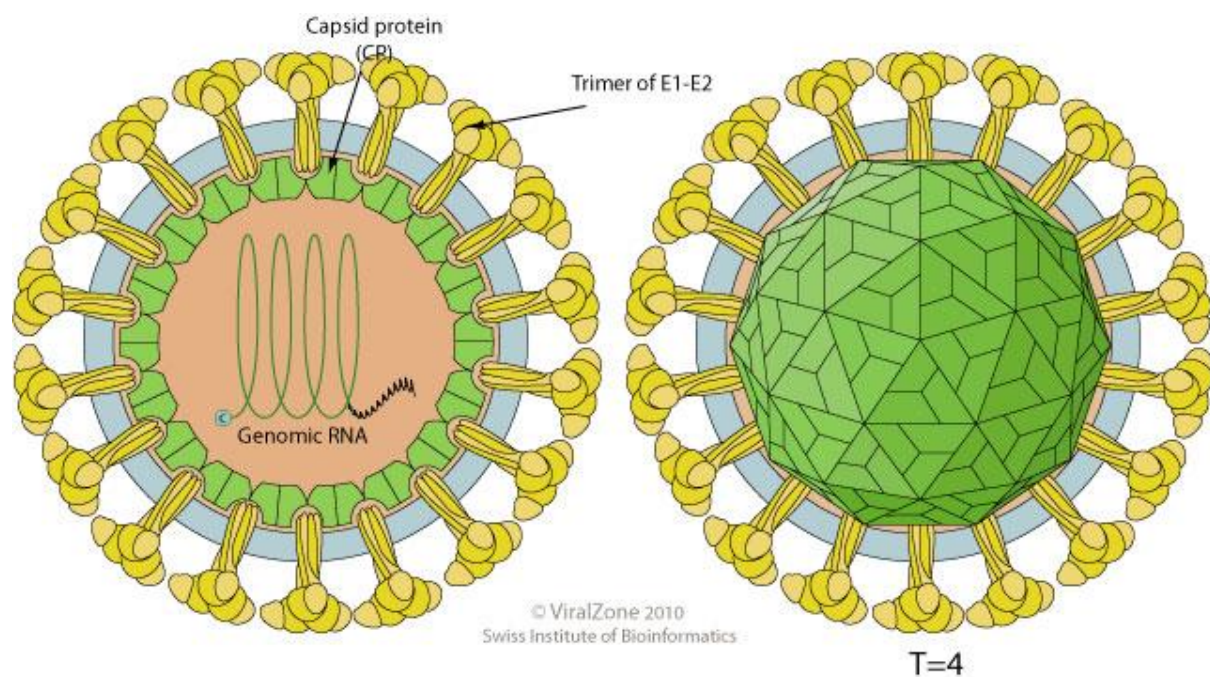


Figure 1.1 The Rubella Virion (ViralZone, 2010)

1.2.3 Genome organisation

The Rubella virus genome has two long open reading frames (ORF), the 5' proximal ORF encodes for the non-structural proteins p150 and p90. Whilst the 3' end ORF encodes the structural proteins capsid (C), E2 and E1 glycoproteins (Figure 1.2) (Dominguez et al., 1990). The 5' is capped and the 3' end is polyadenylated and works as a messenger RNA during virus replication. Amongst the RNA viruses, Rubella virus has the highest G+C content of

69.5% hence the discrepancies in the genomic sequences (Dominguez et al., 1990; Pugachev et al., 1997).

The Rubella virus genomic translation processes illustrated in Figure 1.2, shows a polyprotein precursor (p200) being translated from the 5' open reading frame of the genomic RNA. It then undergoes cleavage to make two non-structural proteins, p150 and p90 (Lee and Bowden, 2000). The positions of the amino acid motifs for the methyltransferase (M), X motif, cysteine protease (P), helicase (H), and replicase (R) are shown on the 5' ORF (Figure 1.2). The Rubella virus structural proteins are synthesized from a 24S subgenomic RNA transcribed from the 3' ORF end. Another polyprotein precursor (p100) is translated from the subgenomic RNA region which undergoes several posttranslational changes to produce the mature capsid (C), E2, and E1. (Lee et al., 2000)

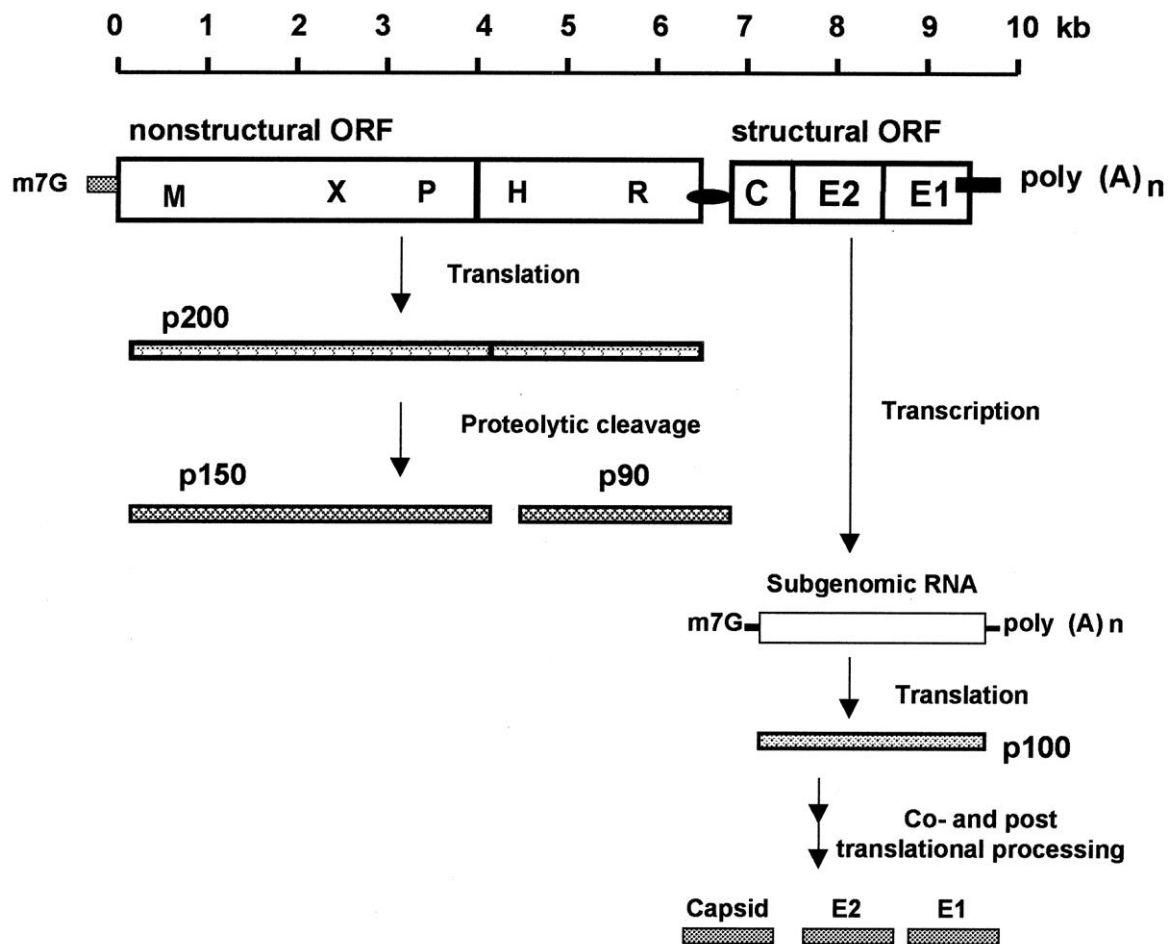


Figure 1.2 Rubella virus genomic structure. (Lee and Bowden, 2000)

1.3 Virus life cycle

1.3.1 Attachment and entry

Rubella virus replication is dependent on the type of cell being infected (Lee et al., 2000; Nahapetian et al., 1986; Ono et al., 2001; Horstmann et al., 1976). It is important not to interpret results from one cell line to another and Rubella virus replication in a cell line cannot be compared to replication of the virus in humans. Attachment of Rubella virus to the cellular receptor is assumed to occur via binding sites on the E2 or E1 glycoprotein molecules, even though the cellular receptor for Rubella virus has not been identified nor

has the receptor-binding site on one or both of the glycoproteins (Katow et al., 1997; Nahapetian et al., 1986; Ono et al., 2001). Katow and Sugiura showed that E1 and E2 glycoprotein conformational change will occur following virus attachment when the fusion of the E1 peptide is exposed. This allows the fusion of viral and cellular membrane allowing the entry of the RNA into the cytoplasm (Lee et al., 2000; Katow et al., 1997). The type of cell infected determines the number and size of Rubella virus-induced cytopathic vacuoles (Nahapetian et al., 1986; Ono et al., 2001). Jin et al., reports of co-localisation of the capsid protein with cytopathic vacuoles during RNA replication, he states that due to poor replication of rubella virus the capsid protein is suggested to be involved in RNA replication. (Jin et al., 2007; Vyse et al., 2002).

1.3.2 Replication

The rubella virus replication has the virus genomic positive, single-stranded RNA act as a template for the translation of the non-structural proteins. The genomic RNA serves as a messenger RNA for non-structural proteins and as a template for the synthesis of negative single-stranded RNA (Fauquet et al., 2005; David et al., 2001; Frey, 1994). The viral RNA replicate the sequences of the structural proteins through a complimentary negative single stranded RNA which acts as a templates and translated as a short mRNA. This short subgenomic RNA is additionally packed in a virion (Atreya et al., 2004; Bosma et al., 1996; Frey, 1994; Lee et al., 2000; Ono et al., 2001).

The long latent viral period by Rubella virus is a result of the slow replication (Frey, 1994; Bowden et al., 1984). Translation can either be early genomic non-structural and late subgenomic structural phases of viral replication which occurs in the cytoplasm (Lee et al.,

2000; Ono et al., 2001). The translation products are polyproteins which are further processed by viral and cellular proteases. The genomic and subgenomic RNAs both have 5' methylated cap and 3' polyadenylated sequences in which the minus-strand intermediate RNA is not capped (Frey et al., 1998; Ono et al., 2001). Thus processing of a separate subgenomic mRNA coding for structural proteins provides Rubella virus with an efficient mechanism to regulate the biogenesis of encapsidation, replication proteins in both temporal and qualitative manner.

1. 4 Structural proteins

1.4.1 Translation, processing and assembly

In the subgenomic mRNA, Rubella virus structural proteins are translated as a polyproteins precursor p110 (Bosma et al., 1996; Frey et al., 1998). The polyprotein precursor p110 is translocated into the endoplasmic reticulum by two separate signal peptides 23 and 20 amino acids in length located at the amino termini of E2 and E1. In the ER the rubella virus capsid protein is cleaved from E2 and E2 is cleaved from E1 (Bosma et al., 1996; Frey, 1994; Frey et al., 1998; Zheng et al., 2003). Rubella virus envelope glycoproteins E1 and E2 are targeted to the golgi as heterodimers (Bernasconi et al., 1996; Ono et al., 2001). While E2 contains a transmembrane golgi retention signal, E1 is retained in a pre-golgi compartment in the absence of E2 and appears to require heterodimerization to enable it to reach the golgi (Bosma et al., 1996; Frey et al., 1998). There were various forms of E1 glycoproteins retained intracellularly with deletions in the ectodomain, lacking the cytoplasmic and transmembrane domains, as well as the 29 C-terminal amino acid residues of the ectodomain (Bernasconi et al., 1996; Best et al., 1995).

1.5 Pathology of Rubella virus and CRS

Rubella is passed from human to human through direct or droplet contact with infected body fluids, most commonly nasopharyngeal secretions and blood through the placenta in pregnant women (Clarke, 1980; Horstmann et al., 1976; Ramsay et al., 1998; Reef et al., 2002). Patients may shed infectious virus from 7 to 30 days after infection, however infants with congenital rubella syndrome may shed the virus for up to a year (CDC, 1994; Glover et al., 1994). The average incubation period is 14 days ranging between 2 to 23 days, in which replication of the virus occurs in the nasopharynx, local lymph nodes and then spreads haematogenously throughout the body of which in pregnant women it goes to the placenta and foetus (Miller et al., 1982; Sadighi et al., 2005; Robertson et al., 1997). Systemic symptoms are caused by viral infection, but some manifestations like rash, thrombocytopenia, and arthritis are caused by an immunological body response (Ballal et al., 1997; Best et al., 1995; Miller et al., 1982).

1.5.1 Vertical transmission and risk of CRS

Viral transmission rate varies with the gestational age, but foetal infection is acquired through the placenta when maternal infection occurs (Forrest et al., 2002; Sadighi et al., 2005). After infecting the placenta, the rubella virus spreads through the vascular system of the developing foetus, causing cytopathic damage to blood vessels and damaging developing organs (White et al., 1994). Although many studies are not clear on the maternal infection rate but some studies show that in the first trimester 80% of foetus are affected of which infection rate drops to 25% in the late second trimester, in week 27-30 it increases to

35% and in week 36 it rises to close to 100% (Gregg, 1941; Grillner et al., 1993; Horstmann et al., 1976).

Congenital Rubella Syndrome (CRS) is the most serious consequence of Rubella virus and hence the public health concern (Gregg, 1941; Robertson et al., 1997). The resulting consequences of rubella infection during pregnancy in the first trimester are miscarriage, abortion, stillbirth and multiple defects (Best et al., 1995; Best, 2007). The common manifestation of CRS in most cases is deafness. At times foetal infection occurs without birth defects this is termed Congenital Rubella infection (CRI) (Robertson et al., 1997; Gregg, 1941; Cochi, 1989).

During the first trimester 90% of cases of rubella infection may develop multiple foetal defects which results in stillbirth or foetal wastage (Miller et al., 1982). At week 16 of pregnancy maternal rubella risk is reduced although sensorinual hearing deficit can still occur up to week 20 (Grillner et al., 1993). CRS is associated with several defects which are ophthalmic for example cataracts, microphthalmia, glaucoma, pigmentary retinopathy, and chorioretinitis. In auditory CRS has sensorineural deafness which is a more pronounced deficit. With the cardiac system CRS causes peripheral pulmonary artery stenosis, patent ductus arteriosus or ventricular septal defects. Aniofacial, microcephaly is the deficit of CRS (CDC, 1998; CDC, 1991). Meningoencephalitis, hepatosplenomegaly, hepatitis, thrombocytopenia and radiolucencies in the long bones are other manifestations presented by CRS in neonates (Ballal et al., 1997; Best, 2007; Bellini et al., 2007). Among the manifestations thrombocytopenia complications can be fatal. Interstitial pneumonitis can also occur in infants with CRS. CRS may infect a neonate but some survive the neonatal

period but will later have developmental disabilities which include visual and hearing impairments. Some infected neonates have an increased risk for developmental delay, including autism, type 1 diabetes mellitus and thyroiditis (Ballal et al., 1997). A progressive encephalopathy resembling subacute sclerosing panencephalitis has been observed in patients with CRS (Plotkin et al., 2004).

1.5.2 Diagnosis of Rubella Infection

Clinical diagnosis of Rubella tends to be unreliable as there are several rash-like illnesses such as Measles virus and Parvovirus B19 mimic Rubella (Best et al., 1995; Best, 2007). Laboratory confirmation of clinically diagnosed patients is essential for the diagnosis of Rubella virus, especially when patient is pregnant or if a neonate is born with CRS (Zuckerman et al., 2000). The absence of Rubella antibodies (IgG) may mean that the person has not been exposed to the Rubella virus or vaccine therefore is not protected against the virus. The presence of IgG antibodies but not IgM antibodies indicates a history of past exposure to the virus or vaccination whereas the presence of IgM antibodies indicates recent or active infection (Goh et al., 2007; Mingle, 1985; Pennap et al., 2009; Sallam et al., 2003).

The occurrence of the typical rash and lymphadenopathy may suggest the clinical diagnosis of rubella (White et al., 1994; Zimmerman et al., 2002). Rubella antibodies are detectable by several methods these include the neutralization test, hemagglutination inhibition, ELISA, and indirect immunofluorescent immunoassay (Jin et al., 2007). The virus can be recovered in cell cultures from respiratory tract secretions and in infants with congenital infection the virus can be culture from urine, cerebrospinal fluid, and blood (White et al., 1994). Viral

interference and immunoperoxidase staining assays can help detection of rubella virus in inoculated cultures. Virus isolation procedures are costly they require a relatively complicated virology laboratory hence they are rarely used except for the diagnosis of congenital rubella (Frey et al., 1998; Jin et al., 2007).

Virus infection and exposure can be accurately be detected using cell culture or the detection of viral RNA by reverse transcription- polymerase chain reaction (RT-PCR) (Zimmerman et al., 2002). Although virus isolation using tissue culture techniques is complicated because wild-type Rubella viruses usually do not produce significant cytopathic effect (CPE). Multiple blind passages of the viral culture are usually recommended hence it is time consuming and expensive (Bellini et al., 2007). Modified Immunocolorimetric assay (ICA) can detect Rubella virus infection in tissue culture, using modified Vero cells expressing the human signalling lymphocyte activation molecule (hSLAM) which has a cellular receptor found to be important for wild-type rubella infection (Frey et al., 1998; Ono et al., 2001).

Congenital rubella in neonates is diagnosed by virus isolation and serologic testing (Jin et al., 2007; Otaigbe et al., 2006; Wang et al., 2004). The affected neonate will have circulating antibodies these include transplacentally acquired maternal IgG antibody, actively produced foetal and neonatal IgM antibody. Maternal IgG antibody is detectable in the neonate and wanes during the first 6 months of life. Therefore, the persistence of IgG antibody beyond 6 months or the demonstration of IgM antibody is diagnostic for congenital rubella infection (Kremer et al., 2006; Pennap et al., 2009).

Diagnosis of maternal infection requires serologic testing for the accurate diagnosis of acute primary rubella infection in pregnancy since an important number of cases are subclinical

(Figure 1.3) (Best, 2007). A convenient, sensitive and accurate ELISA assay is used to measure rubella-specific IgG and IgM. The presence of a rubella infection is diagnosed through a fourfold rise in rubella IgG antibody titre between acute and convalescent serum specimens with a positive serologic test for rubella-specific IgM antibody. A positive rubella culture confirms the presence of the virus in nasopharyngeal excretions (Best, 2007; Plotkin et al., 2008). Serologic studies for rubella virus are best performed within 7 to 10 days after the onset of the rash and should be repeated two to three weeks later as the virus replication takes a long period. Specimens drawn from nasal, blood, throat , urine, or cerebrospinal fluid maybe positive from one week before to two weeks after the onset of the rash (Jin et al., 2007).

Flow chart showing the testing algorithm maternal Rubella infection.

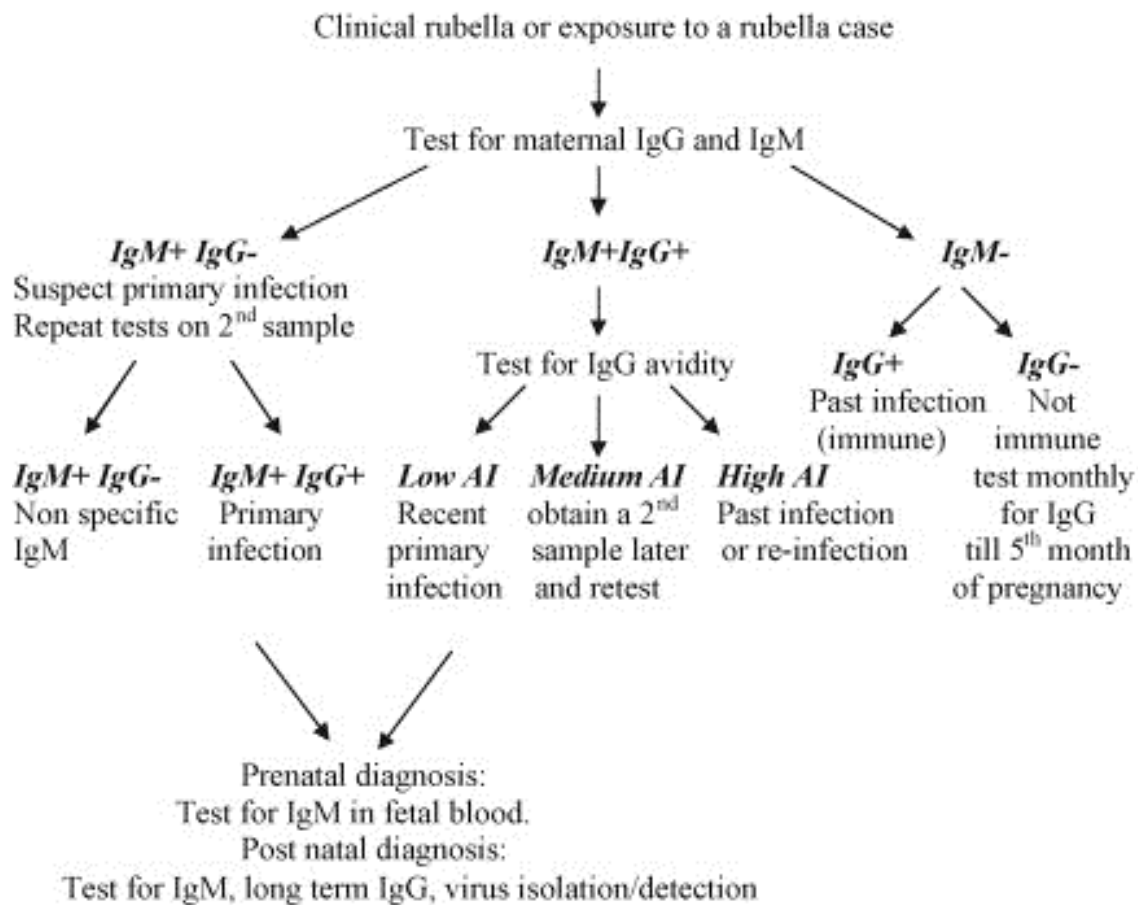


Figure 1.3 Rubella virus screening procedure (adapted from Mendelson et al.,)

It is essential that infected infants be identified as early in life as possible in order to prevent further spread of the Rubella virus. These infants should be considered infectious until they are at least one year old or until two cultures of clinical specimens obtained one month apart after the infant is older than three months of age are negative for Rubella virus (Best, 2007; CDC, 2001). In the Zimbabwean setting a sentinel site for CRS surveillance was initiated in 2009 so as to identify these infants. Early diagnosis of CRS facilitates early intervention for specific disabilities. Results of published reports show significant enhancement of speech and language development and eventual success in school for

children with hearing impairment if they are identified early and intervention begins immediately (Glover et al., 1994; Yoshinaga-Itano, 1995).

1.6 Clinical features

In any individual rubella is usually an infection of minor impact with a mild, self-limiting disease associated with a characteristic rash (Mingle, 1985; Ramsay et al., 1998). Although rubella is asymptomatic in 25% to 50% of cases, some individuals may experience mild symptoms such as low-grade fever, conjunctivitis, sore throat, coryza, head-aches or malaise and tender lymphadenopathy (Bellini et al., 2007; Goh et al., 2007). These symptoms will usually last one to five days before the onset of the scarletin form rash, which may be mildly pruritic. The characteristic rash begins on the face, spreads to the trunk and extremities. It will usually resolve within three days in the same order in which it appeared (Horstmann et al., 1976).

1.7 Vaccines

Since 1969 in the United States, several live attenuated rubella vaccines for the prevention of rubella have been licensed for use (Schoub et al., 1990; WHO, 1994). The vaccine currently in use is prepared from an attenuated rubella virus (RA27/3) which induces immunity by producing a modified mild rubella infection in recipients (Frey et al., 1993; Kremer et al., 2006; WHO, 2004). Two doses are recommended which are administered subcutaneously. The first may be given starting at 12 months (Goh et al., 2007). In countries that have introduced the vaccine, the initial dose is administered as a combined vaccine containing attenuated mumps and measles viruses as well. The second dose is given either at school entry or at entry to middle school or high school. In children Vaccine-induced

infection is usually asymptomatic, but is associated more frequently with rubella-like symptoms in women over the age of 25. Vaccine associated reactions may include fever, lymphadenopathy, arthritis and are usually mild and transient (Goh et al., 2007; CDC, 2009). Although in Zimbabwe we do not immunise routinely in some schools girls in form four are privileged to get the vaccine and those individuals going to countries which require rubella vaccine for visa application get vaccinated. Nationally Zimbabwe is yet to introduce the vaccine to everyone.

Although levels of vaccine induced antibody are lower than those produced by the natural disease, 95% of vaccines are said to seroconvert between 14-28 days following vaccination (CDC, 2009). As with all attenuated vaccines, the duration of protection may be a matter of concern. In 1982, the Centers for Disease Control reported surveillance studies on individuals enrolled in a vaccine study in 1969 (CDC, 1998). During the first 4 years after vaccination, they noted that there was a 50% drop in the hemagglutination inhibition titre, with generally stable titres after that time. Nevertheless, measurable antibody levels persisted in 97% of vaccines over the 10-year study period. In the United States studies reveal that immunity conferred by vaccination was adequate to interrupt the transmission of the rubella virus (Horstmann et al., 1976).

Immunization strategy targeting women of child bearing age who at risk of getting the rubella infection and through them pass it to their unborn children. Using vaccination programs designed primarily to provide widespread childhood immunity to rubella can also help to reduce the occurrence of disease in the community (Schoub et al., 1990; WHO, 1994). A continued downward trend in cases of rubella has been reported by the Centers for

Disease Control, with a record low of 225 cases in 1988 (CDC, 1998). Still of concern, however, is the fact that approximately 6% to 11% of post pubertal women show no serologic evidence of immunity to rubella virus. Additional emphasis is therefore being placed on immunization of this population.

1.8 Epidemiology

Rubella is found worldwide occurring in a seasonal pattern, with epidemics occurring every 5-9 years (CDC, 1998; Goodson et al., 2011; Horstmann et al., 1976). However, the extent and periodicity of Rubella epidemics is highly favourable in both industrialised and developing countries. Humans are the only known hosts for Rubella virus and it has never being detected in any other host (Gomwalk et al., 1989).

A number of studies have described the circulating Rubella viruses in the world and these viruses fall into a relatively small number of phylogenetic groups (Katow et al., 1997; Zheng et al., 2003). The WHO has used the nucleotide sequence inorder to provide the Rubella viruses clades which differ in nucleotide sequence by about 8-10% in their systematic nomenclature proposed in September 2004 which consists of two clades (1 and 2) and seven genotypes (Katow et al., 1997; Frey et al., 1998). Molecular epidemiology has supported control and elimination of vaccine preventable diseases (Frey et al., 1998).

Rubella epidemics are known have been a world-wide phenomenon. Before the introduction of a vaccine in countries such as Australia, United States of America, the United Kingdom and European Countries, rubella epidemics occurred in cycles of 6-9 year interval (Assad et al., 1985). In U.S.A, 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 (Cochi, 1989). Elsewhere, while the immune status of many populations regarding Rubella is less clear, some data have been reported. In Saudi Arabia, the antibody prevalence among girls aged 5-25 years has been reported to be 92% (El-Mekki et al., 1998). Estimates suggest that the burden of CRS in regions that had not introduced rubella-containing vaccines by 2008 may be high. In 1996 approximately 22 000 children with CRS were born in Africa (Robertson et al., 1997).

In some African Countries, 80% of children have been found to be positive for Rubella antibodies by the age of 10 years (Gomwalk et al., 1989). Post-epidemic Rubella virus antibody prevalence in Ghana has been found to be 92% among pregnant women, with susceptibility associated with a younger age (Lawn, 2000). In Eritrea, the prevalence of antibodies to Rubella has been reported to be as high as 99% in some female population (Sallam et al., 2003). In Nigeria, Rubella antibody prevalence in women of child bearing age has been reported to be 77% (Onyenekwe et al., 2005). Some of these studies have reported an early age of exposure to rubella (Gomwalk et al., 1989; Lawn, 2000). The highest sero-prevalence has been seen in age group as young as 5-9 years and in pre-school children (Clarke, 1980; Mingle, 1985). In Southern Africa, Rubella antibodies were found to be highly prevalent in early childhood among the Korekore tribe in Zimbabwe, by 5 years of age 90% of children had Rubella antibodies (Gomwalk et al., 1989).

According to Corcoran *et al.*, a seroprevalence study is an important step in addressing the Rubella infection in women of child-bearing age to determine the feasibility of routine Rubella immunization in South Africa (Corcoran et al., 2005). According to the CG6-NICE Guidelines of 2003, pregnant women should be offered evidence-based information and

support to enable them to make informed decision regarding their care. At first contact, pregnant women should be offered information about; the pregnancy-care services and options available, lifestyle considerations, including dietary information and screening test.

1.9 Treatment

There is no antiviral therapy for rubella virus infection (Horstmann et al., 1976). Patients with congenital rubella need supportive care not only in the neonatal period but also throughout life for such permanent impairments as deafness and heart defects (Atreya et al., 2004; Horstmann et al., 1976). Specific therapies to prevent CRS are not available. Many developed countries have specific vaccination programmes and maternal Rubella is rare. In developing countries or where campaigns of Rubella surveillance about preconception vaccination are inadequate, there are still cases of CRS. Advanced prenatal diagnostic techniques, invasive or not should be offered to the women especially in order to distinguish the cases without foetal damage (Glover et al., 1994).

Interferon and amantadine have been used in individual cases of CRS. Interferon has also been used in the treatment of chronic arthritis secondary to postnatal Rubella infection, again producing indeterminate results (Ahmed, 1992). Isoprinosine has been administered to patients with progressive Rubella panencephalitis but has no apparent therapeutic benefit (Tokugawa et al., 1986).

1.9.1 Management of Rubella infection in pregnant women

If a pregnant woman develops signs or symptoms of a rubella-like illness after she has recently been exposed to rubella, gestational age and her rubella immunity status should be determined (El-Mekki et al., 1998). If immunity status has been known after 12 weeks of gestation no further testing is required. CRS has not been documented after maternal reinfection beyond 12 weeks' gestation (Grillner et al., 1993). Before 12 weeks of gestation if a pregnant woman shows a fourfold rise in rubella IgG antibodies, the clinician should notify them of the chance of secondary reinfection occurring. Foetal risk for congenital infection after maternal reinfection during the first trimester has been estimated at 8% and appropriate counselling should be provided (Dyne, 2008; El-Mekki et al., 1998; Forrest et al., 2002).

Convalescent IgG and IgM rubella antibodies should be detected at gestational age ≤ 16 weeks to determine the unknown immune status. The diagnosis of recent rubella infection should include serologic testing of acute sera for both IgG and IgM rubella antibody. Acute infection is diagnosed when ELISA rubella IgM antibody tests are positive (CDC, 2001). When IgM antibodies are negative or undetectable, testing for both acute and convalescent sera for rubella IgG antibody should be done. During a rubella-like illness a specimen should be collected as soon as possible followed by a convalescent specimen two to three weeks later if the first IgM specimen was negative. Same applies for a suspected rubella exposure, the specimen should be collected immediately and if negative a convalescent specimen 4 to 5 weeks later should be collected (Figure 1.3) (CDC, 2010; Mendelson et al., 2006; Robertson et al., 1997).

Diagnostic difficulty is found with late presentation by a pregnant woman with an unknown immune status. A pregnant woman presenting 5 weeks or more after exposure to a rash illness or 4 weeks or more after onset of a rash presents a diagnostic dilemma. Especially if IgG rubella antibody test is negative and the pregnant woman is susceptible to rubella having no evidence of previous exposure (Vyse et al., 2002; White et al., 1994). If rubella IgG antibody tests positive, it shows that there is evidence of a previous infection. It is then difficult to determine the date of infection and the risk to the foetus, although a low level of antibody suggests more remote infection. Testing for IgM antibody or repeating the test for IgG antibody levels to determine whether there is a significant rise or decline may be considered (Onyenekwe et al., 2000; Pennap et al., 2009).

1.10 Justification of project

There is need to address the seroprevalence of Rubella infection in women of child-bearing age in Zimbabwe due to the teratogenicity effects of primary or secondary Rubella virus infection in the first trimester of pregnancy. Information on the molecular epidemiology of Rubella virus in Zimbabwe is lacking. Rubella infection is not a notifiable disease and there are no available reports of nationwide epidemics or incidence of Congenital Rubella Infections (CRI). Currently there is no national Rubella immunization programme. The percentage of non-immune women of child bearing age is still largely unknown. This study is prompted by the retrospective view revealed by the virology laboratory at Parirenyatwa hospital of the increase in Rubella IgM positive patient samples (683, 22% incidence) after the 2009 Measles outbreak and through the Toxoplasma gondii, Rubella, Cytomegalovirus and Hepatitis routine screen (TORCH) results. Knowledge of Rubella seroprevalence allows

one to model the incidence of CRS, thus providing an indirect measure of the burden of CRS. Rubella vaccine is not yet part of the EPI schedule in Zimbabwe and therefore Rubella virus continues to circulate. The study would assist by providing health policy makers with data when considering the inclusion of the Rubella vaccine in the Expanded Programme for Immunisation (EPI) in Zimbabwe. The seropositivity of pregnant women against Rubella virus in this study will determine the population of pregnant women immune to the virus. Whilst the seronegativity of pregnant women will show which population is susceptible to Rubella virus infection and hence posing a greater chance of passing the infection to the unborn child. This will be the first systematic study of Rubella seroprevalence analysis to be done in Zimbabwe so as to appreciate the burden of circulating Rubella virus before the introduction of the vaccine.

1.10.1 Research Questions

1. What proportion of pregnant women in Harare is immune to Rubella virus infection?
2. What factors influence immunity to Rubella virus infection among pregnant women attending ANC in Harare?
3. What is the incidence rate of Rubella in Zimbabwe.

1.10.2 Aims and Objectives

1. To perform Enzyme Linked Immunosorbent Assay (ELISA) for the detection of Rubella virus.
2. To establish the immune status against Rubella virus in pregnant women in Harare.
3. To characterise the pregnant women that are immune to Rubella virus.

4. To analyse the incidence of Rubella during the Measles/Rubella outbreak of 2009-2011 in Zimbabwe.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study design

Two studies were conducted. First a descriptive cross-sectional study was conducted in Harare involving pregnant women presenting at an antenatal clinic. Second a retrospective analytical study was done on all samples received at the National Measles and Rubella laboratory, Zimbabwe during the 2009-2011 Measles outbreak. Interviewer-administered questionnaires were used to collect socio-demographic, health and laboratory information (Appendix F) in the first study. Venous blood was collected on site, transported to the University of Zimbabwe, Department of Medical Microbiology, and National Virology Laboratory for immunological assays.

2.2 Study Population

In the descriptive cross-sectional study, the population was composed of consenting pregnant women 18-40 years of age at any gestational age of their pregnancy attending antenatal clinic at Rutsanana and Rujeko polyclinics in Harare. In the retrospective analytical study, the population was composed of all a susceptible Measles and Rubella patients presenting at any health care centre in Zimbabwe.

2.2.1 Inclusion criteria

In the descriptive cross-sectional study, any pregnant woman resident in Harare between 18-40 years who consented to take part was included. In this group, any suspicious case presenting at any Harare centre in Zimbabwe with measles or rubella clinical symptoms

were also included in the study. In the retrospective study, any susceptible Measles and Rubella case presenting at any health care centre in Zimbabwe.

2.2.2 Exclusion criteria

In the cross-sectional study, any pregnant woman not residing in Harare not giving consent to participating under 18 or older than 40 years of age. In this group any suspicious case presenting at any Harare centre in Zimbabwe without measles or rubella clinical symptoms.

In the retrospective study, any case without Measles and Rubella clinical symptoms presenting at any health care centre in Zimbabwe.

2.3 Sample size calculation

A sample size of 195 was calculated but due to limited kits for the study I decided to collect just enough samples (51) to use in the cross-sectional study, sample calculation was done using the formula below

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

Where n = sample size

Z = statistic for level of confidence (in this case 1.96)

P = expected prevalence or proportion (in this case 85% in proportion of one; P is 0.85)

d = precision (0.05)

$$n = \frac{(1.96^2)(0.85)(1-0.85)}{0.05^2} = 195$$

The sample size for the retrospective study was not calculated as all susceptible Measles/Rubella cases from the outbreak were included in the study.

2.4 Study area

Two study sites were selected Rutsanana and Rujeko polyclinics which serve high density areas in Harare.

In the Measles/Rubella survey samples were collected throughout Zimbabwe.

2.5 Study samples

2.5.1 Plasma samples (n=51)

Samples for the study were collected from consenting pregnant women attending antenatal care clinic at Rujeko and Rutsanana Polyclinics by a Registered Nurse. A pilot survey for the study was carried in Mutoko district. The specimens were collected from June to July 2012. Whole blood was collected by venepuncture in plain tubes, transported to the National Measles and Rubella Laboratory for processing, aliquoted into 2 cryovials each with at least 500µl of plasma and stored at -30°C awaiting Rubella virus IgG screening using Indirect ELISA method.

2.5.2 Zimbabwe Measles/Rubella 2009-11 serum samples (n=3 003)

In the Zimbabwean Measles/ Rubella outbreak (2009-11) blood specimens were collected routinely by venepuncture at varied Health centres throughout Zimbabwe by an attending health personal. The blood specimen was collected from patients presenting or having history of rash, fever, cough, malaise and mild conjunctivitis. Whole blood was collected in

EDTA or plain tubes was transported to the National Measles and Rubella Laboratory under cold chain (+/-4°C) for processing, aliquoted into 2 cryovials each with at least 500µl of plasma/serum and stored at -30°C awaiting Measles and Rubella virus IgM screening simultaneously using Indirect ELISA method.

2.6 Ethical consideration

The implications of Rubella virus infection was clearly explained to the study participants for them to understand the disease under investigation and type of specimens to be collected. It was explained that Congenital Rubella Syndrome and Infections affect individuals differently and there is no treatment but management is based on individual complications and options available for therapy were discussed. (Appendix D). In the Measles/ Rubella surveillance patients had a clear explanation of the disease and why it is mandatory for a blood sample to be collected so as to confirm Measles virus infection as it is a notifiable disease.

2.6.1 Ethical approval

Approval to carry out the baseline Rubella virus sero –survey from which specimens used in this study were obtained from consenting participants was granted by the Joint Parirenyatwa Hospital and College of Health Sciences Research Committee (JREC ref: IRB 123) and World Health Organisation (WHO) on behalf of Ministry of Health and Child Welfare to use data collected during the Zimbabwe Measles outbreak in 2009-11. Written permission was sought and granted from the City of Harare- Health Services Department to collect samples at the two sites for this study. (Appendix C)

2.7 Specimen collection and transport

We used Dacron swabs to collect the throat specimen. By swabbing the posterior pharynx and tonsillar areas and avoiding the tongue. The mucosa behind the uvula, between the tonsils was gently swabbed with a back-and-forth motion. The swabs were kept moist after collection. Virus can usually be detected 2-3 days before rash and up to about 14 days post rash. The optimal timing for virus isolation is day of rash through day 5 post rash. The swab was returned to the tube and labelled with participant number, age, date. Commercially available kits containing swabs and viral transport media were used (Oracol[®]). The specimen was transported to the Virology Laboratory within 48 hours and stored at -30°C. Maintaining cold chain transport to the Virology laboratory where extraction was done. Extraction buffer was added to the sponge and agitated, the fluid squeezed out and inverted into the tube. The sample was centrifuged at 3000 rpm for 5 minutes. The supernatant was separated into a cryovial, labelled as before and stored at -70°C until virus isolation was to be done.

Venous blood sample of 5ml was collected from the pregnant women into an EDTA vacutainer at the study site. The blood samples identified by the participant study number, age and date of collection were transported to the Virology laboratory under cold chain. At the laboratory the blood was recorded into the log book and centrifuged at 2000 rpm for 5 minutes to separate the serum. Serum was aliquoted into 2 cryovials (routine and backup), labelled with participant identification number, date collected and date processed then stored at -30°C.

2.9 Methods

2.9.1 Detection of Rubella IgM antibodies

Materials: Siemens Enzygnost® Anti-Rubella Virus IgM protocol was used.

In the retrospective study an indirect ELISA technique in which specific antigen was absorbed onto microtiter wells from Siemens ® was used to detect the Rubella antibody (IgM) in serum samples. Serum was treated with blue sample buffer and rheumatoid factor absorbent (Siemens, Enzygnost® Anti-Rubella Virus/IgM) before being added (150µl) to the microtiter well containing Rubella antibody and allowed to react bound to the antigen for 60 minutes in an incubator (Selecta) at 37°C. The wells were washed by an automated washer Biotek (ELx50), USA), five times using wash solution (phosphate buffer with Tween 20) and the presence of antibody bound to the antigen was detected by adding an enzyme-conjugated secondary anti-isotope antibody (100µl) and incubated for an hour at 37°C. After any free enzyme-conjugated secondary anti-isotope antibody was washed away by an automated washer (Biotek), a substrate for enzyme, (100µl) was added and incubated at room temperature for 30 minutes. After that a stopping solution was added, 100µl of 0.5% sulphuric acid and the coloured reaction product was measured using a spectrophotometric plate reader (Biotek (ELx808i), USA) at an absorbance of 450-630nm. The method was done according to the manufacturer's instructions (Appendix A).

2.9.2 Detection of Rubella virus IgG antibodies

Samples were removed from the freezer (-30°C) and reagents from the fridge (4°C) onto a work bench for an hour so that they attain room temperature (25°C). Samples were

prepared by adding 5µl of test sample, positive control, negative control and calibrators to 200µl sample diluent in separate cryotubes. The diluent was at a 1:40 dilution. Placed the required coated strips (58) onto the holder and dispensed 100µl of diluted plasma, calibrators and controls into designated wells. In the blank well 100µl of sample diluent was added in well 1A. After loading the wells were gently tapped to remove bubbles and to mix well then covered the wells. The plate was incubated at room temperature (25-30°C) for 30 minutes. The liquid was decanted from all the wells and washed 3 times with an automated washer using x10 washing buffer. After washing the wells were gently tapped onto a blotting paper to remove residue fluids, 100µl of enzyme conjugate was dispensed to each well using a multi-channel pipette and incubated at room temperature for 30 minutes. The fluid was decanted from the wells and wells were washed 3 times with washing buffer. The wells were blot dried by paper towel and added 100µl of TMB chromogen substrate to each well and incubated at room temperature in the dark. 100µl of hydrochloric acid was added to stop the reaction after 30 minutes. The holding plate was put into the microwell reader and was read at 450-650nm. (Appendix B)

2.9.3 Quality control

The kit had positive and negative controls which were ran with the samples to pass the ELISA run being done after validating the results using the outcome of the controls all the test done were valid. Also included were in-house positive samples stored in the laboratory for quality assurance. These were samples from Zimbabwe which were tested by NICD, South Africa to confirm positive status. Calculation of results was done by a software

package KC4 and transferred to the Dade Behring Excel package which validates the assay by calculating lower and upper limits for the Optometric densities.

2.4 Data analysis

Data from the two studies were entered into an Excel spread sheet and imported to the statistical package (STATA 11.0) for analysis. Descriptive summaries were calculated using proportions for categorical variables (age, parity, education, employment status, vaccination status and gestational age). EPI Info data from the Measles/Rubella surveillance was imported to Excel and then to the STATA package for analysis. Significance of differences was determined using a one-way analysis of variance (ANOVA). Bartlett's test for equal variances was determined within groups.

CHAPTER 3

3.1 Results

Demographic data of pregnant women

Derived from the data collected through interviewer administered questionnaires during the study, the demographic data of respondents shown in the table 3.1 below showed that from the 51 pregnant women sampled in the study, 49.02% (25) were married, 39.22% (20) were single mothers, 9.8% (5) were divorced and 1.96% (1) were widowed. On the level of education, 19.61% (10) reached tertiary education, 49.02% (25) had 'A' Level education, 27.45% (14) having 'O' Level education and 3.92% (2) having reached ZJC Level. On the employment status, 30% (15) of the women were in the public service, 14% (7) were self-employed, 4% (2) were in the private service and 52% (26) were unemployed. Concerning parity out of the 51 women recruited 11.76% (6) had not given birth prior to the study, 29.41% (15) had conceived once, 39.22% (20) had conceived twice, 13.73% (7) had conceived thrice, 3.92% (2) had conceived four times and 1.96% (1) had conceived five times. To add more information not shown in the table below the mean age of participants was 30.9 years with a standard deviation of 6.84. The mean gestational age at antenatal booking among women was 15.9 weeks; the minimum gestational age that women booked was two weeks while the maximum was 36 weeks.

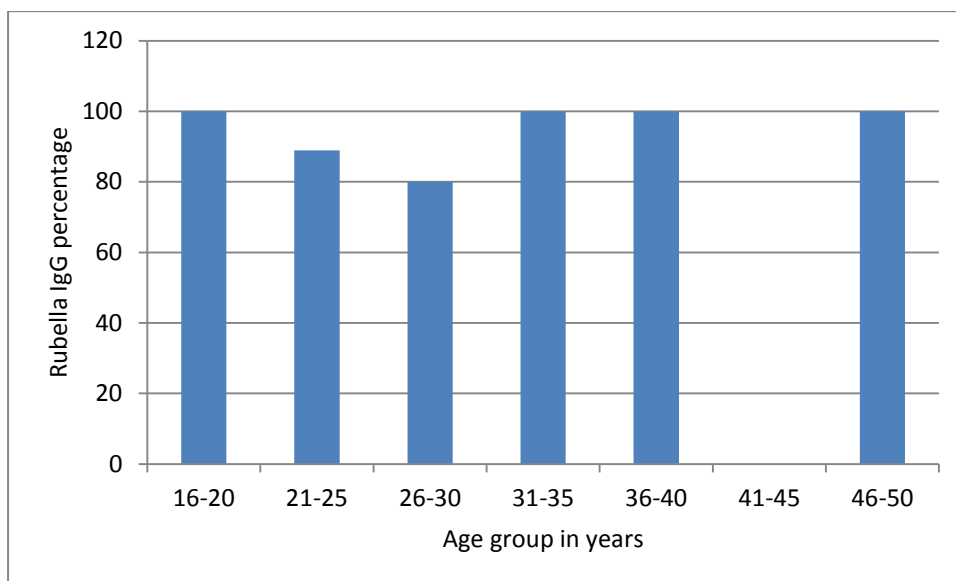
Table 3.1: Demographic data of respondents

Indicator	Frequency	Percentage (%)
Marital status	25	49.02
Married	20	39.22
Single mother	5	9.8
Divorced	1	1.96
Widowed		
Education level	2	3.92
ZJC	14	27.45
'O' Level	25	49.02
'A' Level	10	19.61
Tertiary		
Employment	15	30
Public service	7	14
Self employed	2	4
Private service	26	52
Unemployed		
Parity	1	1.96
5	2	3.92
4	7	13.73
3	20	39.22
2	15	29.41
1	6	11.76
0		

Prevalence of Rubella IgG antibodies amongst pregnant women.

Laboratory results of the blood samples which were collected from the 51 pregnant women in the study were tested for the presence of antibodies (Rubella IgG) showed that 7.8% (4 women) had no detectable antibodies and 92.2% (47) had been previously exposed to the Rubella virus and had detectable antibodies in their blood as shown in Figure 3.1 below. According to figure 3.2 below the age group 16-20 years had detectable antibodies (100%), 21-25 years had 90% level of immunity, 26-30 years had 80% detectable antibodies, 31-35 years had 100% detectable antibodies and > 40 years had 100% detectable antibodies.

Figure 3.1 Prevalence of Rubella IgG according to age group.



Using the two-sample test on the Laboratory ELISA test for Rubella IgG as stated earlier 47 women had detectable antibodies (positive Rubella IgG) and 4 had no detectable antibodies (negative Rubella IgG). On the Rubella negative IgG result it was observed that the mean age was 27 years with a confidence interval of 23-31 years at a 0.05 level of significance. The positive Rubella IgG the mean age was 31 years having a 29-33 years confidence interval at the 0.05 level of significance. Considering both results the average age for detectable antibodies was 30 years having a confidence interval of 29-32 years with the same level of significance as above. The p-value of the above results was at 0.333.

Table 3.2 Two-sample test with equal variances

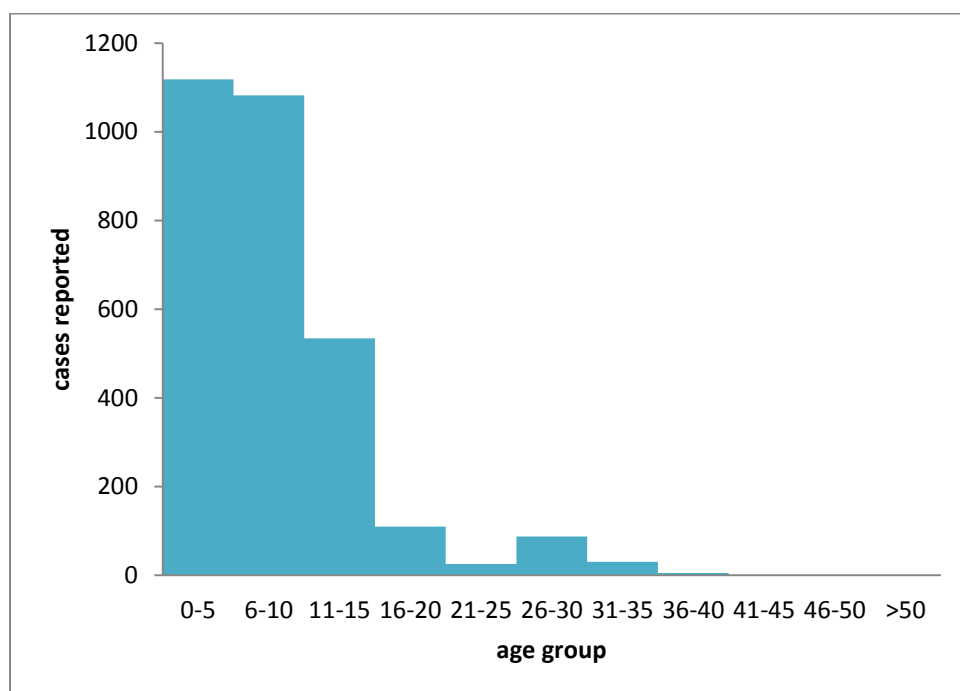
Rubella	frequency	Mean (age)	Std. Err.	Std. Dev.	[95 Conf. Interval]	
Negative IgG	4	27.75	1.3149	2.6299	23.565	31.934
Positive IgG	47	31.234	1.0252	7.0285	29.170	33.297
Combined	51	30.96	0.9575	6.838	29.037	32.884
Diff		-3.484	3.56		-10.644	3.676

P-value = 0.333

Incidence of Rubella in Zimbabwe 2009-2011

During 2009 and 2011, Zimbabwe experienced a Measles/Rubella outbreak and all suspected cases (3 003 cases) had blood samples collected throughout the country. The distribution of the 3 003 susceptible Rubella cases reported was illustrated according to age in the figure below. As shown in the figure; 0-5 age group had 1 118 cases (37%) , 6-10 years with 1 082 cases (36%), 11-15 years with 534 cases (18%), 16-20 years with 110 cases (4%), 21-25 years with 25 cases (1%), 26-30 years with 87 cases (3%), 31-35 years with 30 cases (1%) and > 36 years with 8 cases.

Figure 3.2 Bar graph showing incidence of Rubella according to age.



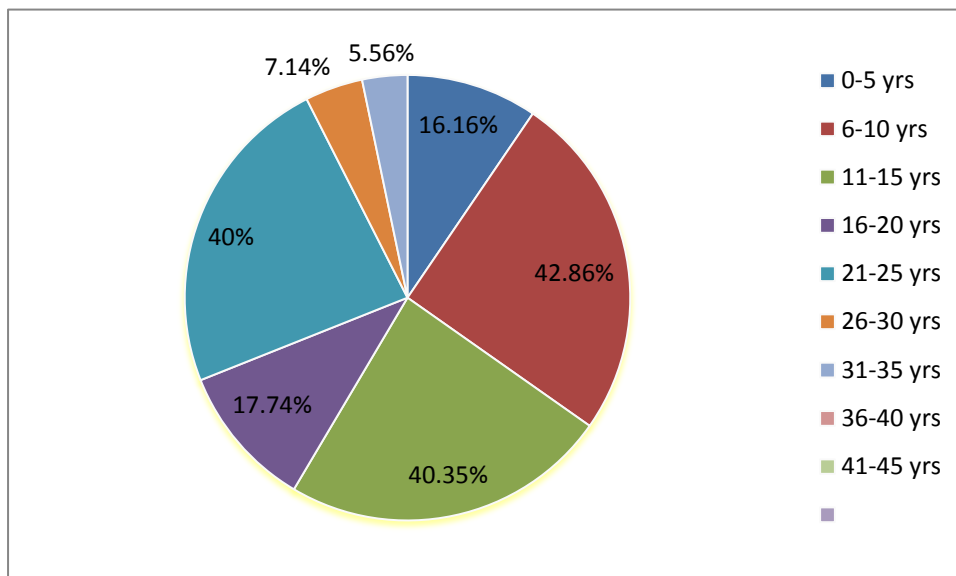
In the Measles and Rubella surveillance of 2009-11 period 3 003 blood samples were collected and 2 805 samples were tested for Rubella virus IgM (recent or active infection). In the surveillance 683 cases (22.74%) were positive confirming recent infection, 1 546 cases (51.48%) were negative, 176 cases (5.86%) were equivocal (indeterminate) and 598 cases (19.91%) were not tested. There was an incidence of 22.74% of Rubella virus infection in the period of 2009-2011 in Zimbabwe. This is illustrated in table 3.3 below.

Table 3.3 Incidence of Rubella virus in 2009-2011

Rubella IgM result	Frequency	Percentage (%)
Positive	683	22.74
Negative	1 546	51.48
Equivocal	176	5.86
Test not Done	598	19.91
Total	3 003	100

A total of 3 003 serum samples were collected and 2 805 were tested for Rubella IgM antibodies during the Measles outbreak. The combined results from all surveillance sites are shown in figure 3.3 below. The Rubella positive results were put according to age groups 0-5 years (16.6%), 6-10 years (42.86%), 11-15 years (40.35%), 16-20 years (17.74%), 21-25 years (40%), 26-30 years (7.14%) and 31-35 years (5.56%). Percentages of Rubella IgM results as recorded in the Measles surveillance data in Zimbabwe 2009-2011.

Figure 3.3 Distribution of Rubella positive IgM according to age groups.



According to the Measles notification forms the data collected was analysed using age stratification. The data had 2 994 recorded cases instead of 3 003 cases due to the fact that 9 cases had missing information on date of birth and age. As shown in table 3.4 below, the age group 0-5 years had 1 118 reported cases (37.34%), 6-10 years had 1 082 reported cases (36.14%), 11-15 years had 534 cases (17.84%), 16-20 years had 110 cases (3.67%), 21-25 years had 25 cases (0.84%), 26-30 years had 87 cases (2.91%), 31-35 years had 30 cases (1%) and > 36 years had 8 cases (0.26%).

Table 3.4 Age distribution of susceptible Rubella cases 2009-2011

Age (years)	Frequency	Percentage %
0-5	1 118	37.34
6-10	1 082	36.14
11-15	534	17.84
16-20	110	3.67
21-25	25	0.84
26-30	87	2.91
31-35	30	1
36-40	5	0.17
41-45	1	0.03
46-50	1	0.03
>50	1	0.03
Total	2 994	100

Anti- Rubella IgM ELISA results.

Using age stratification the Rubella virus IgM results show that 683 reported cases were positive, indicating a 22.81% incidence of Rubella amongst the 2 994 reported cases. 1 543 reported cases were tested and were negative (51.54%), 175 cases had an equivocal result (19.81%) and 593 cases were not tested. The age groups 0-5 years had 134 positive cases (11.9%), 5-10 years with 369 positive cases (34.10%), 10-15 years with 161 positive cases (30.15%), 15-20 years with 11 positive cases (10%), 20-25 years with 4 positive cases (16%), 25-30 years with 3 (3.45%) and 30-35 years with 1 case positive (3.33%). The above information is summarised in the table 3.5 below, showing high rates of new infection in children and a remarked drop in new infections in adults.

Table 3.5 Age distribution and Rubella IgM results 2009-2011

Age (years)	Positive	Negative	Equivocal	Test not done	Total
0-5	134 (11.9%)	695 (62.16%)	33 (2.95%)	256 (22.9%)	1 118 (100%)
5-10	369 (34.10%)	492 (45.47%)	88 (8.13%)	133 (12.29%)	1 082 (100%)
10-15	161 (30.15%)	238 (44.57%)	31 (5.81%)	104 (19.48%)	534 (100%)
15-20	11 (10%)	51 (46.36%)	14 (12.73%)	34 (30.91%)	110 (100%)
20-25	4 (16%)	6 (24%)	3 (12%)	12 (48%)	25 (100%)
25-30	3 (3.45%)	39 (44.83%)	3 (3.45%)	42 (48.28%)	87 (100%)
30-35	1 (3.33%)	17 (56.67%)	3 (10%)	9 (30%)	30 (100%)
35-40	0 (0%)	3 (60%)	0 (0%)	2 (40%)	5 (100%)
40-45	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
45-50	0 (0%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)
>50	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Total	683 (22.81%)	1 543 (51.54%)	175 (5.85%)	593 (19.81%)	2 994 (100%)

In this study, virus isolation was not done on the swabs collected during the Measles/Rubella with a positive Rubella IgM result due to delayed reagent and cell line delivery however the throat swabs were stored at -30°C awaiting viral isolation. Rubella isolation will provide more information on which rubella virus strain is circulating in Zimbabwe. Since the study is still on-going virus isolation will be carried.

CHAPTER 4

4.1 Discussion

In the study the use of interviewer administered questionnaires provided an insight to other factors associated with Rubella virus infection. The study results showed that 49.02% of pregnant women were married, 39.22% were single mothers, 9.8% were divorced and 1.96% were widowed. There was no association between either marital status and Rubella virus infection nor immunity. Although Rubella is a childhood disease as women bear more children they become more prone to Rubella virus infection if they had no previous exposure to the virus. As their children or husbands interact with other infected individuals they can also come home and infect the pregnant woman. In this aspect we also considered parity. Of the 51 pregnant women, 29.41% of the pregnant women had given birth once, 39.22% had given birth twice and 11.79% had not yet given birth. Although we did not make a comparison between age and parity some studies have shown that as age and parity increase a woman has a better chance of attaining Rubella immunity naturally without getting vaccination (El-Mekki et al., 1998; Frey et al., 1998; Onyenekwe et al., 2000).

Although a greater proportion of the pregnant women in the study were educated; 49.02% reaching 'A' Level education, 19.61% having reached tertiary level education, and 27.45% having 'O' Level education. These percentages although high the study participants showed that they had no knowledge about Rubella virus and the complications associated with the virus if infection occurs in the first trimester. Further on with this study it was discovered that most women attending ANC their primary objective was to have a professional (clinician) during their delivery and were unaware of the tests being done on them except for the HIV test. Most of them did not even follow up on the outcome of the test results done and their implications.

In this study the level of education was 100% with a low level of awareness of Rubella infection, despite the majority being educated up to 'O' Level. This emphasises the need for women to be empowered with knowledge of the Rubella infection and its prevention and whenever possible, pregnant women should avoid contact with people that have skin rash.

Information on Rubella should be included in preconception and antenatal educational programmes.

The employment status of the pregnant women showed that 52% were unemployed, 4% were employed in the private service, 30% were working in the public service and 14% were self-employed. Employment status is another exposure factor for pregnant women were they get the Rubella virus through interactions with other possibly infected people. In this study there was no association between employment status and Rubella infection, although I did not come across a health personnel or a teacher in the study it would have had a different outcome.

Of the 51 pregnant women recruited in the study, 4 women (7.8%) had no detectable Rubella virus antibodies (IgG) which was a negative result from the antibody screening. 47 women had detectable antibodies with a 92.2% prevalence from the sampled population. Of much concern were those pregnant women without detectable antibodies as they can get Rubella virus infection as they are not immune. In this study we failed to come up with an average titer to determine immunity against Rubella virus due to lack of reagents and kits to use. Knowing the titer level would rule out chances of reinfection with the wild type Rubella virus. Also with the rise of HIV infections and ARV intake, HIV women might be prone to wild type Rubella virus reinfection even if they have detectable antibodies hence the greater need to know the circulating Rubella virus strain in Harare and Zimbabwe as a whole. The age groups 21-25 and 26-30 years were the ones with pregnant women who had no detectable antibodies of which this is the most reproductive group in the Harare population.

According to Best et al., exposed pregnant women with low level immunity to Rubella can be reinfected in the face of circulating wild type Rubella. The risk of foetal infection is approximately 8% following reinfection in the first 16 weeks of pregnancy, but foetal malformations are rare.

In this study a majority of the pregnant women had developed antibodies in response to the Rubella virus (natural Rubella infection) as there is no vaccination in this country, most respondents did not know their vaccination status (54.9%) and 45.1% acknowledge they have never received the Rubella vaccine. In some private schools in Zimbabwe girls between

the ages of 12-18 receive Rubella virus vaccination but the study was carried in the high density area of Harare and we did not come across such individuals. Even though in the Zimbabwean history there was a time when all school going girls would receive this vaccine. However 7.8% of pregnant women in this study were Rubella seronegative, suggesting that rubella infection during pregnancy can occur and a potential risk exist which leads to CRS.

Minor epidemics of Rubella occur every 6-10 years with major epidemics every 10-30 years (Dyne, 2008; Sadighi et al., 2005; WHO, 2000). It also suggests a sustained Rubella virus circulation at earlier ages, children and adolescents. If the high level of immunity found in this study is not sustained with vaccination, waves of Rubella epidemics will continue to occur whenever immunity wanes in the population with the consequent risk of congenital infection (Otaigbe et al., 2006), hence the need to provide vaccination to seronegative women and pregnant women after giving birth.

The seroprevalence (92.2%) in this study was comparatively higher than what has been found in other studies in Nigeria where, it was 54.1% in pregnant women, 76% in pregnant women from Sri Lanka, 80.5% in pregnant women from Madagascar and 90.1% in women aged 15-45 years from Senegal (Onyenekwe et al., 2000; Pennap et al., 2009; Robertson et al., 1997; Sallam et al., 2003; WHO, 2007). In the afore mentioned studies and mine were conducted on women and pregnant women who were attending ANC or admitted to pregnancy follow up clinics. Thus this is a special group and may not represent the complete female population. The results obtained should be interpreted cautiously and should not be extrapolated to the whole population.

Although the prevalence study of CRS is being done currently at Harare Hospital to determine the burden of Rubella on neonates, it is important to decrease the risk of rubella virus infections among pregnant women and consequent CRS cases. On the CRS surveillance at Harare hospital in Harare by the end of 2012, 45 clinically diagnosed cases were reported with 13 having been confirmed with a positive (Rubella IgM) laboratory result. The World Health Organisation (WHO) recommends introduction of rubella vaccine to be considered (WHO, 2000), this will cater for the seronegative pregnant women and women of child bearing age is indicated by this study that 7.8% pregnant are susceptible to rubella infection.

To appreciate the trends of Rubella virus in Zimbabwe this study further analysed the Measles/Rubella virus laboratory data mainly focusing on the Measles and Rubella outbreak in 2009-11. There was a remarkable increase in Rubella cases during this period (3 003 reported cases). As Rubella virus is a childhood disease 2 002 suspected cases (0-11 years) were reported during this period and 794 cases were reported in the age group 12-35 years. Although this data included both males and females I did not separate what number comprised of women I assumed that the risk of infection confers to both sexes. The number of childhood cases was greater than that of adults but Rubella virus infection during childhood is benign but has more complications in adults.

Of the 3 003 reported Rubella cases, 2 805 cases were tested for Rubella IgM antibodies this was to WHO surveillance policies during an outbreak as they do not test each and every sample received in the laboratory hence the possibility of missing other new infections. According to the WHO surveillance policy once an area/district has had 3 laboratory Measles confirmed cases they will cease to test other suspected cases from that area hence that is not the actual incidence of the disease. The Rubella data is not adequate as its part of the Measles surveillance data and it is not a stand-alone surveillance for Rubella. There is misrepresentation of information. Although from the 2 805 cases reported, 683 cases were Rubella positive and 1 546 cases were Rubella negative. The Rubella negative result does not determine if the patient was exposed to the virus before hence is immune; rather it was to detect recent infection (Rubella IgM).

According to the Measles/Rubella surveillance, the Rubella IgM antibodies were analysed using age group stratification. 0-5 years had 16.6% new cases, 6-10 years had 42.86% and 11-15 years had 40.35% new cases. From the data above the 0-5 years has a lower percentage of a new case but the ages between 6-15 years had a greater percentage suggesting that indeed Rubella is a childhood disease and the age group 6-15 years is the active age group with much interaction and contact. Rubella virus infection spreads much faster in the age group 6-15 hence a remarkable increase in new infections. In the analyses the age group 16-20 years had 17.74% of new cases, 21-25 years had 60% and 26-30 years had 7.14%. For the age group 21-25 years the percentage of the new cases was alarming although they had few reported cases (25), this is the most reproductive group and hence

Rubella virus infection will have complications if it so happened that these infections occurred to pregnant women or women of child bearing age.

Further on the data was analysed, the 0-5 years had an 11.9% of positive Rubella IgM antibodies, 5-10 years with 34.10%, 11-15 years with 30.15%, and 15-20 years with 10% and 20-25 years with 16% Rubella IgM positivity rate. This data suggests that Rubella virus is a childhood disease but if there are non-immune adults they will get the Rubella virus infection as shown with the 16% of the age group 20-25 years age group.

Rubella virus is circulating widely in Africa and primarily infects young children (Goodson et al., 2011). The Zimbabwe Measles/Rubella surveillance data suggest that by the age of 15 most children in Zimbabwe would have developed immunity from natural infection. Although the data suggest that the 54.67% of suspected cases were < 15 years of age, Rubella positive results show that there was an increase of infection in adults as well (> 40%).

This study finding was similar to those observed during the prevaccine era in other regions (Robertson et al., 1997; Rudnicka, 1977). In Europe and the Americas, the age distribution of cases was similar to that in Africa and rubella was primarily a childhood disease that occurred mainly among 5-9 year olds (Assaad et al., 1985). In Africa the proportion of cases was highest among children < 5years of age, suggesting the possibility of infection at a younger age and hence natural infection providing immunity to Rubella virus amongst the pregnant women in this study (Robertson et al., 1997; Rudnicka, 1977).

Rubella vaccine is not part of the EPI schedule in Zimbabwe and Rubella virus continues to circulate freely. Rubella vaccination is not a routine practice in Zimbabwe. This study shows that when women reach child bearing age (taken to be 16 years), they have prior exposure to Rubella virus. As evidenced by the numbers of Rubella childhood infections; 2 734 cases with 1 425 negative Rubella IgM result and 664 positive as highlighted in the survey made by

the WHO and Ministry of Health and Child Welfare most women when they reach child bearing age would have developed natural immunity against Rubella virus (Goodson et al., 2011).

Other seroprevalence studies from Argentina, Nigeria and Sri Lanka, Rubella seropositivity is reported to increase with age (El-Mekki et al., 1998; Frey et al., 1998; Onyenekwe et al., 2000). Results of Rubella IgM during 2009-2011 in this study indicate that most cases were from children and a decrease towards the adults. Robertson et al., conducted a study and found out that Rubella seropositivity seemed to increase in both sexes until the age of 15 years no significant increase was detected after this. Dromigny et al., in their study on women aged 15-45 and Odland et al., who investigated pregnant women and women with miscarriages, could not find any significant difference in seropositivity rates between age groups. While seropositivity did not vary with age in reports on pregnant women and women of reproductive age, in this study it seemed to increase with age in children and adolescents. In general, rubella seropositivity seems to increase with age until the end of adolescence and no significant change occurs thereafter. This finding suggests that exposure to Rubella is high in childhood and adolescence and the rate of infection somewhat stabilises after that.

Rubella and Measles clinical symptoms can be easily be confused and laboratory confirmation is required. A Measles outbreak in Zaria among children turned out to be Rubella (WHO, 2007) and this further supports the findings of this study as there was an increase in Rubella new cases soon after the 2010 period during the Measles outbreak in Zimbabwe although Rubella is not a notifiable disease it was of minimal public concern.

Despite this increase in Rubella new cases there was no CRS surveillance or investigation in Zimbabwe until late 2010 when a sentinel site was established at Harare hospital. Previous surveys of Rubella infection in Africa (Gomwalk et al., 1989) showed a high prevalence of up to 93% by 10 years of age. The high prevalence of Rubella antibodies confers a high rate of immune protection in pregnant women and is associated with low levels of complications.

Although this study did not make a comparison between rural and urban populations, Onyenekwe et al., stated that there was slightly higher prevalence of Rubella antibodies in urban areas although not significant. In this study none of the sociodemographic factors had a significant influence on the prevalence of Rubella.

The study finding should be considered with knowledge of several limitations Rubella cases during 2009-2011 were detected through a surveillance system designed to detect measles and the clinical presentation of rubella may not meet the suspected measles case definitions. 20-50% of rubella infections do not include a rash (CDC, 2010), therefore the descriptive analysis represents a small fraction of all rubella cases in Zimbabwe that occurred during 2009-2011.

CHAPTER 5

5.1 Conclusion

In conclusion, this study has documented that rubella virus circulate widely in Zimbabwe and Harare as shown by the retrospective analytical study of the occurrence of new Rubella infections. In the descriptive cross-sectional survey pregnant women were immune to Rubella virus infection indicative of previous exposure to the virus. Evidence of immunity amongst pregnant women is 92.2% with the remaining 7.8% who are seronegative being susceptible to rubella infection. The limited evidence on CRS burden to date is from the 1970s where Harare reported 18 clinically diagnosed CRS cases following simultaneous epidemics of rubella and measles (Axton et al., 1979). To fully understand the burden of rubella and CRS and to determine the most appropriate strategies for rubella control and CRS prevention, a comprehensive approach is required by integrating rubella active surveillance and measles case based surveillance. Introduction of rubella vaccination should be carefully considered to ensure rubella immunity among women of reproductive age.

Vaccination however is the only way of preventing congenital rubella infection. Two vaccination strategies may be implemented; selective vaccination programme by vaccinating adolescent girls and women while allowing Rubella virus to continue circulating of which this prevents CRS and universal vaccination of children which aims at eliminating both Rubella and CRS.

Before authorities can consider including Rubella vaccine in the EPI vaccination programmes, more information is required on Rubella seroprevalence in women of child bearing age in other Zimbabwean areas. Formal Rubella and CRS surveillance need to be

implemented, and this information will provide a more scientific foundation for recommending that rubella vaccine be included in the national schedule.

5.2 Recommendations

This study recommends that Rubella and CRS be included in the notifiable disease list in Zimbabwe as they are public health problem. As such inclusion of Rubella virus screening during ANC should be made routine. The study show that most pregnant women are unaware of Rubella and CRS, therefore the need to promote awareness that Rubella and CRS occur in Zimbabwe. During the awareness campaigns more focus should be put on groups at high risk for Rubella infection and CRS births. The Ministry of Health and Child Welfare should conduct active surveillance of Rubella virus

5.3 Limitations

This was a small scale cross sectional study due to limited resources hence there is need to carry a large scale survey to determine the seroprevalence of Rubella virus amongst women of child bearing age. The study could not do the Rubella virus IgG titre level assay therefore we could not identify secondary Rubella reinfections. Rubella IgG screening is not done routinely hence acquiring the test kit was difficult therefore a limited number of samples could be tested.

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Appendices

Appendix A

Reagents and Materials for Rubella IgM-ELISA

- i. Supplementary reagents for Enzygnost®/TMB (OUVP)
- ii. Chromogen TMB
- iii. Buffer substrate
- iv. Anti- human IgM conjugate
- v. Enzygnost® Anti Rubella Virus/IgM test plate
- vi. Conjugate buffer microbiol
- vii. Anti- Rubella reference Positive control
- viii. Anti-Rubella reference Negative control
- ix. Sample buffer
- x. RF absorbent
- xi. Reader Optometric
- xii. Automated washer
- xiii. Pipette tips
- xiv. Pipetteman

Rubella IgM Antibody

Kit: Siemens Anti-Rubella-Virus IgM

Supplement: Supplementary Reagents for Enzygnost/TMB

1. Prepare Blue sample buffer; blue colour solution (175µl) and sample buffer (3.5ml) at ratio of 1:21.
2. Prepare RF adsorbent; 5ml distilled water into freeze dried vial (this should be used for samples only).
3. Dilute sample (10µl) with blue sample buffer (200µl) at ratio of 1:21
4. Dilute reference sample (10µl) with blue sample buffer (200µl)
5. Dilute sample in blue buffer (210µl) with RF solution (200µl)
6. Mix gently and absorb reaction for 15 minutes or 4°C overnight.

7. Dispense 150µl (PN, PP₁, Sample and PP₂) into antigen and control wells
8. Incubate at 37°C for 60 minutes (cover with fresh foil).
9. Wash using the wash POD provided using the automated washer; washing POD 10ml + 190ml.
10. Dispense conjugate (100µl) into each well; conjugate (50µl) + conjugate buffer (2.5ml).
11. Incubate for 60 minutes at 37°C (cover with fresh foil)
12. Wash x4
13. Dispense TMB 100µl/well; Chromogen TMB (200µl) + Buffer TMB (2ml).
14. Incubate for 30 minutes at room temperature (cover with fresh foil and protect from light).
15. Add stopping solution 100µl/well.
16. Read at 450nm

Appendix B

Rubella IgG Antibody

Kit: Diagnostic Automation

Materials

- i. Microwell strips: Rubella antigen coated wells (96 wells)
- ii. Enzyme conjugate 12ml
- iii. Negative calibrator 150 μ l
- iv. Cut-off calibrator (Rubella G index) 150 μ l- 15UI/ml
- v. Positive calibrator 150 μ l- 30UI/ml
- vi. Positive calibrator 150 μ l- 100UI/ml
- vii. Negative control 150 μ l
- viii. Positive control 150 μ l
- ix. Washing concentrate 100ml
- x. Sample diluent 22ml
- xi. TMB chromogen substrate 12ml
- xii. Stop solution: 2N HCl- 12 ml

Assay procedure

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 μ l of the test samples, negative control, positive control and calibrators to 200 μ l of sample diluent. Mix well.
3. Dispense 100 μ l of diluted sera, calibrators and controls into the appropriate wells. For the reagent blank, dispense 100 μ l sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from wells. Repeat washing three times with washing buffer.
5. Dispense 100 μ l of enzyme conjugate to each well and incubate for 0 minutes at room temperature.
6. Remove enzyme conjugate from wells. Repeat washing three times with washing buffer.
7. Dispense 100 μ l of TMB chromogenic substrate to each well and incubate for 30 minutes at room temperature.
8. Add 100 μ l of 2 N HCl to stop reaction.(make sure no air bubbles in each well before reading)
9. Read O.D at 450nm with a microwell reader

Appendix C



CITY OF HARARE

Director of Health Services
DR STANLEY MUNGOFA
MD (Cuba) MPH (Zim)

All correspondence to be addressed to the
DIRECTOR OF HEALTH SERVICES
Ref: _____
Your Ref: _____

DIRECTOR OF HEALTH SERVICES
Rowan Martin Building,
Civic Centre,
Penneluther Avenue,
off Rotten Row,
Harare, Zimbabwe.
P. O. Box 596
Telephone: 753326
753330/1/2
Fax: (263-4) 752093

12 March 2012

Tafadzwa Mamvura
Department of Medical Microbiology
P O Box A 178
Avondale
HARARE

Dear Sir

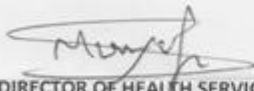
RE: **PERMISSION TO CONDUCT A STUDY AT RUJEKO AND RUTSANANA POLYCLINICS**

I refer to your letter concerning the above.

Permission has been granted to for you to conduct a study on consenting pregnant women attending antenatal care clinic.

For further assistance please liaise with the Sister In Charge Rujeko and Rutsanana Polyclinics.

Yours faithfully


DIRECTOR OF HEALTH SERVICES
SM/rm

C,c, Sister In Charge - Rujeko Polyclinic
- Rutsanana Polyclinic

Appendix D

University of Zimbabwe- College of Health Sciences,
Medical Microbiology Department.

JREC IRB No: 123

Participant Consent Form (English)

Study Title: Prevalence of Rubella virus amongst pregnant women in Harare.

Principal Investigator:

Tafadzwa Mamvura, (BSc Environmental Health, MSc Medical Microbiology Part 2 student)
University of Zimbabwe- College of Health Sciences, Department of Medical Microbiology, B Floor,
Parirenyatwa Hospital, P.O Box A178, Avondale, Harare. Phone: 0773415548/ 04-708093 Ext- 2150

What you should know about this research:

- We give you this consent so that you may read about the purpose, risks and benefits of this research study.
- Routine care is based upon the best known treatment and is provided with the main goal of helping the individual patient. The main goal of the research is to gain knowledge that may help future patients.
- You have the right to refuse to take part, or agree to take part now and change your mind later.
- Whatever you decide, it will not affect your regular care.
- Please review this consent form carefully. Ask any questions before you make a decision.
- Your participation is voluntary

Purpose of study

You are being asked to participate in a research study to determine the prevalence of Rubella virus infection. Rubella infection is a mild self-limiting disease which can be a rash or cold caused by a virus that is transmitted through droplets. Rubella virus affects the unborn child if infection occurs during the first three months/12 weeks of pregnancy. The purpose of the study is to determine what numbers of pregnant women have antibodies against Rubella virus infection in Harare. You were selected as a possible participant in this study because you are pregnant and your unborn baby might be at risk of Rubella infection. We intend to recruit 1 050 pregnant participants from Rujeko and Rutsanana Polyclinics in Harare.

Procedures and Duration

If you decide to participate, a study number will be assigned as your identification. The midwife will give you a physical examination and ask some questions to do with Rubella infection. A routine blood sample of 2-3 millilitres will be collected into a plain tube to check for Rubella virus. Saliva will be collected using a sterile sponge on a stick which shall be rubbed against the cheek and the gum for about one minute this will be for further tests to check for the presence of Rubella virus. Both samples will be collected only on your first maternity clinic visit.

Discomfort and Risks

You might feel discomfort during venous blood and oral swab collection.

Benefits

The benefits of the study are not immediate to participants but to the general population of women of child bearing age in Zimbabwe for the implementation of a vaccine that will protect foetus during pregnancy. Rubella virus complications are managed on individual basis depending on the complication, corrective surgery can be carried.

Alternative procedure or treatment

If you possess antibodies against Rubella virus (positive result) it means that you and your unborn child are not at risk of Rubella infection. If it is discovered that you do not have antibodies against Rubella infection (negative result), you shall receive counselling from your midwife for appropriate measures you must take to safeguard your unborn child as the vaccine is not given to a pregnant woman. In Zimbabwe we do not routinely immunise against Rubella but the vaccine is available and your Clinician will advise you on what to do.

Confidentiality

Information and records concerning your participation are to be used only for the purpose of this study and will not be shared with anyone outside of this study. Samples collected will be identified by the number allocated to you at the clinic. Your results will be sent to this clinic and only you and your clinician can discuss them with you. During the study your sample will be stored at Parirenyatwa Hospital, Virology department and will be destroyed after the study is completed. Publication of the study results will be done after data analysis and will be provided at all study sites.

Voluntary participation

This study is recruiting voluntary participants. If you decide not to participate in this study it will not affect the care you will receive at the clinic in any way. Even if you agree to participate you are free to withdraw from the study at any time.

Participants signed consent

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over. I have been informed verbally and in writing about the study. I understand what is to be done and I agree to participate in this study. I understand I can withdraw from the study at any time if I wish so.

Participant number:.....

Participant signature:..... Date.....

Name of Witness.....Date.....

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research participant or if you feel you have been treated unfairly and would like to talk to someone other than the research team please feel free to contact Joint Parirenyatwa Hospital and College of Health Sciences Research Committee (JREC) University of Zimbabwe- College of Health Sciences, P.O Box A178, Avondale, Harare. Phone: 04-708093 Ext. 2241/2242 Study site _____

Participant # _____

Appendix E

Questionnaire

University of Zimbabwe- College of Health Sciences,
Medical Microbiology Department.
JREC IRB No: 123
Participant Consent Form (Shona)
Gwaro rekunzwisa nekubvuma kupinda muchidzidzo.

Zita rechidzidzo: Chidzidzo chekutsvaga huwandu hwemadzimai akazvitakura vakadzivirwa kubva kune chirwere chegwirikwiti.

Zita remudzidzi:

Tafadzwa Mamvura, (BSc Environmental Health, MSc Medical Microbiology Part 2 student)
University of Zimbabwe- College of Health Sciences, Department of Medical Microbiology, B Floor,
Parirenyatwa Hospital, P.O Box A178, Avondale, Harare. Phone: 0773415548/ 04-708093 Ext- 2150

Chiziviso paChidzidzo ichi:

- Tinokupai mukana uyu kuti muverenge muzive chinangwa chechidzidzo ichi, njodzi dزامungasangana nadzo nezvakanakira dzidzo iyi.
- Rubatsiro rwamagara muchiona maererano nekurapwa kwenyu muchakuwana uye zvichawanika mutsvakiridzo ino zvichabatsirawo vamwe mune ramangwana.
- Mune kodzero yekuramba kukwikwidza muchidzidzo chino kana kuchinja pfungwa dzenyu pamberi kurega kukwikwidza.
- Zvamafunga kuita pafundo ino hazvinei uye yazvichinje kubatsirwa kwenyu pano pachipatara.
- Nyatsoverengai gwaro rino zvakadzama, munotenderwa kubvunza musati masaina.
- Zviri kwamuri kukwikwidza pachidzidzo chino.

Chinangwa chedzidzo

Gwirikwiti rwunokonzerwa neutachiona weRubella. Kune veruzhinji hutachiona uhu hunoonekwa sedziwa kana gwirikwikiti pamuviri. Asi kuna amai vakazvitakura vakabatwa negwirikwiti iri pamuviri panogona kubva, mwana anogona kuberekwa akafa, asingaone, ari matsi kana kuti akaremara nemamwe matambudziko akasiyana. Kuti tione kuti makadzivirwa kubva kune hutachiona uhwu tinoda kuita tsvakiridzo murabhoritori. MuZimbabwe pazvidzidzo zvakamboitwa zvakaonekwa kuti veruzhinji vakadzivirwa kubva kuhutachiona uhwu. Asi chidzidzo chino chinoda kuona kuti madzimai akazvitakura akadzivirwa here kubva kune chirwere chegwirikwiti kuti tichengetedze vana vachazvarwa kubva kune matambudziko egwirikwiti irori.

Zvichaitwa Muongororo

Mukabvuma kupinda muchidzidzo chino, muchapihwa nhamba ichashandiswa sezita renyu. Mbuya Nyamukuta vachakuongorori vokubvunza mibvunzo inochokuita negwirikwiti. Muchatorwa ropa remutsinga 2-3ml richanovhenekwa kuti makadzivirwa here kubva kune hwutachiona hwegwirikwiti. Muchatorwa mate ekumatadza ayo achavhenekwa hwutachiona. Ropa nemate zvichatorwa kamwe chete musi wekutanga wamunoshanyira chipatara chaana mai vakazvitakura. Njodzi nekusagadzika dزامuchasangana nadzo.

Pamuchatorwa ropa remutsinga nemate apa huro pane kakurwadza.

Zvamuchawana muchidzidzo

Chidzidzo ichi chinogona kusabatsira avo vachakwikwidza panguva ino asi kune avo vabereki vamagwana muZimbabwe zvichabatsira kuunzwa kwejekiseni rinodzivira hutachiona uhwu. Uye zvichabatsira vanaChiremba mukurapa kwavo vana vanozvarwa nemataambudziko egwirikwiti.

Zvimwe zvingaitwa kana Kurapwa kwenyu

Kana zvikaonekwa kuti ropa renyu rine umbowo wehutachiona hwegwikwiti zvinoreva kuti imi nemwana wamakatakura hamusi panjodzi yekubatira chirerwe chegwirikwiti. Asi muropa menyu mukashaikwa umbowo hwegwirikwiti zvinoreva kuti hamuna kudzivirirwa kubva nechirwere chegwirikwiti uye imi nemwana wamakatakura muri panjodzi yokuwana hwutachiona uhwu. Mbuya Nyamukuta kana Chiremba vachakurirai zvamunofanira kuita kuti muzvidzivirire. Munyika yeZimbabwe pari zvino veruzhinji havasi kubaiwa kuti vadvivirirwe kubva kune hutachiona wegwikiti asi jekiseni riripo asi haribaiwe vanamai vakazbitakura.

Kuchengetedzwa kwezvakananzika

Zvese zvamataura, humboo nezviwanikwa zvedzidzo rino zvichashandiswa chete muchidzidzo chino chete uye hapana umwe munhu kunze kwechikwata chedzidzo rino achaziva nezvazvo. Ropa nemate enyu achatorwa achapirwa nhamba ichave ndiyo ichashandiswa kuti tizive kuti ndezenyu kwete zita renyu. Zviwanikwa zvedzidzo rino zvichatumirwa kukiriniki ino uye imi nachiremba kana mukoti wenyu muchakurukura nezvazvo. Pafundo ino ropa renyu nemate atorwa zvichachengetedzwa pachipatara cheParirenyatwa, kuchikamu chezeutachiona.

Kusununguka kwenyu kupinda muongororo

Chidzidzo chino chinopinza avo vakasununguka kupinda machiri. Kubvuma kana kuramba kwenyu kukwikwidza muchidzidzo ichi hakukutadzisei kuti muwane rubatsiro pakiriniki pano. Chero mukabvuma kupinda muchidzidzo ichi makasununguka kurega kukwikwidza chero nguva.

Mvumo yenyu yekukwikwidza muchidzidzo

Musati masaina munotenderwa kubvunza zvasusina kunzwisisa maererano nechidzidzo chino. Torai henyu nguva yenyu muchinyatsofunga. Ndatsanangurirwa uye ndaverenga nezvechidzidzo ichi.

Ndanzwisisa zvichaitwa kwandiri uye ndinobvuma kukwikwidza muchidzidzo ichi. Ndinonzwisisa kuti ndinogona kurega kukwikwidza chero nguva ipi, ndazvipira uye handina kumanikidzwa.

Nhamba yepakiriniki yamapirwa:.....

Runyoro rwenyu:..... Zuva.....

Zita rachapupu.....Zuva.....

Muchapirwawo rimwe gwaro rino kuti muchengete.

Kana mune mibvunzo maererano nechidzidzo chino kana mvumo yenyu isina kunyatsojekeswa kubva kune vadzidzi ava. Sunungukai kana madzvinyirirwa uye kana muchida kutura nevamwe vanhu vasiri vechidzidzo chino, ava vanhu munovawana pakero inotevara: Joint Parirenyatwa Hospital and College of Health Sciences Research Committee (JREC) University of Zimbabwe- College of Health Sciences, P.O Box A178, Avondale, Harare. Phone: 04-708093 Ext. 2241/2242

Appendix F

Date recruited -----/____/____ District of
 Residence_____
 City/Town_____ Physical Address_____
 Mobile Number_____
 Religion_____ Age____/____ Date of Birth____/____/____

Socio-economic characteristics. (Tick appropriate)

Married Single mother Divorced Widowed

Marital status

Have you received any form of education Yes/No?

Up to which level of education; specify_____

Are you employed Yes/ No?

What type of Occupation; specify_____

Obstetric History

Gestational age at first visit (weeks) _____ Date of First visit____/____/____

Clinician Name:_____ Designation:_____

Parity Were previous deliveries normal (vaginal) Yes/No

Have you had a miscarriage? Yes/No? If yes how many times_____

Have you had stillbirth? Yes/No? If yes how many times_____

Have you been vaccinated against Measles Yes/No/Don't remember

Have you been vaccinated against Rubella Yes/No/Don't remember

History of fever early in pregnancy Yes/No/Don't remember

History of rash early in pregnancy Yes/No/Don't remember

If yes, for how long (weeks?)

Laboratory Information

Type of specimen collected: Throat swab/Blood sample

Date specimen collected____/____/____ Date specimen received in Lab____/____/____

Date specimen examined____/____/____ Date result sent ____/____/____

Rubella Serology Results

			1=Positive
			2=Negative
IgG	IgM	Virus Isolation	3=Indeterminate
			4=Not Done

Please ensure that all fields are completed and send copy of form to Virology laboratory at
 Parirenyatwa Hospital B-Floor: 0773415548/0772715861