CHAPTER-I

1. INTRODUCTION

1.1 Background

Rubella, commonly known as German measles or three-day measles, is an infection caused by virus that primarily affects the skin and lymph nodes. It is primarily a childhood disease characterized by symptoms of mild upper respiratory infection accompanied by diffuse red maculopapular rash. The rash resembles that of measles, scarlet fever, some cases of mononucleosis, and drug reaction. The disease is usually mild, can even be asymptomatic, self-limited illness characterized by rash, lymphadenopathy, and low grade fever. Young adults who get rubella may get swollen glands in the back of the neck and some pain, swelling, or stiffness in their joints (arthritis). Most people recover quickly and completely from rubella. However, the greatest danger from rubella is not to the children or adults, but to unborn babies. If a non-immune woman acquires a virus during the first trimester of pregnancy, the consequences can be drastic. Fetal abnormalities almost invariably result and include heart defects, cataracts, deafness, mental retardation or even fetal death (Mclean et al, 1997; Banatvala and Best, 1998).

Rubella, which occurs throughout the year with the peak incidence in late winter and early spring, is world wide in distribution (CDC, 1994). Worldwide, it is estimated that there are more than 100,000 infants born with congenital rubella syndrome each year. In 2001, 123 countries/territories reported a total of 836356 rubella cases. Cutts and Vynncychy estimate that there were 110,000 infants (95% confidence interval, 14428 to 308438) affected by congenital rubella syndrome in developing countries (excluding the WHO European region) that had not introduced rubella vaccine. A separate estimate for WHO European region suggests some 4000 congenital rubella syndrome cases occur annually in countries of that region that have not introduced rubella vaccine(Robertson et al,2003). Almost every

country in world have rubella cases been reported. The reported numbers were higher in Bulgaria, Belgium, Kazakhstan, Jordan, Romania, Russia, Ukraine and China (WHO, 2006). In 2005, in South Asia the highest confirmed cases were reported from Bangladesh (609 cases) followed by Nepal (160 cases) and the lowest from India followed by DPR Korea where as nil from Maldives (WHO, 2006).

Rubella is caused by Rubella virus which was first isolated in cell culture in 1962 by Perkman and Weller. Rubella virus is a non-arthropod borne Toga virus which is the only member of the genus Rubi virus. It is enveloped, non-segmented, positive sense RNA virus and replicates in cytoplasm. Only one genetically stable serotype of rubella has been identified (Katow et al., 1997; Frey, 1994).

Rubella is a mild, highly contagious illness which is passed directly from person to person via coughing, sneezing and talking. The disease is most contagious as the rash is appearing, but can be spread from one week before to 5-7 days after rash onset. A pregnant women with rubella can pass the virus onto her baby (or fetus) while it is in womb. Infants with congenital rubella syndrome, who were infected with rubella before birth, may be able to infect others for usually a year, and can therefore transmit rubella to those susceptible persons caring for them. The international classification of disease classifies rubella as two diseases: rubella and congenital rubella syndrome (WHO, 1993; Benenson, 1995).

Rubella has few complications unless it is contracted by pregnant women. Rubella infection in pregnancy can lead to miscarriage, stillbirth, or an infant born with congenital rubella syndrome (CRS). Some defects associated with CRS may be recognizable at birth, while others are detected months or even years later. CRS manifestations may be tranisient (e.g. purpura), permanent structural manifestations (e.g. deafness, central nervous system defects, congenital heart disease, cataract), or late emerging conditions (e.g. diabetes mellitus) (Mellinger et al, 1995).

1.2 Rational and justification of study

Although rubella is generally considered as a childhood illness, people of any age who have not been vaccinated can be infected. Due to the delay in implementation of rubella vaccine programme, most people of the country are still susceptible to Rubella virus infection. The past unpublished result of NPHL showed that the rubella cases in children are significantly high. Moreover, the children below 15 years are more susceptible whose population has crossed 5.4 million. The serious consequences in the rubella infected person such as chronic arthritis, encephalitis, hemorrhagic manifestations, neuritis, panencephalitis though rare but if occurred, have a great health impact. The same rubella if entered in women during first trimester of pregnancy, the consequences can be disastrous. As many as 85% of infants infected in the first trimester of pregnancy would be found to be affected if followed after birth (CDC, 2001). Countries in the measles elimination phase are finding that a high percentage of suspected measles cases are due to rubella.

The Lab based surveillance data in Nepal show a major shift from measles to rubella outbreak after successful measles campaign conducted during 2004-2005 (Ghimire and Patridge, 2006). The unpublished data of NPHL also shows that the large number of women of antenatal clinic visiting women of child bearing age were positive for anti-rubella IgM antibodies which indicates the requirement of urgent attention for vaccination program to introduce rubella containing vaccine in the country.

To the best of our knowledge, from the available published literatures/reports; no countrywide study with inclusion of major population has been done in the past. So the present study has been designed to represent the maximum affected population in the country. This research has been carried out at NPHL, a national measles/rubella laboratory designated by WHO, based on the suspected measles/rubella surveillance samples received through WHO-IPD surveillance network.

CHAPTER – II

2. OBJECTIVES

2.1 General objective

To study the burden of rubella in Nepal.

2.2 Specific objectives

- To estimate the incidence of rubella in Nepal.
- To study the seasonal distribution of rubella in Nepal.
- To study the epidemiological pattern of rubella in Nepal.

CHAPTER-III

3. LITERATURE REVIEW

3.1 Rubella

The name rubella is derived from Latin, meaning "little red". Rubella was initially considered to be a variant of measles or scarlet fever and was called "Third disease". It was not until 1814 that it was first described as a separate disease in the German medical literature, hence the common name "German Measles"(CDC, 2001).

Rubella was first described by German physicians, de Bergan and Orlow, in the mid eighteenth century. At that time it was frequently known by the German name "Rötein", and it was due to the early interest of the German physicians and the general acceptance of a German name that the disease subsequently known as "German measles". For many years German measles was frequently confused with measles and scarlet fever, other infectious disease presenting with rash, and at one time was considered to be a cross between them. The clinical difference between these diseases was recognized in the nineteenth century and rubella was accepted as a distinct disease by international congress of medicine in London in 1881. The disease received comparatively little attention, for infection was generally mild and severe complications were rare, until 1940s when the association between maternal infection and congenital defects such as cataracts, heart disease and hearing loss first recognized. Following the wide spread epidemic of rubella infection in 1940, Norman Gregg, an Australian ophthalmologist, reported in 1941 the occurrence of congenital cataracts among 78 infants born following maternal rubella infection in early pregnancy. This was the first reported recognition of congenital rubella syndrome (Banatvala and Best, 1998).

3.2 Rubella Virus: The Etiological Agent

3.2.1 History

In1914, Hess postulated a viral etiology based on his works with monkeys. Hiro and Tosika in 1938 confirmed the viral etiology by passing the disease of children using filtered nasal washing from person with acute case (Banatvala and Best, 1998).

Rubella virus was not isolated in cell culture until 1962, when Parkman, Buescher and Artenstein (1962) detected the presence of Rubella virus in primary vervet monkey kidney cell culture by means the interference technique and Weller and Neva (1962) reported unique cytopathic effects in primary amnion cell culture (Banatvala and Best, 1998).

The fine structure of Rubella virus was not determined until 1968 as it is difficult to obtain the high titer of virus required by electron microscope. Much of the work on molecular structure and replication of Rubella virus was carried out in the late 1940s and early 1980s, some time after similar work on other viruses. This was probably because Rubella virus is slow to grow in cell culture, high level of virus are difficult to produce consistency and the high G+C constant of the genome has made sequencing difficult (Holmes et al, 1969, Banatvala and Best, 1998).

3.2.2 Classification

Rubella virus, an enveloped RNA virus belongs to the family Toga viridae which is non-arthropod borne and is probably distantly related to the alpha virus. Unlike other Toga viruses, Rubella virus has no known invertebrate host and has been placed by itself in the genus Rubi virus, the only member of this genus (Frey, 1994).

3.2.3 Morphology and structure

The virion has mean diameter of 58nm with a 30nm core (Murphy et al, 1968). The core is surrounded by a lipoprotein envelop with surface spikes 5-8nm in length. The virion is pleomorphic, owing to the delicate non-rigid nature of the envelop. The symmetry of the nucleocapsid has been difficult to establish because of its instability; but rotational analysis of thin sections of rubella virion suggested that the core had a T=3 icosahedral symmetry and 32 capsomes (Matsumo and Higashi, 1974).

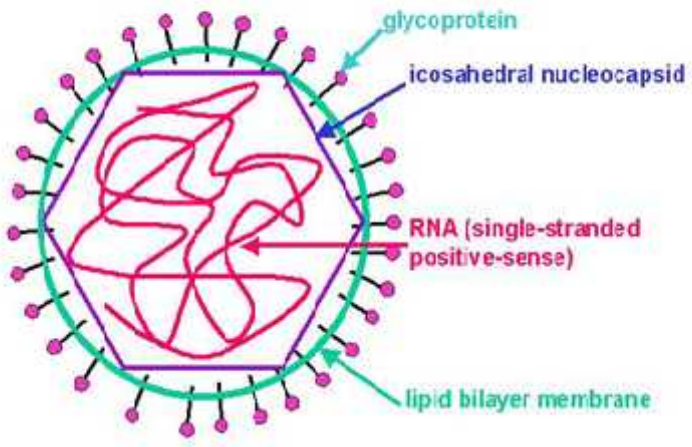


Figure 1: Morphology of rubella Virus

3.2.4 Physical and morphological properties

Physical and morphological properties of Rubella virus are given in the below;

Virus partial

Diameter	40 to 70 nm
Buoyant density	in sucrose 1.16-1.19 dg/ml
Sedimentation coefficient	240S, 242S, 350S ±50S

Nucleocapsid

Diameter	30-40 nm
Symmetry	Icosahedral
Buoyant density	in $CsCl_2$: 1.4±0.4 gm/ml
Sedimentation coefficient	150S
Molecular weight	3200-3500 kDa
Length of surface projection	5-6 nm

Chemical composition

RNA	2.4%
Lipid	18.8%
Protein	74.8%
Carbohydrates	4.0%

Thermal stability

4^0C	stable for >7 days
37^0C	inactivated at 0.1-0.4 $\log_{10}$, TCID 50/ml per hour
56^0C	inactivated at 1.5-3.5 $\log_{10}$, TCID 50/ml per hour
70^0C	inactivated at 5.5 $\log_{10}$, TCID 50/0.1ml per hour
-70^0C	Stable
Freeze drying	stable
pH sensitivity	stable at pH 6.0-8.1, unstable at more alkaline and acid pH
UV sensitivity	inactivated within 40 sec
Photo sensitivity	labile, K= 0.07/min in PBS
Sonication	stable for > 9 min

The stability of virus is enhanced by the addition of proteins to the suspending medium. Thermo stability is improved in the presence of $MgSO_4$. Because of the lipid contents of the viral envelope, Rubella virus is inactivated by detergents and organic solvents (Banatvala and Best, 1998).

3.2.5 Genome structure and function

The genome of Rubella virus is a single strand of RNA. This 40S genomic RNA consists of three structural proteins; E_1E_2 membrane bound glycoprotein and capsid proteins, is infectious but the recovery of the infectivity is poor. The genome is 9759 nucleotide in length excluding the 3^I terminal poly (A) tail and is capped at the 5^1 end. The cap is required for the efficient translation as it serves as a ribosome recognition site (Dominguez et al, 1990).

The base composition of the genome is A 14.9%, U 15.4%, G 30.8% and C 38.7%. The high G+C content (69.5) of the genome has made sequence determination difficult and a number of the original sequences reported (Frey, 1994). The genome is composed of two long open reading frames (ORF) and has some common features with alpha virus. The 5^1 proximal ORF is 6345 nucleotides in length (nuc 41-6385) and codes for the non-structural proteins (NSP). The 3^1 proximal ORF is 3180 nucleotides in length (nuc6509-9697) and codes for the structural proteins. The two ORF are in same translational frame and are separated by 123 nucleotides. The sub genomic RNA which is capped, methylated and polyadenylated, is transcribed from the negative sense subgenomic RNA for which the start site is nucleotide 6433(U). Sequences at the 5^1 and 3^1 ends of rubella virus can form stable stem-loop structure. The structure is thought to be involved with virus replication and may be necessary for efficient translation (Katow et al, 1997; Pogue and Hoffmann, 1996).

3.2.6 Replication

Rubella virus probably enters the cell by receptor-mediated endocytosis. The cellular receptor for the virus has not been identified, but membrane lipid molecules play an essential role .The reproductive cycle takes place in the cytoplasma. It is probable that the process of penetration and uncoating resembles that of alpha virus. The virion is internalized in a coated vesicle and transported to the endosomal compartment. At the low pH in the endosome the C protein becomes lipid soluble and this may allow association of the capsid with viral membrane to uncoat the viral RNA within the viral envelope (Mauracher and Gillam, 1991). The low pH also triggers a conformational change in the envelope glycoprotein and mediates the fusion of the viral membrane and the endosomal membrane to allow the release of viral RNA into the cytoplasm. The viral RNA is translated to produce the 2115 amino acid polyprotein encoded by the 5^{1}(Katow and Sugiura, 1988).

3.3 Pathology

In 1941, N Mc Alister Gregg, an Australian ophthalmologist, published his new famous retrospective study "Congenital cataract following German measles in the mother", in which he showed that, if acquired in early pregnancy, rubella cause congenital malformations. Seventy eight babies, all with a similar type of congenital cataract were born in New South Wales after an extensive rubella epidemic there in 1940 and many of the mothers gave a history of rubella, usually in the first or second months of pregnancy. Congenital defects of the heart were in 66% of cases whose cardiac condition was recorded. These findings were soon confirmed in Australia, and deafness was also noted in many congenitally infected infants. Microcephaly, dental defects and low birth weight were also reported (Gregg, 1944; Wesselhoeft, 1947).

Despite conformation of Gregg’s original observation, an annotation in the Lancet suggested that additional studies were required, as if it could not be proved that the illness

with rash experienced by these mothers was in fact rubella and that it was unlikely that such as association would have previously gone unnoticed. However, Hope-Simpson (1944) reported in the Lancet congenital cataract and heart defects in two babies in England after epidemic rubella. Similar defects had been noted before Greggs original observation but their significance had not been appreciated. Additional retrospective studies reporting congenital defects induced by rubella in early pregnancy were subsequently carried out (Hansaw et al, 1985).

Retrospective studies in which the starting point for investigation was an infect with one or more rubella induced deformities suggested that a very high portion of mothers who had rubella during pregnancy were delivered of infants with congenital malformations. The outcome of pregnancies in which maternal rubella was followed by the birth of unaffected infants was not recorded. Thus, in 1940 when rubella was at its peak incidence in New South Wales Gregg and his colleagues reported that 96% of infants whose mother had had rubella in early pregnancy suffered from congenital defects that were confirmed to cases in which maternal rubella had occurred before the sixteenth week of gestation (Banatvala and Best, 1998).

3.3.1 Post natal acquired infection

3.3.1.1 Clinical features

After an incubation period of 14-21 days the characteristic feature of rubella, rash and Lymphadenopathy may appear. However, only these clinical features can not differentiate rubella with measles. Most often rubella is clinically diagnosed as measles. In young children, the onset of illness is usually abrupt. Such constitutional symptoms as fever and malaise may be present for a day or two before onset of the rash but they usually subside rapidly after its appearance. Older children and adults may experience more pronounced constitutional symptoms 3-4 days before the rash appears and during this prodromal phase and enanthem consisting of erythematous pinpoint lesions on the soft palate may be

present. The exenthem is usually discrete, in the form of pin point maculopapular lesions. It appears first on the face and spreads rapidly to the rest of the body; lesions on the body may coalesce. The rash usually persists for about three days, occasionally longer, but may be fleeting. The mechanism by which rashes induced has not been established. Although immunopathological mechanisms may be responsible, Rubella Virus has been isolated from skin biopsy specimens taken not only from areas with rash but also from the skin without rash and from the skin of patients with sub clinical infection (Heggie, 1978). Furthermore, the development of rash may be prevented by the administration of pooled human immunoglobulin, although this does not prevent viraemia. Patients may complain of tender lymph nodes when or just before the rash appears. Follow-up studies of susceptible people exposed to rubella have revealed that Lymphadenopathy may be present 7-10 days before the onset of rash, and sometimes for an even longer period after it has disappeared. Sub-occipital, post auricular and cervical lymph nodes are most frequently affected (Banatvala and Best, 1998).

Rubella is rarely associated with severe complications. Encephalitis may occur in approximately one in ten thousand cases, but in general prognosis is good (Krugman and Ward, 1968). However, during an epidemic of rubella in Japan in 1987, when complications were reported rather more frequently than in previous epidemics, it was estimated that the incidence was much higher (1:1600 cases) (Moriuchi and Yamasaki, 1990). Very occasionally, rubella is associated with thrombocytopenia which may result in purpuric rash, epitasis, haematuria and gastrointestinal bleeding. The commonest complication of postnatally acquired rubella is joint involvement, although this is rare among children and adult males, it may occur in up to 60% of post pubertal females. Symptoms generally develop as rash subsides and vary in severity from mild stiffness of the small joints of the hands to a frank arthritis with severe pain, joint swelling and limitation of movement. The finger joints, wrists, knees and ankles are most frequently affected. The duration of these symptoms is usually about three days but occasionally they

may persist for up to a month. Rubella induced arthralgia is not associated with a sequelae (Banatvala and Best, 1998; Chantler et al, 2001).

Arthralgia occurs commonly in post pubertal females after administration of rubella vaccine. The mechanism by which natural acquired and vaccine induced infection causes arthralgia is probably complex. Thus, joints symptoms may result from direct infection of the synovial membrane by virus, for Rubella virus has been isolated from the joint aspirates of vaccine with vaccine induced arthritis .Furthermore, studies in vitro have shown that attenuated virus strains will replicate in human synovial membrane cell culture (Grayzel and Beck, 1971). However, an immunomechanism is probably also involved, because, in addition to virus, joint aspirated has been shown to contain rubella specific IgG, which suggests that joint symptoms may be induced by immunocomplexes. It is therefore of interest that the presence of rubella antibody containing immunocomplexes in the serum has been associated with a high incidence of joints symptoms following rubella vaccination (Ogra and Herd, 1971; Mims and Strokes, 1985).

3.3.1.2Pathogenesis

Neonatal, childhood and adult infections of rubella occur through the mucosa of the upper respiratory tract followed by multiplication in the cervical lymph nodes. Viremia develops after seven to nine days and lasts until the appearance of antibody on about day thirteen to fifteen. The development of antibody coincides with the appearance of the rash, suggesting an immunologic basis for rash. The exact mechanism of how the rash is induced is uncertain but an immunopathological mechanism may be present. Lymphadenopathy may precede the rash up to two weeks after the rash has gone. After the rash appears; the virus remains detectable only in the nasopharynx where it may persist for several weeks. In 20%-50% of cases, primary infection is sub clinical (Banatvala and Best, 1998; Herrman, 1985).

3.3.1.3 Reinfection

Natural infection is followed by a high order of protection from reinfection. However, evidence of reinfection may be obtained by demonstrating a significant increase in antibody concentration following the natural and experimental exposure to rubella. Such reinfection is generally asymptomatic (Vesikari, 1972). Reinfection in pregnancy is hazardous only if viremia occurs, and this has rarely been documented in experimental studies (O'Shea et al, 1983). Following maternal reinfection during the first sixteen weeks in pregnancy, the risk of fetal infection has been estimated to be of the order of 8% although fetal damage is rare .Although it is possible that, in such cases, transmission of the virus to the fetus may be due to a specific defect in the maternal immune response, rubella reinfection is not associated with a lack of neutralizing antibodies or persistent impairment of rubella-specific lymphoproliferative response (O'Shea and Corbett, 1994; Best,1993).

3.3.1.4 Immune Responses

Rubella-specific IgG, IgM and IgA responses develop rapidly after the onset of rash. Rubella-specific IgG persists for life, but may decline to low levels in old age. Rubella-specific IgM usually appears within 4 days of onset of rash and persists for 4-12 weeks. Rubella-specific IgM is diagnostic of acute infection. Specific IgM may sometimes persist for up to one year after both naturally acquired infection and rubella immunization. Serum and nasopharyngeal IgA responses are detectable for at least 5 years after infection. Specific serum IgD and IgE responses develop rapidly after onset of infection and persist for at least 6 months. A decrease in total leucocytes, neutrophils and T cells and a transient depression of lymphocyte responsiveness to mitogens and antigens such as purified protein derivative is seen after rubella. MHC class I-restricted CD8+ cytotoxic T lymphocytes have also been demonstrated in rubella-immune individuals (Best and O'Shea, 1995; Banatvala and Best, 1998).

3.3.2 Congenitally acquired Rubella

3.3.2.1 Clinical features

Although the early retrospective enquiries emphasized frequency and importance of such defects as congenital anomalies of the heart, eyes and deafness, it was not until follow up studies had been carried out on infants whose mother had had rubella during the extensive1963/64 outbreak in the USA that it was fully appreciated that congenital rubella frequently caused wide spread multi system disease (Banatvala and Best, 1998).

Maternal viremia associated with rubella infection during pregnancy may result in infection of the placenta and fetus. Rubella virus enters the fetus during the maternal viraemic phase through the placenta. The damage to the fetus seems to involve all germ layers and results from the rapid death of some cells and persistent viral infection in others. Chromosomal aberrations and reduced cell division are present. Only a limited number of infected cells are reduced, resulting in fewer numbers of cells in affected organs at birth. The infection may lead to deranged and hypo-plastic organ development, resulting in structural anomalies in new born. The fetus is almost invariably infected if the mother is infected during the first trimester. After the first trimester, the virus is isolated infrequently from the neonates, probably because the fetal immune mechanism can be activated and infection can be terminated. Following the intrauterine infection in early pregnancy the virus persist through out the gestation and can be isolated from the most organs at autopsy. Virus can also be recovered from nasopharyngeal secretions, urine, stool and cerebrospinal fluid from survivors (Banatvala and Best, 1998).

Infection of Rubella virus can be disastrous in early gestation. The virus may affect all the organs and cause a variety of congenital defects. Infection may lead to fetal death, spontaneous abortion, or premature delivery. The severity of the effects of Rubella virus on the fetus depends largely on the time of gestation at which infection occurs. As many as 85% of the infants infected in the first trimester of pregnancy will be found to be affected if

followed after birth. While fetal infection may occur throughout pregnancy, defects are rare when infection occurs after the 20th week of gestation. The overall risk of defects during the third trimester is probably no greater than that associated with uncomplicated pregnancies (Mellinger et al, 1995).

Timing of the fetal infection determines the extent of teratogenic effect. In general, the earlier infection in pregnancy occurs, the greater the damage to the fetus. Infection during the first trimester of pregnancy results in abnormalities in the infants in about 85% of the cases; where as detectable defects are found in about 16% of the infants who acquired infection during the second trimester. However, birth defects are uncommon if maternal infection occurs after the twenty week of gestation. More recent studies suggest that the actual risk of major fetal damage is much higher than realized. Cardiac and eye defects are more likely to result when maternal infection is acquired during the first eight weeks of pregnancy, that is during the critical phase of organogenesis whereas retinopathy and hearing defects are more evenly distributed through out the first sixteen to twenty weeks of gestation (Hansaw et al, 1985; Banatvala and Best, 1998). In addition, recent studies indicate that infants exposed to Rubella in utero are at increased risk of developing schizophrenia as adults (Brown and Susser, 2002).

Cooper (1975) divided clinical features associated with rubella infection into those that were transient, development and permanent.

Table 1: Clinical features associated with Congenital Rubella Syndrome

SN	Clinical features	Common	Uncommon
1	Transient	Low birth weight Thrombocytopenic purpura Hepatosplenomegaly	Cloudy cornea Hepatitis Generalized Lymphadenopathy

		Bone lesions Meningoencephalitis	Hemolytic anemia Pneumonitis
2	Developmental	Sensorial deafness Peripheral pulmonary stenosis Mental retardation Central language defects Diabetes mellitus	Severe myopia Thyroiditis Hypothyroidism Growth hormone deficiency 'Late onset disease'
3	Permanent	Sensorial deafness Peripheral pulmonary stenosis Pulmonary valvular stenosis Patent ductus arteriosus Ventricular septal defect Retinopathy Cataract Microphthalmia Psychomotor retardation Microcephaly Cryptorchidism Inguinal hernia Diabates mellitus	Severe myopia Thyroid disorder Dermatoglyptic abnormalities Glaucoma Myocardial abnormalities

The pathogenesis of transient lesions is not understood but they are usually present only during the first week of life, do not recur and are not associated with the development of permanent sequelae. Intrauterine growth retardation resulting in low birth weight but at a normal gestational age is among the transient features. Thus Cooper and his colleagues found that about 60% of infected infants fell below the tenth and 90% below the fiftieth percentile (Banatvala and Best, 1998).

A petechial or purpuric rash is also common; particularly among infants whose mother had had maternal rubella in early pregnancy. However, low birth weight and a purpuric rash are seldom the sole manifestations of congenital rubella. These infants may have other anomalies such as congenital heart and eye defects, although they may not always be apparent at birth. Infants with thrombocytopenic purpura generally have a platelet count ranging from 3000 to100, 000 per cubic milliliter, this being associated with a decreased number of megakaryocytes, but of normal morphology, in the bone marrow (Banatvala and Best, 1998).

The classical congenital rubella syndrome triad consists of abnormalities of eyes, ears and hearts. Some of the defects of congenital rubella syndrome are described below;

Deafness

Of the permanent defects, the commonest one is sensorial neural deafness. This results from rubella-induced damage to the organ of corti. However, central auditory impairment may also occur. Hearing loss, which may be unilateral or bilateral, mild or profound, may sometimes be the only rubella- induced anomaly. Peckham (1972) followed up 218 children who were apparently normal at birth but who had been exposed to rubella in utero. When assessed for hearing loss at the age of 1-4 years, 50 (23%) were deaf; when 85 were reexamined between the ages of six to eight years, further hearing defects were detected in another nine children. Of the children with hearing defects, 90% were sero positive. Because rubella antibodies are uncommon before the age of four years, it is particularly important to follow up infants with persistent rubella antibody so that hearing defects can be recognized as early as possible (Banatvala and Best, 1998). The WHO definition of hearing loss in children is permanent unaided hearing threshold level for the better ear of 26 dB or greater (WHO, 1999). Hearing loss occurs in 70-90% of CRS cases, and in 50% of these children it is the only sign of CRS, although it is often not detected initially. There

is a evidence that the amount of hearing loss due to CRS has been underestimated, and mild to moderate hearing impairment due to CRS may be as frequent as severe to profound hearing impairment (Upfold,1984).

Heart disease

Congenital anomalies of the cardiovascular system are responsible for much of the high perinatal mortality associated with congenital rubella syndrome. Numerous studies have shown that the commonest lesions are persistence of a patent ductus arteriosus, proximal (valvular) or peripheral pulmonary artery stenosis, and a ventricular septal defect (Cooper, 1975).

Occasionally, neonatal myocarditis is found, often associated with other cardiac malformations. Rubella-induced damage to the intima of the arteries may result in obstructive lesions of the venal and pulmonary arteries (Rorke and Spiro, 1967; Phelan and Campbell, 1969).

Ocular defects

Many of the ocular defects characteristic of congenital rubella were described by Gregg (1941), who drew particular attention to pigmented retinopathy and cataract. Pigmented retinopathy may be present in up to 50% of the infants with congenital rubella. Cataracts, although usually present at birth, may not be visible until several weeks later. Microphthalmus is often associated with congenital cataract, but glaucoma though rare, is important to recognize because it may rapidly cause blindness. Microphthalmia and glaucoma result from disturbance in organogenesis and retinopathy, and cataract results from intra-uterine tissue destruction. However, delayed manifestations have also been recorded including lens changes, chronic uveitis, glaucoma, choroidal neovascularization, corneal hydrops and keratoconus (Murphy et al, 1968; Menser and Reye, 1974).

Defects in Central Nervous System

About 25% of infants who present at birth with clinical evidence of congenital rubella have central nervous system involvement, usually in the form of a meningoencephalitis. Such infants are often lethargic at birth but may become irritable and often exhibit evidence of photophobia.

Rubella panencephalitis is rare, about 20% cases having been reported as sequel of congenital rubella infection and following postnatally acquired disease (Lebon and Lyon, 1974). Rubella virus has been recovered from the brain both with and without co-cultivation technique; it has also been recovered patients' lymphocytes. The cerebrospinal fluid may contain elevated concentration of protein and immunoglobulin. Oligoclonal band and a high CSF:Serum rubella antibody ratio may be present (Wolinsky,1996). Histological studies show panencephalities with a perivascular inflammatory response as well as vasculitis. Rubella antigens have not been detected in brain sections by immunofluorescence. It has been postulated that post rubella panencephalities may be mediated by immunocomplexes (Waxham and Wollinsky, 1984) or by virus-mediated autoreactivity to brain antigens (Martin and Marquardt, 1989).

Diabetes

Diabetes mellitus was originally believed to be a rare complication of congenital rubella. However, follow-up studies of infants infected in utero during the Australian and US epidemics in 1940 and 1963/64 respectively have shown that 9 of 45 (20%) of Australian and 30 of 242 (12.4%)of US children eventually developed insulin dependent diabetes mellitus. A long latent period is characteristic, the mean age of children developing insulin dependent diabetes mellitus in the US study being nine years; all the Australian patients were in their third decade (Menser et al, 1978).

Bone defects

Bone lesions may be detected by X-ray. Irregular areas of translucency are present in the metaphyseal portion of the long bones but with out any evidence of periosteal reaction in over 20% 0f infants with congenital rubella. These lesions generally resolve within 1-2 months. Cooper (1965) detected these characteristic radiological changes in a fetus of 18 weeks gestation age, suggesting that the process inducing such changes begins in early gestational life (Banatvala and Best, 1998).

Late onset disease

Between the ages of about three and twelve months, some infants may present with such features as a chronic rubelliform rash, persistent diarrhea and pneumonitis. Marshall (1973) referred to this syndrome as "Late onset disease". Although mortality is high, some infants show a dramatic response to treatment with corticosteroids. This syndrome may reflect an immunopathological phenomenon; circulating immuno-complex that appear to certain rubella antigen have been demonstrated in infants with late onset disease (Tardiue et al,1980) and Coyle, Grospierre and Durandy (1982) demonstrated rubella antibody containing immune complexes in children with congenital rubella who developed new clinical problems some years after birth (Banatvala and Best, 1998).

Some developmental defects may take many months or years to become apparent, but then persist permanently. Failure to recognize such defects in early infancy may not always be the result of difficulty in their detection. There is evidence which suggests that such defects as perceptive deafness may actually develop or become increasingly noticeable some considerable time after birth. Thus Peckham (1972) showed that some two year children with apparently normal hearing had severe perceptive deafness when examined later. Menser and Forrest(1974) showed that it might be up to four years before the first rubella defects were recognized; further defects might continue to be recognized up to the age of eight years. The progressive nature of congenitally acquired disease is emphasized by the

finding that children with previously stable congenital rubella-induced defects developed a wide- spread sub acute progressive panencephalities with progressive mortar retardation as late as the second decade in life (Weil et al, 1975).

3.3.2.2 Pathogenesis

The fetus is at risk during the period of maternal viremia, because placental infection may occur at this time. The most likely source of virus is from the maternal viramia. Virus may also be excreted via the cervix up to six days after the onset of rash, and because virus may exist in the genital tract for even longer, placental infection by direct contact or from ascending genital infection can not be excluded. (Sepala and Vaheri, 1974)

After infection in early pregnancy, rubella induces a generalized and persistent virus infection in the fetus which may result in multisystem disease. Tondury and Smith (1966) conducted histopathological studies on the products of conception from mothers clinically diagnosed as infected with rubella; anomalies were present in 68% of 57 fetuses when maternal rubella contracted in the first trimester; and when contracted in the first month of pregnancy, 80% were abnormal, sporadic foci or cellular damage being present in the heart, inner ears, lens, skeletal muscles and teeth. Those authors suggested that Rubella virus enters the fetus via the chorion, in which it induces necrotic changes in the epithelial cells as well as in the epithelial lining of the blood vessels; the damaged endothelial cells are desquamated into the lumen of the vessel and then transported as virus infected "emboli" into the fetal circulation to settle in and infect various fetal organs. Lesions in the chorion were present as early as the tenth day after the onset of maternal rash. Fetal endothelial damage was distributed widely and probably resulted from viral replication rather than from antibody-mediated damage, because the most extensive histopathological changes were present at a gestational period before the fetal immune defense mechanism was sufficiently mature to be activated. Indeed, a characteristic feature of rubella embyopathy

following the maternal rubella in early gestational life is the notable absence of an inflammatory cell response (Tondury and Smith, 1966; Banatvala and Best, 1998).

At least two mechanisms have been suggested for inducing the fetal damage: **a virus-induced retardation in cell division and tissue necrosis**. Studies in vitro on embryonic cell culture and rubella-infected fetuses suggest that Rubella virus may induce chromosomal damage and cause cells to divide more slowly than those are uninfected. This may be due to a specific protein that reduces the mitotic rate of infected cells (Plotkin and Vaheri, 1967). If retardation of cell division occurs during the critical phase of organogenesis, it is likely to result in congenital malformations. It has also been shown that the organs of rubella infected infants are smaller and contain fewer cells than those of uninfected infants (Naeye and Blanc, 1965). The fetal endothelial damage induced by Rubella infection may cause hemorrhages in small blood vessels, leading to tissue necrosis and further damage of malformed organs such as liver, myocardium, organ of corti over a long period. Studies on the products of the conception obtained from virologically confirmed cases of rubella during the first trimester have shown that the fetus is almost invariably infected regardless of the time at which the infection has occurred during this period (Rawls, 1968; Thompson and Tobin, 1970).

3.3.2.3 Persistence of virus

Following intrauterine infection in early pregnancy, Rubella virus persists throughout gestation and can be isolated from the nasopharyngeal secretions, urine, stool, cerebrospinal fluid and tear of the survivors. Rubella virus can be isolated from nasopharyngeal secretions of most neonates with severe congenitally acquired disease, but by the age of three months the portion excreting the virus has declined to 50%-60% and by 9-12 months to 10 % (Cooper and Krugman, 1967). Particularly during the first few weeks after birth, those with severe disease may excrete concentration of virus and readily transmit infection to rubella-susceptible contacts. Rubella virus may persist in infants with

congenitally acquired disease in secluded sites for even longer. Thus, Rubella virus has been recovered from a cataract removed from a 3-year-old child (Menser et al, 1967) and from the cerebrospinal fluid of children with central nervous system involvement up to the age of eighteen months (Banatvala and Best, 1998).

3.3.2.4 Preconceptual rubella

Studies conducted in Germany and U.K. indicates that perconceptual rubella does not result in transmission of rubella virus to the fetus. Thus, Enders and colleagues found no serological or clinical evidence of intrauterine infection in 38 infants whose mothers rash appeared before or with in 11 days after their last menstrual period. However, the fetus of a mother whose rash appeared 12 days after her last menstrual period became infected , and all of 10 mothers who developed rash 3-6 weeks after their last menstrual period transmitted infection to their fetuses (Banatvala and Best, 1998).

3.3.2.5 Immune responses

The serum immune response in CRS differs from that seen in rubella (and from many other viral diseases). At birth, the serum of an infant with CRS contains maternally derived rubella-specific IgG antibodies as well as IgG and IgM antibodies synthesized by the fetus. Maternal rubella-specific IgG is also found in normal infants born to women who are immune to rubella. Therefore, rubella-specific IgM is used to diagnose congenital rubella infection in infants. In infants with CRS, rubella-specific IgM can be detected in nearly 100% at age 0-5 months; about 60% at age 6-12 months; and 40% at age 12-18 months; IgM is rarely detected after age 18 months (Chantler et al, 1982).

3.4 Modes of transmission

Rubella is caused by a virus that passes from person to person hence spreading the disease. The rubella virus passes from person to person through tiny drops of fluid from the nose and throat. It can spread when an infected person coughs or sneezes, or by direct contact

with an infected person's respiratory secretions. A person with rubella is contagious from one week before the onset of the rash until about one to two weeks after the rash disappears. Rubella is also transmitted by infected persons who exhibit no signs or symptoms and 30-50% of all rubella infections are not recognized as rubella disease. It can also be transmitted from a pregnant woman to her unborn child. A pregnant women who catches rubella during the first five months of pregnancy can pass the disease on to her baby(or fetus) while it is in the womb. Infants with congenital rubella syndrome, who were infected with rubella before birth, may be able to infect others for usually a year, and can therefore transmit rubella to those susceptible persons caring for them. They shed the rubella virus in the fluid from nose, pharyngeal secretions and urine for months or even years (Ananthanarayan and Panikar, 2000; McLean et al, 1997).

3.5 Period of communicability

It is most contagious as the rash is appearing, but can be spread a week before and for at least 5-7 days after the onset of rash. Infants born with congenital rubella syndrome shed the virus for long period. Rubella virus can be found in the nasopharyngeal secretions of more than 80% of infants with CRS during the first month of life, 62% at age of 1-4 months, and 33% at age of 5-8 months, 11% at age of 9-12 months and only 3% during the second year of life (Cooper and Krugman, 1967). The mechanism of virus persistence is not known but may be due to defects in cell mediated immunity (McLean et al, 1997).

3.6 Reservoir

Human is the only reservoir of Rubella virus. There is no known animal reservoir.

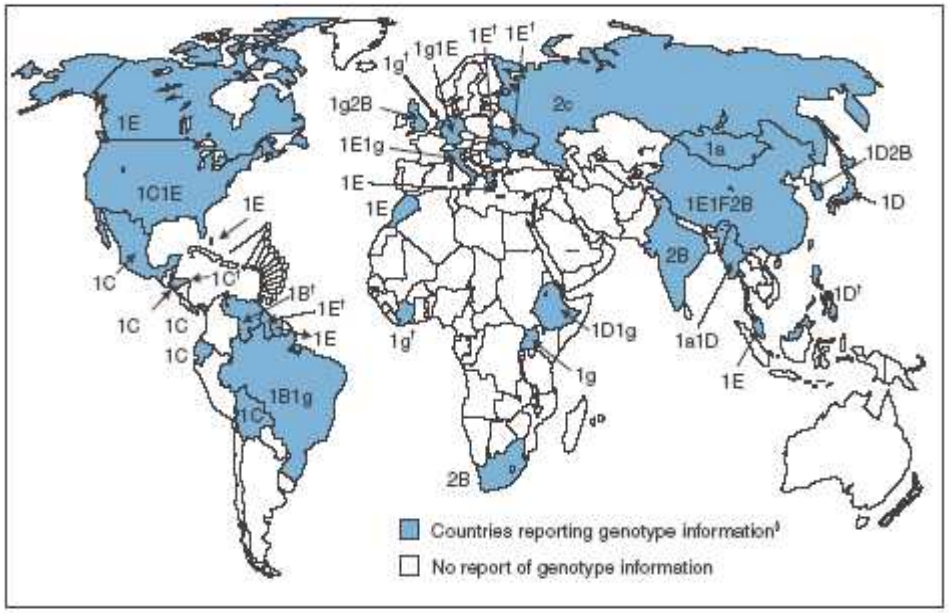
3.7 Epidemiology

Rubella has worldwide distribution. Before the introduction of rubella vaccination, epidemics usually happened in late winter and spring which is now also following the same trend in the countries without having rubella vaccination program. Rubella occurs less

commonly among pre-school children than among school children and young adults. Women of child bearing age were often infected as a result of exposure to their own children or at work (Banatvala and Best, 1998).

Many developing countries have rubella susceptibility rate among the women of child bearing age similar to those reported in developed countries before to the introduction of rubella vaccine; in tropical countries the infection tends to occur at an earlier age than in temperate climate, although out breaks of rubella are seldom reported. However, there is a considerable regional variation. High susceptibility rate may be found among island communities, owing to limited opportunities for the introduction of Rubella virus, as well as among some tribes in remote rural areas (De Freitas and Wong, 1990)

Recent studies of the molecular epidemiology of rubella virus world wide revealed that there are two genotypes, and that genotype I circulating almost worldwide, while genotype II is an Asian prototype restricted to the Asian continent. Genotype I viruses fall into a number of groups, some of which are geographically localized. Antigenically these two genotypes are cross-reactive and immunization with either virus results in immunity to all rubella viruses (Katow, 2004).



* Genotype data represent a summary of information from several laboratories that was made available in July 2005.
† Viruses were characterized after importation into another country.
§ Certain countries reduced indigenous rubella to low levels or have eliminated it during this period (e.g., Canada, Cuba, the United Kingdom, and the United States).

Figure 2: Geographical distribution of Rubella Virus genotypes world wide, 1995-2005 (Source: CDC, 2005)

3.7.1 Disease burden: The global scenario

Though the rubella was first described in the mid eighteenth century, it was accepted as a distinct disease only in 1881. The actual burden of disease came to light after the work of McAlister Gregg in 1941 in which he showed that, if acquired in early pregnancy, rubella could cause congenital malformation (Banatvala and Best, 1998).

In United States, in pre-vaccine era, epidemics of rubella occurred every 6 to 9 years, with the last major epidemic occurring in 1964-1965. The 1964 epidemic resulted in an estimated 12.5 million cases of rubella and 20,000 infants born with CRS. More recently,

there has been moderate resurgence of rubella and a dramatic increase in CRS from1988 to 1990. A provisional total of over 1000 cases of rubella were reported in US in 1990.
Since the mid-1970s, rubella incidence in Canada has remained relatively low. An average approximately 1000 cases (ranging from 237 to 2450) were reported annually from 1986 to 1995; this represents a mean rate of 4.0 per 100,000 populations (CDC, 2001).

In Japan, nation-wide epidemics have occurred every 5 years; 1982, 1987, 1992 and 1997, although 1997 epidemic was small. The 1966 epidemic is considered to be big epidemic in Japan. In 2002, the reported no of cases of rubella was 2984 (Katow, 2004).

In Taiwan there have been four epidemics of rubella, occurring at a rate of once every decade; in 1944, 1957/58, 1968/69 and 1977. After immunization program in 1980 no large epidemics have occurred since 1977, occasionally small outbreaks have been reported (Su and Guo, 2002).

In Singapore, epidemics of rubella occurred in 1969, 1975/76, 1977/79 and 1982. After immunization program in 1976/1982, the incidence of rubella outbreaks decreased until 1987 after which there no data regarding rubella outbreaks (Katow, 2004).

The last 7 years observation shows that rubella had been reported from almost every countries of the world, no matter the number of cases. In Australia, the rubella cases were decreasing since 1999. The reported cases were 379 in 1999, 313 in 2000, 262 in 2001, 253 in 2002, 56 in 2003, 44 in 2004 and 31 in 2005. In Brazil, the rubella reported cases were 11144 in 1999, 8781 in 2000, 3759 in 2001, 1256 in 2002, 319 in 2004 and 318 in 2005. In China, 24015 and 25446 rubella cases were reported in 2004 and 2005. In South Korea, 520 rubella cases in 2002, 520 in 2003, 507 in 2004 and 123 in 2005 were reported. In Japan, 3120 in 2000, 3123 in 2001, 2561 in 2002, and 2794 in 2004 rubella cases were reported (WHO,2006).

3.7.2 Research activities: The global scenario

Rubella and Congenital rubella syndrome is an under-recognized public health problem in many developing countries. Inclusion of rubella vaccine in the national immunization program was found to be implemented in less than one-third of the developing countries in a review conducted by WHO. There is an urgent need for collection of appropriate data to estimate the cost-effectiveness of a potential global rubella control programme (Cutts and Vynnycky, 1999).

A serosurvey of rubella was carried out in Kinshasa (Zaire) by haemagglutination inhibition and IgM assay among 106 newborn infants (91% positive); 101 suckling infants aged 9-18 months (32.7% positive); 100 children aged 2-4 (58% positive); and 100 young girls 9-11 (68% positive), while 93% of mothers showed the presence of protective antibodies (Omanga et al, 1991).

In a serosurvey conducted in southern Italy, serum titers of anti-rubella antibodies were measured in 4,424 babies and children (aged 0-15 years) and in 2,362 females of childbearing age by a microhemagglutination-inhibition technique. Sera were screened for IgM antibodies with an ELISA; positive sera were titrated in a capture immunoenzymatic test. The incidence of serological positive response, high at birth and in the first 6 months of life (65.0%), declined in the older age-groups (60.2% from 6 to 12 months, 57.0% from 1 to 2 years, 54.2% from 2 to 3 years, and 55.2% from 3 to 6 years). Over 6 years, the incidence increased progressively (63.9% from 6 to 9 years and 76.1% from 9 to 15 years). In females aged 15-45 years the seronegativity rate was 8.6% (Leogrande, 1993).

A study that demonstrates prevalence of sensorineural hearing loss due to rubella in Saudi children found positive IgM antibody against rubella virus in the blood of 23 out 1,054 (2.2%) children (age ranged between 12 months and 14 years). 15 out of 23 infected children were found to have bilateral sensorineural hearing loss. Hearing impairment was

bilateral in all cases, profound in 1, moderate to severe in and mild in 5 (Zakzouk and al-Muhaimeed , 1996).

A documented case of rubella reinfection during pregnancy was reported in a previously vaccinated woman with residual antibody titer to rubella of 15 IU/ml in Israel. The reinfection occurred following an exposure to rubella virus (contact with 6-year-old daughter with clinical rubella) between the 7th and 10th week of pregnancy which resulted in transmission of the virus to the fetus. Umbilical cord blood drawn by cordocentesis was found to be strongly positive for rubella IgM antibody. After termination of the pregnancy rubella virus was isolated in cell culture from fetal tissues (Aboudy et al, 1997).

Indirect ELISA assay was used to test 1193 sera for rubella IgG and IgM antibodies in a seroepidemiological survey conducted in Shiraz, Islamic Republic of Iran involving three age- and gender-differentiated sample populations in Shiraz: 203 children aged 2-7 years, 255 paired mothers and neonates (cord blood) and 480 women aged 14-70 years. Seropositivity among women aged 14-70 years was 96.2%. No IgM positive case was found among the 255 tested cord blood samples. Seropositivity among the 203 children was 97.0% (Doroudchi et al, 2001).

To determine the prevalence of rubella antibodies and age of exposure to rubella among schoolgirls in Yemen, the sera samples of 323 female students (age range 11-21 years; mean age 16.26 +/- 1.89 years) drawn from three schools in Sana'a were screened for rubella IgG antibodies using ELISA and, if negative, for IgM in order to exclude the possibility of recent exposure. Of 323 sera, 296 (91.64%) were positive for rubella IgG. All IgG negative sera were also IgM negative. The prevalence of rubella IgG among Yemeni schoolgirls was found to be high, with most becoming immune between the ages of 11 and 21 years (Sallam et al, 2003).

In an investigation carried out in northwestern Brazil, during a large rubella outbreak from April 1 to December 31, 2000, 391 confirmed rubella cases were reported. The incidence among person's ages 12 to 19 years (3.3 per 1000 population) was increased 3.7-fold relative to children ages 1 to 4 years (95% confidence interval, 2.4 to 5.8). Of 21 infants with suspected CRS cases, 17 (91%) were tested for rubella-specific antibodies, of whom 7 were IgM-positive and 5 had confirmed CRS. The peak incidence of confirmed CRS (4.3 per 1000) was in March 2001, 7 months after the outbreak peak, with an annualized incidence of 0.6 per 1000 (Lanzieri et al, 2003).

3.7.3 Rubella situation in SEARO

In SEARO countries, the reported rubella cases have been increasing in recent years. From this region, 966 cases in 1999, 1174 cases in 2000, 993 cases in 2001, 1187 cases in2002, 1481 cases in 2003, 1251 cases in 2004 and 9834 cases in 2005 had been reported. In, 2005 the Measles and Rubella Laboratory Network Serology result showed that out of 3716 serum samples received for test in SEARO region, 914 (25.7%) were positive for Rubella. The highest positive cases were from Bangladesh (609 cases), no case from Maldives and single case were confirmed as Rubella in India (WHO, 2006).

In a study in Bangladesh which investigated the presence of rubella antibody in hearing-impaired children, a total of 198 hearing-impaired children and 200 children without hearing problems were studied. Blood samples were collected from both mothers and children; sera were subjected to ELISA for anti-rubella IgG. Rubella antibody was detected in 74% of the hearing-impaired children and in 18% of those with normal hearing: this finding correlated with the presence of rubella antibody in the mothers (67%) of rubella seropositive hearing-impaired children. In contrast, rubella antibody was observed in only 14% of the mothers of the children without hearing problems. Consistent with the presence of antibody, 41% of the seropositive mothers who had hearing-impaired children gave a

history of fever and rash during early pregnancy. This study indicated a strong association between rubella infection and hearing impairment in children (Rahman et al, 2002).

In a study in India, paired sera of 146 babies with suspected intra uterine infection and their mothers from lower socioeconomic strata was tested for IgM antibodies by commercially available Enzyme immunoassay (EIA) kits. It was seen that out of 146-paired samples evaluated, 15-paired samples (10.27%) were positive for IgM antibodies. The transmission rate of rubella virus from mother to child when the mother was infected was around 55.55% according to this study. CRS prevalence of 10.27% among symptomatic infants is significant as a large majority of rubella infection remains undetected and hence the actual burden of the disease may be higher (Chakravarti and Jain, 2006).

In a study in Manipal, which includes a total of 342 infants suspected of having congenital infections from January 1991-December 1993, 52 (15.2%) were found to be positive for IgM antibodies to rubella virus. The commonest clinical presentation in infants with IgM antibodies to rubella virus was bilateral congenital cataract and hepatosplenomegaly (Ballal and Shivananda, 1997).

3.7.3 Rubella situation in Nepal

WHO-IPD-Government of Nepal integrated Measles surveillance from 2003 as a part of global strategic plan. The Lab based surveillance data in Nepal show a major shift from measles to rubella outbreaks after the successful measles campaign conducted during 2004-2005. The surveillance data of 2003 showed no rubella cases. During 2004, out of 196 outbreaks investigated, 13 were rubella and 11 were mixed rubella/measles outbreaks with 71 rubella cases among 824 serum samples investigated. In 2005, out of 46 outbreaks investigated 36 were rubella and 2 were mixed rubella/measles with 161 rubella cases (Ghimire and Partridge, 2006). The unpublished data of NPHL showed that the rubella

cases in the females of child bearing age was 6.82% in 2060BS, 12.8% in 2061 BS and 14.7% in 2062 BS of the total tested cases.

3.8 Diagnosis

The laboratory diagnosis of rubella depends upon two bases;

a. Clinical diagnosis

b. Etiological diagnosis

3.8.1 Clinical diagnosis

Symptoms of postnatal rubella include the following characters;

In children: Low grade fever; swollen glands; joint pain; headache; conjunctivitis; rash

In adults and children:

- Swollen tender gland or lymph nodes usually in the back of the neck behind ears (may persist for up to a week)
- Fever (usually rises above 38^0C/100.4^0F)
- Headache; Reddened eyes; tiredness; joint pain
-Rash (Firstly appears on face as pink dots under the skin and spreads to the trunk and limb. It appears on the first or third day of the illness but it disappears after about three days with no staining or peeling of skin)
- Forchheimers sign occurs in 20% of the cases, and is characterized by small, red papules on the area of the soft palate.
-Flaking; dry skin
-nerve becomes weak or numb (very rare)

Symptoms of congenital rubella include the following;

- Cataract in eyes
- Heart problems
- Mental retardation
- Growth retardation

- Enlarged liver and spleen
- Skin lesions
- Bleeding problems

Many people with rubella have few or no symptoms, and up to the people who have the disease may not get a rash. In most cases symptoms appear with in 16-20 days after exposure.

Most of such cases are clinically diagnosed measles cases. So, diagnostic test such as serological testing and virus culture are used to confirm the acute or recent rubella infection. Also, many rash illnesses may mimic rubella infection and 20% to 50% of rubella infection may be sub clinical. Laboratory testing is the only way to confirm the diagnosis. (Banatvala and Best, 1998; Ananthanarayan and Panikar, 2000)

3.8.2 Etiological diagnosis

3.8.2.1. Culture

Rubella virus can be isolated from nasal, blood, throat, urine and cerebrospinal fluid specimens from postnatal and congenital rubella cases. Infants with congenital rubella syndrome may shed virus for a prolonged period, so specimen obtained later may also yield Rubella virus. Specimen for virus isolation (urine specimen and pharyngeal swabs) should be obtained monthly until cultures are repeatedly negative (CDC, 2006).

Virus isolation has only limited applicability for the routine diagnosis of rubella. Most clinical situations requiring laboratory diagnosis are better investigated serologically. The few situations in which rubella virus isolation is indicated include the suspected rubella with severe complications, fatal cases for which serological confirmation of the etiology would not be possible, and cases where strain characterization of the infecting agent (i.e. vaccine-like versus wild-like) may be required for epidemiological purposes.

A wide variety of cell types are susceptible to infection by rubella virus. For primary isolation of rubella virus from clinical specimens, however, primary African green monkey kidney (AGMK), Vero, or RK-13 cell cultures are recommended. Isolation of rubella virus in primary cultures of AGMK cells has been considered the standard method since1962. Rubella virus is detected in this cell type by interference with the cytopathic effects of a challenge virus (Herrman, 1985).

Some laboratories use RK-13 or Vero cells for isolation of rubella virus. In these cell systems, rubella virus produces cytopathic effects; however, the cytopathic effect is not always clear on primary isolation, and cell culture fluids may need to be passaged several times for full detection of virus. These cell systems, however, do offer the advantage of direct neutralization for identification of an isolate. Furthermore, an indirect immunoflurescence staining method has been shown to be specific and sensitive for identifying rubella virus isolates in these cells (Schmidt et al, 1966). Molecular typing of Rubella virus isolates is very important for surveillance. Molecular epidemiologic surveillance provides important information about origin of the virus and its circulation. (Banatvala and Best, 1998)

3.8.2.2 Serological diagnosis

Serological techniques for the detection of antibodies to rubella virus provide the approach of choice for laboratory diagnosis of acute and congenital rubella infections and for the determination of rubella immune status.

Methods currently available include hemagglunitation, passive hemagglunitation**,** hemolysis in gel**,** latex agglutination, enzyme immune assay, fluorescent immunoassay, radioimmunoassay, complement fixation and a variety of rubella-specific IgM antibody assays (Herrman, 1985).

Haemaggulutination Inhibition (HI)

Although the rubella HI test remains the benchmark for rubella serology, the numerous more recently developed test systems are now much more widely used than HI for routine rubella antibody testing.

Rubella HI antibody appears very soon after the onset of rubella symptoms and rises rapidly, often reaching peak levels with in seven to ten days. Thereafter, rubella HI antibodies are usually detectable through out life, and thus the presence of HI antibody is a reliable indicator of past rubella virus infection.

HI was the first widely used rubella test. Satisfactory rubella hemagglutinin antigen can be prepared in cultures of BHK_21 or Vero cells or obtained commercially. The minimal acceptable titer of hemagglutinin antigen for this test is 1:64. Erythrocytes to be used in the standard hemagglutinin and HI test are obtained from one to three-day-old unfed baby chicks.The hemagglutinin and HI tests are conviently performed in disposable micro-titration plate (Herrman, 1985).

HI has the advantage that considerable experience has been gained over the past fifteen years regarding the clinical significance of HI titre results. It is well established that the presence of rubella antibody detectable by the HI test accurately correlates with clinical protection of the individual. The HI test does, however, have several disadvantages. It is time consuming and highly technique-dependent for accuracy and it requires pretreatment of serum is assayed for antibody. Failure to completely remove the beta-lipoprotein inhibitors may result in false-positive test results (Herrman, 1985).

Passive hemagglutination

The PHA test is most useful in detecting serological evidence of immunity from past rubella infection. Human erythrocytes for the test are coated with a soluble rubella virus antigen and used in a one-stage PHA system. Standard micro titration techniques are used.

The rubella PHA test is currently available commercially in kit form. These kits are designed to be used for rubella immunity screening; test results are recorded as "Positive" or "Negative" for antibody. Since the sera in the PHA test do not have to be treated to remove non-specific reactants before testing, specimens can be tested rapidly. Immunity screening results obtained with the commercial PHA kits correlate over 98% with HI test results (Herrman, 1985).

Latex agglutination

A passive latex agglutination card test, recently developed commercially for detecting rubella antibody in human serum, offers another alternative system for rubella diagnosis and immunity screening. The test requires no serum pre-treatment and no elaborate equipment and can be performed in a matter of minutes. Rubella antibodies detectable by this latex agglutination test appear with in a few days after onset of acute illness and thereafter persist indefinitely. This test method appears to be most useful for the clinical laboratory performing test in which immediate results are required for patient management (Herrman, 1985).

Hemolysis in gel

The rubella hemolysis-in-gel or radial hemolysis, test is widely used in Europe as a simple and sensitive method for rubella antibody testing. This test has the advantage of simplicity; it requires no special or expensive equipment and can be performed on untreated serum (Herrman, 1985).

Fluorescent immunoassay

An indirect fluorescent-antibody test for rubella antibody was first described in 1964; chronically infected LLC-MK_2 cell cultures were used as the solid phase antigen. Other acutely infected cells are grown either on Leighton tube cover slips or in culture flasks which are then trypsinized and deposited on slides to form smears. The cytoplasm of cell infected with rubella virus contains rubella antigens. These antigens are used to detect specific antibodies by the indirect method in which an anti-human globulin conjugated with fluorescein isothiocyanate is employed. The technique is rapid and relatively inexpensive and allows quantation of IgG and IgM antibodies;however, IFA results are read visually with a fluorescence microscope and are open to subjective interpretation.

More recently, a soluble rubella antigen immobilized on an opaque plastic surface has been used in the indirect fluorescence immunoassay. In this test system, marketed commercially as the FIAX test, the antigen sensitized surface is allowed to react in two step procedure with the serum and fluorescein labeled conjugate, and the resulting fluorescence signal is measured objectively with a fluoremeter. The intensity of the fluorescence signal correlates with the titre of rubella antibody. The sensitivity and specificity of this assay method correlates well with those of the HI test (Herrman, 1985).

Enzyme immunoassay

Solid-phase EIA methods have been successfully applied for the detection and quantitation of rubella antibodies (Gravell et al, 1977). A variety of rubella EIA test kits are available commercially. One distinct advantage of the solid-phase "sandwich" immunoassay techniques, such as EIA, over the standard HI technique is that serum can be tested without pretreatment because no natural serum inhibitors of the reaction are known. These tests are simple to perform and practical for use in clinical laboratories, given the required reagents and equipments (Herrman, 1985).

Specific rubella IgM assays

The presence of specific IgM antibodies to Rubella virus has special diagnostic significance. In patients, the absence of IgG Rubella antibodies means that the person likely has not been exposed to the Rubella virus or been vaccinated and is not protected against it. The presence of IgG antibodies but not IgM antibodies indicates a history of past exposure to the virus or vaccination and indicates that the person tested should be immune to Rubella virus. The presence of IgG antibodies, but not IgM antibodies, in new means that the mother's IgG antibodies have passed to the baby in utero and these antibodies may protect the infant from Rubella infection during the six months of life. The presence of IgM antibodies in new born indicates that the baby was infected during pregnancy (because the mother's IgM antibodies do not pass to the baby through the umbilical cord). The presence of IgM antibodies, with or with out IgG antibodies, in persons indicates a recent infection with Rubella virus.

Specific rubella antibodies in the IgM/IgG class can be detected by various methods, including IFA, EIA, Radioimmunoassay and adsorption of serum IgG with Staphylococcal protein A, or by physical separation of IgM from IgG from density gradient ultra centrifugation or column chromatography followed by HI assay of the fractions. Sucrose gradient ultra centrifugation is considered at present to be the most reliable and specific method for rubella IgM antibody assay. However, only a few diagnostic laboratories have been doing the test.

Some of the more recently developed methods for detecting rubella IgM antibody, such as the IgM-radioimmunoassay and IgM-EIA, offers simpler and more rapid approaches but questions have been raised regarding the specificity of such indirect assay methods. The presence of rheumatoid factor (IgM anti-IgG antibodies) in serum may cause false positive IgM results in these systems. Effective methods for avoiding these nonspecific reactions, including preadsorption of serum to remove IgG and IgG-rheumatoid factor complexes or

IgM captured assays utilizing anti IgM captured antibody on the solid phase carrier, are being incorporated into these procedures to make them acceptable for specific IgM antibody assay in routine diagnosis.Different types of acceptable commercial rubella IgM assays are now available in market.

ELISA

The IgM antibodies against rubella virus which develops after about 7 days up to a month can be detected in the blood. ELISA is a highly sensitive, specific, less time consuming and reproducible method for detection and quantification of many cytokines (Beech et al, 1997; Jung et al, 1998). IgM capture ELISA, requires only one blood sample for case confirmation. In clinically confirmed cases, the specificity of capture assays was 97.3%. The test can be done with minimal training and results may be available within 2–2.5 hours of starting the assay (NPHL-SOP, 2005).

Detection of rubella specific IgM is therefore useful indicator of recent primary infection and becomes the frontline diagnostic test in situation where speed is required or demonstration of rising titre is not possible, e.g. when the first available serum sample is taken at a time when the antibody response had reached a plateau. In situations where test results are equivocal, or maternal infection occurs later than the first trimester, prenatal testing for anti-rubella IgM antibodies in fetal blood may assist in determining whether the fetus is infected.

There are different manufacturers who manufacture IgM/IgG kits. Following are the list of such manufacturers;

Dade Behring (German)

Human (German)

Ranbaxy

Among above various kits available in market, the following kit had been used in this research work.

Test principle of ELISA developed by DADE BEHRING (German)

The RF absorbent binds to the IgG present in the test sample. Any rheumatoid factor in the sample binds to the resulting immune complexes and is thus eliminated. The RF absorbent precipitates up to 15 mg IgG/ml and thus also removes the IgG specific for Rubella virus. This effect enhances the sensitivity of the IgM test.

IgM in the test sample, which is specific for the virus, binds to the virus antigen on the plastic surface of the test plate.The anti-human IgM /POD Conjugate binds to this complex.

The enzyme component of the conjugate catalyses the working chromogen solution (TMB plus hydrogen peroxidase) producing a blue color. This reaction is terminated by the addition of stopping solution POD and a yellow color is formed which is then read at 450 nm. IgM directed against cellular antigens is measured in the same way in the well coated with control antigen.

The differences between the color intensities in the well coated with antigen and in the well coated with control antigen is a measure of the concentration and immunochemical reactivity of the virus antibodies detected in the sample.

IgM capture ELISA, requires only one blood sample for case conformation. In clinically confirmed cases, the specificity of the capture assays was 97.3%. These are considered superior to indirect assays, since they do not require the removal of IgG antibodies.

3.8.3 Molecular virological diagnosis

Although the detection of rubella virus by PCR is not included as confirmatory in the case definition, the test does provide presumption evidence of rubella infection. In the United

Kingdom, there has been extensive evaluation of PCR for detection of rubella virus in clinical specimens, documenting its usefulness. Clinical specimens obtained for virus isolation and sent to CDC are routinely screened by PCR. Further validation is needed for classification of cases that test positive by PCR in the absence of virus isolation (Bosma et al, 1995a). A reverse transcriptase nested PCR (RT_PCR) assay for the detection of rubella virus RNA using a primer for the E_1 open reading frame was found to be sensitive and specific. The PCR provides a very sensitive and unequivocal test for diagnosis of fetal rubella virus infection. RNA extracted from biopsy specimen (chorionic villi), placenta or products of conception was reverse-transcribed using a rubella virus-specific oligonucleotide primer and the cDNA was amplified by PCR (Ho-Terry et al, 1990; Bosma et al, 1995b).

3.9 Prevention and control

3.9.1 Vaccination

Rubella is a vaccine preventable disease. The vaccines are made from live, attenuated viruses which are effective and long-lasting and cause few side effects, except for transient anthralgias in some women. The vaccine has caused a significant reduction in the incidence of both rubella and CRS, making CRS a preventable disease. Vaccines available are BPV77, HPV77-DE5, Condehill, RA2713, To-336, Matsuura etc. RA2713 vaccines are safer and more immunogenic. Vaccines are administered subcutaneously but RF2713 will induce an immune response when administered intranasally. An immune response is induced by Rubella vaccines in approximately 95% of susceptible vaccines. Antibodies develop10 to 28 days after vaccination. Antibodies persist at levels > 1500IU/L (P.000) in most vaccines at least 21 years. Some studies have, however, shown that in approximately 10% of vaccines, antibody titre decline to <1500IU/L with in 5 to 8 years and a small number may become completely seronegative. Antibody concentration and cellular immune response are generally lower than after naturally acquired infection (Banatvala and Best, 1998).

Widespread immunization against rubella is critical to controlling the spread of the disease, thereby preventing birth defects caused by congenital rubella syndrome (Hirsch, 2006). The rubella vaccine is usually given subcutaneously to the children and to unimmunized young adult women if they are not pregnant and will use contractive for next three months. There is no evidence that virus vaccine causes malformations. It should not be given to immunocompromised patients, whether as a result of disease (e.g. malignancy) or of treatment with corticosteroids, radiotherapy or cytoxic drugs or to pregnant women. Children should receive the MMR vaccine between 12 and 15 months of age, and again between 3 and 6 years of age (DoctorNDTV Team, 2004). The prevention of congenital rubella obviously is dependent upon adequate early immunization, resulting in a high prevalence of immunity in women of childbearing age. If there is any doubt that they are immune, women should be screened for rubella immunity at the beginning of pregnancy (Boyer et al, 2004).The rubella vaccine should not be given to pregnant women or to a woman who may become pregnant within 1 month of receiving the vaccine. Pregnant women who are not immune should avoid anyone who has the illness and should be vaccinated after delivery so that they will be immune during any future pregnancies (Hirsch, 2006). Contact isolation is required for neonates suspected to have congenital rubella (Boyer et al, 2004).

3.9.2 Control of transmission

The rubella cases should be excluded from school and childcare for at least four days after onset of rash. Adults should not go to work for the same period of time. Patient with rubella should avoid contact with other people particularly pregnant women. If a woman with suspected rubella is pregnant, the diagnosis should be confirmed serologically and the patient should be referred to a specialist obstetrician for advice, taking care not to expose other pregnant women to possible infection (Banatvala and Best, 1998; CDC, 2001)

3.10 Treatment

No treatment can shorten the course of rubella infection. Most of the time the symptoms are so mild that treatment usually isn't necessary. However, doctors often recommend isolation from others especially pregnant women during the infectious period. If a woman contracts rubella while she is pregnant, she should discuss the risks to the baby with the doctor. If the woman wishes to continue her pregnancy, she may be given antibodies called hyperimmune globulin that can fight off the infection. This can reduce the symptoms but does not eliminate the possibility of the baby developing congenital rubella syndrome. Treatment for congenital rubella syndrome varies depending on the extent of the infant's problems. Children with multiple complications may require early treatment from specialists. (DoctorNDTV Team, 2004).

CHAPTER IV

4. MATERIALS AND METHODS

4.1 Materials

Equipments, chemicals and other supplies available at NPHL were used during the entire study period. List of materials are all given in Annex I.

4.2 Method

4.2.1 Study design

The study was designed as a descriptive cross-sectional study.

4.2.2 Study period

The study was carried for complete one year, January to December 2006.

4.2.3 Study site

The entire test was based at National Public Health laboratory which is the National Referral Laboratory for Rubella, Measles and Japanese Encephalitis in Nepal.

4.2.4 Sample size

The total number of 213 specimens of rubella/measles suspected cases received through WHO-IPD surveillance network for test of rubella in the year 2006 from the whole country were the sample size of the study.

4.2.5 Sample collection, storage and transport

The serum samples during 4-28th day of rash onset from rubella/measles suspected cases from different part of the country were collected stored and transported maintaining the

reverse cold chain to National Public Health Laboratory through WHO/IPD (Immunization Preventable Disease).

Five ml (3 ml from children) of venous blood was collected by vein puncture from each suspected cases after about of 4 days of the appearance of rashes and was put in clean, dry and labeled test tube. For those who visited NPHL for rubella test, blood was collected on the day of lab visit.

The collected blood in test tube was allowed to clot by tilting at 60^{o} in slanted position for 30 minute at room temperature. Then the blood in test tube was centrifuged, the serum was separated and stored at 2-8^{0}C. Those from different part of country were transported to NPHL through IPD/ Surveillance Medical Offices maintaining reverse cold chain. The received samples were checked for their quality (good / haemolysed) and quantity (sufficient/ insufficient) then data were entered into computer and stored at -20^{0}C until tested.

4.2.6 Data collection

Patients details on onset of rash, fever etc were obtained through a questionnaire/case investigation form reached to NPHL through WHO-IPD surveillance system. Laboratory details were recorded at NPHL lab register and required portion of them were recorded for thesis purpose also.

4.2.7 Specimen exclusion

Few of the samples were excluded when they were found with;

- Insufficient specimen for testing.
- Unlabeled samples
- Whole blood

4.2.8 Specimen processing (Laboratory diagnosis of Rubella)

A total of 213 samples received at NPHL from different parts of the country through Measles/Rubella surveillance system were tested at NPHL on weekly basis using standard operating protocol (Annex-II) developed at NPHL utilizing Dade Behring ELISA (German).

4.2.8.1 Protocol of the test

All the required reagents and chemicals were available in the kit provided which were stored as instructed in standard operating procedure .All the specimens received at NPHL were processed and tested using the standard methodology according to the respective protocol. The detail protocols are made available in Annex II.

4.2.8.2 Calculations and quality control

Enzygous anti- Rubella IgM (DADE BEHRING)

Calculations:

On the basis of the difference of optical density (OD) between the OD of test antigen coated well and OD of control antigen coated well, results were interpreted.

OD of specimen = OD of test antigen coated well – OD of control antigen coated well

If OD of the test sample is <0.10 (cut-off value), the test result is **negative.**

If OD of the test sample is >0.20 (retest limit), the test result is **positive.**

If OD of the test sample is between 0.10 and 0.20, the test result is **equivocal.**

Quality control:

The test must comply with the following validation criteria:

1. The absorbance values for each pair of Reference P/P wells must reach or exceed a minimum absorbance value of 0.2ΔA: $\Delta A_{\text{Reference P/P}} \geq 0.2$.
2. The individual reading of the reference P/P at the start and end of the run must not deviate from the mean of these two readings by more than ± 20%.

3. The ΔA for the reference P/N must always be less than 0.1 (≤ 0.99)

4.2.8.3 Interpretation of the result

Enzygous anti- Rubella IgM (DADE BEHRING)

A negative result means the virus specific IgM can not be detected. The patient either is not acutely infected with rubella virus or, if infected or immunized, is (still) unable to produce IgM specific for virus.

When the result is equivocal after a retest also, it indicates that rubella specific antibody have not been formed to the level to give positive result. In such cases, second sample must like wise be collected no later than 7 days later.

If a sample is assessed to be positive this means that virus specific IgM has been detected. These IgM antibodies are formed during primary rubella infection, after vaccination and occasionally as a result of re-infection after a past vaccination. In addition, some patients develop "long-persisting IgM antibodies" after a past infection or vaccination. Furthermore, the patient may have developed specific rubella IgM antibodies as a result of polyclonal response to a primary infection such as Epstein - Barr virus (EBV), Cytomegalovirus (CMV), Hepatitis A virus or even gram negative bacteria.

4.2.9 Data analysis

Collected data was analyzed to find out the age, sex, months, seasons and geographical distribution of the cases. The collected data were analyzed using Epi-info (Version 6.04b-1997)

CHAPTER V

5. RESULTS

A total of 213 samples of suspected measles/rubella cases were received from different part of country through the WHO-IPD surveillance network and analyzed during the study period.

5.1 Suspected Rubella cases and Lab based results:

Table 2: Anti-Rubella IgM positivity

Suspected Rubella cases	Confirmed Rubella cases	Positive percentage
213	105	49.3

Of the total 213 specimens of Rubella/Measles suspected cases received at NPHL, 105 specimens were found to be positive for anti-rubella IgM antibodies which comprise 49.3% of the total tested specimens.

Table 3: Gender wise distribution of rubella cases

Sex	Rubella suspected cases	Rubella confirmed cases	Positive %	% of Total positive cases
Male	118	50	42.37	47.62
Female	95	55	57.89	52.38
Total	213	105	49.3	100

Out of 213 suspected cases of rubella/measles, 118 were from male patients, which accounted 55.40 %of total and the rest 95 (44.60 %) were from female. The ratio of rubella suspected cases in male to female was found to be 1.2:1. Among the 118 male suspected rubella/ measles cases tested, 50(42.37%) were found to be rubella positive which constitutes 47.62% of the total positive rubella cases. Similarly, out of 95 female suspected rubella/measles cases tested, 55(57.89%) were confirmed to be rubella positive which constitutes 52.38% of the total positive rubella cases. The ratio of rubella positive cases in male to female was observed as 0.9:1. The number of rubella positive cases in

female sex was higher than male. The association between the disease and sex is statistically significant **(P<0.05; χ^2 =5.07).**

Table 4: Age wise distribution of rubella cases

Age group (in years)	Suspected Rubella cases	Confirmed Rubella cases	Positive %	% of Total positive
Below 5	**72**	**30**	**41.09**	**28.57**
5-10	**93**	**49**	**52.69**	**46.67**
10-15	**34**	**18**	**52.94**	**17.15**
15-20	**9**	**3**	**33.33**	**2.86**
20-25	**1**	**1**	**100**	**0.95**
25-30	**4**	**4**	**100**	**3.8**
Total	**213**	**105**	**49.3**	**100**

Age wise distribution of rubella cases revealed that the highest number of positive cases (49 cases) was found in the age group 5-10 years, which constitutes 46.67% of the total rubella positive cases. Out of 73 tested suspected cases in the age group bellow 5 years, 30 cases were positive which comprises the 28.57% of the total positive cases. In the age group of 10-15 years, 18 (17.15%) cases were positive out of total 34 cases tested. Out of 14 suspected cases from age group above 15 years, 9 cases were from age group 15-20 years where 3 cases (2.86%) were positive; 1 and 4 suspected cases were from the age group 20-25 years and 25-30 years respectively in which all were positive for rubella. Though the results showed that the positive cases were clustered in lower age, however the association between the age and the occurrence of disease is not found statistically significant **(P>0.05; χ^2 =2.63).**

Table 5: District wise distribution of rubella cases

District	Suspected Rubella cases	Confirmed Rubella cases	% of total positive cases
Baitadi	**8**	**7**	**3.74**
Banke	**1**	**0**	**0.47**
Bara	**10**	**8**	**4.67**
Bhaktapur	**21**	**14**	**9.81**
Chitwan	**6**	**5**	**2.8**
Dhading	**8**	**5**	**3.74**
Dadeldhura	**10**	**8**	**4.67**
Jhapa	**5**	**3**	**2.34**
Kathmandu	**18**	**4**	**8.41**
Kavre	**5**	**0**	**2.34**
Kailali	**14**	**7**	**6.54**
Kapilvasthu	**7**	**0**	**3.27**
Morang	**9**	**7**	**4.19**
Makwanpur	**1**	**0**	**0.47**
Mahottari	**24**	**5**	**11.21**
Nawalparasi	**13**	**4**	**6.07**
Parsa	**1**	**0**	**0.47**
Rautahat	**28**	**10**	**13.08**
Ramechhap	**9**	**7**	**4.67**
Sunshari	**6**	**4**	**2.8**
Saptari	**1**	**0**	**0.47**
Tanahun	**5**	**5**	**2.34**
Terathum	**1**	**0**	**0.47**
Unknown	**2**	**2**	**0.94**
Total	**213**	**105**	**100**

On the study of district wise distribution of suspected rubella cases, it was found that the suspected rubella cases were reported from 23 districts of Nepal. Among these the highest number was reported from Rautahat i e, 28 (13.14%) followed by Mahottari 24 (11.27%), Bhaktapur 21(9.89%) and Kathmandu 18(8.45%). Five districts namely; Banke, Makwanpur, Parsa, Saptari and Terathum each reported only single rubella cases. Out of total tested 213 cases of rubella collected from 23 districts of Nepal, the positivity was found in the samples collected in only 16 districts. The samples of suspected rubella cases reported from remaining 7 districts, namely Banke, Kavre, Kapilbasthu, Makwanpur,

Parsa, Saptari and Terathum were not positive for anti-rubella IgM antibodies. The highest number of positive cases (14 cases) was recorded from Bhaktapur which comprises 13.33% of the total positive cases followed by Rautahat (10, 9.52%), Bara (8, 7.62%) and Dadeldhura (8, 7.62%). The 7(6.67%) positive cases each were found in the reported cases of four districts namely, Baitadi, Kailali, Morang and Ramechhap.

Table 6: Regional distribution of rubella cases

Developmental regions	Suspected Rubella cases	Confirmed Rubella cases	% of Total positive cases
FWDR	**32**	**22**	**20.95**
MWDR	**1**	**0**	**0**
WDR	**25**	**9**	**8.57**
CDR	**131**	**58**	**55.24**
EDR	**22**	**14**	**13.33**
Unknown	**2**	**2**	**1.91**
Total	**213**	**105**	**100**

The highest numbers of rubella cases (131cases) were reported from Central development region, which constitutes 61.5% of the total cases. Far western development region ranked second position with 32 rubella cases (15.02%) followed by western development region with 25 rubella cases (11.73%) and Eastern development region with 22 rubella cases(10.32%). The single number of rubella case was reported from mid western development region. Origins of 2 cases (0.94%) were unknown. Though the specimens from rubella suspected cases were received from all five Development regions however out of total 213 tested cases of rubella, positivity was found in four development regions only. The highest number of positive cases i. e. 58 was found in Central development region, which comprises the 55.24% of the total positive cases. The second highest numbers of Rubella positive cases (22, 20.95%) were recorded from Far western development region followed by Eastern development region (14, 13.33%) and western development region (9, 8.57%). No positive cases were recorded from the mid western development region.

Table 7: Geographical distribution of rubella cases

Geographical regions	Positive cases	Total tested cases	Positive %	% of total positive cases
Terai	53	125	42.4	50.48
Hill	50	86	58.14	47.62
Unknown	2	2	100	1.9
Total	105	213	49.29	100

In the present study, the geographical distribution of the rubella suspected cases were higher in terai than hills, like wise the Rubella positive cases were also higher in terai (50.48% of total positive cases) than hill (47.62%). No reported cases from mountain region, however origin of 1.9% positive cases was unknown.

Table 8: Monthly distribution of rubella cases

Month	Suspected Rubella cases	Confirmed Rubella cases	Positive %	% Of total positive cases
January	26	13	50	12.38
February	28	18	64.28	17.14
March	61	30	49.18	28.57
April	24	18	75	17.14
May	27	13	48.15	12.38
June	17	3	17.65	2.86
July	3	0	0	0
August	0	0	0	0
September	7	2	28.57	1.9
October	1	0	0	0
November	18	8	44.44	7.63
December	1	0	0	0
Total	213	105	49.29	100

Out of 12 months, rubella suspected cases were received in lab in all months except August, however the positive rubella cases were found only in the reported cases of 8 months. No positive cases were found in the remaining four months viz July, August, October and December. The rubella cases were observed increasing from January and reached highest in March, and then gradually decreased on coming months and was nil in

August. The highest number of specimens (61 cases) were collected and tested in March out of which 30 cases (28.57%) were found to be positive and 4 specimens gave the equivocal results. Likewise out of 28 specimens collected in February 18 cases (17.14%) were positive. Out of 24 total tested cases of rubella, 18 cases (17.14%) were positive. Out of 27 and 26 rubella cases reported in May and January respectively, 13 cases (12.38%) showed positive result.

Table 9: Seasonal distribution of rubella cases

Season	Suspected Rubella cases	Confirmed Rubella cases	Positive %	% Of the total positive cases
Winter Season (Dec, Jan, Feb)	**55**	**31**	**60.78**	**29.52**
Spring Season (Mar, Apr, May)	**112**	**61**	**54.46**	**58.1**
Summer Season (Jun, July, Aug)	**20**	**3**	**15**	**2.86**
Autumn Season (Sept, Oct, Nov)	**26**	**10**	**38.46**	**9.52**
Total	**213**	**105**	**49.29**	**100**

Looking at seasonal distribution/epidemiology, it was found that the highest cases and the positivity were found in spring followed by winter season. Of total 213 specimens, 52.58% (112 specimens) were reported in spring season alone out of which 61 specimens were positive which accounts 58.1% of the total positive cases. In winter, out of 55 cases reported, 31 cases (29.52%) showed positive result. The lowest cases were reported in summer where 3 specimens (2.86%) were positive out of 20 specimens collected. Statistically, the association between the season and the occurrence of disease was found significant **($P<0.05$; $\chi^2 = 12.93$)**

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Rubella, a viral disease, is one of the underestimated diseases, but the consequences of the disease showed that if not taken seriously results are drastic. Worldwide, it is estimated that more than 100,000 infants born with congenital rubella syndrome each year. In 2001,123 countries reported a total of 836356 rubella cases (Robertson et al, 2003). The annual WHO report showed that cases have been increasing in recent years which were much more in developing countries where rubella vaccination have not launched yet and in the countries with rubella vaccination, rubella outbreaks have been reported. Understanding of the epidemiology of rubella in Asian countries is relatively limited, because there are many other childhood diseases which are given higher priority to be controlled than rubella in these countries (Katow, 2004). This is exactly what we are facing in our country too.

Some sequalae of rubella such as encephalitis, chronic arthritis, hemorrhagic manifestations and the teratogenic effects when rubella infection is acquired in first trimester of pregnancy relates the public health importance of rubella. Due to lack of vaccination programme against rubella, the outbreaks of rubella have continued to occur, indicating the need for intensified and sustained efforts to reach the goal of controlling rubella and Congenital Rubella Syndrome.

There are various techniques available to diagnose the rubella infection. The present study was conducted based on ELISA technique that was targeted to detect the rubella-anti IgM antibodies which appear in blood from 5 days up to a month after the onset of rash. This technique has been proved to be a reliable and cost effective serological method for rubella infection diagnosis and sero-surveillance of rubella.

The study was an extensive epidemiological study covering all the 75 districts of the country. The present cross-sectional study was carried out during a period from January to December, 2006 based at National Public Health Laboratory.

There is no any document, which states when did rubella tests started in Nepal. However with the collaboration of WHO-IPD, rubella surveillance along with measles had started in Nepal since 2003 where the objective was only focused for measles control. This programme led to the sharp decrease in measles cases, but on the other hand the number of rubella positive cases appears increased in recent years. It is important to note that majority of cases are detected as a result of increased vigilance in the surveillance of measles, since rubella is not notifiable. The increase in the number of rubella cases increased the concern in Nepal. At present, Nepal is not vaccinating against rubella, out breaks of rubella and its burden seems to be overshadowed by measles in the past because of high CFR in measles as compared to negligible rubella complications.

During the study period, a total of 213 rubella suspected cases were received from different hospitals situated in different part of country through surveillance network of WHO-IPD-DoHS for Rubella-anti IgM antibodies test were included in the study. After processing the collected specimens, the study results showed that the significant number of positive cases of rubella.

Out of the total 213 serum specimens received through DoHS-WHO surveillance system from suspected rubella cases, 118 were male which constitutes 55.40% of the total and remaining 96 were female which comprises 44.60% of total. The ratio of male to female was found to be 1.2:1. The present study coincides with the unpublished data of NPHL 2004 where out of total 208 collected cases, 58.65% were male and 41.35% were female.

However, in the unpublished data of 2005, the number was higher in female (52.49) than male (47.51) out of total 261 collected rubella suspected cases.

The age wise distribution of suspected rubella cases received through surveillance network revealed that the highest number (92 cases) was from age group 5-10 years which accounted for 43.19% of total. 73 cases (34.27%) were from age bellow 5 years and 34 cases (15.96%) were from age group 10-15 years. The total number above 15 years was 14 cases which comprise 6.57%. No case was reported above 30 years. The study showed that the most susceptible age group for rubella was 5-10 years followed by age group bellow 5 years. The susceptibility was found low with the increase in age groups. Within the same age group, the suspected cases were higher in male than females

The present data coincides with the past unpublished data of NPHL, 2004 and 2005. In 2004, out of 208 tested cases, the highest cases (38.46%) were from age group 5-10 year followed by age group bellow 5 years (34.62%). In 2005, out of total tested 261 cases, the highest number was from age group 5-10 years which was 49.04% followed by 28.35% in age group bellow 5 years. No suspected rubella cases reported from above 30 years. This might be due to either sub clinical rubella infection where rashes were absent to suspect for rubella or due to naturally acquired immunity. The higher cases in age group of 5-10 years might be due to their schooling and playing age being gathered in group and immature immune system which do not get cared from parents as bellow 5 years where easy transmission of disease occurs.

Out of 75 districts, suspected rubella cases were reported from 23 districts, where highest number (28 cases) was reported from Rautahat which constitutes 13.15% of total followed by Mahottari (24 cases, 11.27%) and Bhaktapur (21cases, 9.86%). Most of the cases (131 cases) were reported from CDR which accounted 61.5% of total where as single suspected from MWDR. The high reported cases from CDR might be due to better awareness about

disease, good reporting system and comparatively better health facilities. The comparatively higher cases in districts might be due to their higher population density. There are some fluctuations of the present data with the past unpublished data. In 2004, suspected rubella cases were reported from 34 districts with the highest number from Morang (32 cases) followed by Bardiya (24 cases) and Sunshari (22 cases). In that year, the highest number was from EDR (45.67%) followed by MWDR (26.92%). Like present distribution, in 2005 the highest cases (59.39%) were reported alone from CDR. In that year, 26 districts had reported the cases where highest was from Kathmandu (60 cases) followed by Bardiya (24 cases) out of 261 total cases. Geographical distribution showed that suspected rubella cases were reported from hills and terai. In this study, 125 suspected cases were from terai which accounted 58.41% and 40.65% were from hills (NPHL, 2006).

The number of suspected rubella cases received through surveillance network in context of month/season wise distribution in this study is following the similar trend of distribution as in other part of world with the cases clustered in late winter and early spring. The highest number was recorded in spring season (52.58%) followed by winter season (25.82%). In month wise distribution the highest was recorded in March (61, 28.63%), followed by February (28, 13.15%), May (27, 12.68%), January (26, 12.21%) and April (25, 11.74%). No case was recorded in month of August. The present data have some discordant with the past unpublished data of 2004 and 2005 (NPHL, 2006). In 2004, out of total tested cases, 23.88% were reported in the month of April followed by March (15.38%). The highest number were recorded in spring season which was 49.04% but unlike present data second highest were recorded in summer season (24.04%). The present is in contrary with the 2005 data where highest number was recorded in summer season (44.06%) followed by spring season (39.repoted8%) and highest number was in June (29.50%) followed by March (17.24%). These slight fluctuations might be due to reporting delay or in collection delay. However the fluctuations, the past data coincides with the present study in that sense that the suspected cases were mostly reported in first five month of the year.

Out of 213 serum samples received through measles/rubella surveillance system, 49.3% were found positive, which is almost similar/ in between the data of 2004 (27.04%) and 2005 (64.75%).

Out of 105 positive cases obtained on this study, 50 were male patients who constitutes 47.62% of total positive cases and 55 were female patients which constitutes 52.38% of total positive cases. Though the suspected rubella cases were higher in males but the confirmed rubella positive cases were higher in females. The ratio of rubella positive cases in female to male was found to be 1.1:1. The association between the disease and sex is statistically significant **($P<0.05$, $\chi^2 = 5.07$).**The present result coincides with the result of 2005, where 55.63% positive cases were female where as 44.37% were male out of total positive cases.

The age wise distribution in this one year showed that the highest positive rubella cases were found in the age group 5-10years which constitutes 46.67%of the total positive case followed by age group bellow 5 years which accounted 28.57% of the age group and 10-15 years constituted 17.15% of the total positive case. The present study is in harmony with the past results unpublished results. In 2004, the highest positive number was from age group which was 45.6% of the total and in 2005, 50.3% of the total positive cases from the same age group. Such pattern of distribution of rubella positive cases might be due to the natural immunity acquired by the rubella infection in early age of life as there is no policy for immunization against the Rubella Virus infection in the country. This is why the positive cases were in decreasing pattern as with the increase in age groups.

The high positive cases in this age group might be due to the schooling age group where playing with their similar friends of same age group led to the higher rate of infection if few of them were infected with rubella. Though the result figures suggest that the rubella

cases were clustered in age group bellow 15 year, however the statistical analysis showed that the rubella cases were independent of age group **(P>0.05, χ^2 =2.63)**.

The age wise distribution of present study coincides with the distribution in other countries. In Bangladesh, Rubella outbreak cases was 35.8% in 5-9 yeasrs,25.9% in 1-4 years, 16.2% in 10-14 years and 12.2% above or equal to 15 years. Like this, in Myanmar, 82.6% in 5-9 yeasrs, 6.5% in 1-4 years, 6.5% in 10-14 years and no case above or equal to 15 years (WHO, 2005). In South Africa in 2004, the study result showed that rubella affected most young children aged 5-9 years, this age group contributed more than 50% of the cumulative total (CDC, 2006).

However, contrary to the present study, a study in Brazil (San Paulo) from April first to December 31, 2001 showed that the incidence among the person of age group 12-19 years was increased 3.7 fold as relative to children of ages 1-4 years (Lanzieri et al, 2003).

The present study showed that the suspected and confirmed rubella cases were clustered bellow 15 years. As the age group increases the, the cases had sharply decreased and no reports above the 30 years. The present result is in accordance as called the rubella as a disease of childhood. The high occurrence in this age is due to the lack of Rubella vaccine and the immune system not fully matured. Moreover, due to its high transmission rate, infected children gathered in school or play ground easily transmit to their company making the high number in this age group. The low reports in higher age group are mostly due to the natural immunity obtained against rubella in early ages of life. When one catches rubella, he/she is life long immune to rubella; this is the cases what makes the difference of rubella cases in the age group below 15 years and above it. Also it might be sub clinical infection in higher age groups where the immune status is strong as 50 % to 60% of Rubella infection is sub clinical. It might be also due to unaware of rubella rashes and not properly reported.

In this study, the rubella suspected cases were reported through out the year except in August, however the positive cases were not found in July, October and November. The total tested cases and positive cases were observed in an increasing pattern till April and gradually decrease to nil in October/November. The highest number of Rubella positive cases (30 cases) was observed in March which constitutes 28.57% 0f the total positive cases. A cumulative of 87.62% of total rubella positive cases in first five months of year indicates the clustering of rubella in these months. The seasonal distribution reveals that the highest positive cases (61 cases) were in spring season which accounts 58.1% of the total positive cases and in winter it was found to be 29.52% of the total positive. The total positive cases in these two seasons alone were found to be 87.62% of the total positive. This result indicates that the rubella cases were dependent on seasons. Statistical analysis showed that there is association between rubella infection and the seasons **($P<0.05$, $\chi^2 = 12.93$).**

The high positive cases in late winter and spring seasons might be due to the fact that the rubella is transmitted through droplet infection (air borne transmission). In this period high transmission is observed due to favorable environment for movement of air droplets containing Rubella virus. However, in summer droplets are too dry and in rainy season rain washes the droplets. Also the gathering of people in these seasons due to suitable environment aids in transmission rate of virus.

In this context, more or less similar pattern of rubella distribution were in the past results (WHO-IPD, 2006). In 2004, 61.4% positive cases out of total positive cases in first five months with the 54.39% in the spring season. However the present study is in contrary with the result of 2005 with the highest positive percentage in June (30.77%) followed by March (17.16).in that year positive cases were higher in summer season (46.15%) followed by spring season (42.6%).

Although the suspected rubella cases were reported from 23 districts of country, the positive cases were observed only from 16 districts. The highest numbers of positive cases were observed in Bhaktapur (13.33% of total positive cases) followed by Rautahat (9.52%), Bara (7.62%) and Dadeldhura (7.62%). In the past unpublished result of 2004, out of 34 rubella reported districts, positive cases were confirmed in 14 districts with the highest in Bardiya (29.82% of total positive) followed by Morang (26.31%). In 2005, out of 26 rubella reported districts, Rubella positive cases were confirmed in 25 districts with highest number in Kathmandu (24.26% of total positive cases). The above distribution indicates that though there are no endemic districts, however cases were reported in similar and neighboring districts due to its easy transmission. The rubella distribution is not constant in constant of district wise distribution. The past unpublished results showed that the outbreaks are haphazardly distributed however in few districts the cases were being occurred in each year. In 2004, most positive cases were from Bardiya, Morang and Sunshari where these 3 districts contributed 73.68% of total positive cases. In 2005, 41 positive cases were observed from Kathmandu alone out of 169 total confirmed positive. In the same year, higher positive cases were also observed in Chitwan (13 cases), Parsa (13 cases) and Bardiya (12 cases).

The regional distribution showed that out of total 213 tested cases, the majority of the cases (131cases) were from CDR followed by FWDR, WDR, EDR and MWDR which constitutes 32, 25, 22 and 1 respectively. The highest numbers of rubella positive cases (58 cases) were alone from CDR which accounts 55.24% of the total positive cases and like wise 22 positive cases (20.95%) from FWDR, 14 positive cases (13.33%) from EDR and 9 positive cases (8.57%) from WDR. In the present study no positive cases confirmed from MWDR. This might be either due to actual decrease in cases or due to lack of proper reporting system and available health facilities. The high rubella positive cases in CDR might be due to high population density and better facilities for reporting and sample

collection system due to higher number of health institutions concentrated in this area. Such increase might also be due to awareness about the disease.

The present result is in contrary with the unpublished past results of NPHL. In 2004, the highest positive cases (59.65% of total positive cases) were from EDR followed by 31.58% in MWDR. However like the present result, in 2005, the highest positive cases were from CDR (65.68% of total positive cases) followed by WDR (13.6%).

In the present study, the geographical distribution of the rubella suspected cases were higher in terai than hills, like wise the rubella positive cases were also higher in terai (50.48% of total positive cases) than hill (47.62%). No reported cases from mountain region, however origin of 1.9% positive cases was unknown. The present result is in perfect harmony with the past unpublished result of 2005, where 50.89% 0f total positive cases were from terai and 47.93% from hills. However in the unpublished result of 2004, most of the positive cases were (85.96% of total positive cases) were from terai and remaining 14.04% from hills. The high positive cases in terai might be due to high population density where transmission of Rubella virus is high in crowded population. The no report from mountains indicates it as a rubella free region. However, in some instances, it might have so happened due to the lack of reporting system and misdiagnosis the rubella as other related diseases due to lack of available health institutions and proper knowledge about it.

6.2 Conclusion

In 2006, out of 213 tested specimens received from different part of country through the WHO-IPD surveillance network, the positivity was found to be 49.3% of the total. The most rubella cases were clustered in the first five months of the year, with the highest cases in March and in context of season it was spring season where the highest positive cases were observed. The rubella positive cases were highest in age group 5-10 years followed

by below 5 years and few cases were from 15 year and above it. More than half of the positive cases were from CDR with highest in Bhaktapur followed by Rautahat Such prevalence of rubella cases (anti-Rubella IgM antibody) stresses the importance of the study on rubella and underlines the importance of introduction of rubella vaccination program in Nepal.

CHAPTER VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. The study work was conducted in National Public Health Laboratory for a complete year, January to December 2006.

2. During the year 2006, a total of 213 specimens of suspected rubella cases received from different part of the country through surveillance network of WHO-IPD 49.3% were confirmed as rubella cases respectively.

3. Of the total 213 suspected cases, 55.14% were male patients and 44.86% were female patients with the confirmed positive cases 47.62% and 52.38% respectively. The ratio of rubella positive case in male to female was observed as **0.9:1**.

4. Of the total, the highest positive cases were from age group 5-10 years (49, 46.67%) followed by age group below 5 years (30, 28.57%), 10-15 years (18, 17.14%) and least from 15 or above 15 years (8, 7.62%).

5. The suspected rubella cases were received from 23 districts of the country. The rubella cases were confirmed in the specimens from 16 districts with the highest positive cases was from Bhaktapur (14, 13.33%) followed by Rautahat (10, 9.52%), Bara (8, 7.62%) and Dadeldhura (8, 7.62%). The highest numbers of positive cases were from CDR with 58 positive cases (55.24% of total positive) and no positive case from MWDR. 125 and 87 suspected cases were reported from terai and hills with 53 and 50 positive cases respectively.

6. The highest rubella cases were confirmed in March (30, 28.57%) followed by February (18, 17.14%), April (18, 17.14%), January (13, 12.38%) and May (13, 12.38%). No positive case observed in July, August October and December. Out of total positive cases, 92 cases (87.62%) were clustered in first five months of the year. The highest positive cases were observed in spring season (61, 58.1%) followed by winter season (31, 29.52%).

7.2 Recommendations

1. Since last few years, rubella surveillance is being carried out with measles with out properly focusing rubella. However, the improved and focused surveillance program for Rubella should be conducted in Nepal to access the actual rubella burden.

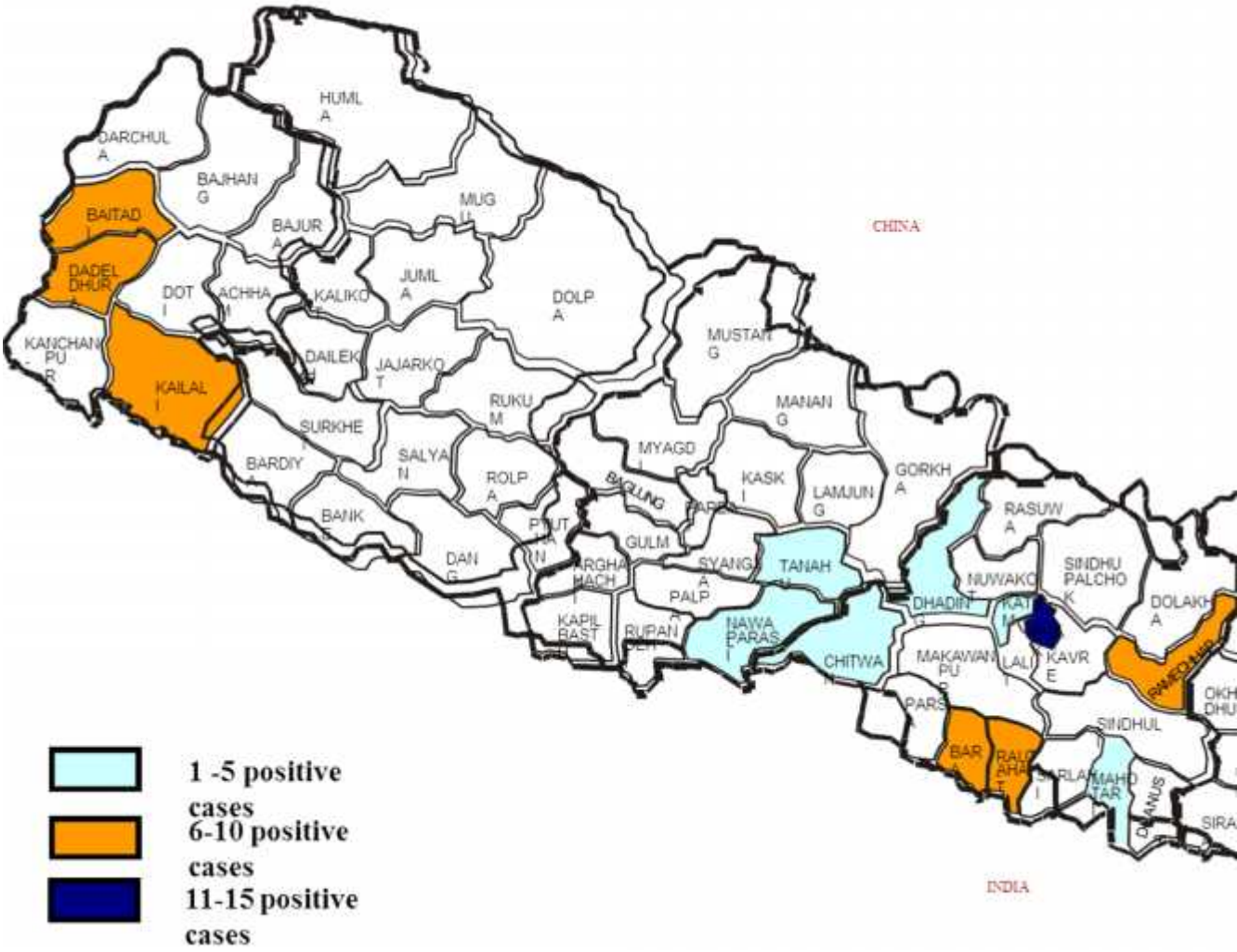
2. Lab testing is limited at NPHL, but for better laboratory based surveillance diagnostic facilities (with skilled man power) should be expanded to other part of the country.

3. A policy for including rubella vaccination in Expanded Program on Immunization is required as early as possible in view of prevention from CRS.

5. Sero-surveillance of women of child bearing age group and adolescent girls is required to understand the actual disease burden of rubella in Nepal.

6. A nation-wide survey for CRS may be an alternate way to establish rubella disease burden in Nepal in order to formulate an appropriate rubella vaccination strategy.

Figure 6 : District wise distribution of Rubella positive cases ,2



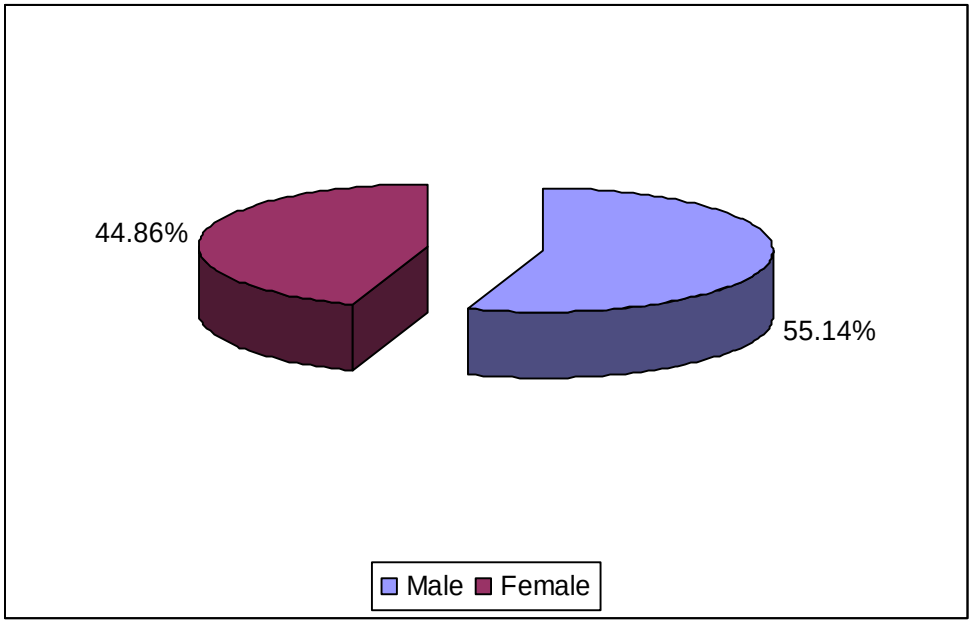


Figure 3: Gender wise distribution of suspected rubella cases, 2006

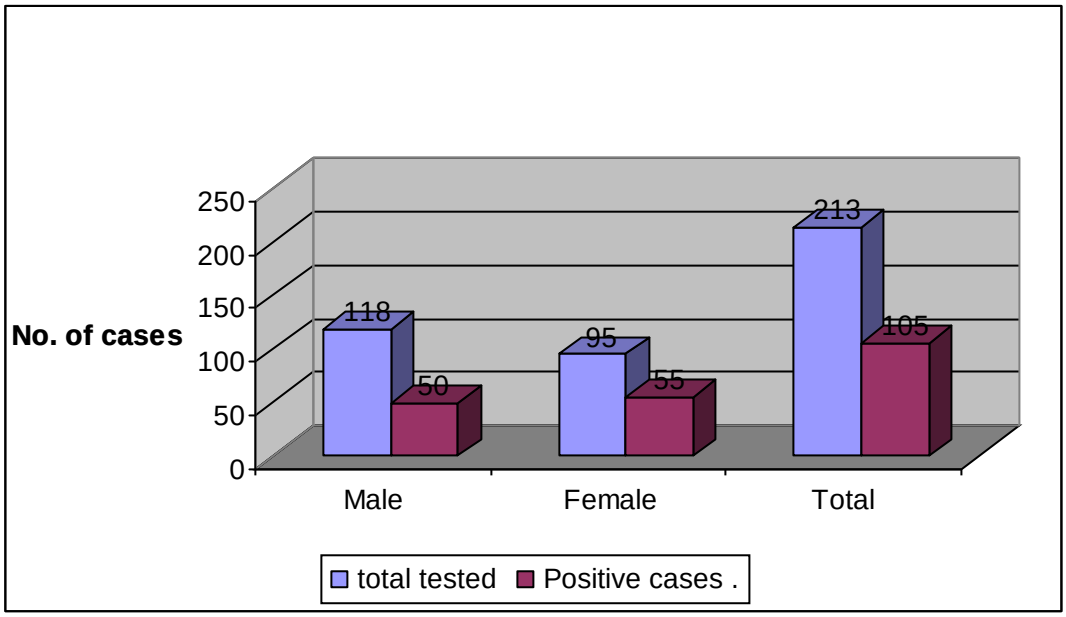


Figure 4: Gender wise distribution of rubella cases, 2006

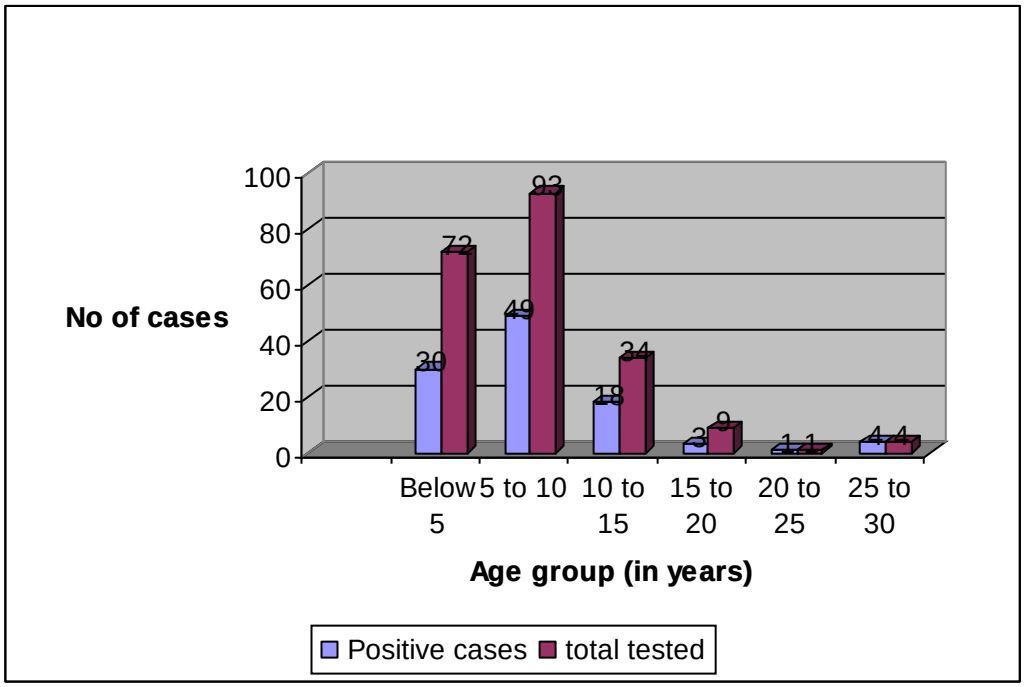


Figure 5: Age wise distribution of rubella cases, 2006

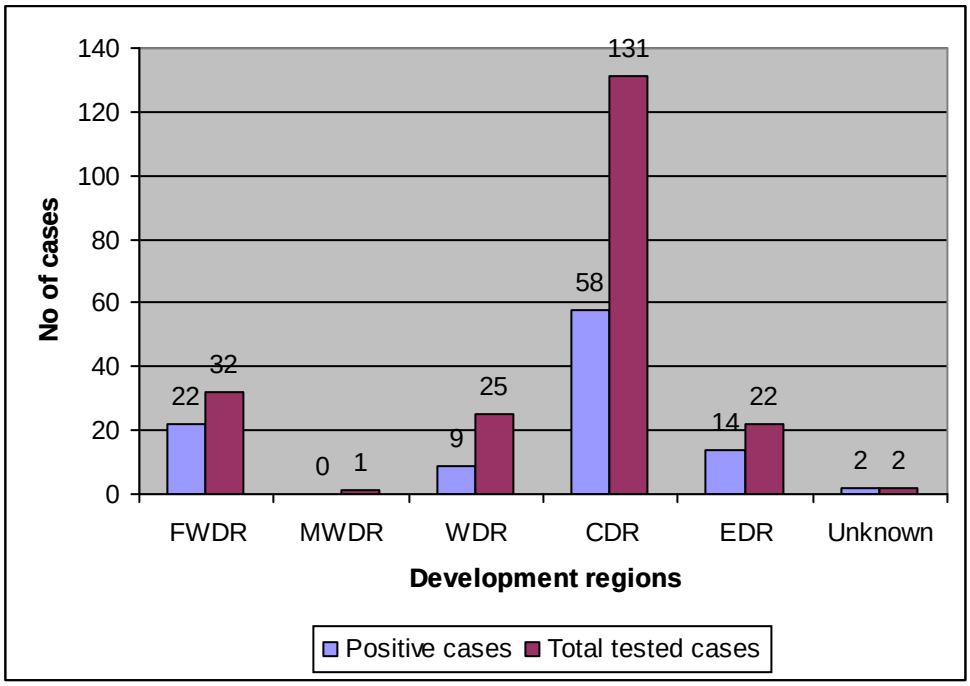


Figure 7: Regional distribution of rubella cases, 2006

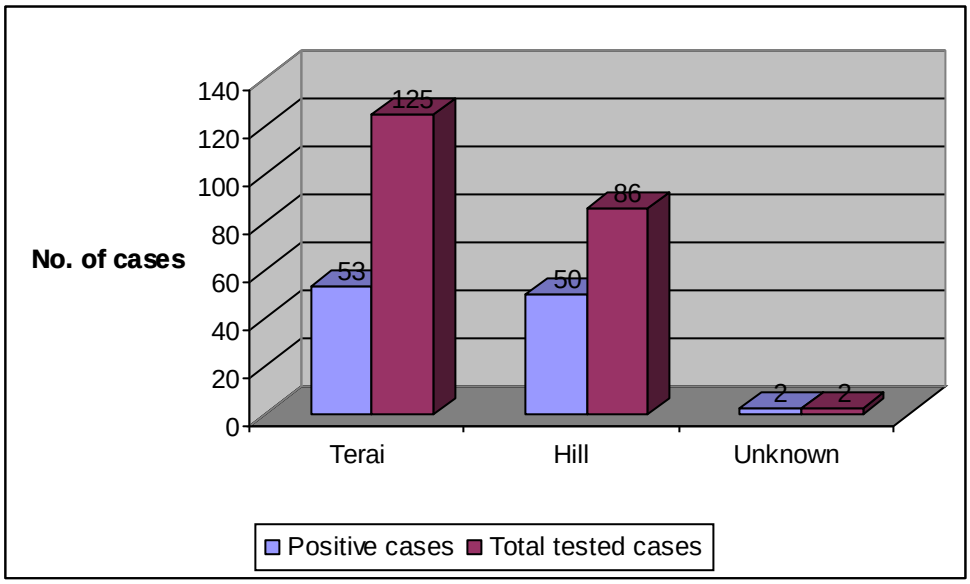


Figure 8: Geographical distribution of rubella cases, 2006

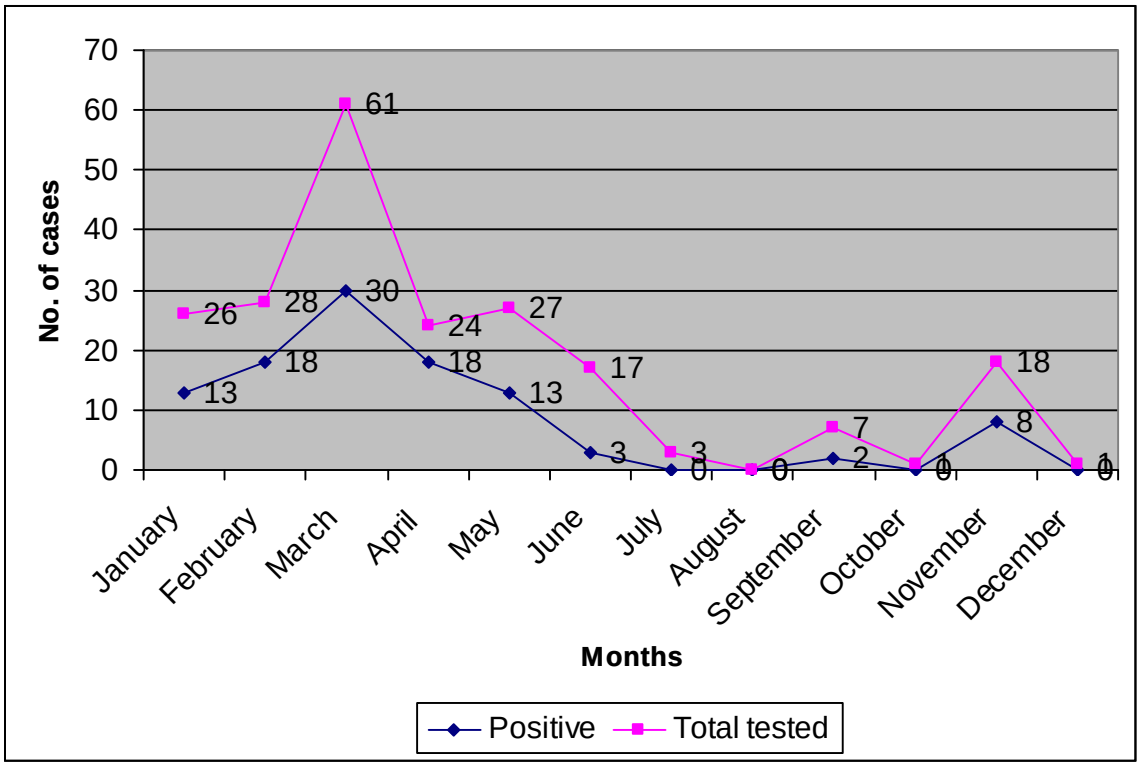


Figure 9: Monthly distribution of rubella cases, 2006

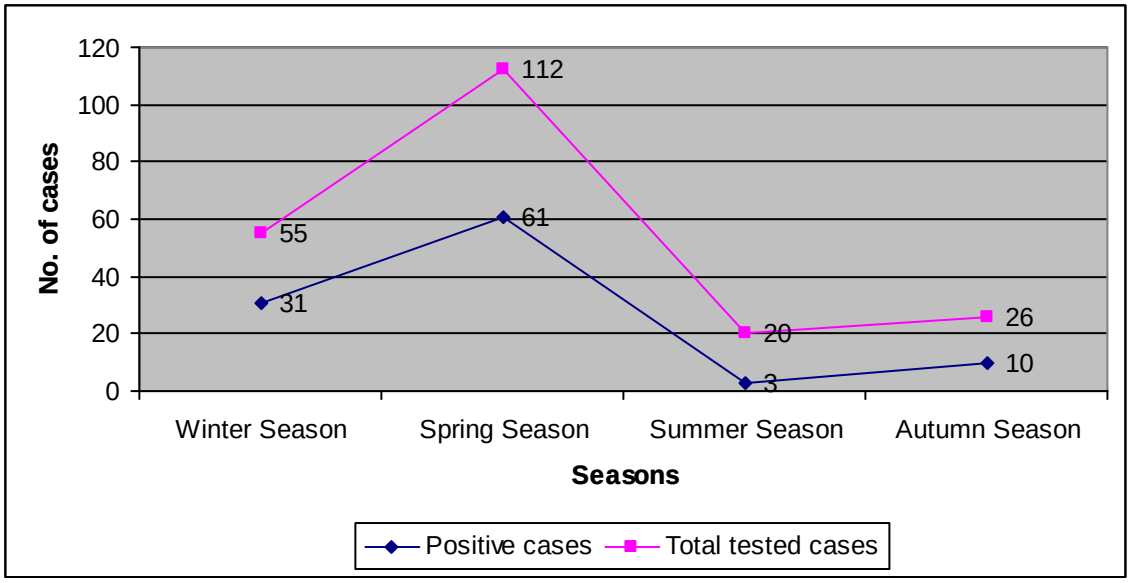


Figure 10: Seasonal distribution of rubella cases, 2006

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