CHAPTER- I

1. INTRODUCTION

Measles is a highly contagious disease with high morbidity and mortality (Chiu *et al*., 1997) caused by Measles Virus, a member of the paramyxovirus family. It is a human disease with no known animal reservoir. The first clinical sign is usually high fever which begins approximately 10-12 days after exposure and lasts 1-7 days. During this initial stage, the patient complains rhinorrhea, cough, red and watery eyes and small white spots inside the cheeks (Koplik spots). After initial symptoms, a characteristic rash develops, usually on the face and upper neck. The rash lasts for 5-6 days, and then fades (Numazaki, 2007).

Before the introduction of Measles vaccines in 1960s, almost everyone contracted Measles, usually during childhood. The result was an estimated 130 million cases and more than 2.5 million deaths due to measles (mainly children) each year. Despite the availability of a safe effective and relatively inexpensive measles vaccine for more than 40 years, Measles kill more children today than any other vaccine preventable disease, mainly in developing countries (WHO, 2007). In 2004, there were an estimated 20-30 million cases of measles worldwide and 453000 deaths (WHO, 2005). Even in countries where measles had been eradicated, cases transported from other countries in the endemic areas remain an important source of infection (Numazaki, 2007).

Measles is one of the most easily transmitted disease. Transmission is primarily by large droplets spread or direct contact with nasal or throat secretions from an infected person (WHO, 2007).

Measles infection is associated with a high fever, rash and cough, affecting mostly children, but also young adults. Children usually don't die directly of measles but from its complications such as pneumonia and diarrhea as a result of the immunosuppression associated with measles infection. The disease can also lead to lifelong disabilities including brain damage, blindness and deafness. Measles is one of the most contagious disease known to man and often occurs in explosive epidemics (WHO, 2007).

Measles is endemic in Nepal; an estimated average of 90 000 cases per year occurred from 1994 to 2002, based on extrapolations from routine surveillance reports (Joshi *et*

al., 2009). Among all vaccine preventable diseases, measles is the greatest cause of morbidity and mortality among children in Nepal. It is estimated that some 150,000 cases of measles occur every year in Nepal with some 5000 deaths (WHO, 2005).

Rubella is a mild illness that presents with fever and rash. The public health importance of Rubella is because infection in the early months of pregnancy usually affects foetal development (Robertson *et al*., 2003). Rubella, commonly known as German measles or three-day measles, is an infection caused by Rubella Virus that primarily affects the skin and lymph nodes. It is primarily 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 is moderately contagious, mostly when the rash is erupting, but is communicable from 1 week before to 5-7 days or more after the onset of the rash. Infants with CRS may shed virus for up to a year after birth (WHO, 2007).

Rubella, which occurs throughout the year with the peak incidence in late winter and early spring, is worldwide in distribution (CDC, 1994). Worldwide, it is estimated that there are more than 100,000 infants born with congenital rubella syndrome each year. Most of these cases occur in developing countries that have not yet introduced rubella vaccine (Robertson *et al*., 2003).

Among all vaccine preventable diseases, measles is the greatest cause of morbidity and mortality among children in Nepal (WHO, 2005). Although Rubella is generally considered a childhood illness, people of any age who have not been vaccinated can

become infected. Due to the delay in implementation of rubella vaccine program, most people of the country are still susceptible to Rubella virus infection. The past unpublished result of NPHL showed the significant number of Rubella positive cases in suspected patients.

Due to similar clinical symptoms of Measles and Rubella, misdiagnosis of suspected cases is a major challenge if diagnosed only according to clinical manifestations. Therefore this study aimed to explore the actual burden of Measles and Rubella in Nepalese population with confirmed laboratory diagnosis of suspected cases for both diseases. This is important to assist in the Measles elimination stratigies along with to plan for the decrease of Rubella burden.

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; very few countrywide studies for epidemiology of measles and rubella with inclusion of major population has been done in the past. Therefore present study aimed to find out the sero-epidemiology of Measles and Rubella in Nepal.

CHAPTER –II

2. OBJECTIVES

2.1 General Objectives

To study the sero-epidemiology of Measles and Rubella in Nepal.

2.2 Specific Objectives

To determine IgM sero-positivity from suspected Measles Rubella patients.

To describe the genderwise and agewise distribution of Measles and Rubella in Nepal.

To describe the ecological and geographical distribution of Measles and Rubella in Nepal.

To study the seasonal variation of Measles and Rubella in Nepal.

CHAPTER-III

3. LITERATURE REVIEW

3.1 Measles and Rubella

Measles and rubella are acute viral infectious diseases, characterized in the pre-vaccination era by endemic pattern with epidemics occurring every 2-4 years. The burden posed by these diseases is not only due to their ability to spread into the population causing high number of cases, but also to the seriousness of clinical complications in a not negligible proportion of cases. Moreover, complications during measles infection may be more serious if the infection is acquired in the first year of life or at adult age. Congenital rubella can occur when the infection is acquired in pregnancy (Gabutti *et al*., 2002).

The name measles is derived from the Latin, misellus, meaning miserable. The disease is also sometimes known as rubeola (from rubeolus, Latin for reddish) or morbilli (from morbus, Latin for disease). There are references to measles as far back as the 7^{th} century. In 10^{th} century, Rhazes, a Persian phuysician, described measles as more dreaded than smallpox. Prior to the introduction of vaccination programs,measles was almost always a disease of childhood. In densely populated areas, measles most commonly affected children aged 3-4 years old. In less crowded urban areas and in rural areas, the highest incidence was among children aged 5-10 years who contracted the disease on entering school (CDC, 2004).

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, 2006).

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 CRS (Banatvala and Best, 1998).

3.2 Measles and Rubella virus: The etiologic agent

3.2.1 History

Paramyxoviruses are so called because they have an affinity for mucous membranes (Greek: myxa=mucus) (WHO, 2007). Measles is a relatively new disease of humans and probably evolved from an animal Morbillivirus. Phylogenetically, measles virus (MV) most closely resembles rinderpest virus (RV), a pathogen of cattle, and it is postulated that MV evolved in an environment where cattle and humans lived in close proximity. Because large numbers of people are required to generate sufficient susceptible individuals to maintain measles in a population, measles probably evolved in the early centers of civilization in the Middle East where populations attained sufficient densities to maintain transmission (Griffin, 2001).

Abu Becr, an Arab physician known as Rhazes of Baghdad, is generally credited with distinguishing smallpox from measles in the 9th century. He dated its first description to the 6th century. Rhazes referred to measles as *hasbah*, "eruption" in Arabic, and regarded it as a modification of smallpox. One distinction noted was that "anxiety of mind, sick qualms and heaviness of heart, oppress more in the measles than in the smallpox". Repeated epidemics of illnesses characterized by a rash are recorded in European and Far Eastern populations between A.D. 1 and 1200. It appears that measles spread across the Pyrenees into France with the Saracen invasion of the 8th century. Repeated epidemics identified as measles are not recorded until the 11th and 12th centuries, and it is first mentioned as a childhood disease in 1224. In the European literature, the name applied was "morbilli," derived from the Italian "little diseases" to distinguish it from plague, *il morbo,* but morbilli included several exanthemata. Sanvages in 1763 defined morbilli as measles but called it rubeola (derived from the Spanish), leading to a common confusion with rubella that persists to the present. Introduction of measles into previously unexposed populations has been associated with high morbidity and mortality (Black, 1992).

Epidemics of rash illnesses were associated with episodes of depopulation in China, India, and the Mediterranean region. There was 26% mortality associated with introduction of measles into the Fiji Islands in 1875. Approximately 56 million people died as a result of European exploration of the New World, largely because of the introduction of Old World diseases, notably smallpox and measles, into native Amerindian populations. Decreases in population are likely to have facilitated the transfer of Spanish culture to South America. Many of the basic principles of measles epidemiology and infection were elucidated by the studies of Peter Panum, a young Danish physician who was sent to the Faroe Islands in 1846 during a large measles epidemic. Panum deduced the highly contagious nature of the disease, the 14-day incubation period, and the lifelong immunity present in older residents, and he postulated a respiratory route of transmission. Complications of measles were first described in the 18^{th} century. In 1790 James Lucas, an English surgeon, described the first case of

postmeasles encephalomyelitis in a young woman who developed paraparesis as the rash was fading. Medical textbooks of the 19th century associated measles with the exacerbation of tuberculosis, and in 1908, while working at a tuberculosis hospital in Vienna, von Pirquet recorded the disappearance of delayed-type hypersensitivity skin test responses to tuberculin during a measles outbreak (Griffin, 2001).

Subacute sclerosing panencephalitis (SSPE) was first described in 1933 by Dawson in a 16-year-old boy with progressive neurologic deterioration. Histologic examination of the brain showed eosinophilic intranuclear and intracytoplasmic inclusions in neurons and glial cells. Reports of paramyxovirus-like particles in the inclusions (Tellez-Nagel and Harter, 1966) were followed rapidly by documentation of elevated MV antibody in serum and cerebrospinal fluid (CSF), staining of the inclusions with antibody to MV and culture of MV from SSPE brains.(Griffin, 2001).

In1914, Hess postulated a viral etiology of Rubella 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. Parkman, Buescher and Artenstein (1962) detected the presence of Rubella virus in primary vervet monkey kidney cell culture by means of 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 1980s and early 1940s, after sometime of 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

Measles virus is a paramyxovirus belonging to genus morbillivirus, is morphologically indistinguishable from para-influenza viruses, mumps virus and Newcastle disease virus (NDV), although there are important functional differences (Peiris and Madeley, 2006).

Rubella virus, an enveloped RNA virus belongs to the family Togaviridae 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 Rubivirus, the only member of this genus (Frey, 1994).

3.2.3 Morphology and Structure

3.2.3.1 Physical and Morphological Properties

Measles virus is viable for less than 2 hours at ambient temperatures on surfaces and objects, while the aerosolized virus remains infective for 30 minutes or more. It is very sensitive to heat and is inactivated after 30 minutes at 56^{o} C. However, the virus appears to survive freeze-drying relatively well and, when freeze-dried with a protein stabilizer, can survive storage for decades at -70^{o}C. The virus is inactivated by solvents, such as ether and chloroform, by acids(pH<5), alkalis(pH>10), and by UV and visible light. It is also susceptible to many disinfectants, including 1% sodium hypochlorite, 70% alcohol and formalin (WHO, 2007).

Measles virus is pleomorphic virus ranging in diameter from 100 to 200 nm, with a mean diameter of 150 nm. Within the morbilliviruses, it is more closely related to the rinderpest virus group, and more distantly related to the canine distemper virus group. Two membrane envelope glycoproteins are important in pathogenesis. These are F(fusion) protein, which is responsible to fusion of virus and host cell membranes, viral penetration, and haemolysis, and the H(Haemagglutinin) protein, which is responsible for binding of virus to cells. Although there is only one serotype of measles virus, there

is genetic variability in wild type viruses. WHO currently recognizes 23 genotypes of MV with 16 genotypes identified since 1990. This genotype variation doesnot appear to be biologically significant, is that there is no change in vaccine efficacy (WHO, 2007).

The ribonucleoprotein helix is readily released from the virion and may, as with the others, be the only identifiable virus structure seen in the electron microscope. The virion structure includes:

a. Spikes, carrying a haemagglutinin but not a neuraminidase function
b. An F protein that is also a haemolysin
c. A matrix protein(M) below the envelope lipid bilayer

There is only one serotype of measles virus and no subtype have yet been recognized, although monoclonal antibodies show that there may be differences between wild and cultivated strains (Peiris and Madeley, 2006).

The measles virus has diameter of 150 nm or more and virion particle is spherical and pleomorphic but nucleocapsid is helical with diameter of 13-18 nm.

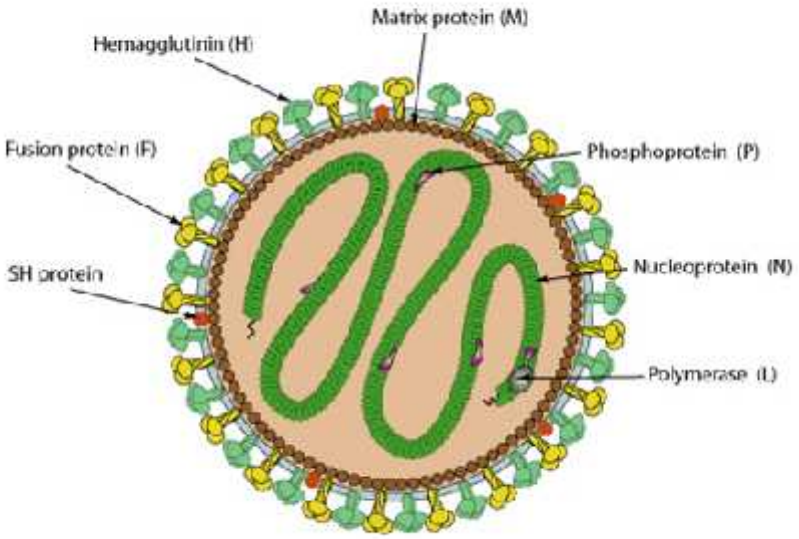

Fig 1: Morphology of Measles virus

(Source: Viral Zone 2009, Swiss Institute of Bioinformatics)

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

Virus particle:	
Diameter	150 nm or more
Symmetry	Spherical
Nucleocapsid:	
Diameter	13-18 nm
Symmetry	Helical
Genome	Single stranded RNA
Genome Size	15 kb
Chemical composition:	
RNA	1%
Protein	73%
Lipid	20%
Carbohydrate	6%

The envelope of Measles virus has Hemagglutinin(H) glycoprotein and fusion (F) glycoprotein (Brooks *et al*., 2004).

The Rubella 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 envelope. 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 icosahedral symmetry and 32 capsomeres (Matsumo and Higashi, 1974).

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

Virus particle:

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

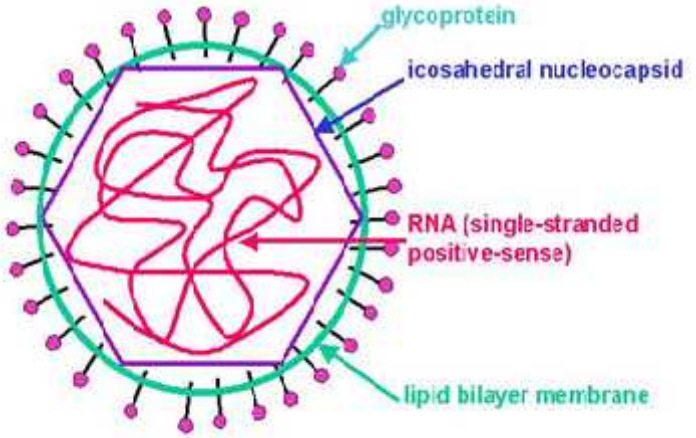

Figure 2: Morphology of rubella Virus

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%

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).

3.2.3.2 Genome structure and function

The measles virus has a non-segmented, negative sense RNA genome with a linear arrangement of genes that are separated by an integrate trinucleotide, GAA. Each gene contains a single open reading frame(except P), transcriptional start and stop signals, and a polyadenylation signal. The entire measles genome consists of 15,895 nucleotides (WHO, 2007).

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 $3^{'}$ terminal poly (A) tail and is capped at the $5^{'}$ end. The cap is required for the efficient translation as it serves as a ribosome recognition site (Dominguez *et al*, 1990).

3.2.4 Replication

Measles virus contains nonsegmented, single-stranded RNA genomes of negative polarity and it replicate entirely in the cytoplasm. Their genomes are 15 to 19 kB in length, and the genomes contain six to ten tandemly linked genes. A lipid envelope containing two surface glycoproteins (H and F), which mediate the entry and exit of the virus from its host cell, surrounds the virions. The nucleocapsid (total length of 1.2 μm) is packed within the envelope in the form of a symmetrical coil (Lund *et al*., 1984).

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' (Katow and Sugiura, 1988).

3.3 Infection

3.3.1 Post Natal acquired infection of Measles and Rubella

3.3.1.1 Clinical features

Infection of the respiratory tract by Measles gives rise to the characteristic cough and coryza, and the less frequent complications of croup, bronchiolitis and pneumonia. Generalized damage to the respiratory tract causes the loss of cilia and predisposes to secondary bacterial infections, such as pneumonia and otitis media. Immune reactions to the virus in the endothelial cells of dermal capillaries cause the measles rash and measles enanthem(Koplik's spots), while interaction between virus infected cells and local cellular immune factors is thought to be involved in measles encephalitis (CDC, 2004). Modified measles occurs in partially immune persons, such as infants with residual maternal antibody. The incubation period is prolonged, prodromal symptoms are diminished. Koplik's spots are usually absent and rash is mild (Brooks *et al*., 2004).

Classic Measles and its Complications

The prominent respiratory symptoms are manifestations of diffuse mucosal inflammation in response to widespread infection of epithelial cells. Interstitial pneumonitis resulting from MV replication and inflammation in the lower respiratory tract is common in uncomplicated disease and frequently is detectable only by radiography or by measuring the alveolar–arterial oxygen gradient (Gremillion and Crawford, 1981; Henneman *et al*.,

1995). Pneumonitis is more likely to be clinically severe during pregnancy (Ali and Albar, 1997; Eberhartphillips *et al*., 1993). Symptomatic giant cell pneumonia is seen primarily in immunocompromised individuals (Enders *et al*., 1959; Forni *et al*., 1994; Radoycich and Zuppan, 1992). Most of the severe pneumonia that complicates measles and leads to chronic pulmonary disease is caused by secondary bacterial and viral infections (Beckford *et al*., 1985; Gremillion and Crawford (1981); Kaschula *et al*., 1983; Morton and Mee, 1986). Other common respiratory complications caused by secondary infections are otitis media and laryngotracheobronchitis. In addition to increasing susceptibility to new infections, previous viral and bacterial infections may be reactivated. Similarly other complications of Measles include Gastrointestinal Disease, Myocardial Disease, Neurologic Disease, Eye Diseases (Griffin, 2001).

After an incubation period of 14-21 days the characteristic feature of rubella, rash and Lymphadenopathy may appear. 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).

3.3.1.2 Pathogenesis

After infection, the Measles Virus invades the respiratory epithelium of the nasophrynx and spreads to the regional lymph nodes. After 2-3 days of replication in these sites, a primary viraemia widens the infection to the reticuloendothelial system. Following further replication, a secondary viraemia occurs 5-7 days after infection and lasts 4-7 days. During this viremia, there may be infection and further virus replication in the skin, conjunctivae, respiratory tract and other organs, including the spleen, thymus, lung, liver and kidney. The viraemia peaks after 11-14 days after infection, and then declines rapidly over a few days (WHO, 2007).

Measles can replicate in certain lymphocytes, which aids in dissemination throughout the body. Multinucleated giant cells with intracellular inclusions are seen in lymphoid tissue throughout the body (lymph nodes, tonsils, appendix). The described events occur during the incubation period, which typically lasts 8-12 days but may last up to 3 weeks in adults (Brooks *et al*., 2004).

During the prodromal phase (2-4 days) and the first 2-5 days of rash, virus is present in tears, nasal and throat secretions, urine and blood. The characteristic maculopapular rash appears about day 14 just as circulating antibodies become detectable, the viremia disappears, and the fever falls. The rash develops as a result of interaction of immune T cells with virus infected cells in the small blood vessels and lasts about 1 week. In patients with defective cell-mediated immunity, no rash develops (Brooks *et al*., 2004).

Neonatal, childhood and adult infections by Rubella virus 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 in 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 Re-infection

Re-exposure to measles virus induces a strong anamnestic immune response with a rapid boosting of IgG antibodies, which prevents clinical disease. It appears that once the immune system has been primed through natural infection, immunity is lifelong. Cellular immunity, consisting of cytotoxic T-cells and possibly natural killer cells, plays a prominent role in immunity and recovery from acute infection. Patients with defects in cell mediated immunity often suffer severe progressive measles infection and have a significantly increases risk of death. Measles-specific immune suppression begins with the onset of clinical disease, before that rash, and continues for several weeks after apparent recovery (WHO, 2007).

Natural infection by Rubella 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

There is only one antigenic type of measles virus. Infection confers lifelong immunity. Most so called second attacks represent errors in diagnosis of either the initial or the second illness. The presence of humoral antibodies indicates immunity. However, cellular immunity appears to be essential for recovery and protection as patients with immunoglobulin deficiencies recover from measles and resist reinfection, whereas patients with cellular immune deficiencies do very poorly when they acquire measles infections. The role of mucosal immunity in resistance is unclear. Measles immune responses are involved in disease pathogenesis. Local inflammation causes the prodromal symptoms, and specific cell-mediated immunity plays a role in development of rash. Measles infection causes immune suppression- most importantly in the cell mediated arm of the immune system- but is observed to affect all components. This is the cause of the serious secondary infections and may persist for moths after measles infection (Brooks *et al*., 2004).

Primary infection with measles virus induces high titres of specific serum antibodies of the IgG and IgM class and also specific secretory IgA antibodies. The IgM and IgA antibody responses are transitory but the IgG antibodies persist throughout the life. In individuals, who have experienced the disease cell mediated immunity can be demonstrated by lymphocyte transformation cytotoxicity assays. The efficacy of parenteral human gammaglobulin as prophylaxis dose, however, suggests that antibodies are of appreciable importance (Pringle and Heath, 1998).

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 extensive 1963/64 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 throughout 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; whereas 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 throughout 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).

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 (Murphy *et al*., 1967; 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 recovered from the brain both with and without co-cultivation technique; it has also been recovered in 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 *et al*.,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 Marquartt, 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 without any evidence of periosteal reaction in over 20% of infants with congenital rubella. These lesions generally resolve within 1-2 months. Cooper et al. (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:

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 viremia. 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 (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 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

Measles is one of the most easily transmitted disease. Transmission is primarily by large droplets spread or direct contact with nasal or throat secretions from an infected person. Less commonly, it is spread by airborne aerosolized droplet nuclei or by indirect contact with freshly contaminated articles. Measles is highly communicable, with a secondary attack rate among susceptible persons of more than 90%. A number of factors tend to increase the severity of measles in developing countries. For example, overcrowding facilitates person to person transmission of the virus and increases the likelihood of

exposure to high viral loads. The measles vaccine virus is not communicable (WHO, 2007).

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

Measles and Rubella are 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 Rubella 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 Measles and Rubella virus. There is no known animal reservoir.

3.7 Epidemiology

In temperate climates, epidemics of measles tended to occur at 2-5 year intervals and lasted 3-4 months. In general, the larger the size of the community, the shorter the interval between epidemics. Measles vaccination programs have had a marked effect on the incidence of the disease and the complications associated with it. There has also been a shift in the age at which measles is most commonly contracted in these populations. After prolonged periods of high vaccine coverage in developed countries, measles transmission now occurs mainly in people that have never been vaccinated and in older children who did not seroconvert following vaccination. Measles outbreak can still occur in countries with high immunization coverage. Such outbreaks demonstrate an immunity gap in the population involved (WHO, 2007).

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 outbreaks 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 worldwide 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).

3.7.1 Global disease burden

Measles is ubiquitous throughout the world and, although a candidate for eradication, this may be difficult to achieve. In tropical areas, particularly Africa, children become infected under the age of 1 year, and the mortality rises in consequence, reaching as high a figure as 42% in children under 4 years of age. Malnutrition is one of the main underlying causes of excess mortality. The attack rate is also high in isolated populations that have not experienced the disease for some years. In the Faroes in the 1840s, three quarter of the population were infected, although the mortality was low. Most of those who were not infected were aged over 65 years, the interval since the last time the disease had been present in the islands, and confirms the infection gives prolonged immunity. In the USA, where a serious attempt to eradicate measles has been made, the number of cases was reduced from over 500,000 per year to about 2000 (a reduction of over 99%), but outbreaks in immigrants and high school students have recently emphasized the problems of preventing imported cases and keeping up a high level of immunization (Peiris and Mandeley, 2006).

Before the introduction of the measles vaccine in 1963, 400,000 to 500,000 cases were reported and an estimated 5 million cases of measles occurred in United States annually. By 1979, 16 years after the introduction of the measles vaccine, the incidence of measles had declined 93% (Wood and Brunell, 1995).

Measles still remains one of the most contagious diseases and a leading cause of child death, despite the development of safe and effective vaccines. Almost all people without immunity can be infected if exposed to the infectious measles virus. Globally more than

30 million people are affected each year by measles. An estimated 454,000 people, the majority of them children, died from measles in 2004 (Table 1). It is estimated that more than 1200 measles deaths occur every day and 50 people die every hour from measles. More than 95% of measles deaths occur in developing countries (Numazaki, 2007).

Table 1: Measles deaths, by region in 2004 estimated by World Bank (Numazaki, 2007).

Sub-Saharan Africa	216,000 [216,000–279,000]
South Asia	202,000 [145,000–264,000]
East Asia and Pacific	32,000 [21,000–47,000]
Middle East and North Africa	4,000 [2,000–5,000]
Europe and Central Asia	<1,000 [–]
Latin America and Caribbean	<1,000 [–]
High Income Countries	<1,000 [–]
Total	454,000 [329,000–596,000]

An outbreak of measles occurred in Fiji starting in February 2006. As of 24 May 2006, a total of 130 measles cases were reported. Over 60% of measles cases were children and infants between 6 months to 6 years of age, with the highest incidence occurring in the 9-11 months age group. Although no deaths were reported, pneumonia was the most common reported complication during the outbreak. From January to May 2006, Australia experienced a large multi-state outbreak, which was responsible for at least 65 of the 97 cases reported. There were a total of 11 cases reported for the same period last year. The outbreak is linked to an importation from India visiting on a spiritual tour. Twenty of the cases are in children aged 1-11 years. Eleven cases were in adults aged from 17 to 58 years. None of the children who contracted measles were immunized and 6 of the adults were not immunized against measles. Cambodia has made substantial progress in reducing incidence of measles through prior national campaigns. The main challenges for Cambodia in achieving measles elimination include improvement in the quality of surveillance as well as improvement in immunization service coverage.

Further, Cambodia is considering adding a second scheduled dose as well as including a school entry check of immunization status. Although the regular measles and rubella immunization has been changed to two-dose schedule, introducing live attenuated MR combined vaccine since April 2006, the first immunization (12-24 months after birth) and the second one (5-6 years, less than 1 year before elementary school entrance), there are still estimated 5-10 thousands measles cases and 50 measles death annually in Japan. China Ministry of Health, the National Immunization Programme (NIP), China and US CDC, WPR EPI conducted a site visit to review the overall status and progress of the Measles Control and Strengthening Routine Immunizations Project. In 2005, no measles deaths were reported compared to over 100 in 2000. Immunization coverage of students entering nursery and elementary school has increased an estimated 40% (Numazaki K, 2007).

Though the rubella was first described in the mid eighteenth century, it was accepted as a distinct disease 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, national-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 ever 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 Measles and Rubella in SEAR

In SEAR countries, the reported measles cases have been decreases in recent years. From this region, measles incidence per 100,000 was 5.12 in 1999 and 4.78 in 2008. Numerical figure of incidence rate in SEAR region from year 1999 to 2008 is shown in table 2.

Table 2: Measles incidence per 100,000 populations, SEAR 1999-2008 (WHO, 2009)

Year	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Incidence Rate	5.12	7.98	6.19	5.22	5.9	6.89	6.64	5.68	4.35	4.74

Similarly in 2008, the confirmed measles positive cases were 1234 out of 4271 specimens tested (positivity rate 28.9%) in SEAR which decreased to 734 confirmed cases out of 3874 specimens tested (positivity rate 18.9%) in 2009 (WHO, 2009).

The data of vaccination rate in SEAR region shows the increasing trend of vaccination coverage and which might be the major cause in decrease of measles incidence (WHO, 2009).

According to WHO, implementation of measles control activities in SEAR countries comprises 3 strategies (WHO, 2009).

1. Implementing elimination strategies (in Bhutan, DPR Korea, Maldives and Sri-Lanka)
2. Advanced stage of mortality reduction (in Bangaladesh, Indonesia, Myanmar, Nepal and Timor-Leste
3. Partial implementation of mortality reduction strategies (in India and Thiland)

In SEAR 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 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).

3.7.3 Measles and Rubella in Nepal

Measles is endemic in Nepal; an estimated average of 90 000 cases per year occurred from 1994 to 2002, based on extrapolations from routine surveillance reports. Routine measles vaccination began in three districts in 1979 and was expanded nationwide by 1989; children receive a single dose of measles vaccine at 9 months of age. The most recent Demographic and Health Survey, conducted in 2001, estimated that coverage with one dose of measles vaccine among children aged 12–23 months was approximately 71% overall, with regional variability from 65% to 79% (Joshi *et al*., 2009).

In 2003, a total of 67 suspected measles outbreaks were investigated using the integrated system; in 2004, a total of 196 outbreaks were investigated. Nearly 70% of these outbreaks were confirmed measles outbreaks. After the start of the SIAs, the number of suspected measles outbreaks detected decreased to 46 in 2005 and to 31 in 2006. In 2005, only one (2%) of the 46 investigated outbreaks was a laboratory-confirmed measles outbreak, whereas 36 (78%) were laboratory-confirmed rubella outbreaks. Similarly, in 2006, two (6%) of 31 outbreaks were laboratory-confirmed measles outbreaks, and 24 (77%) were laboratory-confirmed rubella outbreaks. During 2005 and 2006, three mixed measles and rubella outbreaks were detected: two (4%) in 2005 and one (3%) in 2006. The number of measles cases associated with outbreaks decreased from approximately 1,000 in 2003 to approximately 50 in 2006. During 2005 and 2006, a total of 1,119 suspected measles cases that were not part of any recognized outbreak were reported to SMOs. Serum specimens were collected for 84 of these cases; three (4%) were laboratory confirmed as measles cases (CDC, 2007).

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 of 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 of Measles and Rubella

3.8.1 Clinical diagnosis

Measles is an illness characterized by generalized maculopapular rash lasting 3 or more days with a temperature of 38.3^{o}C (101^{o}F) or higher, and cough, coryza, or conjunctivitis. Clinically, the diagnosis of measles is supported if Koplik's spots are detected and if the rash progresses from the head to the trunk and out to the extremities. The non specific nature of the prodromal signs and the existence of mildcases, however, make clinical signs unreliable as the sole diagnostic criteria of measles disease. As disease prevalence falls, many medical practitioners are inexperienced in recognizing measles, increasing the need for laboratory method of distinguishing measles from other clinically similar diseases. Misdiagnosis of measles is, for example, more common among young infants, and outbreak-associated cases are more likely to be laboratory confirmed than sporadic cases. Measles virus resembles infections with rubella, dengue fever, ECHO, coxackie, parvovirus B19 and herpesvirus 6 viruses, as well as some bacterial and rickettsial disease. Moreover, there are other conditions that may present in a similar form, including Kawasaki's disease, toxic shock and drug reactions (WHO, 2007).

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 within 16-20 days after exposure. Diagnostic test such as serological testing and virus culture are used to confirm the acute or recent Rubella infection or CRS because 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

Measles virus can be isolated from blood, urine and throat swabs by inoculation of different types of cell culture. Human embryo kidney cells are particularly sensitive but monkey kidney or human amnion cells are more often used. In most cell systems the appearance of giant cells suggests the presence of measles virus but identification should ideally be confirmed by immunofluorescent staining with monoclonal antibody. Isolation from the brains of patients with SSPE is difficult because the virus is defective, however, be accomplished by elaborate co-cultivation technique involving layering of brain cells from affected patients onto monolayers of monkey kidney or other susceptible cells (Pringle and Heath, 1998).

The availability of sensitive cell lines for isolation of measles virus from clinical specimens and establishment of routine RT-PCR and sequencing techniques have allowed for rapid genetic characterization of a large number of wild type strains of measles virus. This database of sequence information now makes it possible to use molecular epidemiological techniques to identify the source of wild-type viruses and to differentiate between wild type and vaccine strains (WHO, 2007).

Initially an Epstein-Barr virus-transformed, marmoset B lymphoblastoid cell line, B95a, was the preferred cell line for primary isolation of measles virus. These cells are upto 10,000 times more sensitive for isolation of measles virus from clinical specimens than other commonly used cell lines. B95a cells are relatively easy to maintain in the laboratory and the cytopathic effect(CPE) from measles infection is readily observed. However, this cell line is infected with Epstein-Barr virus, and this presents a hazard to laboratory workers. These cells must be handled as infectious material at all times. For this reason B95a is no longer the preferred cell line for the isolation of measles viruses in Network Laboratories (WHO, 2007).

Another cell line, Vero/SLAM, has recently been evaluated for use in the Global Laboratory Network. These are Vero cells that are transfected with a plasmid encoding the gene for human SLAM (signaling lymphocyte-activation molecule; also known as CDw150), a recently discovered membrane glycoprotein expressed on some T and B cells, which is a cellular receptor for measles virus. The sensitivity of Vero/SLAM cells for isolation of measles virus is equivalent to that of B95a cells. In addition Vero/SLAM cells are sensitive to laboratory-adapted measles strains including vaccine viruses.Vero/SLAM cells have also been reported to be highly sensitive to rubella viruses, although CPE produced may be difficult to discern. The advantage of Vero/SLAM cells is that they are not persistently infected with virus, and therefore present less of a biological hazard than B95a cells. The current disadvantage of the Vero/SLAM cells is that they require culture medium containing Geneticin to retain SLAM expression (WHO, 2007).

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, 2001).

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 Serology

The clinical diagnosis of measles is most often confirmed by serology. Samples ideally consist of acute and convalescent serum pairs, but detection of MV-specific IgM in serum or saliva or low avidity IgG in serum is diagnostic and may require only a single sample. IgM antibody appears at the time of the rash and can be detected by 3 days and up to 4 weeks after the onset of the rash in most individuals. MV-specific IgG peaks approximately 2 weeks after the onset of rash and gradually increases in avidity. EIA allows differential detection of IgM and IgG and is widely used because of its convenience. Plates may be coated with virus or with recombinant MV proteins. For detection of IgM, use of either the antibody capture method or preremoval of interfering IgG from serum is required for reliable results. The HAI test detects antibody primarily to H and correlates well with the neutralization test. The major limitations of the HAI test are the requirement for fresh sensitive monkey erythrocytes, the difficulty of producing sufficient antigen for a large number of tests, and the possible presence of nonspecific HA inhibitors in serum. The CF test has been used to determine measles

immunity. These titers are less stable over time, and the test is more difficult to perform and is not in common use currently. The virus plaque reduction neutralization assay remains the gold standard against which other tests are measured. It is more sensitive than HAI or EIA tests, and it provides the best correlate for protection from infection and therefore the best measure of response to immunization (Griffin, 2001).

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).

3.8.3 Molecular diagnosis

The most important role of molecular diagnosis in measles and rubella control lies the genetic characterization of wild measles and rubella viruses and detecting genomic variation at different time and regions of the world. As RT-PCR can detect inactivated virus particles, the period of time when virus can be detected after rash onset is often several days to weeks longer than for virus isolation. However, RT-PCR presents a number of technical problems related to sensitivity and reproducibility that can invalidate the assay. In addition, cross-contamination during the RT-PCR process is a significant problem unless the strictest laboratory standards are established and maintained. For these reasons it is not recommended that laboratories set up PCR testing unless they have special designated areas for each of the PCR process and have staff that has undergone comprehensive training (WHO, 2007).

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

A highly effective and safe attenuated live measles virus vaccine is available. Measles vaccine is available in monovalent form and in combination with live attenuated rubella vaccine (MR) and live attenuated rubella and mumps vaccines (MMR). However, because of failure to vaccinate children and because of infrequent cases of vaccinate children and because of infrequent cases of vaccine failure, measles has not been eliminated (Brooks *et al*., 2004).

Rubella is also 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 (Banatvala and Best, 1998).

3.9.2 Control of transmission

The measles and 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)

3.9.3 Integrated measles and rubella control

As of early 2006, four WHO Regions had adopted measles elimination goals, and two(the Americas and Europe) had adopted rubella and CRS elimination targets. WHO recommends that four countries undertaking measles elimination should consider taking opportunity to eliminate rubella at the same time, through use of MR or MMR vaccine in their childhood immunization programmes, and also in mass campaigns. An integrated immunization approach to achieving measles and rubella control targets provides an opportunity for increased programme efficiency; the value of this opportunity, however, varies in different circumstances and at different times. Previous immunization activities, logistics and resource availability may make an integrated strategy unfeasible or inappropriate for some countries at present (WHO, 2007).

3.10 Treatment

There is no standard antiviral treatment for measles. Ribavirin inhibits MV replication in vitro and may reduce the severity of symptoms. Administration of high doses of vitamin A during acute measles decreases morbidity and mortality even in the absence of clinical evidence of vitamin A deficiency. In areas of vitamin A deficiency and xerophthalmia, supplementation prevents blindness caused by measles-induced corneal destruction. The World Health Organization (WHO) recommends high-dose vitamin A supplementation for all children with measles in countries where the fatality rate is 1% or more (Griffin, 2001). Similarly 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. (Doctor NDTV 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 one complete year, March 2009 to February 2010.

4.2.3 Laboratory site:

The entire Laboratory work required for the study was done 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 1009 specimens of rubella/measles suspected cases received through WHO-IPD surveillance network for test of Measles and Rubella in the year 2009/10 from the whole country were tested for anti-Measles IgM and anti-Rubella IgM.

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

Five ml (3 ml in case of children) of venous blood was collected from each suspected patients after the appearance of rash in a clean, dry and labeled test tube for those who visited NPHL directly for measles and rubella test.

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 up to 1 week. Those from different part of country were transported to NPHL through WHO-IPD/ Surveillance Medical Offices maintaining 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 2-8^{0} C until tested for one week and at -20^{0}C for longer period.

4.2.6 Data collection:

Patients details on onset of rash, fever and cough, coryza or conjunctivitis were obtained through a questionnaire/case investigation form reached to NPHL through WHO-IPD surveillance system. Laboratory details were recorded at NPHL.

4.2.7 Specimen exclusion

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

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

4.2.8 Specimen processing (Sero-diagnosis of Measles/Rubella)

A total of 1009 samples received at NPHL from different groups of patients were tested at NPHL for anti-Measles and anti-Rubella IgM, on weekly basis using standard operating protocol of NPHL utilizing SIEMENS ELISA test kit (German).

4.2.8.1 Protocol of the test for sero-diagnosis of Measles and Rubella

All the required reagents and chemicals were available in the kit provided which were stored as instructed in SOP (NPHL, 2009).

All the specimens received at NPHL were processed and tested using the standard methodology (Annex II and III). Test Procedure for detection of anti-Measles IgM and anti-Rubella IgM includes following steps,

1. Assay Scheme: The number of wells required ascertained (= number of test samples + number of determinations of the reference P/N and reference P/P).

The reference P/N (Negative Control) was run in one pair of wells at the start of the series (wells A1/A2), or likewise at the start of each plate in the case of large series.

The reference P/P was run as the second (wells B1/B2) and last sample in the series or as the second and last sample on each plate in the case of large series.

2. Dispense Samples (start of test): Appropriate sample was pipetted, 150 µl /well of the prediluted reference P/N and P/P into the initial pairs of antigen (Ag)/ control antigen (CoAg) wells allocated to these samples in the assay scheme. The test dilution was thus 1:21.

The test samples were vigorously shaked after treatment with RF Absorbent and then pipetted 150 µl /well of each sample into one well coated with Ag and one coated with CoAg. The test dilution was thus 1:42.

The samples must be pipetted into the plate rapidly within a maximum of 15 minutes per test plate.

3. Incubate: The finished test plate was covered with foil and incubated in the laboratory incubator at $37\pm1^{O}C$ for 1 hour. Then proceeded immediately to "4x wash".

4. Wash: Foil was removed and all the wells aspirated; introduced approximately 300µl /well of diluted washing solution POD, allowed to act for 1-2 minutes, and then aspirated. The process was repeated two more times. After completing the washing steps, immediately proceeded to the next reagent-dispensing step (otherwise wells may dry out).

5. Conjugate Dispense: Into each well, 100 µl of the working dilution of anti-human IgM /POD conjugate was pipetted.

6. Incubate: The test plate was covered with foil and incubated in the laboratory incubator at $37\pm1^{O}C$ for 1 hour. Then proceeded immediately to "4x wash".

7. Wash: Washing was performed as described in step no 4.

8. Substrate Dispense: 100 µl of Working Chromogen Substrate was added to each well.

9. Incubate: Covering the test plate with foil, incubated at room temperature ($18\text{-}25^{O}C$) for 30 minutes.

10. Stop Reaction: Removing foil, 100 µl of stopping solution POD was added to each well.

11. Photometric Evaluation: Evaluation should be carried out within an hour. The assay wavelength to be used was 450 nm; 650 nm was used as the reference wavelength. For each sample, and also for reference samples, the value to be determined is the difference between the measured absorbance, Δ A (= $A_{antigen}$ – A $_{control\ antigen}$), with the sample diluted as prescribed.

4.2.8.2 Calculations and quality control

Enzygous anti-Measles and anti- Rubella IgM (SIEMENS)

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: $\Delta A_{Reference\ P/P} \geq 0.2$.
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- Measles and anti Rubella IgM (SIEMENS)

A negative result means the virus specific IgM can not be detected. The patient either is not acutely infected with measles or 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 and can not be confirmed as positive. In such cases, second sample must likewise 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.

4.2.9 Data analysis

Data was first entered in Excel Sheet and collected data was analyzed to find out the age, sex, months, seasons and geographical distribution of the cases. Statistical analysis for significance difference between disease and variables was done using SPSS-13 version.

CHAPTER –V

5. RESULTS

Of the total 1020 specimens collected from suspected Measles/Rubella patients; only 1009 specimens were found to be sufficient for testing when received at NPHL. Quantity was not sufficient (QNS) in rest 11 vials.

Sero-positivity of Measles and Rubella

Of the total 1009 specimens tested for anti-Measles and anti-Rubella IgM, 18(1.8%) showed the presence of anti-Measles IgM and 501(49.7%) showed anti-Rubella IgM.

5.1 Genderwise distribution of Measles and Rubella

Among the 540 male suspected Measles cases tested, 10 (1.9%) were identified as Measles positive, which constitutes 55.6% of the total Measles positive cases. Similarly out of 469 female suspected Measles cases, 8(1.7%) were confirmed to be Measles positive which constitutes 44.4% of total positive cases. The ratio of Measles positive cases in male to female was observed as 1.3:1. The no. of Measles positive in male sex was slightly than in female. The association between the disease and the sex is not statistically significant ($P>0.05$, $\chi^2=0.031$).

Table 3: Genderwise distribution of Measles cases

Sex	Suspected Measles cases	Confirmed positive	Positive %	% of total positive cases	Statistics
Male	540	10	1.9	55.6	**$\chi^2=0.031$**
Female	469	8	1.7	44.4	**P=0.861**
Total	**1009**	**18**	**1.8**	**100**	

Among the 540 male suspected Rubella cases tested, 253(46.9%) were identified as Rubella positive which constitutes 50.5% of total Rubella positive cases. Similarly out of 469 female suspected Rubella cases tested, 248(52.9%) were found to be Rubella positive which constituted 49.5% of total Rubella positive cases. The ratio of Rubella

positive cases in male to female was found as 1.02:1. The no. of Rubella positive cases in both the gender was almost similar but positive percentage of the suspected cases was observed higher in female. The association between the disease and the sex is not statistically significant (P>0.05, χ^2=3.41)

Table 4: Genderwise distribution of Rubella cases

Sex	**Suspected Rubella cases**	**Confirmed positive**	**Positive %**	**% of total positive cases**	**Statistics**
Male	540	253	46.9	50.5	**χ^2=3.410**
Female	469	248	52.9	49.5	**P=0.065**
Total	**1009**	**501**	**49.7**	**100**	

5.2 Agewise distribution of Measles and Rubella

Agewise distribution of Measles cases in study of a whole year data revealed that the highest no. of positive cases(8) were from age group 5-15 which constitutes 44.4% of total positive cases followed by 7 cases from age group 1-5 years. Among the total tested specimens, the highest numbers of suspected cases (556) were also reported from the same age group of 5-15 years. In the age group below 1 year, 2 positive cases were detected which constitutes 11.1% of total positive cases. Similarly 1 positive case (5.6%) was confirmed in the age group 15-45 years and none of the positive case was observed in the age group above 45 years. Statistically the association between age group and occurrence of disease is not significant (χ^2=1.711, P>0.05).

Table 5: Agewise distribution of Measles cases

Age group (in years)	Total no. of tested cases	Confirmed positive	Positive %	% of total positive cases	Statistics
Below 1	82	2	2.4	11.1	
1-5	297	7	2.4	38.9	
5-15	556	8	1.4	**44.4**	**χ^2=1.711**
15-45	44	1	2.3	5.6	**P= 0.877**
Above 45	3	0	0	0	
NA	26	0	0	0	
Total	**1009**	**18**	**1.8**	**100**	

Similarly agewise distribution of Rubella cases also showed that the highest no. of positive cases (304) were from the age group 5-15 years which constitutes 60.7% of total positive cases followed by 142 cases from age group 1-5 years which constitutes 28.3% of total cases. Age group 15-45 and above 45 years showed 16(3.2%) and 1(0.2%) positive cases respectively whereas 21(4.2%) cases were observed positive in the age group below 1 year. The association between the age and the rubella infection was found statistically significant (χ^2=31.448, P<0.05).

Table 6: Agewise distribution of Rubella cases

Age group (in years)	Total no. of tested cases	Confirmed positive	Positive %	% of total positive cases	Statistics
Below 1	82	21	25.6	4.2	
1-5	297	142	47.8	28.3	
5-15	556	304	**54.7**	**60.7**	**χ^2=31.448**
15-45	44	16	36.4	3.2	**P<0.001**
Above 45	3	1	33.3	0.2	
NA	26	17	65.4	3.4	
Total	**1009**	**501**	**49.7**	**100**	

Dividing the total no. of Measles cases into two age groups; 17(94.4%) Measles positive cases were from age group up to 15 years and 1(5.6%) positive case was detected from age group above 15 years.

Dividing the total no. of Rubella cases into two age groups; 484(96.6%) Rubella positive cases were from age group up to 15 years and 17(3.4%) cases were from age group above 15 years.

5.3 Monthwise and Seasonal distribution of Measles and Rubella

Samples from suspected Measles/Rubella cases were obtained throughout the year. From the month of February, the samples of suspected cases were in increasing order up to the month of July then gradually decreased till December.

The highest no. of samples (176) was collected in June, out of which only single sample was found to Measles positive. 8 samples were found positive for anti-Measles IgM in July which accounted 44.4% of total positive cases detected throughout the whole year. For the month of March, August and November, 2 cases were confirmed as Measles

positive in each. Similarly only single Measles positive case was confirmed in February, April, June and September each. There was no Measles positive case confirmed in January, May, October and December. Statistically the association between month and occurrence of disease was not found significant.

Table 7: Monthwise distribution of Measles Cases

Month	**Positive**	**Negative**	**Total no. of tested cases**	**Positive %**	**% of total positive cases**	**Statistics**
January	0	22	22	0	0	
February	1	32	33	3.0	5.6	
March	2	172	174	1.1	11.1	
April	1	126	127	0.8	5.6	
May	0	107	107	0	0	**χ^2=18.503**
June	1	175	176	0.6	5.6	**P=0.071**
July	**8**	**155**	**163**	**4.9**	**44.4**	
August	2	95	97	2.1	11.1	
September	1	32	33	3.0	5.6	
October	0	35	35	0	0	
November	2	33	35	5.7	11.1	
December	0	7	7	0	0	
Total	**18**	**991**	**1009**	**1.8**	**100**	

Among the highest no. of suspected samples (176) for Rubella infection in June, 113(64.2%) were known Rubella positive which accounts 22.6% of total cases throughout the year. The no. of Rubella positive cases rapidly increased from March and remained consistent till July and then decreased. Similarly the no. of specimens collected for suspected rubella cases also increased in the same period. During March and July 92(18.4% of total positive) cases in each month were confirmed as Rubella positive.

Statistically the association between Month and occurrence of Rubella infection was found statistically significant.

Table 8: Monthwise distribution of Rubella Cases

Month	**Positive**	**Negative**	**Total no. of tested cases**	**Positive %**	**% of total positive cases**	**Statistics**
January	10	12	22	45.5	2	
February	12	21	33	36.4	2.4	
March	92	82	174	52.9	18.4	
April	71	56	127	55.9	14.2	
May	62	45	107	57.9	12.4	**χ^2=94.074**
June	**113**	**63**	**176**	**64.2**	**22.6**	**P<0.001**
July	92	71	163	56.4	18.4	
August	31	66	97	32.0	6.2	
September	11	22	33	33.3	2.2	
October	6	29	35	17.1	1.2	
November	1	34	35	2.9	0.2	
December	0	7	7	0	0	
Total	**501**	**508**	**1009**	**49.7**	**100**	

Looking at the seasonal distribution of Measles cases, highest number of specimens (437) was obtained in summer season (June, July and August). Among which 11(2.5%) cases were confirmed as Measles positive and this accounts 61.1% of total positive cases throughout the year. Whereas the least number of specimens (62) from suspected Measles cases were collected in winter season and only single case was confirmed as Measles positive in this season. Similarly 3(16.7% of total positive) cases in each season of spring and autumn were confirmed as Measles positive. Statistically the association between season and occurrence of Measles was not significant.

Table 9: Seasonwise distribution of Measles cases

Season	Suspected Measles cases	Confirmed positive	Positive %	% of total positive cases	Statistics
Winter Season (Dec, Jan, Feb)	62	1	1.6	5.6	
Spring Season (Mar, Apr, May)	408	3	0.7	16.7	**χ^2=4.692 P=0.196**
Summer Season (Jun, July, Aug)	**437**	**11**	**2.5**	**61.1**	
Autumn Season (Sept, Oct, Nov)	102	3	2.9	16.7	
Total	**1009**	**18**	**1.8**	**100**	

Seasonwise distribution of Rubella cases revealed that highest no. of positive cases (236) from the suspected Rubella cases (437) were obtained in summer season followed by 225 positive cases from 408 suspected cases in spring season. The positive cases in summer season accounted for 47.1% of total positive throughout the year while the positive cases in spring season accounted 44.9% of total positive. Least no. of specimens (62) was obtained in winter season while least positive cases (18) were obtained in autumn season.

Statistical analysis shows that association between season and Rubella infection is significant.

Table 10: Seasonwise distribution of Rubella cases

Season	Suspected Rubella cases	Confirmed positive	Positive %	% of total positive cases	Statistics
Winter Season (Dec, Jan, Feb)	62	22	35.5	4.4	
Spring Season (Mar, Apr, May)	408	225	55.1	44.9	**χ^2=55.013 P<0.001**
Summer Season (Jun, July, Aug)	**437**	**236**	**54.0**	**47.1**	
Autumn Season (Sept, Oct, Nov)	102	18	17.6	3.6	
Total	**1009**	**501**	**49.7**	**100**	

5.4 Districtwise and regional distribution of Measles and Rubella

A total of 1009 specimens collected from suspected Measles or Rubella infection patients of 60 different districts of Nepal were received and tested at NPHL for anti-Measles IgM and anti-Rubella IgM detection. However Measles positive cases were

reported from 11 districts only while Rubella positive cases were reported from 43 districts. The highest no. of Measles positive cases were confirmed in Kathmandu district (5, 27.8%) followed by Doti (4, 22.2%), and Dang, Darchula, Gulmi, Jhapa, Kailali, Mahottari, Nawalparasi, Salyan, Syangja (1, 5.6% in each). Similarly the highest no. of Rubella positive cases were observed in Mahottari district (78, 15.6%) followed by Dang (71, 14.2%), Kathmandu (57, 11.4%), Bhaktapur (32, 6.4%), Dhankuta (26, 5.2%) and Gorkha (18, 3.6%). These six districts accounted constitute 56.3% of total positive cases. Eight (1.6%) of Rubella positive cases were from unknown districts.

Table 11: Districtwise distribution of Measles and Rubella cases

SN	District	Suspected Measles/ Rubella cases	Measles		Rubella	
			Positive	% of total positive cases	Positive	% of total positive cases
1	Achham	6	0	0	2	0.4
2	Arghakhanchi	10	0	0	8	1.6
3	Baglung	3	0	0	2	0.4
4	Baitadi	6	0	0	1	0.2
5	Bajhang	1	0	0	0	0
6	Bake	6	0	0	2	0.4
7	Bara	9	0	0	0	0
8	Bardiya	12	0	0	10	2
9	Bhaktapur	89	0	0	32	6.4
10	Chitwan	2	0	0	1	0.2
11	Dadeldhura	2	0	0	0	0
12	Dang	120	1	5.6	71	14.2
13	Darchula	1	1	5.6	0	0
14	Dhading	20	0	0	9	1.8
15	Dhankuta	35	0	0	26	5.2

16	Dhanusa	9	0	0	4	0.8
17	Dolkha	1	0	0	0	0
18	Doti	23	4	**22.2**	10	2
19	Gorkha	29	0	0	18	3.6
20	Gulmi	16	1	5.6	7	1.4
21	Ilam	15	0	0	11	2.2
22	Jhapa	16	1	5.6	2	0.4
23	Jumla	2	0	0	0	0
24	Kailali	16	1	5.6	4	0.8
25	Kanchanpur	11	0	0	4	0.8
26	Kapilvastu	2	0	0	0	0
27	Kaski	5	0	0	0	0
28	Kathmandu	136	5	**27.8**	57	**11.4**
29	Kavrepalanchowk	32	0	0	8	1.6
30	Lalitpur	18	0	0	8	1.6
31	Lamjung	2	0	0	0	0
32	Mahottari	138	1	**5.6**	78	**15.6**
33	Morang	9	0	0	7	1.4
34	Mugu	1	0	0	1	0.2
35	Myagdi	1	0	0	0	0
36	Nawalparasi	3	1	5.6	1	0.2
37	Nuwakot	6	0	0	2	0.4
38	Palpa	8	0	0	2	0.4
39	Panchthar	18	0	0	13	2.6
40	Parbat	2	0	0	0	0
41	Parsa	2	0	0	1	0.2
42	Ramechhap	6	0	0	5	1
43	Rasuwa	10	0	0	8	1.6
44	Rautahat	2	0	0	0	0

45	Rolpa	3	0	0	0	0
46	Rupendehi	5	0	0	5	1
47	Salyan	1	1	5.6	0	0
48	Sankhuwasabha	14	0	0	11	2.2
49	Saptari	1	0	0	0	0
50	Sarlahi	11	0	0	7	1.4
51	Sindhuli	13	0	0	10	2
52	Sindhupalchowk	2	0	0	1	0.2
53	Siraha	25	0	0	22	4.4
54	Solukhumbu	5	0	0	1	0.2
55	Sunsari	11	0	0	0	0
56	Surkhet	1	0	0	0	0
57	Syangja	4	1	5.6	1	0.2
58	Tanahu	3	0	0	1	0.2
59	Taplejung	9	0	0	3	0.6
60	Tehrathum	25	0	0	16	3.2
	Unknown(NPHL)	15	0	0	8	1.6
	Total	**1009**	**18**	**100**	**501**	**100**

Out of 1009 suspected Measles cases, majority of cases (506) were from CDR followed by EDR (183), MWDR (141), WDR (93) and FWDR (71) cases respectively. Although the majority of suspected Measles cases were from CDR, positive cases were equal from CDR and FWDR i.e. 6(33.3%) from each region. Lowest no. of Measles positive cases i.e. 1(5.6%) was obtained in EDR while in MWDR and WDR 2(11.1%) and 3(16.7%) confirmed positive cases were found respectively. Positivity rate was highest in FWDR (8.5%).

Table 12: Regional distribution of Measles cases

Region	Suspected Measles cases	Confirmed positive	% of total positive cases	Statistics
FWDR	71	6	**33.3**	
MWDR	141	2	11.1	
WDR	93	3	16.7	χ^2**= 22.127**
CDR	506	6	**33.3**	**P< 0.001**
EDR	183	1	5.6	
Unknown	15	0	0	
Total	**1009**	**18**	**100**	

Similarly regional distribution of Rubella cases showed that highest no. of Rubella positive cases were from CDR (46.1%) followed by EDR(22.4%), MWDR(16%), WDR(9%) and FWDR(5%).

Table 13: Regional distribution of Rubella cases

Region	Suspected Rubella cases	Confirmed positive	% of total positive cases	Statistics
FWDR	71	25	5	
MWDR	141	80	16	
WDR	93	45	9	χ^2**= 21.899**
CDR	506	231	46.1	**P=0.001**
EDR	183	112	22.4	
Unknown	15	8	1.6	
Total	**1009**	**501**	**100**	

Majority of Measles positive cases (12 out of 538) were obtained from Hill region which covers 66.7% of total positive cases. Terai and Mountain region accounted 27.8% and 5.6% of total positive cases respectively. The highest positivity rate (2.2%) was also observed in Hill region followed by Mountain and Terai region. Total no. of specimens

from suspected Measles cases were also highest from hill region followed by Terai and Mountain region.

Table 14: Geographical distribution of Measles cases

Geographical region	Suspected Measles cases	Confirmed positive	Positive %	% of total positive	Statistics
Terai	410	5	1.2	27.8	
Hill	538	12	2.2	**66.7**	**χ^2= 1.670**
Mountain	46	1	2.1	5.6	**P= 0.644**
Unknown	15	0	0	0	
Total	**1009**	**18**	**1.8**	**100**	

Geographical distribution of Rubella cases also revealed the highest no of Rubella positive cases (249 out of 538) were observed from Hill region which constitutes 49.7% of total Rubella positive cases followed by Terai(43.7%) and Mountain(5%) region. But the highest sero-positivity rate was detected as 54.3% in Mountain region followed by Terai(53.4%) and Hill(46.3%).

Table 15: Geographical distribution of Rubella cases

Geographical region	Suspected Rubella cases	Confirmed positive	Positive %	% of total positive	Statistics
Terai	410	219	53.4	**43.7**	
Hill	538	249	46.3	**49.7**	**χ^2= 5.252**
Mountain	46	25	54.3	5	**P= 0.154**
Unknown	15	8	53.3	1.6	
Total	**1009**	**501**	**49.7**	**100**	

5.5 Measles in vaccinated and unvaccinated patients

Highest sero-positivity rate (7.6%) was observed in suspected Measles patients who did not have history of Measles vaccination whereas sero-positivity rate was only 1.2% in suspected Measles patients who had history of Measles vaccination. However, the no. of suspected Measles cases were higher among vaccinated for Measles. Statistical analysis shows the association between vaccination and occurrence of Measles is highly significant.

Table 16: Measles positive cases by measles vaccination history

Measles vaccination	**Suspected Measles cases**	**Confirmed positive**	**Positive %**	**Statistics**
Yes	829	10	1.2	
No	66	5	**7.6**	**χ^2= 14.682**
Unknown	114	3	2.6	**P= 0.001**
Total	**1009**	**18**	**1.8**	

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Measles is still the leading cause of childhood death and is a vaccine-preventable disease globally. Even countries in which measles had been eradicated still remain at risk of importation from countries that have not yet eliminated the disease. Globally more than 30 million people are affected each year by measles. An estimated 454,000 people, the majority of them children, died from measles in 2004. It is estimated that more than 1200 measles deaths occur every day and 50 people die every hour from measles. More than 95% of measles deaths occur in developing countries (Numazaki, 2007). Measles is endemic in Nepal; an estimated average of 90 000 cases per year occurred from 1994 to 2002, based on extrapolations from routine surveillance reports. Routine measles vaccination began in three districts in 1979 and was expanded nationwide by 1989; children receive a single dose of measles vaccine at 9 months of age (Joshi *et al*., 2009). Although a massive measles vaccination program has been established measles outbreaks are still happening.

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 was 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). Nepal is standing with the similar situation.

The present cross-sectional study was carried out during a period from March 2009 to February 2010 based at National Public Health Laboratory. The study was an extensive epidemiological study covering all the 75 districts of the country along with suspected Measles/Rubella patients visiting NPHL.

With the collaboration of WHO-IPD, Measles surveillance along with Rubella 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.

Out of total 1020 samples 11 samples were QNS for testing and 1009 samples were in sufficient quantity for testing. Among the total no. of suspected cases, 540 cases were from male patients which constituted 53.5% of total cases while 469 cases were from female patients which accounted 46.5% of total cases. The present study also agrees with the 2006 results of NPHL, which reported 55.4% male cases and 44.6% female cases (Kc, 2007).

The agewise distribution of suspected Measles/Rubella cases revealed that the highest number (556) cases were from age group 5-15 years which accounted for 55.1% of total. Similarly 297 cases were from age group 1-5 years which accounted 29.4% of total, 82 (8.2%) cases were from age group below one year while 45 (4.5%) cases were from age group 15-45 years. Only three cases were from age group above 45 years. The study showed that the most susceptible age group for Measles/Rubella was 5-15 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 is similar to the past results of September 2004 to January 2005 which also shows most cases were children aged 5-14 years (56%) or 1-4 years (32%) (Joshi *et al*., 2009). This data also agrees with the past unpublished results of NPHL, 2004, 2005, and 2006. 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. Similarly in 2006, 43.19% suspected cases were from age group 5-10 years. Very few suspected Measles/Rubella cases were reported from above 45 years. This might be due to either sub clinical infection where rashes were absent to suspect for Rubella or due to naturally acquired immunity. The higher cases in age group of 5-15 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 of Nepal, suspected Measles/Rubella cases were reported from 60 districts, where highest no. of cases (138) were reported from Mahottari district followed by Kathmandu (136 cases) and Dang district (120 cases). Most of the cases (506 cases) were reported from CDR which accounted 50.1% of total followed by EDR (183 cases, 18.1%) where as 71 suspected cases 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. Geographical distribution of suspected Measles/Rubella cases shows that highest number (558 cases) was reported from Hill region which accounted 55.3% of total cases followed by Terai region (410 cases, 40.6%). Only 46 cases (4.6%) cases were reported from Mountain region.

Monthwise and seasonwise distribution of suspected Measles/Rubella cases shows the pattern of increasing no. of cases from late winter to spring and cases mostly clustered in spring and summer and it is following the similar trend of distribution as in other parts of world. Highest no. of suspected cases were reported in summer season (43.3%) followed by spring season (40.4%). The least no. of suspected cases were obtained in winter season (6.1%). In monthwise distribution, the highest no. (113 cases, 11.2%) was reported in June followed by March and July (92 cases, 9.1% in each). This study is in contrast to the study of 2006 which showed highest no. of suspected cases (52.58%) in spring season followed by winter season (25.82%) (Kc, 2007). But this finding shows the similar pattern of seasonal distribution with the NPHL data in 2005 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 1009 specimens tested, 1.8% was positive for Measles whereas 49.7% cases found positive for Rubella infection. It shows the decreased of Measles positivity than that of past results, as 2.8% positivity was observed for Measles in 2006 and 6.8% in 2007. But the Rubella positivity rate shows slight increase compared to past results of 2006, which showed 49.3% positivity for suspected Rubella cases (NPHL, 2009).

Out of the 18 Measles positive cases obtained, 10 were male patients which constitute 55.6% of total cases and 8 were female patients (44.4%). The suspected Measles cases were also high in male and similar trend was also observed in the positive cases. The association between disease and sex is statistically insignificant ($P>0.05$, $\chi^2= 0.861$).

Similarly out of 501 Rubella positive cases observed, 253 were male patients which constitutes 50.5% of total cases and 248 were female patients which constitutes 49.5% of total. Although the no. of positive cases was similar in two sexes, positivity rate was

slight higher in female (52.9%) compared to male (46.9%). The association between disease and sex is not statistically significant ($P>0.05$, $\chi^2= 3.410$).

The agewise distribution of Measles cases revealed that highest no. of positive cases were observed in age group 5-15 years which constituted 44.4% of total cases followed by age group 1-5 years (38.9%). The association between occurrence of Measles and age group is not found statistically significant ($P>0.05$, $\chi^2= 1.711$).

Similarly agewise distribution of Rubella cases also showed that the highest no. of positive cases (304) were from the age group 5-15 years which constitutes 60.7% of total positive cases followed by 142 cases from age group 1-5 years which constitutes 28.3% of total cases. Dividing the total cases in to two age groups up to 15 years and above 15 years, most of the rubella positive cases were clustered in the age group up to 15 years but 9 positive cases were observed in the female patients of age group above 15 years which is also the age of child bearing. This clearly signifies the risk of congenital rubella syndrome in the population. The association between occurrence of Rubella and age group is statistically significant ($P<0.05$, $\chi^2= 31.448$).

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 Measles or Rubella. 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 years, 6.5% in 1-4 years, 6.5% in 10-14 years and no case above or equal to 15 years (WHO, 2005).

The present study showed that the suspected and confirmed Measles and Rubella cases in were clustered bellow 15 years. As the age group increases the, the cases had sharply decreased and very few reports above the 45 years (No measles positive and single Rubella positive case). The present result is in accordance as called the Measles and

Rubella as a disease of childhood. The high occurrence of Rubella in this age is due to the lack of Rubella vaccine and the immune system not fully matured and high Measles cases in this age group might be due to high contact rate among children and immunity gap in the population. 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 Measles and Rubella in early ages of life. When one catches Measles or Rubella, he/she is lifelong immune to respective disease; this is the cases what makes the difference of Measles and 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 infection is sub clinical. It might be also due to unaware of rashes and not properly reported.

In accordance to this study, suspected Measles/ Rubella cases were obtained throughout the year. Out of 18 total positive Measles cases, 8 cases were found positive for anti-Measles IgM in July which accounted 44.4% of total positive cases detected throughout the whole year. For the month of March, August and November, 2 cases were confirmed as Measles positive in each. Similarly only single Measles positive case was confirmed in February, April, June and September each. There was no Measles positive case confirmed in January, May, October and December. Although positive Measles cases are distributed in few months, association between occurrence of disease and month is not statistically significant ($P>0.05$, $\chi^2= 18.503$). Looking at the seasonal distribution of Measles cases, highest number of specimens (437) was obtained in summer season. Among which 11(2.5%) cases were confirmed as Measles positive and this accounts 61.1% of total positive cases throughout the year. Similarly out of total 501 Rubella positive cases, 113 were known Rubella positive in June which accounts 22.6% of total cases throughout the year. The no. of Rubella positive cases rapidly increased from March and remained consistent till July and then decreased. Similarly the no. of specimens collected for suspected rubella cases also increased in the same period. During March and July 92(18.4% of total positive) cases in each month were confirmed as

Rubella positive. Seasonally highest no. of positive cases were observed in summer season (47.1% of total positive) followed by spring season (44.9%). Statistically the association between Month and occurrence of Rubella infection was found statistically significant ($P<0.05$, $\chi^2= 94.074$). Similarly association between season and occurrence of Rubella is also statistically significant ($P<0.05$, $\chi^2= 55.013$).

The present study is similar with the result of 2005 with the highest positive Rubella 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%) (NPHL, 2009).

Although the suspected Measles/Rubella cases were reported from 60 districts of country, the Measles positive cases were observed only from 11 districts while Rubella positive cases were reported from 43 districts. The highest no. of Measles positive cases were confirmed in Kathmandu district (27.8%) followed by Doti (22.2%), and Dang, Darchula, Gulmi, Jhapa, Kailali, Mahottari, Nawalparasi, Salyan, Syangja (5.6% in each). Similarly the highest no. of Rubella positive cases were observed in Mahottari district (78, 15.6%) followed by Dang (14.2%), Kathmandu (11.4%), Bhaktapur (6.4%), Dhankuta (5.2%) and Gorkha (3.6%). These six districts accounted 56.3% of total positive cases. Eight (1.6%) of Rubella positive cases were from unknown districts.

Regional distribution of Measles cases presented that out of total 1009 suspected Measles cases, majority of cases (506) were from CDR followed by EDR (183), MWDR (141), WDR (93) and FWDR (71) cases respectively. Although the majority of suspected Measles cases were from CDR, positive cases were equal from CDR and FWDR i.e. 6(33.3%) from each region. Lowest no. of Measles positive cases i.e. 1(5.6%) was obtained in EDR while in MWDR and WDR 2(11.1%) and 3(16.7%) confirmed positive cases were found respectively. Positivity rate was highest in FWDR (8.5%). The association between occurrence of Measles cases and region is statistically significant ($P<0.05$, $\chi^2= 22.172$). Similarly regional distribution of Rubella cases showed that that highest no. of Rubella positive cases were from CDR (46.1%) followed by EDR(22.4%),

MWDR(16%), WDR(9%) and FWDR(5%). The association between occurrence of Rubella and region is also statistically significant ($P<0.05$, $\chi^2= 21.889$). 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. Least no. of cases from FWDR might be either due to actual decrease in cases or due to lack of proper reporting system and available health facilities.

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%).

Geographical distribution of Measles shows that majority of Measles positive cases (12 out of 538) were obtained from Hill region which covers 66.7% of total positive cases. Terai and Mountain region accounted 27.8% and 5.6% of total positive cases respectively. The highest positivity rate (2.2%) was also observed in Hill region followed by Mountain and Terai region. The association between disease and geographical region was not statistically significant ($P>0.05$, $\chi^2= 1.670$). Similarly geographical distribution of Rubella cases also revealed the highest no of Rubella positive cases (249 out of 538) were observed from Hill region which constitutes 49.7% of total Rubella positive cases followed by Terai(43.7%) and Mountain(5%) region. But the highest sero-positivity rate was detected as 54.3% in Mountain region followed by Terai(53.4%) and Hill(46.3%). The association between occurrence of Rubella and geographical region is also not statistically significant ($P>0.05$, $\chi^2= 5.252$). The distribution of Measles/Rubella in Hill and Terai is almost similar but less report from mountains indicates it as less burden region for Measles and Rubella. However, in some instances, it might have so happened due to less population density and also might be 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.

According to Measles vaccination status, higher sero-positivity rate (7.6%) was observed in suspected Measles patients who did not have history of Measles vaccination where as sero-positivity rate was only 1.2% in suspected Measles patients who had history of Measles vaccination. Statistical analysis shows the association between vaccination and occurrence of Measles is highly significant ($P<0.05$, $\chi^2= 14.682$).

6.2 Conclusion

Out of 1009 specimens tested, 1.8 %(18) were confirmed as Measles positive and 49.7% (501) were confirmed as Rubella positive. Highest no. of Measles positive cases (44.4%) were observed in July whereas most Rubella cases were clustered in the five months of the year which include March, April, May, June and July with the highest cases in June and in context of season it was summer season when the highest positive cases of both Measles and Rubella were observed. Both Measles and Rubella positive cases were highest in age group 5-15 years followed by age group 1-5 years. Measles positive cases were equal in CDR and FWDR, which was the highest among all regions with highest positive cases in Kathmandu district. Almost half of the Rubella positive cases (46.1%) were from CDR with highest in Mahottari. Geographical distribution of Measles and Rubella showed that highest cases were reported from Hill region and very few cases were reported from Mountain region. Measles vaccination shows protective effect against Measles as positivity rate for suspected Measles cases in vaccinated population was significantly lower compared to that of non-vaccinated. High positivity rate of Rubella cases underlines the importance of introduction of Rubella vaccination program in Nepal.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. The present study was conducted in National Public Health Laboratory for a whole year March 2009 to February 2010. During the study period, a total of 1020 specimens from suspected Measles/Rubella cases were received through WHO-IPD network along with few direct samples at NPHL, of which 1009 specimens were tested for anti-Measles IgM and anti-Rubella IgM. Out of 1009 suspected Measles/Rubella cases, 53.5% were male patients and 46.5% were female patients.

2. Of the total suspected cases tested by ELISA technique, 1.8% cases showed the presence of anti-Measles IgM whereas 49.7% cases showed the presence of anti-Rubella IgM.

3. Among all confirmed Measles positive cases, 55.6% cases were from male patients and 44.4% cases were from female patients respectively and ratio of Measles positive cases in male to female was observed as 1.3:1. Similarly out of total confirmed Rubella positive cases, 50.5% cases were male patients and 49.5% were female patients and ratio of Rubella positive cases in male to female was observed as 1.02:1.

4. Highest no. of Measles positive cases were from age group 5-15 years (44.4%) followed age group 1-5 years (38.9%), below 1 year (11.1%) and 15-45 years (5.6%). Collectively 94.4% Measles positive cases were from age group up to 15 years and rest 5.6% from above 15 years.

5. Highest no. of Rubella positive cases were also from age group 5-15 years (60.7%) followed by 1-5 years (28.3%), below 1 year (4.2%), 15-45 years (3.2%)

and above 45 years (0.2%). Collectively 96.6% Rubella positive cases were from age group up to 15 years and rest 3.4% from above 15 years.

6. The highest no. of Measles positive cases was obtained in July (8, 44.4%) followed by March, August and November (2, 11.1% each). Seasonwise distribution of Measles positive cases showed that most of positive cases were clustered in summer season (61.1%).

7. The highest no. of Rubella positive cases were obtained in June (113, 22.6%) followed by March and July (92, 18.4% each). Seasonwise distribution of Rubella positive cases revealed that most of the positive cases were observed in summer season (47.1%) followed by spring season (44.9%).

8. Suspected Measles/Rubella cases were reported from 60 districts of country but the Measles positive cases were observed only from 11 districts while Rubella positive cases were reported from 43 districts. The highest no. of Measles positive cases were confirmed in Kathmandu district (27.8%).

9. Although the majority of suspected Measles cases were from CDR, positive cases were equal from CDR and FWDR i.e. 6(33.3%). Similarly regional distribution of Rubella cases showed that that highest no. of Rubella positive cases were from CDR (46.1%) followed by EDR(22.4%).

10. Higher sero-positivity rate (7.6%) was observed in suspected Measles patients who did not have history of Measles vaccination whereas sero-positivity rate was only 1.2% in suspected Measles patients who had history of Measles vaccination.

7.2 Recommendations

1. Detection of significant no. of Measles positive cases despite of massive vaccination strategies signifies the need of further strengthening the vaccination strategies with more coverage in population. On the same time, detection of Measles positive cases who had history of vaccination suggests the need to evaluate the sero-conversion rate in population with proper time of vaccination and maintaining the proper cold chain up to the remote villages of Nepal to meet the goal of Measles elimination.

2. Changing pattern of suspected cases from Measles to Rubella with high no. of Rubella positive cases in population signifies for need of a policy to immunize all children against Rubella which would help in preventing CRS.

3. Lab testing is limited in very few places of Nepal, but for better laboratory based surveillance, diagnostic facilities (with skilled man power) should be expanded to other part of the country.

4. Sero-surveillance of women of child bearing age should be done and immunization policy needs to be developed for adolescent girls and/or women of child bearing age group before conception to avoid CRS problem in new borne ones. In addition, 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.

5. Out of 1009 suspected Measles/Rubella cases tested, only 51.4% were confirmed either Measles or Rubella. The etiology of rest of the patients (48.6%) remained unknown. As all cases were with rash syndrome, further investigations in future are recommended to know other etiologies of rash disease including Dengue and other paramyxovirus infections.

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