CHAPTER I

1. INTRODUCTION

1.1 Background

Vector-borne diseases are among the main causes of illness and death and constitute a major public health problem in the countries of the South Asian region. Nepal has been found endemic for Japanese encephalitis (JE), malaria and kala-azar (KA). Approximately, 64.6 % of the total population of Nepal is at a constant risk of acquiring vector borne diseases and hence, JE has been considered as one of the most important vector borne diseases in Nepal (Bajracharya, 2001).

JE is an acute viral infection of the central nervous system. Patients with JE typically present a few days of non-specific febrile illness followed by headache, vomiting and a reduced level of consciousness, often heralded by convulsion and may progress to a serious infection of the brain (encephalitis) (Solomon, 1997).

JE is a mosquito borne arboviral infection caused by Japanese encephalitis B virus (JEV). JE virus (JEV), a member of the genus *Flavivirus* (family Flaviviridae), is transmitted naturally in an enzootic cycle among mosquitoes and vertebrate amplifying hosts, chiefly domestic pigs and Ardeid (wading) birds (Burke *et al.*, 1988). Culex mosquitoes, primarily *Culex tritaeniorhynchus*, are the principal vectors. Humans accidentally/mechanically acquire the infection by mosquito-bites only when they encroach upon this enzootic cycle (Solomon, 2003) but JE virus infected persons do not have high titer viremia and are therefore considered as “dead-end” hosts. They do not contribute to perpetuation of virus transmission (Brooks *et al.*, 2004).

Numerically, Japanese encephalitis is one of the most important causes of viral encephalitis worldwide, with an estimated 50,000 cases and 15,000 deaths annually (Tsai, 1997; Solomon, 1997). Most of the sporadic and epidemic cases of JE are

reported annually from the People's Republic of China (PRC), Korea, Japan, Southeast Asia, the Indian subcontinent, and parts of Oceania. Viral transmission occurs across a much broader area of the region than is recognized by epidemiologic surveillance (CDC, 1993). Approximately, 3 billion people and 60 % of the world's population live in the JE endemic regions (Kabilan, 2004). JE usually is severe, resulting in a fatal outcome in 25% of cases and residual neuropsychiatric sequelae in 30% of cases (Burke *et al.*, 1998). Some hyper endemic districts of Nepal represent the paddy field ecosystem with abundant *Culex* species and amplifying hosts like pigs and migratory birds indicating the potential epidemics in these districts. High humidity, summer temperature of 24^{o}C-38^{o}C and paddy field ecosystem of the terai region are the favourable conditions for breeding of *Culex* mosquitoes, the principal vector of JE in Nepal. Therefore, a high prevalence of JE has been identified in the terai and inner terai regions where cross-border transmission is also possible around the border areas (EDCD, 2005). Sporadic cases have also been reported from other non endemic districts.

Humans become infected after the bite of an infected mosquito. Viral infection rates in the mosquitoes range from <1% to 3%. Because the paddy–breeding *Culex* mosquitoes, which transmit JEV, are unavoidable, the majority of the population in rural Asia has been infected with the virus by early adulthood (Solomon, 2003).

These species are prolific in rural areas where their larvae breed in ground pools and especially in flooded rice fields. All elements of the transmission cycle are prevalent in rural areas of Asia, and human infections occur principally in this setting. Because vertebrate-amplifying hosts and agricultural activities may be situated within and at the periphery of cities, JE cases occasionally are reported from urban locations (CDC, 1993).

The occurrence of JE is mainly observed in children and its severity increases with two age extremities. The epidemiological pattern and the geographical distribution of JE

have been found changing throughout Asia. There is the reduction of JE cases in the developed countries such as China, Japan and Korea with widespread use of JE vaccine and proper vector control systems. In those countries where children are immunized against JE, the disease incidence is shifted towards the elderly (Vaughn and Hoke, 1992).

The present records of developing countries indicate the rising trends of JE occurrence and expansion of disease into JE non-endemic areas (Kabilan, 2004). The similar trend is also seen in Nepal, which can't be ignored (Bista *et al.*, 1999; Rai *et al.*, 1987).

JE outbreaks are usually circumscribed; do not last more than a couple of months and the outbreaks terminate after the majority of amplifying hosts have become infected. JE cases in Nepal start to appear in the month of April-May, reach its peak during late August to early September and start to decline from October (EDCD, 2005). There is always some periodic oscillation but when favouring climatic condition coincides with the natural period, fulminating outbreak of the vector borne disease occurs (MacDonald, 1957).

Health services are far from the reach of most of the people living in JE endemic areas, especially in the rural communities. Poor socioeconomic status of people, low literacy rate, and the absence of voluntary agencies are also identified as the major hindrances to disease control in most areas of Nepal (Sherchand *et al.*, 1996).

1.2 Rationale and Justification of the Study

The plain region (terai and inner terai) of Nepal is always found to be endemic for JE. Twenty four districts of terai and inner terai regions are affected by JE and around 12.5 million people are estimated to be at the risk of disease (EDCD, 2005). Moreover, the population of the high risk group (1-15 years) has reached 5.4 million, which reveals the scope of problem in Nepal. Since the first outbreak in 1978, seasonal outbreaks have been reported annually (EDCD, 2005) with 1000-3000 cases and 200-400 deaths in

Nepal. Although 24 districts are considered to be at constant risk of disease, sporadic cases have also been reported from other non endemic districts including some of the hill districts. More serious side of the disease is that still the no. of cases and deaths have been found in an increasing pattern (EDCD, 2005; Joshi *et al.*, 2005).

In recent years, outbreaks of JE have occurred in a large no. of areas, and the high case fatality rate and frequent residual neuropsychiatric sequalae in survivors make JE a significant public health problem in Nepal. Serological surveys have revealed that about 10 % of the people living in JE endemic areas are infected with the virus and most of them are infected before the age of 15 years (CDC, 1993; EDCD, 2005).

JE has a great economic impact on the Nepalese people, primarily the poor people of rural areas. On an average, the combined cost due to the disease (direct: medical, transportation, food; indirect: work/wage earning time lost due to the disease) was US $ 136 per household (Adhikari *et al.,* 2002). This expenditure invites immediate economic crisis in the concerned households and a long term economic burden on local health system and the society. This type of research can reveal the latest picture of the national disease burden, which ultimately provides guidelines to policy makers of health, economic and social sectors.

Very few focused studies have been carried out in the past to find out the burden of the disease in particular areas. The present study with national coverage can reveal the changing epidemiological pattern and the geographical distribution of the disease.

In the surveillance of Acute Encephalitis Syndrome (AES) including Japanese encephalitis which is a collaborative program of Ministry of Health and World Health Organization (WHO), WHO-Program for Immunization Preventable Diseases (WHO-IPD) is involved since last few years (Personal communication). This study is also a collaborative study of Central Department of Microbiology, Tribhuwan University

(CDM, TU)/National Public Health Laboratory (NPHL)/WHO focused mostly on sero-epidemiology of JE.

CHAPTER II

2. OBJECTIVES

2.1 General Objective

To study the sero-epidemiological pattern of JE in Nepal in 2005.

2.2 Specific Objectives

2.2.1 To know the burden of Acute Encephalitis Syndrome (AES) in Nepal.

2.2.2 To know the burden of JE in Nepalese population.

2.2.3 To find out the case fatality rate (CFR) due to AES and JE in Nepal.

CHAPTER III

3. LITERATURE REVIEW

3.1 Japanese Encephalitis

Japanese encephalitis (JE) is a mosquito borne arboviral infection caused by Japanese encephalitis B virus (JEV). JE is the most important cause of endemic encephalitis and annually a remarkable number of deaths due to the same are recorded worldwide. JE is an acute viral infection of the central nervous system. In recent decades, outbreaks of JE have occurred in a large number of countries of Asia, and the high case fatality rate and frequent residual neuropsychiatric sequalae in the survivors make JE a significant public health problem (Solomon, 1997; Burke *et al.,* 1988).

3.2 JE Virus: The Etiological Agent

3.2.1 History:

Encephalitis due to JE virus (JEV) was reported in Japan as early as 1871, but JEV was isolated from a clinical case in the first reported epidemic in Japan in 1924 (Miyake, 1964). Nakayama strain of JEV was first isolated from human cases in 1935 (Monath, 1985). This strain is used in the development of mouse brain inactivated vaccine. Mosquito, as the vector for JEV transmission was, identified in 1950 only, 25 years after the recognition of JEV.

The term type B encephalitis was originally used to differentiate summer epidemics form Von Economo's encephalitis lathergia (sleeping sickness, known as type A), but the B has since been dropped (Solomon *et al.,* 2000). In 1933, a filterable agent was transmitted from the brain of a fatal case to cause encephalitis in monkeys; the virus was identified as the prototype Nakayama strain of JEV after it was isolated in 1935. This virus was later classified as a member of the genus *Flavivirus* of family flaviviridae.

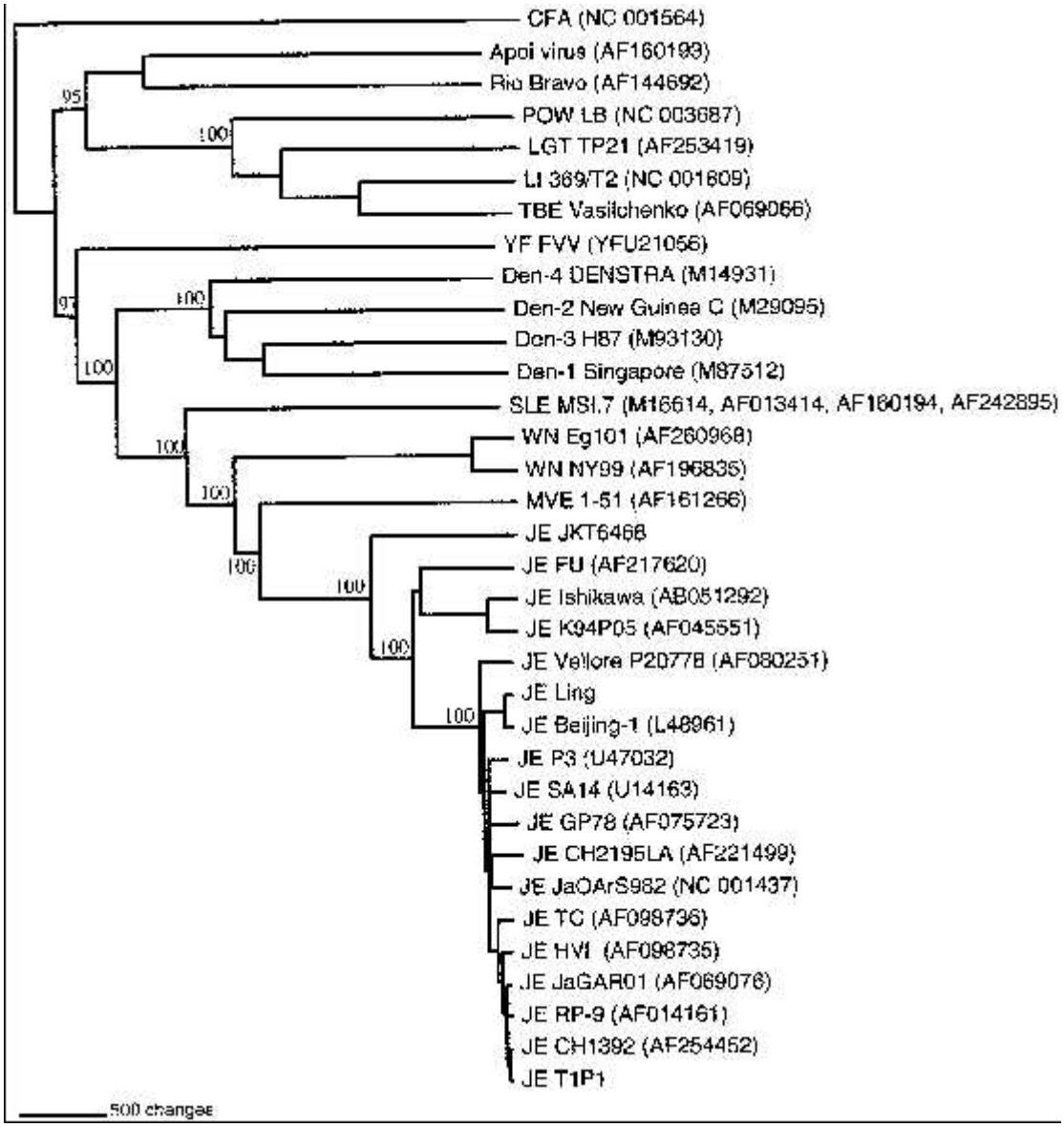


Figure 1: Phylogenetic tree showing the relationship between the flaviviruses. The complete nucleotide sequence of every strain of JEV for which the complete sequence is available, plus representative strains of other important flaviviruses, were aligned using the Vector NTI sequence analysis program, and %age differences calculated. The phylogenetic tree was then constructed using the maximum likelihood method (PAUP* version 4.04a; Sinauer Associates, Sutherlan, MA). The robustness of phylograms was evaluated by 1000 bootstrap replicates. Strain identities are given following the name of

the virus, and GenBank accession numbers, or the relevant publications, are shown in parentheses. The tree was outgrouped using cell fusing agent (CFA). Horizontal branch lengths are proportional to the number of changes. POW = Powassan virus; LGT = Langat virus; LI = louping ill virus; TBE = tick-borne encephalitis virus; YF = yellow fever virus; Den = dengue virus; SLE = St Louis encephalitis virus; WN = West Nile virus; MVE = Murray Valley encephalitis virus; JE = Japanese encephalitis virus. **Source:** Chiou and Chen, 2001; Jan *et al.,* 1996.

3.2.2 Morphology

JE virus, a member of *Flavivirus* genus is antigenically related to St. Louis encephalitis (SLE) virus, Rocio virus, Murray Valley encephalitis (MVE) virus, West Nile virus (WNV) and several other flaviviruses (Gubler *et al.,* 1989). Molecular virological studies suggest that all flaviviruses derived from a common ancestor some 10-20,000 years ago, and are rapidly evolving to fill ecological niches (Gould, 1997). Like other flaviviruses, JE virus consists of a single strand of positive-sense RNA (11kb) wrapped in a nucleocapsid and surrounded by a glycoprotein containing envelope (Solomon, 2003). The diameter of the envelope is about 50 nm.

The RNA comprises 3 regions: a short 5' untranslated region (UTR), a longer 3' UTR, and between them a single open reading frame (Chambers *et al.,* 1990). The open reading frame encodes for 3 structural proteins [capsid protein (C), pre-membrane protein (PrM) and envelope protein (E)] and 7 non-structural (NS) protein (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Thomas *et al.,* 1990). E protein, the longest structural protein (contains about 500 aminoacids) is the major target of the host antiviral humoral immune response (Heinz, 1986; Mason *et al.,* 1991) and is also thought to be the cell receptor binding protein, and a mediator of membrane fusion and cell entry (Monath *et al.,* 1996)

E protein constitutes the major immunogen and is also expressed on the plasma membrane of infected neurons (Russell *et al.,* 1980). This envelope protein is the major

antigen responsible for cross reactivity of virion with other closely related arboviruses (Edleman *et al.,* 1976).

3.3 Clinical Outcomes/Disease Spectrum

3.3.1 Pathogenesis

After transmission to man by an infected mosquito vector, JEV multiplies locally and in regional nodes (Ahmed, 1999). Before the invasion of central nervous system (CNS), a phase of transient viremia occurs. Some data obtained from the experiment in mice and macaque monkeys suggest that the site of peripheral amplification is dermal tissue and then lymphocytes (Solomon *et al.,* 2002). In animal models, JEV strains differ in both their neuroinvasiveness (following peripheral inoculation) and neurovirulence (following intracranial inoculation) (Solomon, 2003). The means by which JEV crosses the blood-brain barrier is unknown (Solomon *et al.,* 2002). JEV is thought to invade brain via vascular endothelial cells by endocytosis (Liou and Hsu, 1998).

Immunochemical staining of human post mortem material has shown diffused infection throughout the brain, indicating a haematogenous route of entry (Desai *et al.,* 1995; Johnson *et al.,* 1985). Experimental studies with related flavivirus (SLE virus) suggest the olfactory route as an important route of viral entry (Monath *et al.,* 1983). Replication within the endothelial cells followed by the passive transfer into brain seems a more likely mechanism for JEV entry (Dropulie and Masters, 1990; Liou and Hsu, 1998).

Other factors which compromise the integrity of the blood-brain barrier have also been considered as risk factor for neuroinvasion (Solomon *et al.,* 2002). It has also been suggested that head trauma (for example, due to road traffic accident) during the transient viremia could facilitate the viral entry into the CNS (Shiraki, 1970).

In the neurons, JEV replicates and matures in the neuronal secretory system, mainly the rough Endoplasmic Reticulum and Golgi apparatus, eventually destroying them (Hase *et al.,* 1990).

3.3.2 Immunological aspect:

Both humoral (particularly against E and NS1 proteins) and cellular (including cytotoxic T lymphocytes) arms of immune system are involved in immunity to JEV (Johnson *et al.,* 1985; Konishi *et al.,* 1995). The humoral immune response in JE has been well characterized (Solomon *et al.,* 2002). But the relative contribution of individual components has not been well understood (Tiroumourougane *et al.,* 2002).

3.3.2.1 Humoral immunity

After primary infection with JEV, a rapid and potent monotypic IgM response occurs in serum and cerebrospinal fluid (CSF), usually within 7 days of infection (Burke *et al.,* 1985). The role of antibodies in protection is not yet clearly understood. However, disappearance of neurological signs has been noted in the presence of IgM antibodies (Edleman *et al.,* 1976). Antibodies to JEV probably protect the host by restricting viral replication during the viremic phase, before the virus crosses the blood-brain barrier (Hammon and Sather, 1973).

Evidence form other flaviviruses suggests that antibodies to JEV may also limit the damage during established encephalitis by neutralizing extracellular virus and facilitating lysis of infected cells by antibody dependent cellular cytotoxicity (ADCC) (Carmenaga *et al.,* 1974). Attempts to isolate virus during raised antibody titers, are usually negative.

Within 30 days of infection the survivors exhibit IgG in the serum and CSF. Asymptomatic infection with JEV is also associated with raised IgM in serum, but not in CSF (Burke *et al.,* 1985). An anamnestic antibody response with early rise in IgG and

slow rise in IgM, has been noted in patients with secondary infection (previously infected with antigenically related flaviviruses).

3.3.2.2 Cell mediated immunity

The importance of cell mediated immunity was recognized of late. In animal models of Japanese encephalitis, the cellular immune response seems to contribute to the prevention of disease during acute infection by restricting virus replication before the CNS is invaded (Solomon *et al.,* 2002). Though cytotoxic T-lymphocyte response to flaviviral infection has been noted in men and mice, their role in JE is not very clear (Hill *et al.,* 1992; Kulkarni *et al.,* 1991). Immunization with inactivated JE vaccine induces T-cells activation in vivo (Aihara *et al.,* 2000). These studies reflect the protective role of cell mediated immunity (CMI) in JE.

3.3.2.3 Interferon

In addition to humoral and cellular immune responses, endogenous interferon α has been detected in the plasma and CSF of humans with JE (Burke and Morrill, 1987).

3.3.2.4 Apoptosis

Recent attention has focused on the role of apoptosis in the pathogenesis of arboviral encephalitis. Apoptosis has been shown in-vitro in a range of cell lines for different flaviviruses, including JEV (Jan *et al.,* 2000; Liao *et al.,* 1997; Parquet *et al.,* 2002).

3.3.3 Clinical features

Infection with JEV is often asymptomatic. The ratio of asymptomatic to symptomatic infection varies between 21:1 and 1000:1 (Halstead and Grosz, 1962; Thongcharoen, 1989). The mean is one symptomatic case for every 300 infections (Kalyanarooj, 1995). Both humoral and cellular immune responses attenuate the selective infection and destruction of neurons (Johnson, 1987). Grey matter is the principal target of JEV.

Patients with JE typically present after a few days of non-specific febrile illness, which may include coryza, diarrhoea, and rigors (Solomon, 1997). This is followed by headache, vomiting and a reduced level of consciousness, often heralded by a convulsion. Convulsions occur often in JE, and have been reported in upto 85 % of children (Kumar *et al.,* 1990) and 10 % of adults (Dickerson *et al.,* 1952; Poneprasert, 1989). In some patients, particularly older children and adults, abnormal behaviour may be the only presenting clinical feature (Solomon *et al.,* 1997).

The onset of illness can be abrupt, acute, sub-acute or gradual. The course of disease can be conveniently divided into the following three stages:

a. a prodromal stage preceding CNS feature,

b. an encephalitis stage identified by CNS symptomatology, and
a late stage noticeable by recovery or persistence of signs of CNS injury (Webb and Perriera, 1956).

The prodromal stage (2-3 days), is characterized by high grade fever with or without rigors, headache, general malaise, nausea and vomiting. Definitive clinical diagnosis is not possible in the prodromal stage (Tiroumourougane *et al.,* 2002). Encephalitis stage (3-5 days) manifests with altered sensorium, convulsions, neck stiffness, muscular rigidity, mask like facies, abnormal movements, dehydration, weight loss and thick and slow speech. Death usually occurs due to neurological illness in the first week.

Children, who survive, slowly regain the neurological function over several weeks. Mild cases may make a complete recovery (Bista and Banerjee, 2000). Only one third of the cases recover normally (so called abortive encephalitis). Residual neurological impairment includes thick, slow speech, aphasia and paresis. Intellectual involvement may be found in 30 % of cases, speech disturbance in 34 % and motor deficits in 49 % (Tiroumourougane *et al.,* 2002). Other sequelaeae in the patients recovering from JE may include:

- Behavior sequelae (aggressiveness 72 %, depression 38 %, attention deficits 55 %).
- Intellectual sequelae (abnormal intelligence 44-72 %, borderline intelligence 33 %, mild mental retardation 11 %, moderate mental retardation 11 %).
- Other neurological sequelae (epilepsy 16-20 %, memory deficit 46 %, cranial nerve paralysis 16 %, blindness 2 %).

JEV specific antibodies and JEV antigen can be detected in serum or CSF. Topographic distribution of tissue associated antigen in thalamus, hippocampus, substantia nigra and white matter of basal ganglia and medulla oblongata explains the evolution of post JE sequelaeae (Desain *et al.,* 1995).

After chemotaxis, JEV may be degraded by neutrophils with the help of respiratory brust and toxic radical generation. JEV induces human peripheral blood monocytes to secrete a chemotactic cytokine (human macrophage derived factor or hMDF) that causes chemotaxis of neutrophils (Singh *et al.,* 2000).

Hypoferraemia (decrease in iron concentration) is associated with the accumulation of iron in spleen, accompanied by transient anaemia (Mc Callum, 1991).

Recently, poliomyelitis like acute flaccid paralysis (AFP) has been identified in a sub-group of patients infected with JEV (Solomon *et al.,* 1998). A small proportion of children may present with features of aseptic meningitis with no other clinical features of encephalopathy (Solomon *et al.,* 1996). Seizure occurs in more than 75 % of pediatric patients but is less frequently observed in adults (Tsai and Yu, 1994). Viral load may play a vital role in innate immunity of host to restrict the initial JEV infection in CNS.

Limited data has also indicated that JE acquired during the first or second trimesters of pregnancy can cause intrauterine infection and miscarriage (Mathur *et al.*, 1985). Infection during third trimester has not been associated with adverse effect in newborns.

3.4 Vectors

Japanese encephalitis is a vector borne disease transmitted by mosquito, an arthropod. Thirty species of mosquitoes belong to five genera of *Culex, Anopheles, Aedes, Mansonia and Amergeres* harbor JEV (EDCD, 2005). They are mostly zoophilic or feed on animal blood. Entomological surveys carried out from 1981 through 1984 in Nepal indicated that the culicine mosquitoes namely, *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. Vishnui* and *Cx. fuscocephalus* as the vector suspected of transmittinig JEV to both animals and humans (Pradhan, 1981; Regmi and Joshi, 1985; Khatri *et al.*, 1983).

Eight species of the *Culex* mosquito live in rural rice growing and pig-farming regions, and breed in flooded rice fields, marshes, and standing water around planted fields (Rao, 2000). *Culex* mosquitoes predominantly feed on cattle (85-88%), pigs (4-5%) and human (2-6%) blood. *Culex* mosquitoes can fly upto 5 kilometers indicating the JE epidemics limited to particular areas.

The virus is transmitted in an enzootic cycle among mosquitoes and vertebrate amplifying hosts, chiefly domestic pigs and Ardeid (wading) birds (Burke and Leake, 1988). All elements of the transmission cycle are prevalent in rural areas of Asia, and human infections occur principally in this setting. Because, vertebrate amplifying hosts and agricultural activities may be situated within and at the periphery of cities, JE cases occasionally are reported form urban locations.

Some hyper endemic districts of Nepal represent the paddy field ecosystem with abundant *Culex* species and amplifying hosts like pigs and migratory birds. Because, the female *Cx. tritaeniorhynchus* is found abundant in rice field-ecosystem of the endemic

areas during the transmission season, and because JEV isolates have been obtained only from a pool of *Cx. tritaeniorhynchus* females; this species is suspected to be the principal vector of JE in Nepal (Gubler *et al.*, 1989; Darsie and Pradhan, 1989).

This was also supported by a survey carried out in six districts of Nepal, which revealed that 45 % of the total vector population was found to be *Cx. tritaeniorhynchus* (MoH, 2003). An unpublished report of environmental health project (EHP) stated that there is clear evidence of JE vectors (*Cx. tritaeniorhynchus* and *Cx. quinquefasciatus*) in the Kathmandu valley, located at 1300 m above sea level (MoH, 2003).

The mosquito borne mode of JE transmission was elucidated with the isolation of JEV in 1983 and subsequently in other field studies that also established the role of aquatic birds and pigs in the viral enzootic cycle (Tsai, 1994).

Cx. tritaeniorhynchus has been implicated as the most important vector in south India (NIV, 1980). Veneral transmission of JEV occurs in *Cx. bitaeniorhynchus* mosquitoes.

Mosquito become infective 14 days after the entry of JEV from the viremic host.

3.5 Reservoir Hosts

It is well established that swine and varieties of birds, both wild and domestic, are amplifying hosts of JE and serve as a source of infection for those mosquitoes that transmit JE to humans (Buescher *et al.*, 1959; Carey *et al.*, 1968; Dhanda *et al.*, 1977). The virus does not cause any disease among its natural hosts and the transmission continues unnoticed through mosquitoes (Rao, 2000).

Pigs are the most important reservoirs. Though they do not manifest the disease, they develop very high titers of virus in circulating blood and infect mosquitoes (Rao, 2000). Thus, pigs are the amplifying hosts in which JEV are proliferated. Asymptomatic

viremia remains for several days in pigs. But piglets (below 6 months age) show non suppurative encephalitis and nervous signs because maternally acquired antibodies do not remain for long period.

Culicine mosquitoes have been found to be breeding and growing in close association with pigs and fowl (Joshi, 1983). Serological studies carried out in Nepal showed the JEV infection in pigs and ducks (Joshi, 1984). In some areas, 100% of the pigs examined have tested positive for JE antibodies and remain as carrier of JEV. A serological survey conducted in Nepal demonstrated anti-JEV antibodies in the samples of 40% of pigs, 35% of pond herons and cattle egrets, and 7% of duck. Potential reservoir population in the endemic districts also reflects towards the possible endemic situation of JE in Nepal

Table 1: Potential Reservoir Population in Nepal, 2001

Particulars	Pig population	Duck population
Nationwide (75 districts)	912,350	411,410
In 24 endemic districts	358,850	331,646
Percentage in endemic districts	39 %	81 %

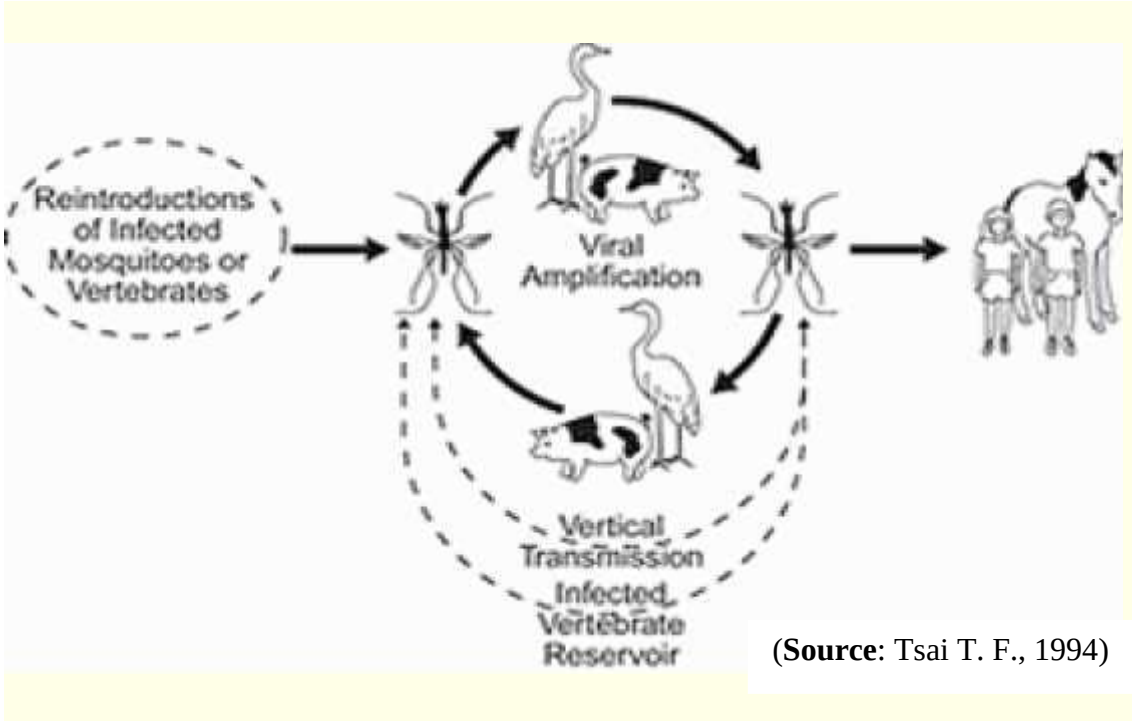
Source: EDCD, 2005

Although JEV has been recovered form human blood, the low level of viremia is not sufficient to infect mosquitoes. By the time patients exhibit the signs and symptoms of JE, virus disappears from the blood (Chan and Loh, 1966; Herman and Anandarajah, 1974).

Domestic animals like horses are also the victims of JEV infection and show the signs of encephalitis. Under experimental conditions, Gould *et.al.* (1964) demonstrated horse to horse transmission by *Cx. tritaeniorhynchus*. Cattle are frequently infected in enzootic areas (Sakai *et al.*, 1990) but do not develop sickness or viremia (Ilkal *et al.*,

1988). Bovines, ovines and caprines can be infected with JEV but do not serve as amplifying and reservoir host due to insufficient virus titers (Carey *et al.,* 1968; Hayashi *et al.,* 1970; Nandi *et al.,* 1982). Bats, pigeons and sparrows can develop viremia and can infect mosquitoes.

3.6 Transmission Cycle



(**Source**: Tsai T. F., 1994)

Figure 2: Generalized transmission cycle of Japanese encephalitis virus

The virus is maintained in nature in a transmission cycle which involves susceptible hosts and blood feeding mosquitoes. Mosquitoes transmit the virus to many species of birds and swine (Buescher and Scherer, 1959; Scherer *et al.,* 1959). When the mosquito bites and takes the blood meal, JEV are shed into its saliva and eventually injected into the victim's blood stream. The risk for developing JE after a mosquito bite can be factored into a series of probabilities. Only bites of vector mosquitoes pose a risk and fewer than 3% of vector mosquitoes are likely to be infected (CDC, 1993).

The maintenance and spared of JE virus appear to be mainly through a pig-mosquito-pig cycle (Gould *et al.,* 1974; Johnson *et al.,* 1974) and bird-mosquito-bird cycle (Joshi *et al.,* 1998). Some studies suggest that virus may be transmitted transovarially in vector mosquitoes (Soman *et al.,* 1985). Man is incidental and dead end host. The epidemiology of the arboviral encephalitides must account for the maintenance and dissemination of the viruses in nature in the absence of humans (Brooks *et al.,* 2004).

In tropical climates, where mosquito populations are present throughout the year, the cycle continues between mosquitoes and reservoir animals (Brooks *et al.,* 2004). The mechanism of maintaining the virus over the winter in temperate areas has not been elucidated. Overwintering in the mosquitoes is a possibility either in infected hibernating mosquitoes or by transovarial passage (Rosen *et al.,* 1980). Possible but unproved overwintering mechanisms include the following: (1) hibernating mosquitoes at the time of their emergence may re-infect birds; (2) the virus may remain latent in winter within birds, mammals, or arthropods; and (3) cold-blooded vertebrates (snakes, turtles, lizards, alligators, frogs) may act as winter reservoirs (Brooks *et al.,* 2004).

3.7 Diagnosis

3.7.1 Clinical diagnosis:

The clinical symptomatology of all viral encephalitides is similar and therefore clinical diagnosis at best can only be an educated guess and is made by the association of encephalitis and some symptoms and signs with possible viruses (Rao, 2000).

In JE, the leukocyte count is often raised. Differential counts reveal neutrophilia ranging between 51 % and 90 % whereas CSF examination shows a raised opening pressure, cell count of 10-980 X 10^6/litre, protein < 900 mg/liter and normal glucose level (Tiroumourougane *et al.,* 2002). The generalized changes in an electro encephalalogram (EEG) may help in differentiating JE from herpes encephalitis (Misra and Kalita, 1998).

3.7.2 Etiological diagnosis

Etiological diagnosis of JE is based on virus isolation or demonstration of virus specific antigen or antibodies in CSF/Serum.

3.7.2.1 Culture

Intra-cerebral inoculation in suckling mouse brain is a conventional method to isolate JEV. Various cell cultures such as primary chick, duck embryo cells, and cell-lines of Vero, LLCMK2, C6/36, and API cells are more often used to isolate JEV. Recently, sensitive mosquito inoculation techniques have been described for the isolation of JEV (Gajanana *et al.,* 1995).

However, isolation of virus from a clinical specimen is generally considered a rare occurrence (Shope and Sather, 1979) probably because of low viral titers, rapid production of neutralizing antibodies, frequent freezing/thawing of clinical materials, and the logistic difficulty in transport of specimens in the developing countries (Mohan Rao *et al.,* 1988; Leake *et al.,* 1996). Lack of skilled manpower and virus culture laboratories are also major hindrances to viral isolation in these countries. For these reasons, serological diagnosis is more emphasized in the developing countries.

3.7.2.2 Antigen detection

Various studies have proved the efficacy of JEV antigen detection in CSF using reverse passive haemagglutination (Ravi *et al.,* 1989), immunofluorescence (Raghava and Badrinath, 1998) and staphylococcal co-agglutination tests using polyclonal and monoclonal antibodies (Zhang *et al.,* 1989) in rapid diagnosis of JE.

3.7.2.3 Antibody detection

Laboratory diagnosis of human arboviral encephalitis by serology has changed greatly over the last few years. In the past, identification of antibody relied on four tests namely haemagglutination inhibition (HI) test, complement fixation (CF) test, plaque reduction neutralization test (PRNT) and indirect fluorescent antibody (IFA) test. However, these

tests are still in use in some laboratories. These assays are reliable when done and interpreted properly (Beaty *et al.,* 1989). Positive identification using these assays requires paired sera to demonstrate four fold rise in antibody titer between acute and convalescent serum samples (Bista and Banerjee, 2000). In recent decades, these conventional tests have been replaced by antibody capture enzyme linked immunosorbent assays (ELISAs) which are more sensitive and specific.

In 1980s, IgM and IgG ELISAs were developed which have become the accepted standard for diagnosis of JE (Solomon *et al.,* 1998; Burke *et al.,* 1982). After the first few days of illness, the presence of anti-JEV IgM in the CSF has a sensitivity and specificity of > 95% for CNS infection with the virus (before this, false negative may occur) (Burke *et al.,* 1985). Nearly in all patients, after 7 days of onset of symptoms, specific anti-JEV IgM can be detected in CSF or in serum or in both. IgM antibody capture ELISA (MAC-ELISA) is the method of choice to demonstrate virus specific antibody in both blood/serum and CSF. Detection of JEV specific IgM is one of the most reliable indicators of JEV infection (Bundo and Igarashi, 1985). In IgM detection technique, single sample of serum or CSF is adequate for diagnosis of JE and the single positive test is confirmatory. Moreover, presence of IgM in the CSF indicates local antibody formation associated with brain infection and is not seen in person with asymptomatic infection with JEV.

ELISA is a highly sensitive, specific, less time consuming and reproducible method for detection and quantification of many cytokines (Beech *et al.,* 1997; Jung *et al.,* 1998). The sensitivity of ELISA for JE was 89 %, specificity 91 % and accuracy 92 % with reproducible results and was also able to detect a minimum concentration of 23 ng human macrophage derived factor (hMDF) per ml in test samples (Singh *et al.,* 2000).

Avidin biotin system (ABC MAC-ELISA) (Chow *et al.,* 1992), biotin labeled immunosorbent assay to sandwich ELISA (Chang *et al.,* 1984), nitrocellulose membrane based IgM capture dot enzyme immunoassay (MAC DOT) (Solomon *et al.,*

1998), and antibody capture radioimmunoassay (ACRIA) (Burke *et al.,* 1982) are some of the newer modifications of MAC-ELISAs that have been used in antibody detection.

Evaluation of a new commercially available IgM/IgG antibody capture ELISA for diagnosis of JE showed sensitivity of 88 % with serum, 81 % with CSF; specificity of 97 % with sera from patients with primary and secondary dengue virus infections whereas specificity was 100 % when samples from non flavivirus infections were tested (Cuzzubbo *et al.,* 1999).

Yamamoto *et al.* (2000) described hydroxyapatite coated nylon beads (Ha-Ny beads) to be applicable for the development of a new JEV antibody-detection kit, which is simple, inexpensive and does not require specific laboratory facilities.

JEV specific IgM persists even till 116 to 350 days after acute illness (Edelman *et al.,* 1976), which suggests that IgM antibody persistence is related to acute virulence rather than chronicity of JEV infection.

Twenty four CSF samples with anti-JEV IgM negative status were tested for the presence of JEV specific IgG by ELISA, which revealed that almost all samples exhibited IgG1 suggesting its role in the clearance of virus from CNS (Thakare *et al.,* 1991).

3.7.2.4 Principle of MAC ELISA developed by AFRIMS

This MAC ELISA technique is designed to detect JEV specific IgM antibodies in the specimens (serum or CSF). IgM antibodies are captured by goat anti-human IgM previously coated on the solid phase (wells of ELISA plate). Then, the JEV antigen which can bind only with the specific anti-JEV IgM, is applied over the captured IgM. Non specific antibodies are removed during the washing step. If the JEV antigen binds to the JEV specific antibody, its presence in the specimen is detected with the addition of enzyme linked conjugate (horse redox peroxidase enzyme and human anti-flavivirus

IgG). This enzyme changes the substrate to coloured compound; the intensity of colour produced can be detected with the help of ELISA reader (AFRIMS, 2005).

3.7.2.5 Molecular virological diagnosis

The molecular fine mapping of important antigenic regions in JE over last few years has paved way for the future development in laboratory diagnosis (Tiroumourougane *et al.*, 2002). The reverse transcriptase polymerase chain reaction (RT-PCR) amplification of viral RNA may help in specific and rapid detection of JEV in various samples (Meiyu *et al.*, 1997; Paranjpe and Banerjee, 1998). However, its reliability as a routine diagnostic test has yet to be shown.

3.7.3 Nepal: National guidelines for JE diagnosis

Diagnosis of JE at national level is guided by the following case definitions (Based on Recommendations of the National Workshop on Vector-Borne Diseases, 1997).

- Patients having an elevated temperature (over 38^0C), altered consciousness, or unconsciousness are considered as "**possible meningitis/encephalitis**" cases and are referred for a lumber puncture.
- If the suspected case has between 50-1000 cells (predominantly lymphocytes) per mm^3, the case is diagnosed as having "**probable viral encephalitis.**"
- If a case of "probable viral encephalitis" as defined above presents a positive specific anti-JE IgM in the CSF or serum at the time of illness, the case is classified as a **confirmed case of JE**.

3.8 Prevention and Control

3.8.1 Control of mosquito vector

Risk of human JEV infection is closely tied to vector abundance, which in turn is associated with, agricultural practices. Vector density and viral infection rate in vectors

coincided with the mensal distribution of cases in hyper endemic areas (Gajanana *et al.*, 1996).

Surveillance of the adult mosquito population has to be carried out throughout the year. Novel water management and irrigation practices such as periodic lowering of water level, intermittent irrigation and constant flow systems prevent or inhibit the larval development of mosquitoes (Rao *et al.*, 1995). Growing of the water fern *Azolla microphyla* in rice field was evaluated as a biological agent against mosquitoes breeding in rice fields (Rajendran and Reuben, 1991).

The principal vector of JE, *Cx tritaeniorhynchus,* has been found susceptible to deltamethrin and lambdacyhalothrin, but more information is required on the insecticide resistance in the JE vector and behaviour towards different insecticides (EHP, 2004). This mosquito is also resistant to DDT in India. Although spraying of an appropriate insecticide in the resting places of mosquitoes is an easy method, environmental pollution due to insecticide and development of resistance by mosquitoes against an array of insecticides have discouraged the use of insecticides in vector control programs.

3.8.2 Prevention of mosquito bite

Use of nets (normal and insecticide impregnated) and mosquito repellents (coils, creams and mats) by the population at risk and avoidance of outdoor sleeping in the tropics during evening hours, staying in screened houses, and wearing long sleeved shirts and long trousers (Tsai, 1992) reduce the risk of exposure to vectors. Pyrethroid impregnated curtains were found to be effective and proved its efficiency in the control of JE vectors (Theodore, 1990). Gurung *et al.* (2003) reported that mosquito-net non-users are at 2.6 times greater risk of developing JE in comparison to net-users in Nepal.

3.8.3 Immunization of reservoirs

Building of piggeries away from human dwellings in the countries where pigs are reared near human settlement and making them mosquito proof would be desirable. Immunization of pigs may reduce viral transmission by limiting or preventing viremia in pigs. SA 14-14-2 virus grown in cells is used to immunize pigs.

3.8.4 JE awareness programs

To increase knowledge about JE, awareness programs can be useful in the developing countries like Nepal. These programs focus on the hygiene and sanitation practices of the people to reduce the mosquito breeding and also encourage the use of mosquito repellents and bed nets for personal protection. Use of broad casting media (radio, television etc.), news papers, hording boards and pamphlets can be implemented as the tools of public awareness programs.

3.8.5 Immunization against JE

JE is an immunization preventable disease (IPD). Vaccination of the population at risk is the method of choice for prevention of JE. The three JE vaccines (Theodore, 1990) in widespread production and in worldwide use for this purpose are: 1. inactivated mouse brain-derived JE vaccine; 2. inactivated primary hamster kidney (PHK) cell-derived JE Vaccine, and 3. live attenuated JE vaccine. Post vaccination neurological complications such as encephalitis and peripheral neuropathy have been reported in only 1-2.3 per million vaccinees (CDC, 1993; WHO, 1998).

3.8.5.1 Inactivated mouse brain-derived JE vaccine

The inactivated JE vaccines available for immunization purpose against JE are derived from infected mouse-brain, which was licensed in Japan in 1954 (CDC, 1993). This vaccine is manufactured and commercially available in India, Japan, Korea, Taiwan, Thailand, Vietnam & United states of America (USA).

3.8.5.2 Inactivated primary hamster kidney (PHK) cell derived vaccine:

In order to avoid brain antigens and allergic reactions associated with crude antigens and to ease production, tissue culture-derived vaccines were attempted in China. Although this enhanced inactivated vaccine was reported to be immunogenic, there were no reports on efficacy or persistence of immunity (Theodore, 1990).

3.8.5.3 Live attenuated vaccine:

Attenuated JE viral strains have been generated through a large number of passages in various cell culture systems. SA 14-14-2 vaccine is an example of live attenuated JE vaccine. Efficacy trials in children of 1-10 years have yielded high protective rates. Recent advances in molecular biology have emphasized to explore novel approaches for developing recombinant vaccines based on proteins, viruses and DNA (Kabilan, 2004).

In general vaccination is indicated in the following groups:

a. People living in endemic areas
b. Travelers spending 30 days or more in an endemic area.
c. Travelers spending less than 30 days during epidemics or if extensive outdoor activity in rural areas is expected.
d. Lab workers with potential risk of exposure to JEV; since twenty-two cases of laboratory-acquired JE have been reported (CDC, 1988)

3.8.6 JE vaccination in Nepal:

Mass vaccination campaigns in JE endemic areas are effective in controlling the disease in human. In 1983, 1152 subjects were immunized against JE using BIKEN killed lyophilized vaccine at British Military Hospital, Dharan.

In 1999, a live attenuated BHK vaccine trial was conducted in 3 districts (Bardiya, Banke and Kailali) with SA 14-14-2 single dose vaccine imported from South Korea. Out of 492,442 children between 1 and 15 years, only 224000 (45.5%) were vaccinated.

Vaccine coverage was 83.5 % in Bardiya followed by Banke (41.3 %) and Kailali (22 %) (EDCD, 2001).

During the year 2001 and 2002, two million doses of inactivated cell culture JE vaccines were donated by china. Although 4 doses were planned, only 3 doses were administered to the children of 6 months to 10 years age (EDCD, 2001).

Single dose of SA 14-14-2 was proved to be highly effective in context of Nepal too (Bista *et al.*, 2001; Ohrr *et al.*, 2005).

Disease morbidity was decreased in the vaccinated districts and was lower as compared to the non vaccinated districts. But there was again rise in morbidity due to discontinuation of the vaccination program (**Annex V**). Because the vaccines were given to the children, disease prevalence has been shifted to old age from under 15 years (Joshi *et al.*, 2005).

3.9 Disease Burden

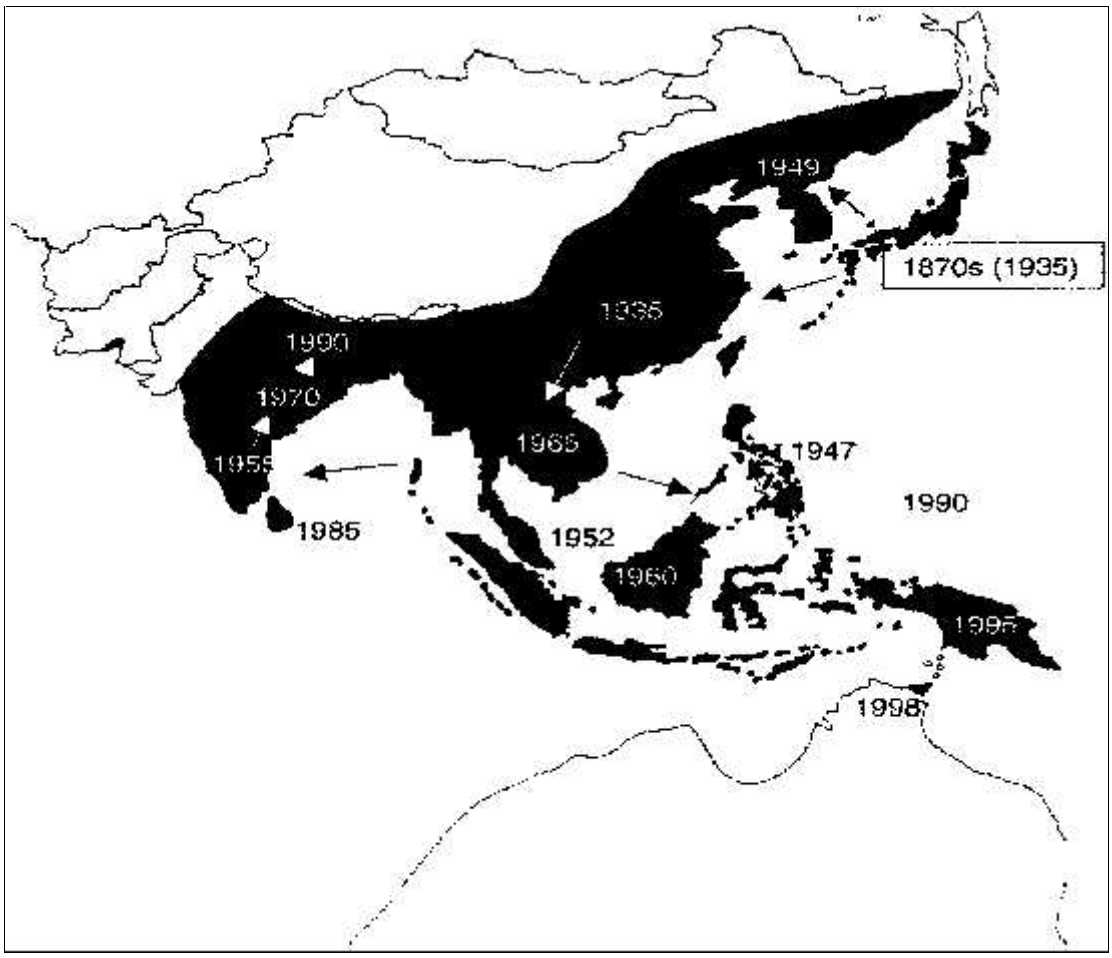
3.9.1 JE burden and research activities: The global scenario

The history of the disease goes back to 1871. Epidemics of encephalitis were described in Japan from the 1870s onwards. Major epidemics were reported about every 10 years with more than 6000 cases reported in the 1924 epidemic (Miyake, 1964). During 1970s and 1980s, JE was endemic to only a few countries of East Asia like Japan, Korea and China. Then, it spread from East Asia to South East Asia (SEA) and then to South Asia. The virus was isolated in Japan in 1935, and has been recognized across Asia since then (Solomon, 2003).

Epidemic and sporadic cases of JE occur in many Asian countries including Cambodia, China, Indonesia, India, Japan, Malaysia, Myanmar, Nepal, Pakistan, Philippines,

Korea, Sri Lanka, Thailand and Vietnam, and in the South Eastern Russian Federation (Tiroumourougane *et al.*, 2002).

Figure 3: Current distribution of Japanese encephalitis with the approximate dates of the first major outbreaks.



(**Source:** Solomon, 2003)

Although considered by many in the west to be a rare and exotic infection, Japanese encephalitis is numerically one of the most important causes of viral encephalitis worldwide, with an estimated 50,000 cases and 15,000 deaths annually (Tsai, 1997; Solomon, 1997). The outcome of JE ends with the death of 25% of patients and residual severe neuropsychiatric squelae in 30 to 60% of the survivors. Approximately, 60 % of

the world's population lives in the JE endemic regions (Kabilan, 2004). Therefore, it has been considered as a global public health problem.

In the past 50 years, the geographical area affected by JEV has expanded. The timing of the first reported cases or new epidemics in each area gives an impression of the relentless spread of JE (Solomon *et al.,* 2002). The disease has occurred on the western pacific islands with outbreaks in Guam in 1947 (Hammon *et al.,* 1958) and Saipan in 1990 (Paul *et al.,* 1993). In that year in Saipan, 10 cases occurred among a population of 40,000 and the prevalence of antibody to JEV among 234 lifelong Saipan residents surveyed after outbreak was 4.2% while the sero-prevalence in pigs was 96% (n=52).

Gradual spread of disease to other non Asian regions for example, Torres strait of Australian mainland has been reported recently (Hanna *et al.,* 1996). The first outbreak of JE (two clinical cases) in Australia was reported in 1995. These cases were identified on an island in the Torres Strait. No new case of JE was notified in Australia in 2002. An entomological investigation of an outbreak of JEV in the Torres Strait, Australia in 1998 recovered 43 isolates of JEV from adult mosquitoes (42 form *Cx. sitiens* and one from *Ochlerotatus vigilax*) and also identified 2 confirmed human JE cases in that area and Cape York Perinsula in Northern Queensland (Johansen *et al.,* 2001). Because of these outbreaks, mosquito borne arboviruses causing human diseases have been considered as important public health issues in Australia also.

From 1978-1993, 12 cases of JE occurred in the US. In the US, JE mostly occurs among military personnel, expatriates, and, rarely in returning travelers. There have been American and Australian military cases reported following the Korea and Vietnamese wars and postings to South East Asia (Burdon *et al.,* 1994).

Typical and atypical form of JE was reported in the citizens of Russia visiting JE endemic Asian countries (after 5 months, returning to Russia form China) (Pogodina *et al.,* 1996).

Two cases of JE in UK travelers have been documented. The first was a British woman who had been living and working in Hong Kong and was diagnosed with JE in 1982; she died as a result of cardiac and respiratory complication (Rose *et al.,* 1983). Another case was a woman who had been to Thailand; she recovered completely after 4 months.

Since, most of the JE cases (35-50,000/year) are reported form the Asian countries (specially, South East Asian countries) where more research activities are also focused. More about JE scenario is, hence, discussed in the following section under the heading: JE in Asia/South East Asia.

3.9.2 JE burden and research coverage in Asia/South East Asia

In northern temperate region of Asia, JEV causes larger summer epidemics, whereas in southern tropical regions, it causes endemic disease year round (Vaughn and Hoke, 1992). Cross sectional serological surveys have shown that in rural Asia, most of the populations are infected with JEV during childhood or early adulthood. About 10 % of the susceptible population is infected each year (CDC, 1993); however, most infections are asymptomatic.

In Japan, annually, 6000 JE cases with upto 60 % case fatality rate was reported during 1960s. In recent years Japan has decreased the JE cases to less than 100 patients per year after 1972. This may be due to development of inactivated JE vaccines and national immunization programs. An investigation conducted during 1967 to 1973 suggested that swine vaccination was not effective enough in Japan for the control of JE among humans because during this period, 10 human JE cases were reported. Out of 10 confirmed JE cases, 4 died in the Kurume region of Japan form 1984 to 1990 (Shoji *et al.,* 1994). Three JE cases in the non vaccinated US marines stationed on Okinawa were identified in 1991 (Saito *et al.,* 1999). During the outbreaks of 1991-1993, 4 JE patients were reported in Kyushu island of Japan and all of them recovered completely (Shoji *et al.,* 2002). Six patients unexpectedly presented with JE from early August to mid September 2002 in the Chungoku district of Japan which indicates that JE in Japan is

still a threat to adults and elderly with decreased or absent immunity to JEV (Ayukawa *et al.,* 2004).

The ratio of subclinical to clinical infection in vaccinated population was estimated to be 20,00,000:1, which was 2000-80,000 times higher than the ratio previously reported for unvaccinated population (Konishi and Suzuki, 2002).

Korea demonstrated more than 1000 JE cases/year before 1969 but after the vaccination started in 1960 onwards the number of cases decreased dramatically. Vaccine coverage reached almost 100 % in the 3-15 years age group in 1985. The widespread use of vaccine in children has been associated with a higher incidence of JE in those over 15 years (Vaughn and Hoke, 1992).

Although epidemics in northern Vietnam followed by 1965; currently, 1000-3000 cases/years are reported. In northern Vietnam, seasonal pattern of JE epidemic has been found as in other temperate areas whereas in southern Vietnam, sporadic cases have been reported throughout the year. During 1976-1991, Vietnam reported AES cases from all provinces with the highest number of cases (936) in 1980 and highest number of deaths (339) in 1977 (Ha *et al.,* 1995). A serological study carried out in Gia Luong district of Vietnam after vaccination during 1993-1994 showed 71.66% JE positive cases out of 85 clinical encephalitis cases (Nga *et al.,* 1995). None of the JE positive case was previously vaccinated which also supported the efficacy of vaccination in Vietnam. Virological and serological study conducted in Laos during 1993-1995 showed an increase in JE positivity with age. A case-control study on adult and pediatric AES patients admitted to Bach Man Hospital, Vietnam, detected 67% positive cases out of 46 pediatric AES cases as compared to only 6% JE positive cases out of 33 adult AES patients (Lowry *et al.,* 1998). A study conducted in 1998 in Vietnam detected 12 (55%) of the 22 children with acute flaccid paralysis (AFP) had evidence of acute JEV infection (Solomon *et al.,* 1998).

Hong Kong also reported 2000 JE cases in the year 2000.

In the history of China the first JE case was reported in 1935 with the first virus isolation in 1940. There are currently 10,000-20,000 cases/year, although in the early 1970s it was over 80,000 cases per year (Vaughn and Hoke, 1992). Some studies conducted in China suggested early diagnosis, treatment and universal JE vaccination for all susceptible populations as the key reasons for decreasing incidence of sequelaeae and death due to acute JE. JE vaccination is encouraged in China. Effectiveness of live attenuated JE vaccine (SA 14-14-2) for single dose was 80 % and that for two doses was 97.5 % (Hennessy *et al.,* 1996).

In Thailand, 1500-2500 cases are reported annually. An antibody prevalence survey conducted in Thailand during 1989 studied 3089 blood samples of children (aged 6 months to 14 years); out of which 27.45 % of children possessed neutralizing antibody to JEV (Rajanasuphot *et al.,* 1992). Strickman *et al.* (2000) studied distribution of dengue and JE among the school children of rural and suburban Thai villages where, out of the 1,477 children, 33/1000 had recent dengue and 7/1000 had recent JE infection. In a study on acute undifferentiated fever caused by infection with JEV, JE was reported in 22 (14%) individuals out of 156 adults; indicating JE as an underappreciated cause of acute undifferentiated fever in Asia (Watt and Jongsakul, 2003). This study was carried out in Chiangrai Regional Hospital, Thailand. Chokephaibulkit *et al.* (2001) conducted a perspective study of childhood encephalitis in Bangkok from 1996 to 1998. Among 26 (65%) children with identifiable viral agents, JE was reported in 6 children.

In Taiwan, the first clinical case of JE was recorded in 1931. The case incidence rate of JE during 1966-1997 showed a sharp decrease from 2.05/10,000 in 1967 to about 0.03/10,000 in 1997 reflecting the efficacy of JE vaccination started in 1968 onwards in Taiwan (Wu *et al.,* 1999).

After JE was first reported in 1951 in Malaysia, only occasionally epidemics have been documented (in 1974, 1988 and 1992) with more than three forth of the cases being

children. The total number of cases is seemed to be too low, although the actual number of cases could probably be more (Haw, 1995). Out of 195 children with CNS symptoms admitted to pediatric ward of Penang hospital, Malaysia, 38.5 % demonstrated the anti JEV IgM in their CSF (Cardosa *et al.*, 1995).

Some countries in the South East Asia, including India, Nepal demonstrated a remarkable increase in the number of JE cases since 1970s. In 1948, Sri Lanka became the first country to report JE cases in South East Asia (SEA) region (Tsai, 1994).

India is a JE endemic region that borders Nepal. Four Districts of Uttar Pradesh (along Indo-Nepal border) have reported JE cases with frequent outbreaks (EHP, 2004). The pattern of JE epidemics reported in India correlates well with Nepal (Kubo *et al.*, 1996). Although the first epidemic of JE in India was recognized around Vellore in 1948 (Sehgal SJ, 1989), the recognition of JE based on serological surveys, was first made in 1955 in Tamilnadu. Since then, many major outbreaks form different parts of the country have been reported, predominantly in rural areas. Subsequent surveys carried out by the National Institute of Virology (NIV), Pune indicated that about half of the population in South India had neutralizing antibodies to this virus (Park, 2002).

Twenty four states of India have reported JE including some states bordering to southern Nepal. In India, children are mostly affected with the morbidity rate of 0.3 to 1.5/100,000 population and case fatality rate of 10 % to 60 % (Reuben and Gajanana, 1997). National data of 1996 to 2000 shows an average of over 2500 JE cases and about 550 deaths per year in India (Park, 2002).

First outbreak of JE in Haryana state was recorded in 1992 with 294 cases and 205 deaths (Sharma *et al.*, 1992). A prospective serological community based study of subclinical flavivirus infection in children during 1989-1991, showed an overall incidence of 15 JE cases per 10,000 children for age group 5-9 years (Gajanana *et al.*, 1995). Gajanana *et al.* (1996) conducted a study during the transmission seasons of

1993-1995 and found 62.4 % JE cases out of 85 acute encephalitis or other related CNS disorders.

A serological study carried out during the period of June to December 1997 in Maharastra identified 5 JE cases among 52 suspected cases of viral encephalitis admitted at the Singli Government Hospital (Thakare *et al.,* 1999). Rao *et al.* (2000) studied the epidemic of JE in Andra Pradesh during October to November 1999, and recorded 873 cases and 178 deaths.

Among suspected cases of JE recorded in the hospitals of Arunachal Pradesh form 1986 to 1995, 162 cases were diagnosed as JE with predominance in lower age group and male sex (Chattopadhay, 2001). Dash *et al.* (2001) reported 40 % JE cases and 17 % acute Dengue cases in 1993 in Orissa.

CSF and serum samples of 348 viral encephalitis patients admitted to different hospitals of Assam during the period of June to August from 2000 to 2002 were tested, and out of which 53.7 % were found to be JE positive (Phukan *et al*., 2004).

As of 18 September 2005, 3551 cases of JE had been reported form 30 districts of Uttar Pradesh (India), with 764 deaths (CFR = 22%); moreover 56 cases and 15 deaths were reported alone on 18th September (WHO, 2005). The adjoining state of Bihar had reported 238 cases and 58 deaths (CFR = 24 %) during the same period with 13 cases and 4 deaths reported on 18 September (WHO, 2005).

3.9.3 Tsunami and JE

After the Tsunami, implication for JE in combination with monsoon rains is much more speculative than those for malaria and dengue. In receptive disaster zones, pigs are likely to have been wiped out. As the monsoon rains in the south east, Sri Lanka gradually changes the flooded areas form brackish to fresh water, culicine mosquito

populations may build up which, in the absence of domestic animals, will revert to biting humans (WHO, 2005).

JE may contribute to a public health disaster in the tsunami stricken areas. It is recommended that the health sector should be prepared to deal with JE cases once large stretches of flooded areas have changed from brackish to fresh water and build up of the indicated culicine mosquito population is observed (WHO, 2005).

3.9.4 JE among travelers

Although JE is a substantial public health problem in Asian countries, transmission to short-term travelers to JE endemic countries has rarely been reported (CDC, 1993; Geraghty and McCarthy, 2004). Monthly incidence of JE in travelers is less than one per one million among short term and urban travelers but 0.25 to 1 per 5000 among rural travelers to endemic regions (Halstead and Grosz, 1962). Since 1981, only 5 cases of JE among Americans traveling or working in Asia are known to have occurred.

Although the overall risk for infection among travelers is low, risk varies substantially by season (e.g. risk is highest in the rainy season), geographic location, duration of travel, outbreak presence and activities of the travelers (CDC, 1993; Shlim and Solomon, 2002).

In general, vaccine should be offered to persons spending 1 month or more in JE endemics areas during the transmission season, especially if travel will include rural areas. Under specific circumstances, vaccine should be considered for persons spending less than 1 month in JE endemic areas (e.g. travelers to areas experiencing epidemic transmission and persons' extensive outdoor activities in rural areas).

3.9.5 JE situation in Nepal

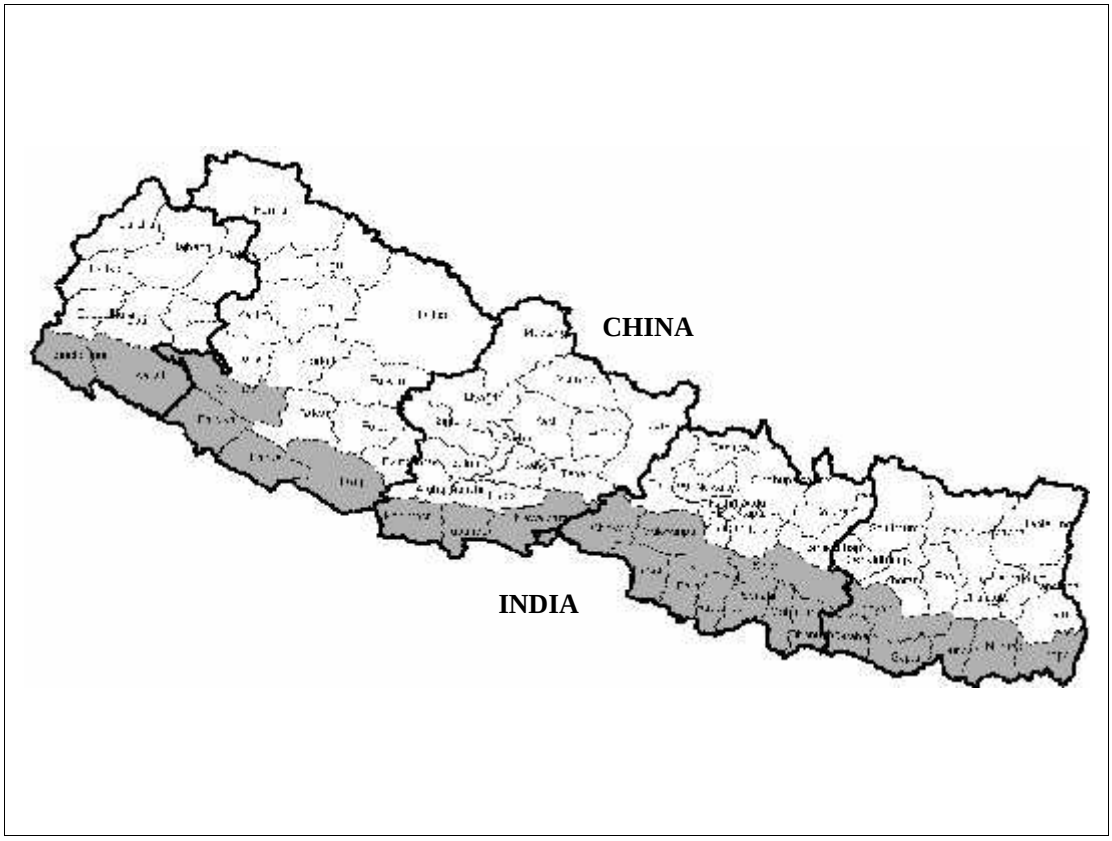
In Nepal, JE has been recognized as a significant public health problem because of its severity and the increasing incidence rates. Clinical cases of JE were reported in Nepal even before 1975; however, the first epidemic was identified in Rupandehi district of Western Development Region (WDR) from adjoining Uttar Pradesh state of India during 1978 (Joshi, 1986; Bista and Shrestha, 2005). Subsequently, JE epidemics occurred in Morang district of Eastern Nepal from adjoining Bihar state of India and thus, the disease is gradually spread into other districts in the successive years.

The mosquito, *Cx. tritaeniorhynchus* is considered as the principal vector of JE in Nepal. Three different strains of JEV isolated from Nepal are: Nep-1/90, B-2524 and B-9548.

The number of JE cases and deaths that occurred due to JE in Nepal during the thirteen-year period (1978 to 1990) correlated well with the findings of India (Kubo *et al.,* 1996). This was also proved by Kubo's antigenic study and is attributable to the free and frequent travel of people of both countries through open border.

In Nepal, JE is endemic in 24 districts (20 terai and 4 inner terai districts) starting from Jhapa in the east to Kanchanpur in the far west; however, sporadic cases from other districts have also been reported in recent years (EDCD, 2005). The plain areas (<1000m) were seen to be endemic, while the hills (1000-3000m) and mountains (>3000m) seen to be affected sporadically in Nepal (Kubo *et al,* 1996).

Figure 4: Japanese encephalitis endemic districts (n = 24) of Nepal



Source: EDCD, 2005

Environmental conditions of paddy field ecosystem in the terai region are the most favorable for the breeding of *Culex* mosquitoes. There is considerable seasonal variation in the number of JE cases each year, though the disease has been recorded throughout the year from endemic areas. Epidemic/outbreak generally starts in the month of April/may, reaches its peak during August and September, declines in October and levels off in November (Joshi *et al.*, 2005; EDCD, 2005).

Table 2: JE cases and deaths in Nepal: 1978 to 2004

Year	No. of cases	Deaths	CFR %
1978	422	119	28.2
1979	182	49	26.9
1980	622	231	**37.1**
1981	54	16	29.6
1982	843	390	**46.3**
1983	242	36	14.9
1984	142	45	**31.7**
1985	629	183	29.1
1986	1615	**415**	25.7
1987	502	140	27.9
1988	1403	380	27.1
1989	868	227	26.2
1990	365	102	27.9
1991	650	145	22.3
1992	702	127	18.1
1993	446	108	24.2
1994	1836	383	20.9
1995	1246	257	20.6
1996	1450	263	18.1
1997	**2953**	**407**	13.8
1998	1161	149	12.8
1999	**2924**	**434**	14.8
2000	1729	169	9.8
2001	1908	277	14.5
2002	842	168	20.0
2003	931	161	17.3
2004	1538	131	8.5
Total	**28205**	**5512**	**19.5**

Source: EDCD, 2005; Joshi *et al.*, 2005

Since 1978, several epidemics have been occurred and each successive epidemic has been found to be larger than the previous one. A total of 28,205 cases and 5512 deaths with the average CFR rate of 19.5 % has observed in Nepal from 1978 through 2004 (EDCD, 2005; Joshi *et al.*, 2005). The highest CFR (46.3 %) was observed in the year 1982 and the lowest (8.5 %) in 2004. The largest epidemic in the history of Nepal was reported in 1997 with 2953 cases and 407 deaths (CFR = 13.8 %) whereas the known

smallest epidemic was reported in 1981 with 54 cases and 16 deaths. The highest no. of casualty was reporded in 1999 with 424 deaths out of 2942 cases and the least no. of casualty was reported again in the year 1981 with only 16 deaths. A comprehensive result of 1978 to 2003 showed that more than 50 % morbidity and 60 % mortality have occurred in the age group below 15 years (Bista and Shrestha, 2005).

The major epidemics in Nepal were identified in the years; 1986, 1988, 1994, 1995, 1996, 1997, 1999, 2000, 2001, and 2004 with 1615, 1403, 1836, 1246, 1450, 2953, 2924, 1729, 1908 and1538 cases respectively (EDCD, 2005; Joshi *et al.,* 2005).

The annual case incidence (CI) rate has been found to be increasing (leaving some exceptions) in Nepal over last few decades. CI rate (per 1,00,000) ranged from 0.2 in 1981 to 25 in 1997 (Joshi *et al.,* 2005). Data analysis report of 1993 to 1997 showed the increasing pattern of CI rate in each successive year except in the year 1995 (Bista *et al.,* 1999). During this period, the CI rate reached its peak (25.3) in 1997 with high CI in Mid-western and Far-western regions. The cumulative result of this 5 years period indicated below 15 years children as the most vulnerable group with 4802 cases (61 % of the total cases within this period) and male sex (57 %) dominated the female (42 %) cases (Bista *et al.,* 1999).

From 1998 through 2004, there was an increase in CI from 1998 (13) to 1999 (31.8) but 1999 onwards, the CI rate followed the clear-cut decreasing pattern except in the years 2001 and 2004 (Joshi *et al.,* 2005). Also during this period, Mid-western and Far-western regions represented the most affected regions.

Although four laboratories namely NPHL (Teku), VBDRTC (Hetauda), BPKIHS (Dharan) and JE laboratory (Nepalgunj) were established for confirmatory diagnosis of JE using MAC ELISA technique, only two laboratories (NPHL and BPKIHS) are being conducted till date. For external quality assurance of these laboratories, Armed Force

Research Institute of Medical Sciences (AFRIMS), Department of Virology, Bangkok, Thailand has been participated, specially for JE.

Of the 204 samples tested in 1999, 137 (67 %) were found to be positive for JE whereas 47 samples would not be confirmed due to unavailability of the second samples (Bista and Banerjee, 2000). In the same year, some samples which were positive for JEV but showed low conversion titres, were tested against other flaviviruses at AFRIMS, Thailand and some samples showed extremely high titres against West Nile Virus (WNV) (Bista and Banerjee, 2000). In 1998 and 2000, 70 % and 62 % of cases were conformed as JE positive (Joshi *et al.*, 2005). In 2001, 43 % (374/880) cases were found to be JE positive whereas the year 2002 detected 32.7 % (290/888) JE positive cases but in 2003, quite high percentage (89.9 %) of JE cases were confirmed among 277 tested cases (Bista and Shrestha, 2005).

An unofficial data obtained from NPHL demonstrated 389 (35.3 %) JE positive cases among 1101 specimens tested in 2004 (Personal communication).

CHAPTER IV

4. MATERIALS AND METHODS

4.1 Materials

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

4.2 Methods

4.2.1 Study design

This study was designed as a cross-sectional descriptive epidemiological study.

4.2.2 Study period

The present study was carried out for a year period from January through December 2005.

4.2.3 Study site

This study consisted of two components; in the first stage, specimens were collected, stored and transported to National Public Health Laboratory (NPHL), Kathmandu and B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan for testing from all over the country. This component of this study was completed with the technical support of World Health Organization-Program for Immunization Preventable Diseases (WHO-IPD), Nepal under its JE surveillance activities. The second component of the study was laboratory testing and analysis which was conducted at NPHL and BPKIHS. NPHL is also the referral Laboratory for JE and other viral diseases such as Measles and Rubella investigation in Nepal. Similarly, BPKIHS (one of the largest health institution in the

eastern part of Nepal) was also established as one of the JE diagnostic laboratories in Nepal. For this reason, NPHL and BPKIHS were chosen as the sites for laboratory diagnosis of JE.

4.2.4 Sample size

The no. of reported AES cases from Nepal in the year 2005 was considered as the sample size of the study and in context of laboratory based analysis, the no. of specimens from AES cases received for testing at NPHL and BPKIHS were considered as the sample size.

4.2.5 Data collection

Primary data collection was made by interview through standard questionnaire **(Annex VIII)** at the concerned health institution where AES patients were admitted or visited. The collected data were obtained through WHO-IPD labline list at NPHL (**Annex IX**). Laboratory data were obtained during specimen testing at NPHL and BPKIHS. Population data were obtained from Health Management Information System (HMIS) and Epidemiology and Disease Control Division (EDCD), Department of Health Services (DoHS), Ministry of Health and Population (MoHP).

4.2.6 Specimen collection, storage and transport

4.2.6.1 Serum

Following aseptic precautions, 5 ml (3 ml from children) of venous blood was collected by vein puncture from each AES cases during acute phase of illness and was put in a labelled, clean and dry test tube. The blood in the test tube was allowed to clot for 30 minutes at room temperature then at $4^{o}C$ to retract the clot. The test tube was centrifuged and serum was separated. One ml of clear serum was transferred into a provided screw capped (labelled) serum vial and transported to IPD/Surveillance Medical Offices (SMOs) where standard case identification (epid. no.) was given to each AES case and serum vials were placed accordingly in the suitable cryo-boxes. Until transported to NPHL/BPKIHS, specimens were stored at $2\text{-}8^{o}C$. The specimens

were transported to NPHL/BPKIHS in an ice box following standard reverse cold chain protocol. The received specimens and their corresponding forms were checked thoroughly, data entered into the computer and stored at -20^{o}C until tested. Few specimens were also received directly at NPHL from different hospitals situated at Kathmandu valley. Same protocol was followed for those specimens too.

4.2.6.2 CSF

CSF specimens were collected by the attending medical doctors of the concerned health institutions. CSF was transferred in a provided screw capped (labelled) vial and transported to NPHL/BPKIHS as for serum specimens mentioned above.

4.2.6.3 Specimen rejection criteria

Few of the specimens were rejected when they were found with:

- Insufficient specimen for testing
- Whole blood (except those blood specimens which were received/collected at NPHL/BPKIHS)
- Sample vials without lid/labelling

4.2.7 Specimen processing (Laboratory diagnosis of JE)

Out of 2256 specimen vials received, only 2239 specimens were found to be acceptable for testing. Seventeen vials were rejected because of insufficient quantity. All the acceptable specimens were tested for the presence of anti-JEV IgM following the MAC ELISA technique developed by Armed Force Research Institute of Medical Sciences (AFRIMS), Department of Virology, Bangkok, Thailand.

The advantages of the MAC ELISA technique over other serological techniques is the requirement of only single properly timed serum sample, instead of paired sera. During the testing procedure, the protocol provided by the mentioned institute (**Annex II**) was strictly followed to achieve high level of accuracy.

4.2.7.1 Protocol of the test

According to the method of preparation mentioned in the provided protocol, all the required reagents and chemicals were prepared in the laboratory prior to sample processing. The reagents and chemicals were stored at the corresponding temperatures as instructed in the protocol. Storage temperature for each chemical and reagents are made available in **(Annex II)**. Methods of preparation and the composition of the required reagents and chemicals are available in the Standard Operating Procedure (SOP) **(Annex II)**.

All the serum and CSF specimens received at NPHL and BPKIHS through surveillance system were processed and tested using standard methodology. The MAC ELISA technique developed by AFRIMS, which is a three days-procedure was implemented to test the specimens. The detail protocol of the test is made available in **Annex III**.

4.2.7.2 Calculations

On the basis of the average optical density (OD_{av}) of the duplicate wells, EIA units of samples were calculated to interpret the result as JE positive (P) or negative (N).

Binding Index (BI) was calculated by using the following formula:

Binding index (BI) = **[**OD_{av} (test sample) - OD_{av} (NC) **] / [**OD_{av} (WPC) - OD_{av} (NC) **]**
EIA units (U) = BI × 100

According the protocol, the weak positive control was defined as 100 units. All the calculations were done using simple software in the computer (**Annex X**)

4.2.7.3 Interpretation of the result

According to the provided protocol, value of **≥ 40** units are considered as positive; however the results should be correlated with the clinical findings. In case of CSF specimen, **≥ 40** units is confirmatory of JEV infection whereas **≥ 40** units in context of serum specimen is considered as confirmatory of JEV infection only for surveillance purpose.

4.2.8 Data analysis

Data analysis was made with a view to determine the national scenario of the AES/JE case incidence (CI), case fatality rate (CFR), and the anti-JE IgM positivity among AES cases in Nepal. CI and CFR were calculated using standard formulas (**Annex VII**). Data were statistically analysed by using **Epi Enfo 6** (Version 6.04b - January 1997).

CHAPTER-V

5. RESULTS

This study was conducted at NPHL and BPKIHS to know the sero-epidemiological pattern of Japanese encephalitis in Nepal.

5.1 Surveillance Data Based Analysis

During the year 2005, a total of 2952 AES cases were reported from different hospitals/health institutions situated at different parts of the country (**Annex IV**). However, only 2239 specimens could be collected, tested and evaluated.

Of the 2952 AES cases, 1647 were male which accounted 55.8 % of total reported cases and the rest 1305 (44.2 %) were female.

Table 3: Sexwise distribution of AES cases in Nepal, 2005

Sex	No. of AES cases	% of total AES cases
Male	1647	55.8
Female	1305	44.2
Total	**2952**	**100**

To know the distribution pattern of AES cases according to the epidemiological week, the whole year was divided into 52 epidemiological weeks and designated as

epidemiological week no. 1 for the first week of January and epidemiological week no. 52 for the last week of December.

All 52 weeks of the year 2005 recovered AES cases in the range of 1 to 498 cases per week. The highest no. of AES cases 498, 432, 372, 295, 237, 191, 139 and 92 were reported in the epidemiological week no. 34, 35, 33, 36, 37, 32, 38 and 31 which constitute 16.9 %, 14.6 %, 12.6 %, 10 %, 8 %, 6.5 %, 4.7 % and 3.1 % of the total AES cases. Although, all 52 weeks recovered AES cases in the range of 1 to 498 per week, epidemiological week no. 31 to 38 reported majority of cases in the range of 92 to 498 per week. This pattern clearly revealed that the majority of AES cases i.e. 2256 (76.4% of the total AES cases) were identified in the period of 8 weeks. AES cases were reported in an increasing pattern to reach its peak in the week no. 34 and again after the week no. 35, decreasing pattern of AES cases was observed.

Table 4: Distribution of AES cases in Nepal by epidemiological week, 2005

Epid. week no.	No. of AES cases	% of total AES cases
1	2	0.1
2	6	0.2
3	4	0.1
4	9	0.3
5	7	0.2
6	1	0.0
7	3	0.1
8	3	0.1
9	3	0.1
10	6	0.2
11	6	0.2
12	8	0.3
13	3	0.1
14	8	0.3
15	9	0.3
16	6	0.2
17	6	0.2
18	9	0.3
19	9	0.3
20	10	0.3
21	11	0.4
22	16	0.5
23	12	0.4
24	30	1.0
25	25	0.8
26	21	0.7
27	36	1.2
Epid. week no.	**No. of AES cases**	**% of total AES cases**
28	37	1.3
29	41	1.4
30	43	1.5
31	92	**3.1**
32	191	**6.5**
33	372	**12.6**
34	498	**16.9**

35	432	**14.6**
36	295	**10.0**
37	237	**8.0**
38	139	**4.7**
39	56	1.9
40	46	1.6
41	29	1.0
42	24	0.8
43	16	0.5
44	31	1.1
45	18	0.6
46	16	0.5
47	11	0.4
48	11	0.4
49	15	0.5
50	16	0.5
51	10	0.3
52	7	0.2
Total	**2952**	**100.0**

From Kailali district alone, 435 (14.7 %) AES cases were reported followed by Dang (303, 10.3 %), Bardiya (274, 9.3 %), Kathmandu (259, 8.8 %), Banke (219, 7.4 %), Kachanpur (149, 5 %), Kapilvastu (111, 3.8 %), Nawalparasi (112, 3.8 %) and Sunsari (108, 3.7 %). More than 66 % (1535 cases) of the total AES cases were reported from 9 districts which can be considered as endemic districts. Twenty districts reported 2-10 AES cases each. Eight districts namely; Panchthar, Bhojpur, Tehrathum, Okhaldhunga, Rasuwa, Rolpa, Kalikot and Doti each reported only single AES case.

Table 5: Districtwise distribution of AES cases in Nepal, 2005

S. N.	District	No. of AES cases	% of total AES cases
1	Panchthar	1	0.0
2	Ilam	12	0.4
3	Jhapa	85	2.9
4	Sankhubasabha	3	0.1
5	Bhojpur	1	0.0
6	Dhankuta	4	0.1
7	Tehrathum	1	0.0
8	Morang	94	3.2
9	Sunsari	108	3.7
10	Saptari	28	0.9
11	Siraha	34	1.2
12	Udayapur	27	0.9
13	Okhaldhunga	1	0.0
14	Sindhuli	13	0.4
15	Sarlahi	26	0.9
16	Dhanusa	43	1.5
17	Mahottari	17	0.6
18	Ramechhap	2	0.1
19	Dolakha	9	0.3
20	Rautahat	44	1.5
21	Bara	53	1.8
22	Parsa	29	1.0
23	Chitawan	48	1.6
24	Makawanpur	16	0.5
25	Kathmandu	259	**8.8**
26	Lalitpur	56	1.9
27	Bhaktapur	28	0.9
S. N.	**District**	**No. of AES cases**	**% of total AES cases**
28	Kavrepalanchowk	30	1.0
29	Sindhupalchowk	5	0.2
30	Dhading	15	0.5

31	Nuwakot	6	0.2
32	Rasuwa	1	0.0
33	Gorkha	3	0.1
34	Kaski	7	0.2
35	Lamjung	4	0.1
36	Tanahun	10	0.3
37	Syangja	8	0.3
38	Kapilvastu	111	3.8
39	Rupandehi	112	3.8
40	Nawalparasi	64	2.2
41	Palpa	9	0.3
42	Gulmi	3	0.1
43	Arghakhanchi	13	0.4
44	Parbat	3	0.1
45	Baglung	2	0.1
46	Rukum	2	0.1
47	Rolpa	1	0.0
48	Salyan	8	0.3
49	Pyuthan	3	0.1
50	Dang	303	**10.3**
51	Banke	219	**7.4**
52	Bardiya	274	**9.3**
53	Surkhet	28	0.9
54	Kalikot	1	0.0
55	Doti	1	0.0
56	Kailali	435	**14.7**
57	Bajhang	2	0.1
58	Kanchanpur	149	5.0
	Unknown (NPHL)	78	2.6
	Total	**2952**	**100.0**

The highest no. of AES cases (839 cases) were recorded from Mid Western Development Region (MWDR) which constitutes 28.4 % of the total cases. Central Development Region (CDR) ranked second position with 700 AES cases (23.7 %) followed by Far Western Development Region (FWDR) with 587 cases (19.9 %) whereas the least no. of cases (349, 11.8 %) was identified in Western Development Region (WDR). Origins of 2.6 % cases were unknown.

Table 6: Regional distribution of AES cases in Nepal, 2005

Region	AES Cases	% of total AES cases
FWDR	587	19.9
MWDR	839	**28.4**
WDR	349	11.8
CDR	700	23.7
EDR	399	13.5
Unknown	78	2.6
Total	**2952**	**100.0**

Geographical distribution of AES cases showed terai region (20 districts) as the most endemic region with reported 2232 cases which account for 75.6 % of the total AES cases. This is followed by hill region that reported 558 (18.9 %) cases. Inner terai, which comprises only 4 districts, reported 84 (2.8 %) cases.

Table 7: Geographical distribution of AES cases in Nepal, 2005

	No. of AES cases	% of total AES cases
Terai	2232	**75.6**

Inner terai	84	2.8
Hill	558	18.9
Unknown	78	2.6
Total	**2952**	**100.0**

5.2 Laboratory Based Analysis

The specimens were collected and transported properly to NPHL and BPKIHS through WHO-IPD from all over the country as described in the methodology part.

Of the total 2256 specimens collected from the AES patients; only 2239 specimens were found to be sufficient for testing when received at the laboratories. Out of the total specimens received, 254 were tested at BPKIHS (also retested at NPHL) whereas rest 1985 were tested at NPHL. Quantity was not sufficient (QNS) in rest 17 vials (Table 8). Of the total 1935 serum specimens received for testing, 1750 (90.4%) were in good condition and 185 (9.6%) serum samples were haemolysed (Table 8).

Table 8: Quality and quantity of samples

Tested samples	Serum samples	Good		Haemolysed		QNS	
		No.	%	No.	%	No.	%
2239	**1935**	**1750**	**90.4**	**185**	**9.6**	**17**	**.008**

A total of 1935 (86.4%) of serum specimens (paired and single) and 304 (13.6%) cerebrospinal fluid (CSF) specimens were studied in the year 2005. Out of the 1935 serum specimens, 1826 (94.4%) were first specimens (acute sera) whereas 109 (5. 6%) were obtained as second specimens (convalescent sera). Only one out of 304 CSF specimens was received as second specimen (Table 9).

Table 9: Nature of samples according to collection time

	First	%	Second	%	Total	%
Serum	1826	94.4	109	5. 6	1935	86.4
CSF	303	99.7	1	0. 3	304	13.6
Total	**2129**		**110**		**2239**	**100**

During the study period of one year, a total of 11 samples (3 sera and 8 CSF) were collected from the Indian citizens during their visit to Nepal.

Genderwise distribution of JE suspected cases (Table 10) shows that out of 2239 cases investigated, 1269 (56.7%) cases were male and 970 (43.3%) were female. Number of male cases is slightly higher than female (Table 10).The ratio of male cases to female was found to be 1.3:1.

Table 10: Sexwise distribution of tested cases in Nepal, 2005

Sex	No. of cases	%
Male	1269	**56.7**
Female	970	43.3
Total	**2239**	**100**

During the present study period, AES patients between the age range of 10 days to 90 years were investigated. The highest no. of cases i.e. 828 (37%) belonged to the age group 5-15 years followed by 776 (34.7%) cases from the age group 15-50 years whereas the least no of cases were from the age group above 50 years. In all the age groups, the no. of male patients was predominant (Table 11).

Table 11: Age and sexwise distribution of cases in Nepal, 2005

	Below 5 Years		5-15 Years		15-50 Years		Above 50 Years	
Sex	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Male	238	10.6	472	21.1	434	19.4	125	5.6
Female	198	8.8	356	15.9	342	15.3	74	3.3
Total	**436**	**19.4**	**828**	**37.0**	**776**	**34.7**	**199**	**8.9**

Dividing the whole no. of cases in two major age groups, i. e. below 15 years and above 15 years, the majority of cases (59.1%) were found within the age group below 15 years, while 915 (40.9%) cases belonged to the age group above 15 years.

Table 12: Distribution of cases in two age groups in Nepal, 2005

	Up to 15 Years		Above 15 Years	
Sex	**No.**	**%**	**No.**	**%**
Male	748	33.4	521	23.3
Female	576	25.7	394	17.6
Total	**1324**	**59.1**	**915**	**40.9**

5.2.1 Laboratory results

Of the total 2239 specimens (serum/CSF) of the AES cases tested by MAC ELISA technique, 723 (32.3%) showed the presence of anti-JEV IgM. Serum of 1935 and CSF of 304 JE suspected cases were studied; out of which 663 serum and 60 CSF specimens were found to contain anti-JEV IgM. In the serum samples, 34.3 % sero-positivity was detected whereas the CSF samples demonstrated the sero-positivity of 19.7 % which is below the overall positivity rate of 32.3 %.

Table 13: Positivity of anti-JEV IgM in the tested specimens in Nepal, 2005

	Total	Positive	Positive %
Serum	1935	663	34.3
CSF	304	60	19.7
Total	**2239**	**723**	**32.3**

Out of 1516 negative samples, 1281 (84.5 %) demonstrated below 20 EIA units. Among the rest negative samples, 145 (9.6 %) showed 20-30 units and 90 (5.9 %) samples demonstrated 30 to below 40 units. Altogether, 235 clinically diagnosed AES cases showed anti-JEV IgM titers in the range of 20 to 40 units. This may be regarded as doubtful result.

Table 14: Evaluation of antibody titres of negative specimens

EIA units	No. of samples	% of total negative sample
Below 20	1281	**84.5**
20-30	145	9.6

30-40	90	5.9
Total	**1516**	**100**

In both the age groups (below 15 years and above 15yaers), higher no. of JE positive cases showed the antibody titre between 40 and 100 units. However, out of 273 cases with high titre antibody (above 100 units), 173 (63.4 %) were from the age group below 15 and only 100 (36.6 %) were from the age group 15 years and above.

Table 15: Age groupwise antibody titres of positive specimens

Age group (in years)	**Antibody titres**		**Total positive**
	40-100 units	**Above 100 units**	
Below 15	234 (52 %)	173 (63.4 %)	407
15 and above	216 (48 %)	100 (36.6 %)	316
Total	**450**	**273**	**723**

Among the 1269 male AES cases tested, 420 (33.1%) were identified as JE positive which constitutes 58.1% of the total JE positive cases. Similarly, out of 970 female AES cases tested, 303 (31.2%) were confirmed to be JE positive which constitutes 41.9% of the total JE positive case. The ratio of JE positive cases in male to female was observed as 1.4:1. The no. of JE positive cases in male sex was higher than in female. The association between the disease and the sex is not statistically significant (**P> 0.05; χ^2 = 0.87**).

Table 16: Genderwise distribution of JE cases in Nepal, 2005

Sex	**Total no. of cases**	**Positive**	**Positive %**	**% of total positive**	**Statistics**
Male	1269	420	33.1	**58.1**	**χ^2 = 0.87**
Female	970	303	31.2	41.9	**P = 0.351**
Total	**2239**	**723**	**32.3**	**100**	

Eleven samples from the Indian cases received at NPHL, which were not included above on epidemiological data, were also analysed and found a positivity of 36.4 %.

Agewise distribution of JE cases in 2005 revealed that the highest no. of positive cases (298) were found in the age group 5-15 years which constitutes 41.2 % of the total JE positive cases. Among the tested specimens, the highest no. of AES cases was also reported in the same age group. In the age group 15-50 years, 247 positive cases were detected which accounts for 34.2 % of the total positive cases. Similarly, 109 positive cases (15.5 %) and 69 (9.5 %) were confirmed in the age groups below 5 years and above 50 years respectively. The highest sero-positivity (36 %) was also found in the age group 5-15 years followed by the age group above 50 years (34.7 %), 15-50 years (31.8 %) and below 5 years (25 %). Statistically, the association between the age and the occurrence of the disease was found highly significant (χ^2 **= 152.09; P = 0.00**).

Table 17: Agewise distribution of JE cases in Nepal, 2005

Age group (in years)	No. of tested AES cases	Positive	Positive %	% of total positive	Statistics
Below 5	436	109	25	15.1	χ^2 = **152.09** **P = 0.00**
5-15	828	298	36	**41.2**	
15-50	776	247	31.8	34.2	
Above 50	199	69	34.7	9.5	
Total	**2239**	**723**	**32.3**	**100**	

Dividing the total no. of cases into two age groups; 426 (58.9 %) JE positive cases from the age group upto 15 years and 297 (41.1 %) JE positive cases from above 15 years were detected. Among the tested AES cases and the confirmed JE cases, the no. of male cases dominated in both the age groups, upto 15 years and above 15 years.

Table 18: Age and sexwise distribution of JE cases in Nepal, 2005

	Upto 15 Years		Above 15 Years	
Sex	**Positive**	**Total**	**Positive**	**Total**

Male	260	748	160	521
Female	166	576	137	394
Total	**426 (58.9 %)**	**1324**	**297 (41.1 %)**	**915**

Specimens from suspected JE cases (AES) were obtained throughout the year. From the month of May, the no. of JE positive cases were observed in an increasing trend till September, and then gradually decreased to meet zero in December. The highest no. of specimens (1035) were collected and tested in September, out of which, 436 were found to be positive for anti- JE IgM which accounts for 60.3 % of the total JE positive cases detected in the year 2005. During August, 172 cases (out of 477 AES cases) were confirmed as JE positive which constitutes 23.8 % of the total positive cases. After the month of September, the no. of AES as well as the JE positive cases followed a decreasing pattern. A cumulative of 96.5 % of total JE positive cases were confirmed in the period of four months, namely August, September, October and November. Statistically, the association between season/months and the occurrence of the disease was found highly significant (χ^2 **= 162.61; P = 0.00**).

Sero-positivity rate was highest in the month of September (42.1 %) followed by August (36.1 %), October (27.2 %), November (16.6 %) and others.

Table 19: Monthwise distribution of JE cases in Nepal, 2005

Month	**Positive**	**Negative**	**No. of tested cases**	**Positive %**	**% of total positive cases**	**Statistics**
January	5	14	19	26	0.7	χ^2 = **162.61** **P = 0.00**
February	1	13	14	7.1	0.1	
March	5	18	23	2.2	0.7	
April	0	22	22	0	0.0	
May	1	24	25	4	0.1	

June	3	37	40	8.1	0.4	
July	10	100	110	9.1	1.4	
August	172	305	477	**36.1**	**23.8**	
September	**436**	**599**	1035	**42.1**	**60.3**	
October	58	155	213	**27.2**	**8.0**	
November	32	161	193	16.6	4.4	
December	0	68	68	0	0.0	
Total	**723**	**1516**	**2239**	**32.3**	**100.0**	

A total of 2239 the serum and/or CSF specimens collected from AES patients of 55 different districts of Nepal were received and tested at NPHL for anti-JE IgM detection. However, JE positive cases were reported from 41 districts only. Out of 2239 specimens collected from the AES patients of 55 districts, no JE positive case was detected from 14 districts namely; Panchthar, Sankhubasabha, Tehrathum, Okhaldhunga, Ramechhap, Sindhupalchowk, Dhading, Nuwakot, Rasuwa, Gorkha, Kaski, Parbat, Rolpa and Baglung.

The eight districts that recorded a single JE positive case in each were Dolakha, Lamjung, Tanahun, Syangja, Gulmi, Rukum, Salyan and Kalikot. The highest no. of JE positive cases were confirmed in Bardiya district (111, 15.4 %) followed by Kailali (106, 14.7 %), Banke (96, 13.3 %), and Dang (89, 12.3 %). These 4 hyper-endemic districts constitute 55.7 % of the total positive cases. Similarly, 31 (4.3 %) JE positive cases from Kathmandu, 30 (4.1 %) from Kapilvastu, 27 (3.7 %) from Rupandehi, 22 (3 %) from Kanchanpur, 21 (2.9 %) from Sunsari, 19 (2.6 %) from Bara and 18 (2.5 %) from Nawalparasi were also recorded. These 11 endemic districts of Nepal accounted for 78.8 % of the total JE positive cases. Eighteen (2.5 %) positive cases were from unknown districts.

Table 20: Districtwise distribution of JE cases in Nepal, 2005

S.	District	Positive	Negative	No. of tested	% of total

N.				cases	positive cases
1	Panchthar	0	1	1	0
2	Ilam	3	7	10	0.4
3	Jhapa	11	48	59	1.5
4	Sankhubasabha	0	3	3	0.0
5	Tehrathum	0	1	1	0.0
6	Morang	12	42	54	1.7
7	Sunsari	**21**	47	68	2.9
8	Saptari	3	14	17	0.4
9	Siraha	6	14	20	0.8
10	Udayapur	2	10	12	0.3
11	Okhaldhunga	0	2	2	0.0
12	Sindhuli	3	10	13	0.4
13	Sarlahi	7	19	26	1.0
14	Dhanusa	8	22	30	1.1
15	Mahottari	3	6	9	0.4
16	Ramechhap	0	3	3	0.0
17	Dolakha	1	7	8	0.1
18	Rautahat	13	34	47	1.8
19	Bara	**19**	47	66	2.6
20	Parsa	10	21	31	1.4
21	Chitawan	3	41	44	0.4
22	Makawanpur	3	16	19	0.4
23	Kathmandu	**31**	262	293	4.3
24	Lalitpur	7	51	58	1.0
25	Bhaktapur	4	30	34	0.6
26	Kavrepalanchowk	7	31	38	1.0
27	Sindhupalchowk	0	5	5	0.0
28	Dhading	0	15	15	0.0
29	Nuwakot	0	7	7	0.0
30	Rasuwa	0	1	1	0.0
31	Gorkha	0	2	2	0.0
32	Kaski	0	5	5	0.0
33	Lamjung	1	5	6	0.1
34	Tanahun	1	12	13	0.1
35	Syangja	1	6	7	0.1
36	Kapilvastu	**30**	14	44	4.1
37	Rupandehi	**27**	27	54	3.7
38	Nawalparasi	**18**	28	46	2.5
39	Palpa	3	3	6	0.4
40	Gulmi	1	3	4	0.1
41	Arghakhanchi	2	3	5	0.3
42	Parbat	0	3	3	0.0

43	Baglung	0	2	2	0.0
44	Rukum	1	1	2	0.1
45	Rolpa	0	1	1	0.0
46	Salyan	1	4	5	0.1
47	Pyuthan	3	2	5	0.4
48	Dang	**89**	125	214	12.3
49	Banke	**96**	101	197	13.3
50	Bardiya	**111**	132	243	15.4
51	Surkhet	12	14	26	1.7
52	Kalikot	1	0	1	0.1
53	Kailali	**106**	100	206	14.7
54	Bajhang	2	1	3	0.3
55	Kanchanpur	**22**	45	67	3.0
	Unknown (NPHL)	18	60	78	2.5
	Total	**723**	**1516**	**2239**	**100.0**

Zonal analysis of JE cases in 2005 showed that the specimens from AES cases were collected from all 14 zones. Bheri zone topped in the no. of tested AES cases i. e. 466, of which 219 were confirmed as JE positive (sero-positivity = 47%). From Bagmati zone 451 AES cases were tested but only 49 were diagnosed as JE positive indicating a low sero-positivity rate (sero-positivity = 10.9%). Bheri zone demonstrated the highest percentage i. e. 30.3 % of the total positive cases; followed by Seti zone (108, 14.9 % of total positive), Rapti zone (94, 13 %) and Lumbini zone (81, 11.2 %). The least no. of JE positive case (i. e. 1 case) was confirmed in Karnali zone whereas no positive case was detected in Dhawalagiri zone.

Table 21: Zonewise distribution of JE cases in Nepal, 2005

Zone	Positive	Negative	No. of tested AES cases	% of total positive cases
Mechi	14	56	70	1.9
Koshi	33	93	126	4.6
Sagarmatha	11	40	51	1.5
Janakpur	22	67	89	3.0
Narayani	48	159	207	6.6

Bagmati	49	402	451	6.8
Gandaki	3	30	33	0.4
Lumbini	81	78	159	11.2
Dhawalagiri	0	5	5	0.0
Rapti	**94**	133	227	**13.0**
Bheri	**219**	247	466	**30.3**
Karnali	1	0	1	0.1
Seti	**108**	101	209	**14.9**
Mahakali	22	45	67	3.0
Unknown	18	60	78	2.5
Total	**723**	**1516**	**2239**	**100.0**

Out of 2239 AES cases tested, majority of the cases i. e. 747 cases were from CDR followed by MWDR, FWDR, EDR and WDR with 694, 276, 247 and 197 cases respectively. Although the majority of AES cases were from CDR, majority of the JE positive cases (314 cases) were recorded from MWDR which accounts for 43.4 % of the total positive cases. Eighteen percent (130) of the total positive cases were recorded from FWDR which is higher than that recorded from CDR (16.5 %). Positivity rate was highest in the FWDR (47.1 %) followed by MWDR (45.2 %), WDR (42.6 %) and EDR (23.1 %). The least sero-positivity rate was detected in CDR (15.9 %).

Table 22: Regional distribution of JE cases in Nepal, 2005

Region	Positive	Positive %	Total tested AES cases	% of total positive cases	Statistics
FWDR	**130**	**47.1**	276	**18.0**	
MWDR	**314**	**45.2**	694	**43.4**	χ^2 =
WDR	84	**42.6**	197	11.6	**193.85**
CDR	119	15.9	747	16.5	**P = 0.00**
EDR	58	23.5	247	8.0	
Unknown	18	23.1	78	2.5	

Total	**723**	**32.3**	**2239**	**100.0**	

Majority of the JE positive cases (615 out of 1542) were obtained from terai region which covers 85.1 % of total positive cases. Hill and inner terai region comprised 9.7 % and 2.8 % of total positive cases respectively. The highest sero-positivity rate was detected as 39.9 % for terai region. However, the no. of tested AES cases was seemed to be higher in hill region than in inner terai, anti-JE IgM positivity rate was just reverse i.e. 28.6 % for the inner terai and only 12.8 for hill region.

Table 23: Geographical distribution of JE cases in Nepal, 2005

	Positive	**Positive %**	**No. of tested AES cases**	**% of total positive Cases**	**Statistics**
Terai	**615**	**39.9**	1542	**85.1**	
Inner Terai	20	**28.6**	70	2.8	χ^2 **= 140**
Hill	70	12.8	549	9.7	**P = 0.00**
Unknown	18	23.1	78	2.5	
Total	**723**	**32.3**	**2239**	**100.0**	

5.3 Disease Morbidity and Mortality due to AES and JE in 2005

Disease morbidity was calculated in terms of case incidence (CI) per 100,000 population. Population data of 2005 was obtained from HMIS and EDCD divisions of DoHS, MoHP.

In the year 2005, a total of 322 deaths were reported among the total 2952 AES cases reported from different parts of Nepal. Case fatality rate (CFR) of AES was found to be **10.9 %** whereas the case incidence (CI) rate was detected as **12.9** per 100,000 people. Among the tested AES cases, 134 deaths were reported which accounts for 41.6 % of the total deaths due to AES cases. Out of 723 laboratory confirmed JE positive cases, 43 deaths were reported. For confirmed JE positive cases, CFR and CI were calculated as **5.9 %** and **$3.2/10^5$**.

Table 24: Deaths due to AES and JE in Nepal, 2005

Population at risk	Cases	Total cases	Deaths	CFR %	CI/10^5
22,948,646	Total AES cases	2952	322	10.9	12.9
22,948,646	Tested AES cases	2239	134	6	9.8
22,948,646	JE positive cases	723	43	5.9	3.2

Age specific analysis showed that both the highest no. of deaths (188) due to AES and highest CFR (13.9 %) were observed in the age group 15 years and above. But in contrast to the CFR, the CI rate was found to be highest (18.1) in the age group 5-15 years followed by 16.4 in the age group below 5 years and the least (9.8) among 15 years and above.

Table 25: AES cases, deaths, CFR and CI by age group in Nepal, 2005

Age group (in years)	Population	No. of AES cases	Deaths	CFR %	CI/10^5
Below 5	3,260,641	534	49	9.2	**16.4**
5-15	5,863,866	1061	85	8.0	18.1
15 and above	13,824,139	1357	188	13.9	9.8
Total	**22,948,646**	**2952**	**322**	**10.9**	**12.9**

Again, the age specific analysis revealed the same pattern of CFR and CI rate among the laboratory confirmed JE positive cases. The highest CFR (8.9 %) and the least CFR (2.8 %) were observed in the age groups 15 years and above, and below 5 years respectively whereas the highest CI rate (5.1) and the least CI rate (2.3) were found in the age groups 5-15 years and above 15 years respectively.

Table 26: JE positive cases, deaths, CFR and CI by age group in Nepal, 2005

Age group (in years)	Population	No. of JE positive cases	Deaths	CFR %	CI/105
Below 5	3,260,641	109	3	2.8	3.3
5-15	5,863,866	298	12	4.0	**5.1**
15 and above	13,824,139	316	28	8.9	2.3
Total	**22,948,646**	**723**	**43**	**5.9**	**3.2**

Table 27 shows the districtwise at-risk population, cases, deaths, CFR and CI rates. Although the CFR reached upto 100 % in some districts, the no. of AES case was quite low (i.e. only 1 case) and the CI rate in those districts was also very low. The highest CI rate (65.1) was observed in Bardiya district followed by Kailali (63.5), Dang (60.1), Banke (51.6), Kanchanpur (35.7), Kathmandu (21.6) and Kapilvastu (21.1). Most of the terai districts showed the CI rate to be above 5.0. The lowest CI rate (i.e. 0.4) was calculated for Rolpa district whereas in contrast to CI, 100 % CFR (1 AES case and 1 death) was reported in the same district, which can not be defined statistically.

Table 27: Cases, deaths, CFR and CI by district in Nepal, 2005

S. N.	District	Population	No. of AES cases	Deaths	CFR %	CI/10^5
1	Panchthar	216,999	1	0	0.0	0.5
2	Ilam	307,363	12	1	8.3	3.9
3	Jhapa	745,069	85	11	12.9	11.4
4	Sankhubasabha	170,320	3	0	0.0	1.8
5	Bhojpur	215,876	1	0	0.0	0.5
6	Dhankuta	178,604	4	0	0.0	2.2
7	Tehrathum	121,186	1	0	0.0	0.8
8	Morang	914,799	94	10	10.6	10.3
9	Sunsari	683,032	108	9	8.3	15.8
10	Saptari	617,042	28	4	14.3	4.5
11	Siraha	619,271	34	6	17.6	5.5
12	Udayapur	314,988	27	2	7.4	8.6
13	Okhaldhunga	166,330	1	0	0.0	0.6
14	Sindhuli	302,495	13	0	0.0	4.3

15	Sarlahi	688,160	26	4	15.4	3.8
16	Dhanusa	728,555	43	5	11.6	5.9
17	Mahottari	597,790	17	3	17.6	2.8
18	Ramechhap	228,300	2	0	0.0	0.9
19	Dolakha	219,817	9	1	11.1	4.1
20	Rautahat	590,554	44	1	2.3	7.5
21	Bara	608,484	53	3	5.7	8.7
22	Parsa	541,383	29	0	0.0	5.4
23	Chitawan	516,098	48	2	4.2	9.3
24	Makawanpur	426,897	16	0	0.0	3.7
25	Kathmandu	1,200,294	259	15	5.8	21.6
26	Lalitpur	367,150	56	4	7.1	15.3
27	Bhaktapur	243,304	28	2	7.1	11.5
28	Kavrepalanchowk	412,019	30	2	6.7	7.3
29	Sindhupalchowk	327,610	5	0	0.0	1.5
30	Dhading	364,772	15	1	6.7	4.1
31	Nuwakot	310,452	6	1	16.7	1.9
32	Rasuwa	48,340	1	0	0.0	2.1
33	Gorkha	307,968	3	0	0.0	1.0
34	Kaski	414,584	7	0	0.0	1.7
35	Lamjung	187,810	4	1	25.0	2.1
36	Tanahun	340,113	10	0	0.0	2.9
37	Syangja	338,791	8	1	12.5	2.4
38	Kapilvastu	527,145	111	22	19.8	21.1
39	Rupandehi	774,849	112	18	16.1	14.5
40	Nawalparasi	617,188	64	6	9.4	10.4
41	Palpa	287,309	9	2	22.2	3.1
42	Gulmi	317,840	3	0	0.0	0.9
43	Arghakhanchi	223,890	13	3	23.1	5.8
44	Parbat	169,092	3	0	0.0	1.8
45	Baglung	287,146	2	0	0.0	0.7
46	Rukum	202,802	2	0	0.0	1.0
47	Rolpa	223,880	1	1	100.0	0.4
48	Salyan	228,913	8	1	12.5	3.5
49	Pyuthan	227,460	3	0	0.0	1.3
50	Dang	503,821	**303**	38	12.5	**60.1**
51	Banke	424,152	**219**	20	9.1	**51.6**
52	Bardiya	420,863	**274**	31	11.3	**65.1**
53	Surkhet	314,867	28	6	21.4	8.9
54	Kalikot	113,030	1	0	0.0	0.9
55	Doti	221,107	1	1	100.0	0.5
56	Kailali	684,718	**435**	64	14.7	**63.5**
57	Bajhang	178,816	2	1	50.0	1.1
58	Kanchanpur	417,139	**149**	19	12.8	**35.7**

	Unknown		78			
	Total	**22,948,646**	**2952**	**322**	**10.9**	**12.9**

In the table below, regional pattern of AES cases, deaths, CFR and CI rate are summarized. Ninety seven deaths due to AES were reported from MWDR which constitutes the highest percentage (30.1 %) of the total deaths due to the same. Eighty five deaths (26.4 %) were reported from FWDR and the least no. of deaths (43, 13.4 %) was from EDR. CFR was found to be highest (15.2 %) in WDR followed by 14.5 % in FWDR, 11.6 % in MWDR and the least (6.3 %) in CDR. CI rate was calculated to be the highest (39.1) in FWDR followed by 31.5 in MWDR and the least (7.3) in WDR.

Table 28: AES cases, deaths, CFR and CI by regions in Nepal, 2005

Region	Population	No. of AES cases	Deaths	% of total deaths	CFR %	CI/10^5
FWDR	1,501,780	587	**85**	**26.4**	**14.5**	**39.1**
MWDR	2,659,788	839	**97**	**30.1**	11.6	**31.5**
WDR	4,793,725	349	53	16.5	**15.2**	7.3
CDR	8,722,474	700	44	13.7	6.3	8.0
EDR	5,270,879	399	43	13.4	10.8	7.6
Unknown		78				
Total	**22,948,646**	**2952**	**322**	**100.0**	**10.9**	12.9

Among the confirmed JE positive cases, the highest no. of deaths (19 deaths) were reported from MWDR followed by 16 from FWDR and the least (3) from CDR but the highest CFR (12.3 %) was found in FWDR followed by 6.1 in MWDR and the least (1.7 %) in CDR. CI rate reached its peak in MWDR (11.8) followed by 8.7 in FWDR and the least 1.1 in EDR.

Table 29: JE cases, deaths, CFR and CI by regions in Nepal, 2005

Region	Population	JE positive cases	Deaths	CFR %	CI/10^5
FWDR	1,501,780	**130**	**16**	**12.3**	**8.7**
MWDR	2,659,788	**314**	**19**	**6.1**	**11.8**
WDR	4,793,725	84	3	3.6	1.8
CDR	8,722,474	119	2	1.7	1.4
EDR	5,270,879	58	3	5.2	1.1
Unknown		18			
Total	**22,948,646**	**723**	**43**	**5.9**	3.2

Out of tested AES cases (2239 specimens), 134 deaths was reported in Nepal. The highest no. of deaths was reported from MWDR (57) alone which constitutes 42.5 % of total deaths among tested AES cases. FWDR reported 33 deaths (24.6 %) and WDR reported the least no. of deaths (7, 5.2 %) among the tested AES cases.

Table 30: Regional distribution of deaths among tested AES cases in Nepal, 2005

Region	No. of tested AES cases	Deaths	% of total deaths
FWDR	276	33	**24.6**
MWDR	694	57	**42.5**
WDR	197	7	5.2
CDR	747	23	17.2
EDR	247	14	10.4
Unknown	78		
Total	**2239**	**134**	**100.0**

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Japanese encephalitis is numerically one of the most important causes of viral encephalitis worldwide, with an estimated 50,000 cases and 15,000 deaths annually (Tsai, 1997; Solomon, 1997). Approximately, 60 % of the world's population lives in the JE endemic regions (Kabilan, 2004). The high fatality rate and the frequent residual neuropsychiatric sequalae in survivors make JE a significant public health problem. This is true in context of Nepal too. Since, it was first reported in 1978 in Nepal, it has been considered as one of the major public health problems because of its severity, high mortality and increasing pattern of morbidity (EDCD, 2005; Joshi *et al.*, 2005).

There are various techniques available to diagnose JE. Present study was conducted using IgM capture ELISA (MAC ELISA) technique. This method has been proved to be a reliable serological method for JE diagnosis (Bundo and Igarashi, 1985; Cuzzubbo *et al.*, 1999).

The present cross-sectional study was carried out during a year period from January through December 2005 at NPHL, Kathmandu and BPKIHS, Dharan in collaboration with WHO-IPD. This study, an extensive epidemiological study covering all 75 districts of the country, has made an attempt to know morbidity, mortality, vaccination status and outcome of the disease throughout the year covering all seasons. However, there were few focused data available from the country in the past.

During the study period, a total of 2952 AES cases were reported through different hospitals situated at different parts of the country. The present study revealed that the year 2005 reported one of the largest epidemic in Nepal with 2952 cases in its history. The largest epidemic in the history of Nepal (1978-2004) was reported in 1997 with 2953 cases, the smallest epidemic reported in 1981 was only with 54 cases (EDCD, 2005; Joshi *et al.*, 2005).

Of the total reported AES cases, 1647 were male which accounted 55.8 % of total reported cases and the rest 1305 (44.2 %) were female. The cumulative data of 1993-1997 also shows the predominance of male cases (4495) over female (3358) (Bista *et al.,* 1999). The present result also coincides with the results of 1999 and 2000, both of which reported 54 % male and 46 % female cases (Bista and Banerjee, 2000; Joshi *et al.,* 2005). The present result also agrees with the cumulative results of 2002 and 2003 which demonstrated 55 % male and 45 % female cases (Bista and Shrestha, 2005). Males have higher chances of being bitten by mosquitoes due to their extensive outdoor activities whereas females are commonly restricted to household works (indoor activities).

Weekly distribution showed the highest no. of AES cases 498, 432, 372, 295, 237, 191, 139 and 92 were reported in the epidemiological week no. 34, 35, 33, 36, 37, 32, 38 and 31 which constitute 16.9 %, 14.6 %, 12.6 %, 10 %, 8 %, 6.5 %, 4.7 % and 3.1 % of the total AES cases. Although all 52 weeks recovered AES cases in the range of 1 to 498 per week, epidemiological week no. 31 to 38 reported majority of cases (92 to 498 per week). This pattern clearly revealed that the majority of AES cases i.e. 2256 (76.4% of total) were identified during the period of 8 weeks. This distribution pattern is also similar with that of the previous years. During 1997 to 2004, it was seen that the epidemic appeared to start from 30^{th} week, reached its peak and almost ended after 40^{th} week with some variation in individual year (Joshi *et al.,* 2005; Bista and Banerjee, 2000). In Nepal, about 90 % of the cases are concentrated in the period ranging from mid-July to September (EDCD, 2005).

From Kailali district alone, 435 (14.7 %) AES cases were reported followed by Dang (303, 10.3 %), Bardiya (274, 9.3 %), Kathmandu (259, 8.8 %), Banke (219, 7.4 %), Kachanpur (149, 5 %), Kapilvastu (111, 3.8 %), Nawalparasi (112, 3.8 %) and Sunsari (108, 3.7 %). More than 66 % (1970) of the total AES cases were reported from 9 districts which can be considered as hyper-endemic districts. All these districts (except

Kathmandu) are among the 24 endemic districts identified by EDCD (EDCD, 2005). Higher no. of cases from Kathmandu district may be due to the good reporting system, a number of health institutions concentrated in this area and high level of public awareness.

Among these endemic districts namely, Kailali and Kanchanpur belong to FWDR whereas Dang, Bardiya, Banke belong to MWDR suggesting these two regions as the most affected areas. In 2005, 28 % (839) and 20 % (587) cases were reported from MWDR and FWDR respectively. These results are similar with the cumulative report of 1993-1997 which showed 39 % and 22 % of the cases were identified in MWDR and FWDR respectively (Bista *et al.*, 1999). MWDR reported highest no. of cases among the 5 regions in 1998, 1999, 2001, 2002 and 2003 followed by FWDR (except in 1998 and 2003) whereas in 2000, the pattern reversed and FWDR reported the highest no. of cases followed by MWDR (Bista and banerjee, 2000; Joshi *et al.*, 2005). In the present study, CDR ranked the second position with 700 AES cases (23.7 %).

Geographical distribution showed terai region (20 districts) as the most endemic region with 2232 cases which accounts for 75.6 % of the total AES cases. Environmental conditions of paddy field ecosystem in the terai region are most favourable for the breeding of *Culex* mosquitoes indicating its higher incidence in that region. Considerable no. of cases (558) were also reported from hill region. It indicates the possibility of expansion of the disease from regular endemic areas i. e. terai region to other non endemic areas.

Of the total 2256 specimens collected from the AES patients, only 2239 specimens were found to be sufficient for testing when received at NPHL/BPKIHS. Quantity was not sufficient in rest 17 vials (0.008 %). This provides an evidence of proper storage and transportation of sample to the laboratories during the study period. The credit goes to WHO/IPD for its technical support during the surveillance program. About 9 % of the serum samples out of 1935 were found haemolysed. This may be due to insufficient time provided for clotting or shaking the sample vials before being clotted. These minor

problems could be solved by refresher trainings on proper sample collection and serum separation to the concerned laboratory personnel.

Of the total 2239 specimens (serum and CSF) tested by MAC ELISA technique, 723 (32.3%) showed the presence of anti-JEV IgM above cut-off level. This is in accordance with some of the previous reports from Nepal. Studies carried out by Bista and Shrestha, (2005) and Pandey *et al.* (2003) in Nepal also showed more or less similar positivity rate. During the year 2002, 422 specimens were tested at NPHL, out of which 30 % were JE positive (Joshi *et al.,* 2005) and an unofficial data obtained from NPHL demonstrated 389 (35.3 %) JE positive cases among 1101 specimens tested in 2004 (personal communication). Both of these results are closer to the present positivity rate.

This result is in contrary to some of the previous findings from Nepal. A study carried out in Bheri Zonal hospital by Bajracharya *et al.* (2001) demonstrated a positivity rate of 47.2 % which is quite higher than the present result. The difference observed could be due to the reason that the study was solely conducted in the hyper endemic region of Nepal where the positive detection rate could be higher than the national figure. In 1999, 67 % of the tested samples out of the 204 were JE positive (Bista and Banerjee, 2000) whereas in 1998 and 2000, 70 % and 62 % of cases were conformed as JE positive (Joshi *et al.,* 2005). In 2001, 43 % (374/880) cases were found to be JE positive and in 2003, quite high percentage (89.9 %) of JE cases were confirmed among 277 tested cases (Bista and Shrestha, 2005).

The positivity difference between the previous years' reports and the present finding could be due to small sample size and localised nature of study in contrary to wide coverage of surveillance samples received during this study through WHO-IPD technical support.

The present finding does not correlate with 13.2 % (using N test) positivity reported by Kubo *et al.* (1993), 15.4 % (using N test) by Kubo *et al.* (1996) and 13.3 % using HI and 22 % using N tests by Ogawa *et al.* (1992). The present finding shows quite higher

positivity rate than the above reports which could be due to variation in geographical distribution and the techniques used.

Out of 1935 serum and 304 CSF specimens studied, 34.3 % and 19.7 % positivity rates were detected for serum and CSF respectively. Positivity rate of CSF was below the overall positivity rate (i. e. 32.2 %). This result clearly indicates that serum specimens are good enough to diagnose JE for surveillance purposes.

Out of 1516 negative samples, 1281 (84.5 %) demonstrated below 20 EIA units while among the rest negative samples, 145 (9.6 %) showed 20-30 units and 90 (5.9 %) samples showed 30 to 40 units. Altogether, 235 clinically diagnosed AES cases showed anti-JEV IgM in the range of 20 to 40 units. This suggests that the actual no. of JE positive cases may go higher than that detected in the present study with the present cut-off value. The higher antibody titers among the negative samples could be due to timing of sample collection prior to sufficient IgM antibodies production in the body against JEV i. e. within 10 days of infection. The level of anti-JEV IgM in the serum is variable with 68 % in day 1, 100 % in day 7, 96 % in day 30 and 72 % in day 180 (Burke *et al.*, 1985). The actual picture could be revealed if paired sera (acute and convalescent) from all specimens were made available. Paired sera were obtained from only 109 (5.6 %) cases among the total 1826 cases whose serum specimens were tested. Hence, these points strongly support for the higher positivity rate than detected in the current study.

In both the age groups (below 15 and above 15 years), majority of JE positive cases showed the antibody titers between 40 and 100 units. However, out of 273 cases with high titers antibody (above 100 units), 173 (63.4 %) were from the age group below 15 and only 100 (36.6 %) were from the age group 15 and above years. The high antibody titer against JEV was seemed to be prevalent in children (below 15 years) than in adults. The reason behind this could not be assessed in the current study.

Among the total JE positives, 420 were male which constitutes 58.1% whereas 303 were female which constitutes 41.9% of the total positive cases. The no. of JE positive cases in male sex was higher than in female with the ratio of 1.4:1. This result also correlates well with the findings of the study conducted by Ogava *et al*. (1992) where about 60 % of the total positive cases were male. The higher number of male cases may be due to the more exposed body parts of males leading to higher chances of mosquito bite. Males also have higher chances of being bitten by mosquitoes due to their extensive outdoor activities whereas females are commonly restricted to household works (indoor activities). Females of the endemic region do not roam around the rice fields and jungles during evening time, which males usually do. The sleeping habit of males outdoors and young females indoors in the terai region may be another factor contributing for the higher number of male cases Ogava *et al*. (1992). Because of these reasons, males are more exposed to mosquitoes and acquire JEV infection. In the remote areas of Nepal, still the priority is given to males than females regarding health services which can contribute to the high no. of male cases. High mobility of males across the border areas could help to transport the epidemic strains of JEV from India during the outbreak season. The association between the disease and the sex is not statistically significant (**$P > 0.05$; $\chi^2 = 0.87$**). The sero-positivity rate within the individual sex was almost similar which does not agree with the result of Ogava *et al*. (1992).

The finding of present study differs with the results of Joshi (2004) (2 female and 1 male positive case) and Bajracharya *et al*. (2001) (60.9 % female) which stated more positive cases in female than in male. This may be due to very low no. of samples and the localized nature of study which can not be statistically defined.

In 2005, the agewise distribution revealed that the highest no. of JE positive cases (298) were found in the age group 5-15 years which constitutes 41.2 % of the total positive cases. Statistically, the association between the age and the occurrence of the disease was found highly significant (**$\chi^2 = 152.09$; $P = 0.00$**). Also, among the lab confirmed

cases, the highest no. was detected in the same age group. The present finding closely correlates with the national figure of previous years (EDCD, 2001) and also with the study of Bista and Banerjee (2000) both of which showed above 40 % cases from the age group 5-15 years. Five to fifteen years children are the most active and they always roam outside home to play around water loaged areas and rice fields. Because of high temperature of terai region during the mosquito breeding season, children play outside the home in the evening time unknowingly putting themselves at the risk of mosquito bite. These activities increase the chances of mosquito bites. Adult people of age group 15-50 are also active and go outside the home to fulfil their home requirements and hence, the chances of mosquito bites are also increased. Slightly low no. of cases in this age group could be due to the improved awareness about the mosquito bite.

Similarly, 69 (9.5 %) JE confirmed cases from above 50 years represented the least no. of positive cases among the age groups. The possible reasons for this may be that aged people rarely leave their home and spend almost all the time at home. Children of the age group below 5 years showed very few positive cases because they are under strict supervision of their parents for their protection until they are grown up. These two extremities usually do not leave home, and a good care and mosquito nets are provided by the family to prevent them from mosquito bites. These environments protect them from being infected.

Analysis of the total no. of cases by dividing into two age groups revealed 426 (58.9 %) JE positive cases from the age group upto 15 years and 297 (41.1 %) from above 15 years. In both the age groups, the no. of male cases predominated. This indicates that the risk of acquiring JEV infection is considerably high among children than adults. No or low degree of knowledge about the disease and hygienic conditions, and low immunity may be the reasons for high prevalence among the children. This result shows similarity with the national figure of previous years (Bista *et al*., 1999; EDCD, 2005; Joshi *et al.*, 2005 and Bista and Shrestha, 2005).

Some similar studies carried out in India and aboard also reported a considerable no. of confirmed JE cases among the children. Rao *et al.* (2005) reported that the age group 1-14 years including infants had been affected but nearly 86.8 % of them were from 1-9 years age group in Andhra Pradesh epidemic during 1999. Chokephaibulkit *et al.* (2001) reported JE in children during 1996-1998 in Thailand. Lowry *et al.* (1998) reported acute JE in 31 of the 46 paediatric AES, compared with only 2 of 33 adult AES patients admitted to a hospital in Veitnam during 1995. Out of 195 children with CNS symptoms admitted to pediatric ward of Penang hospital, Malaysia, 38.5 % demonstrated the anti-JEV IgM in their CSF (Cardosa *et al.*, 1995).

However, the present result is in contrary to the previous findings of Bajracharya *et al.* (2001), Kubo *et al.* (1996) and Joshi (2004); all of which showed higher prevalence rate among the adults than children. This difference could be due small sample size, variation in the test procedure and localized nature of the above studies.

Specimens from suspected JE cases (AES) were obtained throughout the year. From the month of May, the no. of JE positive cases were observed in an increasing trend till September, and then gradually decreased to meet zero in December. The highest no. specimens (1035 specimens) were collected and tested in September, out of which, 436 were found to be positive for anti- JE IgM which accounts for 60.3 % of the total JE positive cases. During August, 172 (out of 477 AES cases) were confirmed as JE positive which constitutes 23.8 % of the total positives. After the month of September, the no. of AES as well as the JE positive cases followed a decreasing pattern. A cumulative of 96.5 % of total JE positive cases were confirmed in the period of four months, namely August, September, October and November. Statistically, the association between season/months and the occurrence of the disease was found highly significant (χ^2 **= 162.61; P = 0.00**).

In this regard, the similar pattern of JE distribution in Nepal has been described by EDCD (2005) in its annual report of 2002 and 2003. Hence, the result is very much

similar to the previous years' data. These findings also clearly reflect the seasonal variation in JE cases. August, September and October are favourable for mosquito breeding because of paddy cultivation. Moreover, in August and September, heavy rain changes most of the dry areas to water-loaged areas which are appropriate for mosquito breeding. These could be the possible reasons for high incidence of disease in particular season/months. The present monthwise pattern of JE showed a little bit difference with the average distribution pattern of 1993-1997 when the peak of epidemic was observed in the month of August (Bista *et al*., 1999) rather than September. This variation could be due to late starting of the monsoon in the year 2005. Data obtained from NPHL shows that the specimens tested were all negative during the period of January to July 2004. In this regard, the present result does not match with the result of 2004.

Specimens from suspected JE/AES cases were obtained throughout the year but no positive case was detected during April and December. This pattern also supports the seasonal trends of the disease in Nepal.

Although AES cases were reported from 58 district of Nepal, specimens were received from 55 districts. However, JE positive cases were detected from only 41 districts. The highest no. of JE positive cases were confirmed from Bardiya district (15.4 %) followed by Kailali (14.7 %), Banke (13.3 %) and Dang (12.3 %). These 4 endemic districts constitute 55.7 % of the total positive cases. These districts have been identified as the most affected districts of Nepal since last few years. Report of 1999 (Bista and Banerjee, 2000) and cumulative report of 1993-1997 (Bista *et al.*, 1999) also prove this fact except in context of Morang district in which fewer cases were reported in the year 2005. Morang reported higher no. of cases in the previous years.

Similarly, 4.3 % JE positive cases from Kathmandu, 4.1 % from Kapilvastu, 3.7 % from Rupandehi, 3 % from Kanchanpur, 2.9 % from Sunsari, 2.6 % from Bara and 2.5 % from Nawalparasi were also confirmed. Altogether, these 11 districts of Nepal account for 78.8 % of the total JE positive cases. All these districts (except Kathmandu) are among the 24 endemic districts identified by EDCD (EDCD, 2005). Higher no. of cases

from Kathmandu district may be due to the good reporting system, a number of health institutions concentrated in this area and high level of public awareness. Even though the sero-positivity rate was quite low (10.6 %), such a large no. of positive cases in the hill district like Kathmandu reflects an alarming situation. This also shows the actual risk of JE in Kathmandu. Presence of *Cx. tritaeniorhynchus* in Kathmandu (According to MoH source) and isolation of JEV by Ogawa *et al.* (1992) from a pig raised in Kathmandu are indicators of the real presence of disease. The result of studies carried out by Kubo *et al.* (1996) and Bista and Banerjee (2000) also support the present finding. The regular contact of the inhabitants of Kathmandu with people from different part of the country also signals towards the high positive cases.

Eight districts that recorded single positive case were Dolakha, Lamjung, Tanahun, Syangja, Gulmi, Rukum, Salyan and Kalikot. This could be due to the extensive travelling of the people from these districts to endemic regions (terai) for the fulfilment of their daily requirements, and India seeking for job. They could have acquired the disease during these activities. No JE positive case was detected from 14 hill districts.

The most endemic districts like Bardiya, Kailali, Banke, Dang, Kapilvastu, Rupandehi and Nawalparasi demonstrated the positivity rate of 40-68 % which is quite higher than the overall positivity rate (32.3 %). This indicates that the positivity rate could be much higher if the districts having 1 or 0 positive case were omitted.

Bheri zone demonstrated the highest no. of JE positive cases (219) which accounts for 30.3 % of the total positive cases followed by Seti zone (14.9 %), Rapti zone (13 %) and Lumbini zone (11.2 %). High no. of positive cases in particular zones is due to some endemic districts belonging to these zones. Sero-positivity rate was found highest in Seti (51.7 %), followed by Bheri (47 %) and Rapti (41.4 %). This pattern suggests that some non-endemic zones were responsible for the low overall positivity rate otherwise, it could be very high.

Out of 2239 tested AES cases, majority of the cases (747) were from CDR followed by MWDR, FWDR, EDR and WDR which constitute 694, 276, 247 and 197 cases respectively. The highest no. of the JE cases (314 cases) was reported from MWDR which accounts for 43.4 % of the total positive cases and FWDR (18 % of the total positives); both of which are higher than that reported from CDR (16.5 %). Positivity rate was highest in the FWDR (47.1 %) followed by MWDR (45.2 %), WDR (42.6 %) and EDR (23.1 %). The least sero-positivity rate was detected in CDR (15.9 %). These results are similar to the national cumulative report of past few years (Bista *et al.*, 1999; Joshi *et al.*, 2005; EDCD, 2005; Bista and Banerjee, 2000). Some hyper-endemic districts (and most affected Zones) belong to FWDR and MWDR to establish these two regions as the most vulnerable areas. Akiba *et al.* (2001) also confirmed 78 % of suspected cases as JE positive in the south western part of Nepal.

The highest no. of tested cases with least positivity was identified in CDR. The reasons behind this may be the good reporting system, a number of health institutions concentrated in this area, high level of public awareness and visit of complicated /referred cases in referral hospitals. Some terai districts belonging to CDR may also contribute to the increased no. of JE positive cases in this region.

Both the majority of positive cases (85.1 %) and the highest sero-positivity rate (39.9 %) were identified in the terai. The present result also agrees with the previous findings that showed hyper-endemicity of JE in the terai. This could be due to those districts belonging to this region, which reported major proportion of JE cases. In terai, most of the people have farming as their major occupation. Hence, presence of paddy fields also provides a suitable environment for mosquito-breeding. The open border system with India can also contribute to high no. of JE cases there. Rai *et al.* (1987), Kubo *et al.* (1993), Kubo *et al.* (1996) and Pandey *et al.* (2003) also reported similar pattern in Nepal. These results support the present finding. The economic activities in these areas are related to farming, fishery, cattle raising and jungle roaming for woods. Extensive

outdoor activities, longer stay outside the home and exposed body parts due to poor clothing put them at a high risk of mosquito bites.

A few positive cases identified in hill region could be either imported from terai and inner terai regions or originated there. Positive cases from hill districts may widen the JE burden beyond the terai belt of Nepal and this can not be ignored. These areas should not be considered as risk free and entomological surveys should be conducted to prove.

In the year 2005, a total of 322 deaths were recorded among 2952 AES cases reported from different parts of the country. An overall case fatality rate (CFR) of AES was found to be **10.9 %** whereas the case incidence (CI) rate was observed as **12.9** per 100,000 population. Although the present epidemic was seemed to be the second largest since 1978, the no. of deaths was comparatively fewer than that was recorded in the previous large epidemics. The present CFR was also lower than the average CFR (**19.5 %**) of the cumulative of past epidemics. To support the present finding, decreasing pattern of CFR was also observed in 2002, 2003 and 2004 (Bista and Shrestha, 2005; Joshi *et al.*, 2005). This could be due the intermittent vaccination programs, improved public awareness and good case management system developed in the hospitals. Another reason for low no. of deaths could be the missed deaths because people of some rural communities still do not prefer to go to health institutions rather believe in local healers. Reduced time interval in between the onset of illness and hospitalization also plays a vital role to minimize the CFR. The CFR of present finding is less as compared to the study done by Parajuli *et al.* (1992) during 1989 (CFR = 6.6 %) and Joshi *et al.* (1995) during 1990, 1991, 1992 and 1993 with CFR 36 %, 38 %, 35 % and 32 % respectively. A great fluctuation in the CI rates has been observed since 1978, but in the recent years, it has decreased. Slightly higher CI in 2005 may be related to changing epidemiological pattern of JE in Nepal.

For confirmed JE positive cases, CFR and CI were calculated as **5.9** % and **$3.2/10^5$**. Around 60 % of the total deaths were observed among the not tested AES cases; most

deaths among not tested cases could be due to JE which remained unassessed. In the remote rural areas, people bring their patients to the health institution only when the condition is very severe. Before visiting the health institutions, they frequently visit the traditional local healers (Dhami, Jhakri, Guruwa and others) and usually waste their time. This could be a possible reason for higher no. of deaths among the not tested cases.

Age specific analysis showed that CFR of both AES and JE was highest in the age group 15 years and above. This finding is in accordance with the results of 2001, 2000 and the cumulative report of 1993-1997 where the highest CFR was stated in the same age group i. e. 15 years and above (Bista *et al.*, 1999; Joshi *et al.*, 2005). People of this age group usually ignore the minor symptoms (low fever, headache, nausea etc.) and do not go to the hospital for treatment rather consult the local healers. By the time they reach the hospital, the condition becomes quite critical. This may ultimately lead to death related to higher CFR in this age group. In contrast to adults, children are usually brought to the hospital by their parents immediately after the appearance of minor symptoms and get recovered due to timely treatment.

In contrast to the CFR, the CI rates of both AES and JE were found to be highest in the age group 5-15 years followed by below 5 years and the least among 15 years and above. National figures of 2000 and 2001 regarding CI rate also support the present finding (Joshi *et al.*, 2005). Five to fifteen years children are the most active and they spend most of their time outside home to play around water loaged areas and rice fields. These activities increase the chances of mosquito bites leading to higher incidence of the disease due to low immunity. On the other hand, elder people have comparatively better knowledge about the disease and the vectors which help to protect them from infection. This could be the possible reason for high CI rate among children.

The highest CI rate (65.1) was observed in Bardiya district followed by Kailali (63.5), Dang (60.1), Banke (51.6), Kanchanpur (35.7), Kathmandu (21.6) and Kapilvastu

(21.1). Most of the terai districts showed the CI rate above 5.0 with considerably high CFR. This shows that the prevalence is concentrated in particular districts. These districts showed the increased CI rate in the previous years also (Joshi *et al.*, 2005; EDCD, 2005). Higher CI in Kathmandu could be due imported cases or due to presence of the disease in the indigenous population or due to more consciousness of the people toward their health.

In the present study, 97 deaths due to AES were reported from MWDR which constitutes 30.1 % of the total deaths followed by 85 deaths (26.4 %) from FWDR. CFR and CI rate were also found highest in FWDR followed by MWDR. These facts reveal that the two regions were the most affected areas of Nepal. This is also supported by various studies previously conducted in Nepal. These two regions also topped the CI rates from 1993 through 2004 agreeing with the present finding (Bista *et al.*, 1999; Joshi *et al.*, 2005); however regional CFR was fluctuating in these years. This fact concludes that the regional distribution pattern of the disease has not changed yet in Nepal. The districts with high CI and CFR fall in these two regions. This distribution pattern is not only true regarding AES cases but also true in context of confirmed JE cases in the year 2005.

Intermittent vaccination against JE was conducted in some endemic districts of Nepal from 1999 through 2002. Efficacy of vaccination in Nepal has already been proved by a couple of case-control studies conducted in Nepal regarding JE vaccination (Bista *et al.*, 2001; Ohrr *et al.*, 2005). Therefore, expanded programme of immunization (EPI) in JE endemic areas should be strongly emphasized.

6.2 Conclusion

In Nepal, among total AES patients (2952), only **2239** specimens were collected and tested in 2005. Out of which, **32.3 % (723)** were confirmed as JE positive using the present cut-off value. The etiology of rest **66.7 %** JE negative AES cases was unknown which should be tested for other possible viral diseases. Majority of the JE positive

cases (about 56 %) were found to be concentrated in few districts of terai namely Bardiya, Kailali, Banke and Dang. Around **79 %** positive cases were confirmed in **11** districts including these 4 districts. A considerable no. of positive cases (including the indigenous cases) were also identified in Kathmandu district which could be due to referred cases but some confirmed cases with no history of journey to endemic region before infection indicates possible risk of JE. As in the previous years, MWDR and FWDR remained the most affected regions. More than **85 %** positive cases were solely from terai. Children below 15 years were the most vulnerable to JE infection. Although AES cases were reported throughout the year, August, September and October reported majority of JE cases (**92.1 %**) showing the seasonal trend. CFR and CI rate due to AES/JE has been found decreasing since past few years. Of the negative specimens, 235 clinically diagnosed AES cases showed anti-JEV IgM in the range of 20 to 40 EIA units (using the preset cut-off value of **≥40 units**) which is an indication of early sampling and should not be excluded from possible JEV infection.