EPIDEMIOLOGICAL STUDY OF JAPANESE ENCEPHALITIS IN KANCHANPUR DISTRICT DURING OUTBREAK SEASON

A THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE MASTER'S DEGREE OF SCIENCE IN ZOOLOGY

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TO

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KIRTIPUR, KATHMANDU
NEPAL
2004

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ABBREVIATION

AES	:	Acute Encephalitis Syndrome
AIDS	:	Acquired Immunodeficiency Syndrome
Anti-JE	:	Anti-Japanese Encephalitis
BI	:	Binding Index
C	:	Core
CFR	:	Case Fatality Rate
CI	:	Case Incidence
CNS	:	Central Nervous System
CSF	:	Cerebro Spinal Fluid
CT	:	Computed Tomography
Cx	:	*Culex*
DDPN	:	District Demographic Profile of Nepal
E	:	Envelope
ECG	:	Electro-Cardiogram
EDCD	:	Epidemiology and Disease Control Division
ELISA	:	Enzyme Linked Immuno Sorbent Assay
H_2O_2	:	Hydrogen Peroxide
H_2SO_4	:	Sulfuric Acid
HIV	:	Human Immunodeficiency Virus
HMG	:	His Majesty's Government
ICP	:	Intrancranial Pressure
IF	:	Immuno Flurorescence
IgM	:	Immunoglobulin M
JE	:	Japanese Encephalitis
JELVP	:	Japanese Encephalitis Live Vaccine Protocol
JEV	:	Japanese Encephalitis Virus
KAP	:	Knowledge, Attitude and Practice
MRI	:	Magnetic Resonance Imaging
N	:	Neutralization
NC	:	Negative Control

NHS	:	Normal Human Serum
NS	:	Nonstructural
NZFHRC	:	National Zoonoses and Food Hygiene Research Centre
OD	:	Optical Density
OPD	:	Ortho-Phenylene Diamine
PBS	:	Phosphate Buffer Solution
PHC	:	Primary Helath Centre
PrM	:	Pre-Membrane
RNA	:	Ribose Nucleic Acid
SEA	:	South East Asia
TUTH	:	Tribhuvan University Teaching Hospital
UTR	:	Untranslated Region
VBDs	:	Vector Borne Diseases
VDCs	:	Village Development Committees
WHO	:	World Health Organization
WNV	:	West Nile Virus
WPC	:	Weak Positive Control

ABSTRACT

Japanese encephalitis has been one of the most serious public health problem in Nepal since 1978 because of the severity of the disease and high mortality rate. The present study, aimed to determine the case incidence and case fatality rate due to Japanese encephalitis, to know the public awareness about the disease, and to assess the effect of Japanese encephalitis vaccination, was carried out from April, 2003 to November, 2003 in Kanchanpur district. During the study period, a total of 25 suspected encephalitis cases were recorded with their sera samples from Mahakali Zonal Hospital, Kanchanpur and tested by anti-Japanese encephalitis Immunoglobulin M capture enzyme linked Immuno assay. The results were analysed in three steps: questionnaire survey, clinical aspects, and laboratory diagnoses. The overall case incidence was 6.61 per 100,000 population, the highest being among 15-44 years age group (9.55/100,000) followed by 45 and above years (7.64/100,000) and 0-14 years (3.16/100,000). But statistically, the difference was found not to be significant (χ^2=3.17, p >0.05). The case incidence was high among male (8.33/100,000) than female (4.83/100,000) but not statistically significant (χ^2 = 0.93, p > 0.05). Maximum cases were of Chhetri (44%) followed by Tharu (24%) and lower caste (24%) and Brahmin (8%). Statistically, the difference was not found to be significant (χ^2=6.52, p>0.05). Further, the highest number of cases were recorded in the month of September (52%) followed by August (40%), and July (4%) and October (4%). Children of age group 0-14 years constituted 20% of total case but all were not vaccinated. The overall case fatality rate was 16%. Among 25 serum samples, 12% of samples were found to be positive for anti-Japanese encephalitis immunoglobulin M antibody. All the 3 positive cases belonged to 15-44 years age group. But statistically, there was not significant difference among age groups (χ^2 = 3.31, p > 0.05). One positive case belonged to male sex and two to the female sex. Among 3 serologically confirmed cases, one case developed JE sequelae (left side hemiplegia) and one had mixed infection with Plasmodium falciparum. Among total clinical cases, 48% of cases were found to be aware and 52% unaware about mosquito vector. 56% of cases used to do outdoor activity and 44% indoor activity at down and dusk. 60% of cases were found to be mosquito-net users and 40% non-users. 96% of cases had paddy cultivation around houses during rainy-season and 4% did not have. 8% of cases had reared pigs in open coop by traditional method and 92% did not have pigs. Among 90 interviewed respondents, only 23% of respondents were aware about mosquito borne mode of the disease and 18% had correct knowledge regarding transmission season and symptoms of the disease. 60% of respondents visited the health care facilities for medical treatment. Upgrading of hospital laboratory, use of insecticide impregnated mosquito net, conduction of public health education campaigns and environmental studies in relation to JE are discussed.

I

INTRODUCTION

Nepal's topographical and socio-ecological diversification help to promote periodic epidemics of infectious diseases, epizootics and natural disasters. Millions of people are at risk of infection and hundreds of them die every year due to communicable diseases, malnutrition and other health related events. As also majority of the population is rural area based and illiterate, economic and demographic changes coupled with sudden occurrence of epizootics might possibly have contributed to the disease outbreaks. Japanese encephalitis (JE), malaria, kala-azar, HIV/AIDS etc. have been affecting large number of people in successive years.

JE is a mosquito borne viral zoonosis caused by *Flavivirus*. It causes an acute *Flaviviral* neurological infection of the central nervous system (CNS). It is primarily a disease of swine, equine, and wading birds. The disease accidently affects human population and no viraemia in human thus man plays no role in perpetuating the virus (Pavri, 1979).

Though many genera and species of mosquito vectors are belived to be responsible for the disease transmission, Culex mosquito (*Cx. tritaeniorhynchus*) is the principal vector in Nepal. It often breeds in paddy ecosystem.

JE is principally a disease of rural agricultural areas, where vector mosquitoes live and grow in close association with the main vertebrate hosts. Thus residents of rural areas in endemic locations, active duty military deployed to endemic areas, expatriates and travelers living for prolonged periods in rural areas where JE is endemic or epidemic are at the greatest risk. People practicing conventional type of pig rearing at home, with outdoor and open latrines and living in mud or wood houses are mostly affected by the disease.

The important factors governing spillover of the disease to man are the availability of amplifying host, the density and absolute number of mosquitoes, adequate man mosquito contact and longevity of vector. JE does not usually occur in urban areas. So the risk to short term travelers and those who confine their travel to

urban centers is very low. Residents of developed countries usually have no natural immunity to the Japanese encephalitis virus (JEV) and travelers of all ages are equally susceptible to infection with the virus.

JE is wide spread in temperate and tropical Asian countries (South East Asia), the Indian subcontinent, China, Korea, Japan and parts of Oceania and is thus the most important causes of epidemic encephalitis worldwide. The annual incidence of clinical infection in endemic areas ranges from 10 to 100 per 100,000 population. Approximately 3 billion people and 60% of the world's population live in endemic regions and about 35,000 to 50,000 cases are notified annually, with 10,000 to 15,000 deaths (Tsai, 2000). More than 50% of the affected populations are children of less than 15 years of age. Case fatality rate range from 0.3 to 60%. There is nearly universal exposure to the virus by adulthood. Ratio of affected males and females is 1.5:1. In developed countries of Asia and in areas where children are protected by immunization, JE occurrence is increased in the elderly, consistent with waning immunity with age.

JE is transmitted to humans via the bite of infected mosquitoes. The virus initially propagates at the site of the bite and in regional lymphnodes. Subsequently, low viremia develops, leading to inflammatory changes in the heart, lungs, liver and reticuloendothelial system. Most infection are cleared before the virus can invade the CNS, leading to sub-clinical disease. However, neurologic invasion can develop, possibly by growth of the virus across vascular endothelial cells, leading to involvement of large areas of the brain, including the thalamus, basal ganglia, brain stem, cerebellum, hippocampus and cerebral cortex. Certain neurotransmitter receptors are believed to be involved in the binding of JE virions to cells in the CNS. The affinity of JE virus for neural tissue leads to propagation in the brain.

JE outbreaks are usually circumscribed. It usually does not last more than a couple of months and dying out after the majority of amplifying hosts (pigs) have become infected. Birds are also the natural hosts for JE. Epidemics occurs when the virus is brought into the peridomestic environment by mosquito bridge. For the prevention of the disease in human, safe and efficacious vaccines are available

❖ Statement of Problem

In Nepal, 24 districts of Terai and inner Terai regions are mostly affected by JE and 12.5 millions people are estimated to be at the risk of the disease. Annually 2,000-3,000 total cases and 200-400 deaths occur. Total 26,094 cases and 5,334 deaths have been reported with average case fatality rate of 20.44% in an aggregate since 1978 to 2003. Thus in terms of morbidity and mortality this disease is the major public Health problem in Nepal (EDCD).

After the first epidemiological surveillance of JE conducted from 1978-1984 in Kanchanpur and other JE endemic district by His Majesty's Government, Department of Health Services, Epidemiology and Statistics Division, Zoonotic Disease Control Section in collaboration with WHO, most of the zonal hospital including Mahakali Zonal Hospital of Kanchanpur district reported JE cases annually on the basis of clinical diagnosis due to lack of laboratory facilities. The cases are generally reported in high numbers in every alternate year since 1997 to 2002 in Kanchanpur district as revealed by the data 51, 1, 50, 43, 21, 95, 85, 138, 118, 160 and 34, cases in the year 1992, 1993, 1994, 1995, 1996, 1997, 1998, 2000, 2001 and 2002 respectively. Few studies have been done to know the knowledge, attitude and practice (KAP) of people in Kanchanpur district about JE. Assessments have not been carried out yet to know the effect of JE vaccination. So this study is an attempt to find out the actual situation of JE in Kanchanpur district.

❖ Significance of the Study

In Nepal, where socio-economic condition of majority of the population is poor and are uneducated, people do not know about the recently introduced JEV infection. Even at many times, Mahakali Zonal Hospital reported high JE cases. Thus, one of the most important significance of the study is to shed light in its epidemiology, public awareness and importance of immunization.

II

AIMS AND OBJECTIVES

❖ General Objective

To study the epidemiology of Japanese encephalitis (JE) in Kanchanpur district of Far Western Region.

❖ Specific Objectives

- To determine the present case incidence and case fatality rate (CFR%) due to JE in different age groups, sexes and ethnic groups in Kanchanpur district.
- To know the level of public awareness i.e. knowledge, attitude & practices (KAP) of people about JE.
- To assess the effect of JE vaccination carried out in the past.

III

LITERATURE REVIEW

❖ JE Situation in Different Countries

The history of JE goes back to 1871 AD. Even though cases and outbreaks of clinically resembling JE have been observed since 19th century, the first outbreak of JE was occurred in Japan during 1924. Then it spread from East Asia to South East Asia (SEA) and then to South Asia. It was called "summer encephalitis" till identified in 1936 after the JE virus was isolated from human cases in Japan during 1935.

During the 1950s and the early 1960s, severe outbreaks of JE in human with more than 6000 cases were recorded in **Japan**. The case fatality rate was as high as 60%. Since 1972, however, the number of cases has rapidly decreased to fewer than 100 cases per year, following the development of inactivated JE vaccines in the mid-1960s and a nationwide immunization programme.

In the Republic of Korea, more than 1000 cases were reported annually until 1969. The majority of the victims were children under 14 years of age, as in other countries experiencing JE epidemics. During 1955–1965, the highest rates of incidence were observed among children from four to seven years of age. Immunization started in the late 1960s and there have been fewer than 1000 cases a year since 1969. The Government started an intensified immunization programme in the early 1980s and the number of cases has dramatically decreased since 1985. Vaccine coverage reached almost 100% in the 3–15 age group in 1985.

China reported more than 20,000 cases annually until 1992. Since 1993 the number of reported cases has been decreasing. In 1996, for the first time during the last two decades, the number of cases fell below 10,000. China is also strengthening its JE immunization programme.

In Vietnam, the first big JE outbreak was reported in 1965 in northern region. Since then, 2,000–3,000 cases have been reported annually. The JE epidemic in northern Vietnam is seasonal, which follows the pattern of countries in the temperate

zone, while in southern Viet Nam sporadic cases of JE is reported through out the year. The most affected age group in Viet Nam is children under nine years.

The first outbreak of JE (two clinical cases) in **Australia** was reported in 1995. The cases occurred on an island in the Torres Strait. During the outbreak, the JE virus was also isolated from healthy humans (asymptomatic infection). In Papua New Guinea, two children were confirmed as having contracted JE in 1997 (http: www.wpro.who.int) .

The earliest documented clinical record of JE in **Taiwan** can be dated back to 1931, although under the name of summer encephalitis. Kobayasi first succeeded in isolating JEV from fatal JE cases in 1938. The ecology of JE in Taiwan was first studied and addressed by Gryaston and others in 1962. It was promulgated in 1955 that JE was designated a notifiable disease in the island. A mass vaccination program against JE for children was implemented in 1968 (Wu *et al.,* 1999).

In Malaysia, the first report of JE was published by Chuikshank in 1951, describing an outbreak among British prisoners during the second world war. The number of cases from 1985-1993 totaled 273 but the actual number of cases could probably be more (Haw, 1995). Three outbreaks have been reported: first during 1974 in Pulau Langkawi (10 cases; 2 deaths); second during 1988 in Pulari Pinnag (9 cases and 4 deaths); and third during 1992 in Serian, Sarawak (9 cases, 4 death). 31% of cases between 1989-1993 were in children aged 0-4 years, 52% in the 7-14 years age group, 9% in the 15-24 years age group and 8% in adults aged >25 years. Cases have occurred principally in children below 14 years accounting for 83% of the total cases. More males (62%) are affected than females (38%). The current outbreaks in northern Perak has resulted in 9 deaths (www.vadscorner.com/jelsk.html).

There have been two documented cases of JE in **UK** travellers. 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 complications (Rose, *et al.,* 1983). The second was a woman who had been to **Thailand** in 1994, she recovered fully after 4 months (Rose, *et al., 1*983). There have also been **American** and **Australian** military cases reported following the **Korea** and **Vietnamese** wars and postings to South East Asia (Burdon *et al.,* 1994).

Map 1: Current Distribution of Japanese Encephalitis (The approximate dates of the first major outbreaks, or first virus isolations)

Some countries in the South-East Asia Region of WHO (**India, Nepal, and Thailand**) have reported a marked increase in the number of JE cases since 1970s.

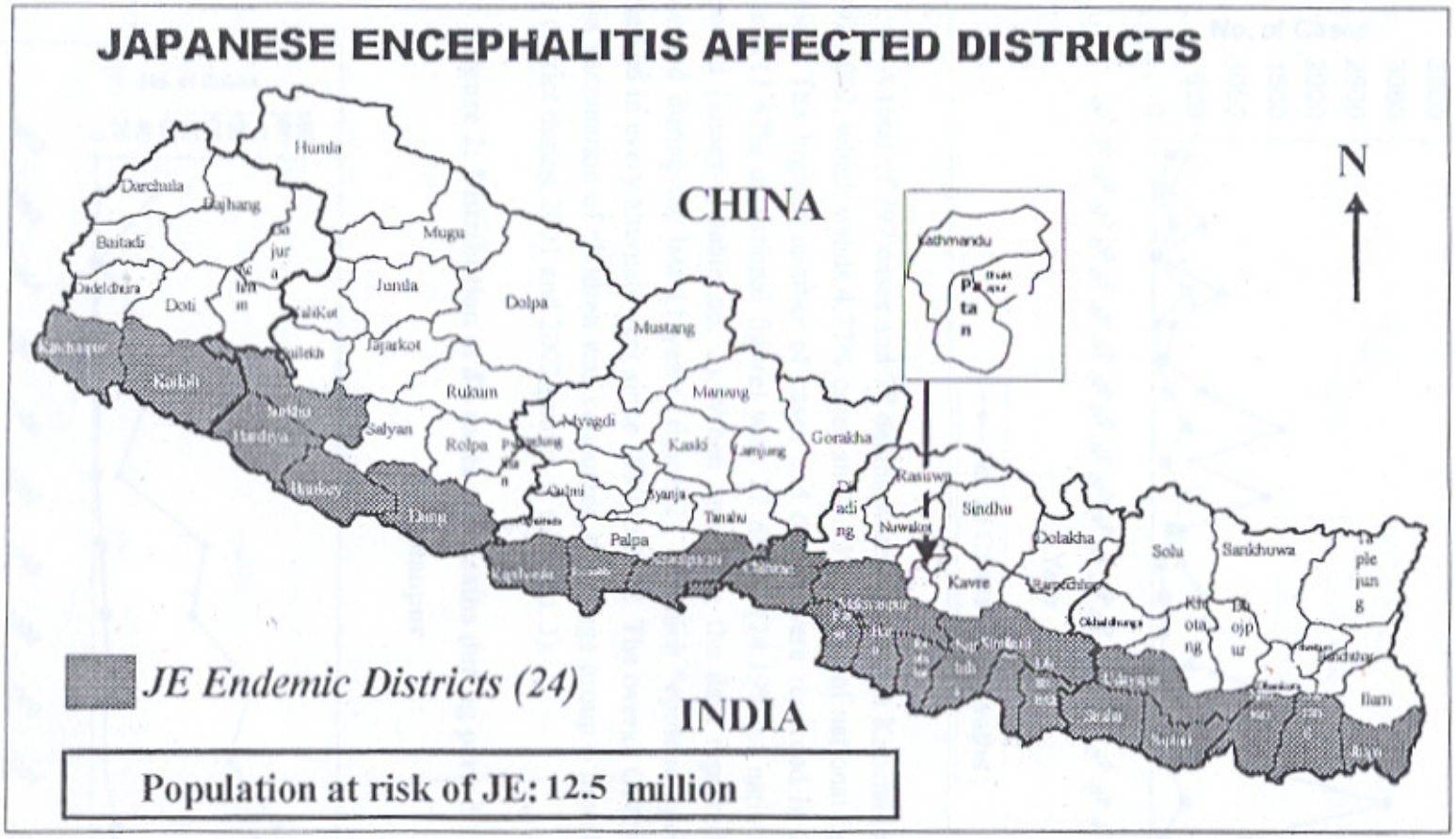
The first indication of JE transmission in SEA region was found in **Sri Lanka** where an outbreak was apparently reported in 1948 (Tsai, 1994). In **India**, epidemics of JE were first recognized around Vellore in 1948 (Sehgal, 1989) and 85% of donkey sera were anti-JE antibody positive.

❖ JE Situation in Nepal

In Nepal, it is said that clinical cases were reported even before 1975. An epidemic of JE was first introduced in Rupendehi district of the western development region (WDR) from adjoining Uttar Pradesh State of India during 1978 and subsequently 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 successive years (Annual report, 2001, EDCD).

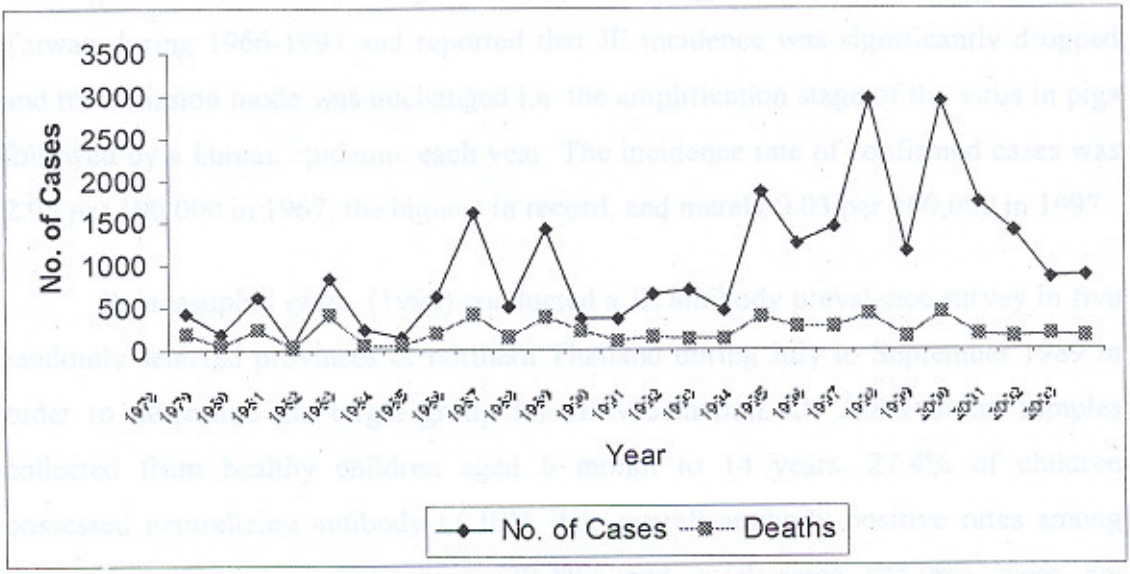
In Nepal, outbreak of JE consistently corresponds with the rainy season. Number of cases of encephalitis starts increasing with the onset of the rainy season (June-July) and reaches its peak in August-September and thereafter gradually subsides (Bista, 2001) to null off by October.

A total of 26,094 cases and 5,334 deaths were recorded in Nepal through different hospitals since 1978 to 2003. The highest number of cases were reported in 1997 when 2,953 cases reported with 407 deaths occurred. The highest number of deaths were reported in 1999 when 434 death occurred out of 2,924 cases reported (annex-1, table no. 1). When analyzing the data reported to the pattern observed during the last 26 years (figure 1), in which "epidemic peaks" have been observed every 3 to 5 years. The overall CFR% was 20.44%.



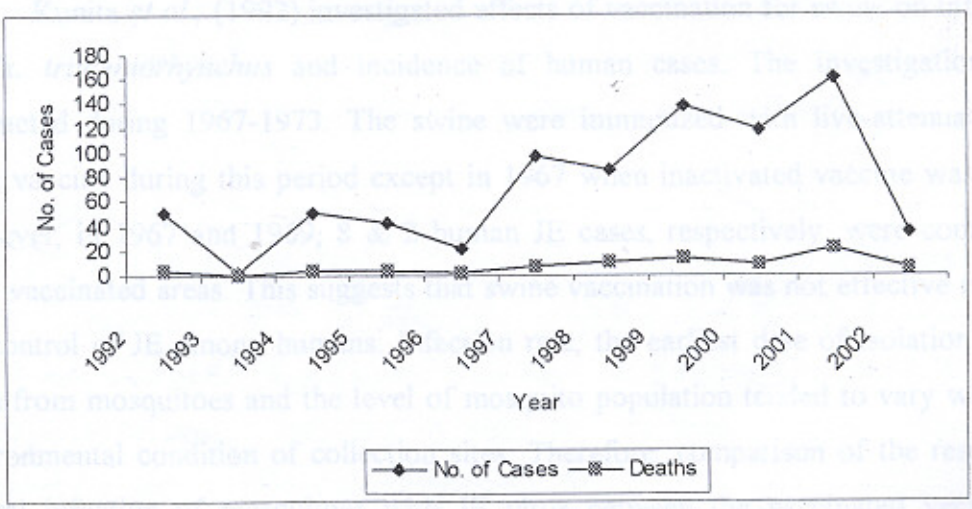
Map 2: Japanese Encephalitis Endemic District of Nepal

Figure 1: Distribution of JE cases and deaths during period 1978-2003, Nepal



A total of 797 cases and 77 deaths were recorded in Kanchanpur district since 1992-2002, which stands 4.77% cases and 2.93% deaths of national figure during that period. The highest number of cases and deaths were recorded in 2001 when 160 cases (11.42% of national figure) with 22 deaths (14.19% of national figure) were reported (annex-1, table no. 2). When analyzing the data reported to the pattern observed during the last 11 years (figure 2), in which "epidemic peaks" have been observed in every alternate year since 1997 to 2002. The overall CFR% was 9.67%. A mass vaccination of children was conducted in the age group 6 month to 10 years in the district during 2001 and 2002 (annex-1, table no. 3).

Figure 2: Distribution of JE cases and deaths during period 1992-2002, Kanchanpur



❖ JE Epidemiological Research in Global Perspective

Wu *et al.*, (1999) analysed the epidemiology of Japanese encephalitis on Taiwan during 1966-1997 and reported that JE incidence was significantly dropped and transmission mode was unchanged i.e. the amplification stage of the virus in pigs followed by a human epidemic each year. The incidence rate of confirmed cases was 2.05 per 100,000 in 1967, the highest in record, and merely 0.03 per 100,000 in 1997.

Rajanasuphot *et al.*, (1992) conducted a JE antibody prevalence survey in five randomly selected provinces of northern Thailand during July to September 1989 in order to determine the target group for JE vaccination. Of 3,089 blood samples collected from healthy children aged 6 month to 14 years, 27.4% of children possessed neutralizing antibody to JEV. The overall antibody positive rates among children residing in urban areas (29.4%) and rural areas (25.9%) were not significantly different. Inapparent JEV infection occurred early in life with an age-dependent increase. Approximately 351 human were inapparently infected by JEV for each apparent JE case, thus it denotes that the clinical attack rate for JEV in 1:351.

Sharma *et al.*, (1992) reported first outbreak of JE in Haryana State, North India. A total of 294 cases with 205 deaths were diagnosed based on clinical manifestations to have JE. Females suffered more than males. 5-9 years age groups were the mostly affected population. During outbreak of JE, cases were found to be scattered over comparatively large area. 183 villages were affected covering the 1.02 million population in 38 blocks.

Kunita *et al.*, (1992) investigated effects of vaccination for swine on infection of *Cx. tritaeniorhynchus* and incidence of human cases. The investigation was conducted during 1967-1973. The swine were immunized with live-attenuated JE virus vaccine during this period except in 1967 when inactivated vaccine was used. However, in 1967 and 1969, 8 & 2 human JE cases, respectively, were confirmed from vaccinated areas. This suggests that swine vaccination was not effective enough for control of JE among humans. Infection rate, the earliest date of isolation of JE virus from mosquitoes and the level of mosquito population tended to vary with the environmental condition of collection sites. Therefore, comparison of the results of natural infection of mosquitoes with JE virus between the vaccinated years and

unvaccinated years in areas, which are favorable for the breeding of *Cx. tritaeniorhynchus* and infected mosquitoes, will reveal the effectiveness of swine vaccination.

Paul *et al.*, (1993) reported an outbreak of JE on the island of Saipan during October 1990. Ten cases occurred among a population of 40,000. The prevalence of antibody to JE virus among 234 lifelong Saipan residents surveyed after the outbreak was 4.2%. Age, household crowding and lack of air conditioning were risk factors for infection. The seroprevalence in pigs was 96% (n=52).

Shoji *et al.*, (1994) reported a high mortality due to JE, 4 of 10 patients, in the Kurume region on Kyushu Island, Japan, from 1984 – 1990.

Ha *et al.*, 1994 (1995) conducted a study on current situation of JE in the south Vietnam from 1976-1992 and reported that cases of Acute Encephalitis Syndrome (AES) and deaths occured annually in all 17 provinces in the south of Vietnam. The highest morbidity of 936 patients was recorded in 1980, while highest mortality of 339 deaths in 1977. The lowest figure of morbidity was 197 cases in 1990 and the mortality was 34 deaths in 1985. Sporadic cases were reported throughout the year but outbreaks with low peaks were seen during February and July annually.

Makino *et al.*, 1994 (1995) conducted a virological and seroepidemiological survey of arbovirus infections in Pilot areas in Laos under the WHO- JICA- LTPHC project since 1993-1995. Human sera were obtained at the laboratory in Khammouane Provincial Hospital, and at Sok Yai village in Vientiane municipality. In Khammouane area, antibody-positive rates to DEN-4 and JE increased with age and reached over 50% by 21-30 and 31-40 years old respectively. JE antibody survey in swine sera indicated that JE virus was active during the rainy season.

Nga *et al.*, (1995) studied on transmission of Japanese encephalitis virus in Gia Luong District, Ha Bac Province, Vietnam, after JE vaccination. The study was carried out from 1993-1994. Serodiagnosis by IgM- capture ELISA (MAC-ELISA) carried out on 60 of 85 clinical encephalitis cases and detected 43 positive (71.66%). All these serologically confirmed JE cases had not been vaccinated. The result supported the vaccine efficacy to prevent overt JE.

Map 3: Global Epidemiology of Japanese Encephalitis, 2003

Map 4: Distribution of Japanese Encephalitis in Asia, 1970-1998

(Source: Tsai TR, Chang GW, Yu YX. Japanese encephalitis vaccines. In Plotkin SA and Orenstein WA, eds., *Vaccines* - 3rd edition, WB Saunders, Inc., Philadelphia, PA, 1999;672-710.)

Dapeng *et al.*, (1995) carried out a clinical case control study to identify prognostic factors present at hospital admission associated with early sequelae and fatal outcome of actual JE in Gusi county, Henan Province, central China from June to September 1991. A total of 70 patients with laboratory confirmed acute JE were studied, of whom 3 cases died and 33 cases had neurological or psychiatric sequelae at the end of 3 months follow up. A history of the vaccination was not correlated with the early sequelae and fatal outcome of the disease. The paper suggested that early diagnosis and treatment and universal JE vaccination for all susceptible populations are key for decreasing incidence of sequelae and fatal outcome of acute JE.

Cardosa *et al.*, (1995) carried out a study to determine if JE virus is an important causative agent of viral encephalitis among pediatric admissions in Penang, Malaysia. 195 children with CNS symptoms and 482 children with non-specific febrile illness admitted into the pediatric ward of Penang Hospital during 16 months period were taken into the study. It was determined that 5 of 13 children with a discharge diagnosis of viral encephalitis had JEV specific IgM in CSF, indicating that 38.5% of the viral encephalitis cases was due to JEV. They also screened 482 nonspecific febrile cases for IgM to JEV and to Dengue viruses and found that 2 (0.4%) had IgM specific for JEV and 9 (1.9%) has IgM specific for Dengue virus.

Gajanana *et al.*, (1995) conducted a community based study of subclinical flavivirus inflections in children in an area of Tamil Nadu, India, where JE is endemic. They carried out a 3 years prospective serological study between 1989 and 1991 in the primary health centre in the cohort of school children aged 5-9 yrs. The overall incidence of JE cases was 15 per 10000 children aged 5-9 years and the estimated ratio overt: inapparent infection was 1:270.

Gratier *et al.*, (1996) conducted a serological survey of arboviruses infection during military mission (Apronuc) in Cambodia. 3,883 sera collected from military men before and after their mission in Cambodia (Apronuc mission, 1992) were analysed to study arboviruses circulation in that country. Vaccination against JE did not assure a entire safety since 19 men were infected by the virus during their stay in Cambodia.

Hanna *et al.*, (1996) studied an outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995, to determine the distribution of virus infection during the outbreak of JE and to describe the environmental factors facilitating the outbreak. They performed human and porcine serological surveys for JE virus activity throughout the Torres Strait and mosquito and household survey in the island of Badu (where the clinical cases occurred), Australia, during April-May 1995. The serological surveys identified recent JE virus infection among residents or domestic pigs on at least nine outer Torres Strait island. A JE virus, confirmed by nucleotide sequencing, was isolated from two asymptomatic Badu residents.

Pogodina *et al.*, (1996) reported typical and atypical forms of JE in the citizens of Russia visiting Asian countries endemic for JE. They reported JE in patients after 5 months returning to Russia from China; in a pregnant women (6 months gestation) who lived in Birma for 3 years, mildly manifest pyramidal signs in her one year old infant with a normal mental status and in a patient one month after arrival in Japan. None of the patients was vaccinated against JE. Indications to prophylactic vaccination of subjects leaving for countries endemic for JE are discussed.

Hennessy *et al.*, (1996) measured the effectiveness of live attenuated Japanese encephalitis vaccine (SA-14-14-2) by conducting a case-control study in rural Sichuan Province, China. They found that the effectiveness of one dose was 80%, that of two doses was 97.5%. Thus they concluded that a regimen of two doses of live-attenuated JE vaccine, administered 1 year apart, is effective in the prevention of clinically important diseases.

Reuben and Gajanana (1997) studied on Japanese encephalitis in India and reported that children were mainly affected, with morbidity rate estimated at 0.30 to 1.5 per 100,000 population. Case fatality rate ranged from 10% to 60%, and upto 50% of those who recover might be left with neurologial deficits. Reported incidence were generally higher in males than in females, but subclinical infections occurred equally in both sexes. Diagnosis at the PHC level was based on clinical symptoms only.

Lowry *et al.*, (1998) performed a cohort and case – control studies on all adult and pediatric acute encephalitis syndrome (AES) patients admitted to the neurology service of Bach Mai Hospital, Hanoi, Vietnam, between June 5 and August 3, 1995.

Among pediatrics AES patients, 31 (67%) of 46 had acute JE, compared with only two (6%) of 33 adult AES patients.

Neogi *et al.*, (1998) studied on HIV seropositivity during epidemic outbreak of JE mid 1995 in Manipur. Of the sixteen serum samples from patients with history of febrile headache, convulsions, mental confusion, neck rigidity, etc., concomitant JEV and HIV infection could be detected in 3 cases.

Solomon *et al.*, (1998) studied on poliomyelitis-like illness due to JEV. The study was carried during 1995. 12 (55%) of the 22 children with acute flaccid paralysis had evidence of acute JEV infection, compared with only one (1%) of 88 age matched hospital controls to a referral centre in Ho Chi Minh city, Vietnam.

Saito *et al.*, (1999) reported three Japanese encephalitis cases in US marines stationed on Okinawa island, Japan, 1991. None of the patients had been administered JE vaccine. This report underscores the importance of JE vaccination.

Yoshida *et al.*, (1999) gave first report on human cases serologically diagnosed as JE in Bali, Indonesia, using IgM- captue ELISA both on serum and CSF of the patients. They examined serum specimens from 12 patients with clinical diagnosis of viral encephalitis, meningitis or dengue haemorrhagic fever (DHF), and found 2 JE cases.

Thakare *et al.*, (1999) carried out a study on Japanese encephalitis in Singli district, Maharashtra. The study was carried out during the months June to December, 1997 and reported that of the 52 cases of suspected viral encephalitis admitted at the Government Hospital, Singli, IgM antibodies to JE virus were detected in the sera of 5 cases. These cases were from the congested area of Singli and the adjoining villages. All age groups and both genders were affected.

Victor *et al.*, (2000) reported three cases of JE for the first time from two villages of Dharmapuri district (Krishnagari Health Unit) in Tamil Nadu during November 1999. Two children died and one developed neurological sequelae. Out of 146 sera samples from children below 15 years, the presence of HI antibodies to JEV,

WNV and Den-2 virus were found to be 8.9, 3.4 and 6.85% respectively and three children had IgM antibodies to JEV.

Myint *et al.*, (2000) investigated a Japanese encephalitis virus infection and the possibility of JE outbreak in Bogalay township (Nyi-Naung-Wa village), Myanmar in 1999. JE virus antibody was determined among the pigs and the people living near the pig farms in that village and at an adjacent village as a control. Homotypic or monotypic JE antibodies were detected in 33% of the pigs tested. Neither homotypic nor monotypic JE virus infection among the people, because of the presence of JE virus infection among the pigs and the presence of *culex* mosquito vector in that area.

Russell and Dwyer (2000) investigated arboviruses associated with human disease in Australia and reported that mosquito-borne arboviruses are an important public health issue in Australia.

Strickman *et al.*, (2000) studied distribution of dengue and Japanese encephalitis among children in rural and suburban Thai villages. The study was carried out in the rainy season of 1989. Students in three schools in the largely non-agricultural, sub-urban community of Bang Bua Thong, Nontaburi Province were sampled in late June and July. Of 1,477 children, 33/1000 had recent dengue infection and 7/1000 had recent JE infection. Serum samples were taken in late August in the agricultural community of Hua Samrong, Chachoengsao province. Of 748 students in two schools, 95/1000 had signs of recent dengue infection and 32/1000 had signs of recent JE infection.

Rao, *et al.*, (2000) studied the epidemic of JE in Andra Pradesh which occurred during October-November, 1999 affecting 15 out of 23 districts. In total, 873 cases with 178 deaths had been recorded. During the epidemic, area of 47 PHCs had been affected in Anatapur district in western Andhra Pradesh. On an average 4.5% of 3175 villagers had been affected.

Vijayarani and Gajanana (2000) reported low rate of Japanese encephalitis infection among rural children in Thanjavur district (Tamil Nadu), an area with extensive paddy cultivation. Among children aged 5-12 years, the infection rates for

JE in two consecutive transmission seasons of 1991-92 and 1992-93, were 1.8 and 5.1% respectively. A high cattle to pigs ratio (400:1) could possibly be an important factor for the low JE infection rate in children in the district.

Chokephaibulkit *et al.*, (2001) performed a prospective study of childhood encephalitis in Bangkok from 1996 to 1998. Of the viral agents identifiable in 26 (65%) of 40 children, JE was reported in 6 children.

Chattopadhay, (2001) studied the status of Japanese encephalitis infection in Arunachal Pradesh. Suspected cases of JE were recorded in the hospitals of Arunachal Pradesh from 1986 to 1995. 162 cases were diagnosed as JE with predominance in male sex and lower age group. Maximum cases were recorded between June to October. 11.3% pig sera showed JE antibodies when tested by ELISA test.

Johansen *et al.*, (2001) conducted an entomological investigations of an outbreak of JEV in the Torres Strait, Australia, in 1998, and recovered 43 isolates of JE virus from adult mosquitoes (42 isolates from *Culex sitiens* and one from *Ochlerotatus vigilax*). They also reported two confirmed human JE cases in that area and Cape York Perinsula, in northern Queensland.

Dash *et al.*, (2001) carried out a retrospective analysis of epidemiological investigation of Japanese encephalitis outbreak occurred in Rourkela city of Orissa, India. The investigation was carried out during an outbreak of JE in July/August of 1993. Among the serum samples, 40% of the sera showed antibodies against JE, while 17% of the sera showed recent infection due to Dengue virus.

Shoji *et al.*, (2002) reported a low mortality of JE, none of 4 patients, in the Kurme region on Kyushu Island, Japan during the outbreaks of 1991-1993.

Wong, (2002) reported a total of 2000 JE cases in Hong Kong, China, during the period of January – March in Quarterly epidemiological report (http: //www.afcd.gov.hk/quarantine/vetnews/download/qer 1302 .PDF).

Thakare *et al.*, (2002) studied on prevalence of West Nile virus infection in India. During the course of the virological investigation of cases of suspected viral fevers carried out at the National Institute of Virology (NIV), Pune India. Seven cases

of encephalitis were reported in which WN virus-specific IgM class antibodies were detected in CSF samples.

Konishi and Suzuki (2002) estimated a ratio of sub-clinical to clinical infections in vaccinated human populations who acquired natural infection with JE virus, and evaluated protective capacity of the currently approved inactivated JE vaccine by comparing the ratio with those reported for unvaccinated populations. The ratio of subclinical to clinical infections in vaccinated populations was estimated to be 2,000,000: 1, which was 2000-80,000 times higher than the ratio previously reported for unvaccinated populations.

Padbidri *et al.*, (2002) conducted a serological survey of arboviral disease among the human population of Andaman and Nicobar Islands, India. 2,401 sera were collected from six major localities. The prevalence of HI antibodies against JE virus was 5.9%. The result of N-tests indicated a prevalence of JE virus 2.1%.

Watt and Jongsakul (2003) studied on acute undifferentiated fever caused by infection with JEV. The study was performed in a cohort of 156 adults presenting to a hospital in Chiangmai, Thailand and reported JE in 22 individuals (14%).

Ayukawa *et al.*, (2004) reported an unexpected outbreak of Japanese encephalitis in the Chugoku district of Japan during the period from early August to mid September, 2002. They reported 6 patients of JE. Five patients, except for one without sequelae, had a severe outcome, including one death. The mean age was 67.5 years (range 42 – 89 years). The report indicated that JE in Japan is still a threat to adults and the elderly with decreased or absent immunity to the JE virus.

❖ JE Epidemiological Research in Context to Nepal

Rai *et al.*, (1987) conducted a serological study of JE in Kathmandu, Nepal, during 1987. A total of 154 serum samples were collected from the staff and students of Tribhuvan University, Teaching Hospital(TUTH). 4 out of 154 subjects were confirmed to have JE virus infection and were all from the Terai area. There were no subjects with confirmed JE virus infection from Kathmandu and Hill areas.

Parajuli *et al.*, (1992) carried out an epidemiological study of JE in all epidemic districts of Nepal during the year 1989 and reported that of 868 total JE cases, 227 died. All ages and both sex groups were affected from the disease.

Joshi *et al.*, (1995) carried out an epidemiological survey of JE in all endemic areas of Nepal through National Zoonoses and Food Hygiene Research Centre (NZFHRC) from 1990 to 1993. The CFR were 36.0%, 38.0%, 35.2% and 31.7% in 1990, 1991, 1992 and 1993, respectively.

Kubo *et al.*, (1996) studied changing seroepidemiological pattern of JEV infection in Nepal. During the study period seropositivity was observed in 15.4% (98/638) of the individuals visiting TUTH.

Zimmerman *et al.*, (1997) reported a first outbreak of JE in Kathmandu valley, Nepal during September and October, 1995 and treated 15 patients with meningoencephalitis.

Akiba *et al.*, (2001) conducted an epidemiological study of JE outbreak in the south-western part of Nepal in 1997 and reported a high density of JE infections. It was estimated that 27.9% of the total population were infected with JE virus in the study area.

Bista *et al.*, (2001) conducted a case control study to measure the efficacy of single-dose SA-14-14-2 vaccine against JE. During the study period, of several cases of JE admitted to hospitals from early August, 20 children, aged 1-15 years, were identified whose illness was suspected as on JE case definition and were resided in the villages receiving the vaccine. None of 20 JE cases had received JE vaccine compared with 326 of 557 age-sex matched village controls.

Gurung and Singh (2003) studied on factors associated with JE in Nepal. Total of 142 number of confirmed JE cases and 142 controls from Banke, Bardia and Dang district were interviewed. They concluded that non-immunization status, presence of household pigs, non-use of mosquito-net and out-door sleeping are the risk factors related with the occurance of JE.

IV

INFECTIOUS AGENT, VECTORS, HOSTS, SEASONALITY, TRANSMISSION CYCLE, CLINICAL SPECTRUM, DIAGNOSIS, TREATMENT AND PREVENTION OF THE DISEASE

❖ Infectious Agent of JE

The infectious agent is Japanese Encephalitis Virus (JEV). JEV is one member of 70 viruses in the *Flavivirus* genus of the Flaviviridae family. It is antigenically related to St. Louis encephalitic (SLE) virus, Rocio virus, Murray valley encephalitis virus, West Nile virus and several other flaviviruses (Gubler, 1989).

Morphologically, the JEV virion is spherical, approximately 40-50 nm in diameter, with a lipid membrane enclosing isometric 30nm diameter nucleocapsid core comprised of a capsid (C) protein and a single stranded messenger (positive) sense viral RNA of approximately 11 kb (JELVP, 1999). The RNA comprises a short 5' untranslated region (UTR), a longer 3' UTR and between them a single open reading frame (ORF) of approximately 10 kb (Chambers *et al.*, 1990). This codes for a single polyprotein which is co- and posttranslational cleaved by viral and host proteases into three structural proteins (core-C, pre-membrance-PrM; and envelope-E) and seven nonstructural (NS) proteins (NSI, NS2a, NS2B, NS3, NS4A, NS4B, NS5). The C protein is highly basic and combines with the RNA to form the nucleocapsid. The PrM is closely associated with the E protein, forming a heterodimer and is thought to act as a 'Chaperone' to it, impairing its function until after virion release. Immediately prior to virion release, the PrM protein is cleaved by a furin like protease to its mature M protein form. This allows the formation of E protein homodimers, which are thus 'activated' (Stadler *et al.*, 1997). The E protein is the largest structural protein, consisting of nearly 500 amino acids with up two potential gylcosylation sites. It is the mature target for the humoral immune response, and is thought to be important in viral entry into host cells (Solomon, 2003). Thus biological activities like mediation of viral bindings to susceptible cells, haemagglutination, possible

participation in endosomal viral function and induction of host protective immune response are ascribed to "E" protein of the virus.

The virus can be inactivated at 56° C temperature in 30 minute. It can survive at 0° C upto 3 weeks, at -70° C upto several months, and very long time in 10% serum or milk (Shrestha, 2003).

The molecular phylogeny of JE viruses, based on the 240 base nucleotide sequence of viral PrM, divides JE isolates into four distinct genotypes, with a maximum divergence of 21% among the isolates. The largest genotype consist of viruses from Japan, Okinawa, China, Taiwan, Vietnam, Philippines, Sri Lanka, India and Nepal. A second genotype consist isolates from northern Thailand and Cambodia, and a third, from southern Thailand, Malaysia, Sarawak, Australia and Indonesia. Five Indonesian isolates, two from Java, two from Bali and one from Flores, similar to each other and distinct from other Indonesian isolates, form the fourth genotype. Co-circulation of multiple genotypes was observed only in Thailand and Indonesia (JELVP, 1999).

Until now prevalence of three different strains of JEV have been reported in Nepal (Nepal 1/90, B-2524 and B-9548). A study on sero-epidemiology of JEV infection carried out by Takashi Kubo *et al.*, in 1996, found out that the number of JE cases and deaths that occurred due to JE in Nepal for the last two decades correlated well with the findings in India.

❖ Vectors

Thirty species of mosquitoes belonging to five genera of *Culex, Anopheles, Aedes, Mansonia* and *Amergeres*, harbour the viruses of JE (annex-2). They are mostly zoophilic. Mosquitoes become infective 14 days after the entry of JEV from the viremic host i.e. domestic pigs and wild birds infected with JEV (Annual report, 2001). Viral infection rates in the mosquitoes range from less than 1% to 3%. In temperate zones, the vectors are present in greatest numbers from June through September and are inactive during winter months. *Culex* mosquitoes can fly upto 2 kms radius.

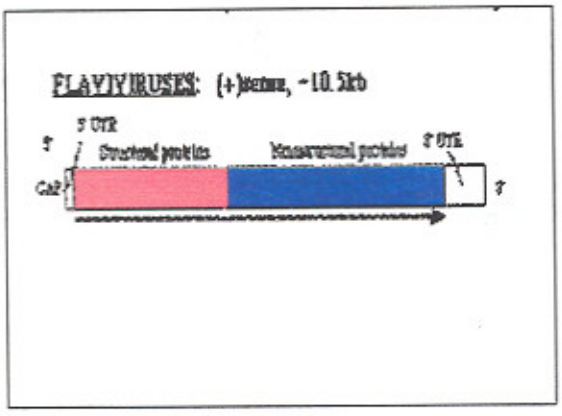


Plate:1
Genome of *Flavivirus*

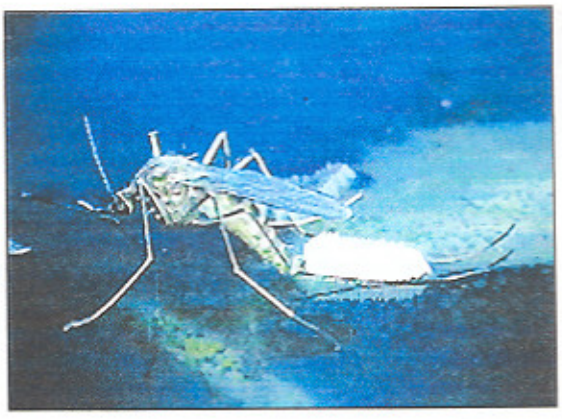


Plate:2
Culex Mosquito Laying Eggs

Plate:3
Amplifying Host (pig) of Japanese Encephalitis (JE)

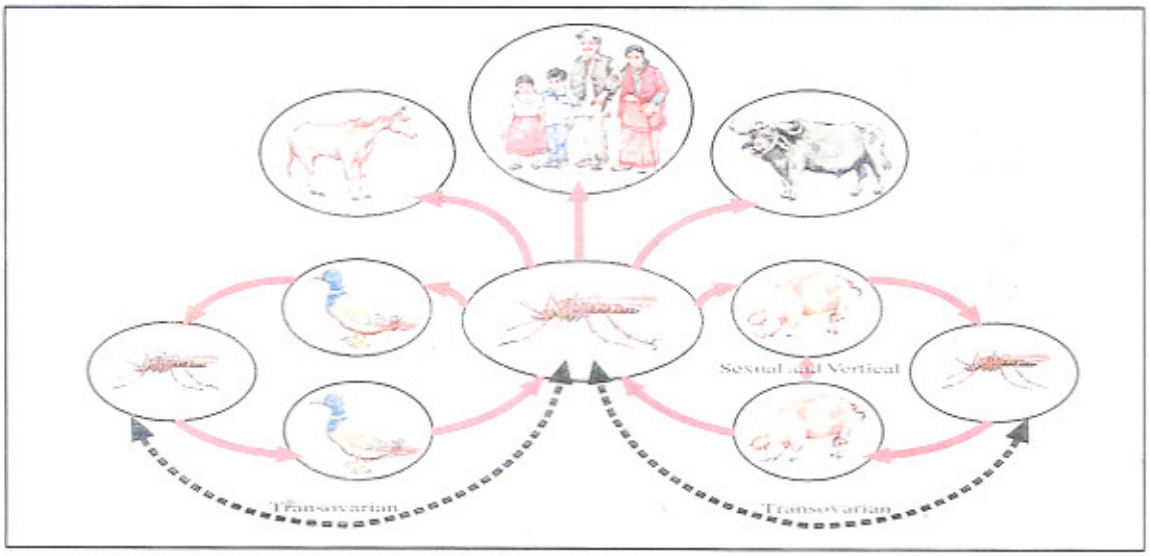


Plate:4
Japanese Encephalitis Transmission Cycle

The mosquito borne mode of JE transmission was elucidated with the isolation of JE virus 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). Entomological studies carried out during the outbreaks of 1981-1984, have shown *Culicine* mosquitoes namely *Cx tritaneorhyncus, Cx gelidus, Cx vishnui, Cx pseudovishnui and Cx fuscocephala,* as suspected vectors of transmitting JE virus both in animals and human (Pradhan, 1981; Regmi *et al.*, 1985; Khatri *et al.*, 1983). Since, *Cx tritaneorhyncus* is found in abundance in the rice field ecosystem of the endemic areas during the transmission season, and because JE virus isolate have been obtained only from a pool of *Cx tritaneorhyncus* females, this species is suspected to be the principal vector of JE in Nepal (Gubler *et al.*, 1989; Darsie *et al.*, 1989).

Culicine mosquitoes breed in irrigated rice fields, shallow marshes, ponds, pools and ditches with fresh or polluted water with grass or aquatic vegetation in partial shade or full sun. Breeding preference of *Cx tritaneorhyncus* and epidemics of JE associated with paddy field ecosystem have been adequately substantiated by different studies. Experts believe that rice fields are the probable predominant source of larval breeding in this country because they have demonstrated abundant presence of potential JE vectors in the rice fields.

Culex mosquitoes prefer to feed outdoors (exophagy) principally on vertebrate host other than human beginning at dusk and during evening hours until dawn. They feed predominantly on cattle (85-88%); 4-5% of blood meals from pigs, and 2-6% from humans. Humans are the incidental hosts.

❖ Vertebrate Hosts

It is well documented 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). Pigs are important amplifying host of JEV. Infected pigs generally do not manifest overt symptoms of illness. Virus of JE proliferates in pigs. It remains viremic for several days so that the biting mosquitoes become infected. In some places, upon 100% of pigs have anti JE antibodies.

JE virus has been recovered from human blood, but it is assumed that the level of viremia titers is not sufficiently high to infect mosquitoes. By the time patients develop signs and symptoms of JE infections, the virus disappears from the blood (Chan *et al.*, 1966; Hermon *et al.*, 1974). This might be the reason that transmission of JEV from humans has not been proved yet. They probably serve as dead end hosts.

Though Bovines, ovines and caprines have been found to be infected with JEV and the major vectors of JE feed on them, they do not appear to serve as amplifying and reservoir host as they do not develop significant virus titers (Carey, 1968; Hayashi *et al.*, 1970; Nandi *et al.*, 1982).

Horses are the only domestic animals so far known which show the signs of encephalitis due to JE virus infection. Since they are not abundant in large parts of South East Asia, they don't play a significant role in the transmission.

Bats have been shown to develop viremia.

Among birds, pond herons and cattle egrets may play an important part in the natural history of JE virus. There is no convincing evidence that migratory birds can transfer the virus from one region to another. Pigeons and sparrows can develop viremia and can infect mosquitoes.

In Nepal infection in pigs and ducks has been proved through serological studies (Joshi, 1984 and Joshi *et al.*, 1994). *Culicine* mosquitoes have been found to be breeding and growing in close association with wading birds and ducks. In a sero-survey conducted in Nepal, 40% of pig, 35% of pond herons and cattle egrets and 7% of ducks from the collected samples are found to be positive for anti JE antibody. In contrary, bovine, sheep, equines and goats are also found to be positive for anti JE antibody but there is no viremia (in too low amount in blood meal). Potential reservoir population in Nepal is given in annex-3.

❖ Seasonality of the Disease

The JEV is transmitted seasonally. In temperate regions, it is transmitted during summer and early fall, approximately from May to September. In subtropical and tropical areas, seasonal patterns of viral transmission are correlated with the

abundance of vector mosquitoes and of vertebrate amplifying hosts. These, in turn, fluctuate with rainfall, with the rainy season, and with migratory patterns of avian-amplifying hosts. In some tropical locations, however, irrigation associated with agricultural practices is a more important factor affecting vector abundance, and transmission may occur year round (annex-4).

❖ Transmission Cycle

JE is transmitted to human beings through the bite of infected mosquitoes. The maintenance and spread of JE virus appears to be mainly through a pig-mosquito-pig cycle (Gould *et al.*, 1974; Johson *et al.*, 1974) and ardeid bird-mosquito-ardeid bird cycle (Joshi *et al.*, 1998).

Mosquitoes become infected by feeding on domestic pigs and wild birds infected with JE virus. Studies suggest that virus may be transmitted transovarially in vector mosquitoes (Soman, *et al.*, 1985). Infected mosquitoes then transmit JE virus to humans and animals during the feeding process. The JEV is amplified in the blood systems of domestic pigs and wild birds.

JE virus is not transmitted from person-to-person. Only domestic pigs and wild birds are carriers of the JE virus.

❖ Clinical Spectrum

• Clinical Illness in Man

Incubation period ranges between 5-15 days. Clinical attack rate is low (1:20-1:1000) with mean rate 1:300 (Kalyanarooj, 1955). Clinical manifestations of JE vary from a mild self-limited febrile illness with headache, aseptic meningitis, to a most severe form of illness with encephalitis (Thongcharoen, 1989; Kumar, *et al.*, 1990). Illness starts with an abrupt onset of high fever and headache. More severe infection is marked by quick onset, severe headache, high fever, neck rigidity, stiffness, stupor, disorientation, coma, tremors, occasional convulsions (especially in infants) and spastic (but rarely flaccid) paralysis.

Gastrointestinal symptoms like anorexia, nausea and abdominal pain are common in children. Irritability, vomiting and diarrhoea or an acute convulsion may occur in early hours and days in infants and children. Seizure occurs in more than 75% of pediatric patients but are less frequently observed in adults (Tsai, 1994).

Although symptoms suggest raised intracranical pressure (ICP), papilledema, dexamethasone does not improve outcome (Hoke, *et al.*, 1992). Signs of extra-pyramidal involvements including tremors, mask-like facing, rigidity and chorea athetoid movements are characteristic of JE. The illness resolves in 5 to 7 days if there is no CNS involvement. The mortality in most outbreaks is less than 10%, but the rate is higher and can exceed 30% in children. Neurological sequelae are reported in upto 30% of the recovered patients (annex -5). Case fatality rates range from 0.3% to 60%. There have been reports of recurrence of symptoms of JE several months after resolution of acute illness.

Limited data indicate that JE acquired during the first or second trimesters of pregnancy causes intrauterine infection and miscarriage (spontaneous abortion). Infections that occur during the third trimester of pregnancy have not been associated with adverse outcomes in newborns.

Clinical laboratory examination shows moderate peripheral leucocytosis with neutrophilia and mild anaemia. Neutrophils may predominate in early CSF samples but a lymphocytic pleocytosis is typical. CSF protein is moderately elevated in about 50% cases. CT and MRI scans reveal low density areas and abnormal signal intensities in thalamus, basal ganglia and putamen which correlate with clinical findings of tremor, rigidity and abnormal movements that are common in the acute phase of illness. Persistent ECG abnormalities are common in the children.

- **Clinical Illness in Animals**

Infected pigs generally do not manifest overt symptoms of illness. Piglets (below 6 months of age) show non-suppurative encephalitis and nervous signs. Pregnant sow may abort or give still birth. Abnormal fetus are found in the litter with the arrest of the growth at different critical periods. Hydrocephalus interna and defect of the brain is seen in the fetuses or still births. There will be congestion and oedema

(dropsy) of scrotum of the affected boar. Perivascular infiltration of lymphocytes at mid brain and proliferation of glia cells at the cerebrum are seen in abnormal new-born piglets.

Bovines, ovines and caprines are symptomless and dead end hosts.

Horses are the only domestic animals so far known which shows the signs of encephalitis due to JEV infection. Signs and symptoms of paralysis of mouth lips, paddling of legs, unable to stand, circling, in-coordination, high fever, jaundice, rigid neck, petichiation of nasal mucosa, paralysis, convulsions are seen. Litters in the horse stable shows trace of circling movement of diseased horse. Lesions like marked congestion or haemorrhages under pia matter of the cerebrum, dilation of the blood vessels under pia matter and in parenchyma of the cerebrum and perivascular infiltration of the cells consisting of mononuclear cells at the white matter of the cerebrum are seen. Glia nodules are found at the white matter of the cerebrum. Neuronophagy are found at the grey matter of cerebrum.

❖ Diagnosis

In an area endemic to JE, when clinical features of a fever patient during transmission season resembles with JE infection, a case can be suspected as JE. Virus can hardly be recovered from blood but can be recovered from CSF in about one third of patients. The most widely used diagnostic method is IgM capture ELISA. Specific anti-JE IgM can be detected in CSF or in serum or in both in approximately 75% of patients within first four days of illness and nearly in all patients after 7 days of onset of symptoms. Moreover, presence of IgM in the CSF indicates local antibody formation associated with brain infection and is not seen in persons with asymptomatic infection with JE virus (Manath, 1988).

Conventional serological procedures like haemagglutination inhibition, complement fixation, immunofluorescence (IF) or neutralization (N) are also in practice until now. Assays for JE viral genomic sequences by polymerase chain reaction (PCR) have been developed, but clinical studies have not been reported (Tanaka, 1993). In many patients who die during the first week after onset of symptoms, the virus may be isolated from the brain, or viral antigen may be

demonstrated by IF. Virus may be isolated from CSF during the early phase of an acute illness, in such cases fulminating infection is present and prognosis is poor. Isolation from blood is uncommon.

Diagnosis of JE at National Level is guided by the following case definitions (based on recommendations of the National workshop on VBDs, 1997 and National recommended case definitions and surveillance strands, 1999):

"Any case having elevated temperature (over 38^0C), altered consciousness or unconsciousness, will be considered as **POSSIBLE MENIGITIS/ENCEPHALITIS** and be referred for Lumber Puncture."

"If the suspected case has between 50 and 1000 cells (predominantly lymphocytes per mm^3) in the CSF, it will be 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 will be considered as a **CONFIRMED CASE OF ENCEPHALITIS DUE TO JANAPESE ENCEPHALITIS VIRUS**."

❖ Treatment

There is no specific anti-viral treatment against JE. Intensive supportive therapy is indicated. Interferon-alpha, ribavirin and 6-azauridine have proven to have in vitro efficacy against pathogenic flaviviruses (Cranee, *et al.*, 2003; Solomon *et al.*, 2003)

❖ Prevention

The risk of acquiring JE can be reduced by reducing exposure to mosquito bites, particularly at dusk and dawn, when the *Culex* mosquito vector is most active.

JE vaccine is recommended for those intending to stay for long periods in rural areas where JE is prevalent (endemic or epidemic) during a transmission season,

or whose planned activities will increase the risk. The mass vaccination of children against JE has shown as an effective measure by reducing population at risk.

Though WHO does not recommended vaccination of swine population as the preventive strategy for JE saying that it is reared for 8 to 12 months only. In Nepal, most of the pig farmers are not commercialized. So, vaccinating the pigs in JE endemic areas are being suggested as it prevents the disease in amplifying host, thereby reducing the infection rate drastically in human.

V

MATERIALS AND METHODS

❖ Chemicals and Reagents Required for Anti-Japanese Encephalitis IgM Enzyme Linked Immunoassy

- Sodium Chloride (NaCl)
- Potassium Chloride (KCl)
- Sodium Carbonate (Na_2CO_3)
- Sodium bicarbonate ($NaHCO_3$)
- Sodium hydroxide (NaOH)
- 3% Hydrogen peroxide (3% H_2O_2)
- 4M Sulfuric acid (4M H_2SO_4)
- Citric acid
- Potassium Phosphate, monobasic
- Sodium phosphate, Dibasic, anhydrous
- Acetone
- Tween 20
- Goat anti-human IgM
- JE antigen
- Weak anti- JEV IgM
- Normal human serum(NHS)
- Human anti-flavivirus IgG-HRP
- O-Phenylene diamine (OPD) powder
- 13.5 % Bovine Serum albumin
- Distilled water

❖ Apparatus Required

- Microtitre ELISA plate
- Eppendorf tubes

- Micropipettes P 20, P 50, P 100, P 500
- Pipette tips
- Syringes
- Centrifuse machine
- Measuring cylinder
- Humified reaction Chamber
- Analytical or electric balance, 0.001 readability
- Incubator
- Deepfreezer/Refrigerator
- Spectrophotometer (ELISA plate reader)
- Timer
- PH meter
- Paper towels. (Blotting paper)

❖ Study Area

The study was carried out in Kanchanpur district. It is situated in the terai belt (south-western part) of Mahakali Zone between $80^0 03'$ E to $80^0 33'$ E longitude and $28^0 33'$ N to $29^0 08'$ N latitude. The total area is 1,610 km^2 (i.e. 1.09 % of national figure) and elevation ranges from 176-1,528 meters from sea level. The eastern and northern boundaries of the district are linked with Kailali and Dedeldhura district respectively, whereas western and southern boundaries with Uttaranchal Pradesh of India.

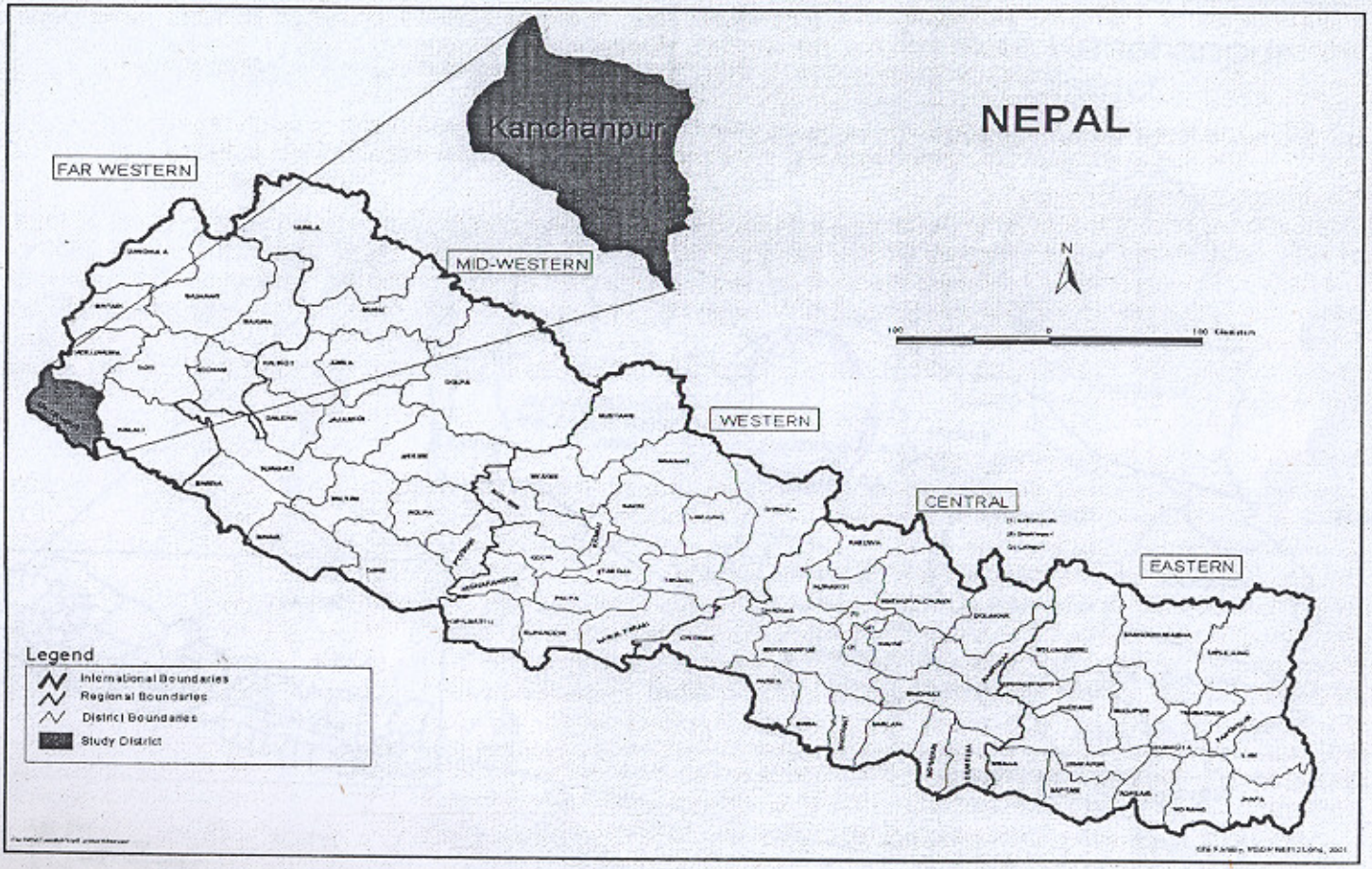
The district has tropical and subtropical type of climate. The average annual minimum and maximum temperature records are 17.5^0C and 30.5^0C respectively. During the month of December–January the minimum temperature goes 3^0C to 5^0C and in the month of May-June the maximum temperature goes up to 41^0C. Monsoon sets in the month of June and continues until September with greatest amount of rainfall in the month of July and August. The average annual rainfall is 1575 mm.

The district is divided into a Mahendranagar Municipality and 19 VDCs. District headquarter is Mahendranagar, which is 709 km far from Kathmandu and takes about 18 hours journey by bus from Kathmandu. The total population is 377,899

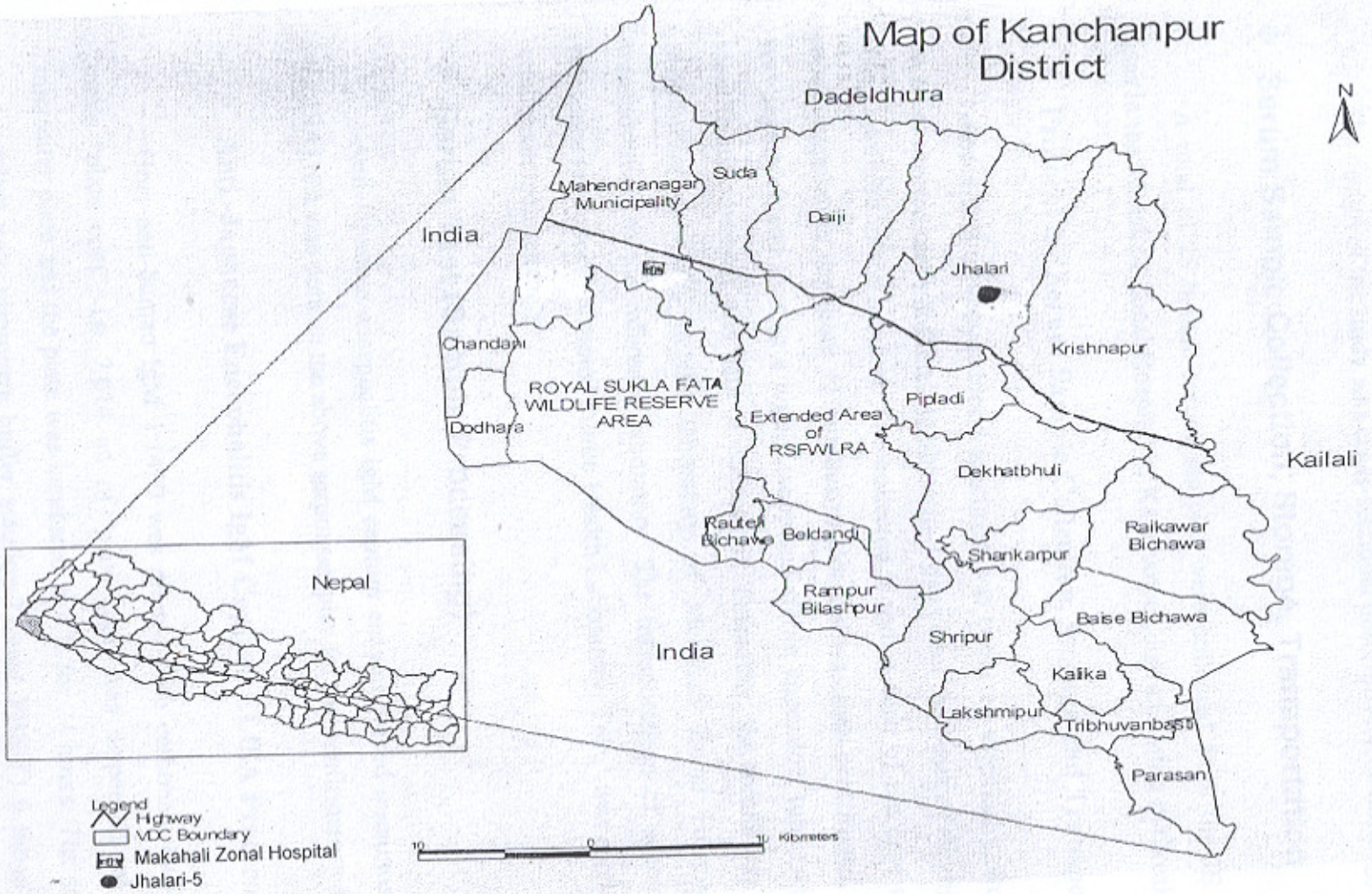
(191,910 males and 185,989 females) which stand 1.63% of national figure. population below 15 years of aged is 158,017. Annual growth rate is 3.9%. The literacy rate is 59.65% (72.07% males and 46.91% females). Total number of households are 60,158, out of which 571 have livestock, land and poultry, 209 have poultry only and 17,567 have livestock and poultry. The population of pigs/ducks in 2000 and 2001 was 20,186 and 6,445 which accounted 2.21% and 1.57% of national figure respectively. Of total land use 36.2% is cultivated land and 19.7% is irrigated land. In rural areas, each house is surrounded by paddy cultivation during rainy season. Thus, the agriculture is the backbone of the district. The district is inhabited by 80 caste/ethnic groups, majority of which are Chhetri (102,713), followed by Tharu (88,155) (DDPN, 2002).

There is a zonal hospital of 50 beds capacity in the district headquarter, which shortage of professional manpower. The district has two primary health centers without much needed physical as well as technical resources, eight health posts and ten sub-health posts. Health care delivery system is integrated through general health services (Kayastha and Shrestha, 1997). Population per doctor is 20,994 and population per hospital bed is 7,558 (DDPN,2002)

In the present study, human serum samples were collected in the Mahakali Zonal Hospital, which is located in the south-east of Mahendranagar city (district headquarter). Interviews through questionnaires were carried out among the patients able to respond or the closer guardians of JE suspected cases in the zonal hospital, and to the elders of 65 sample households in the freed Kamaiya community of ward no. 5 of Jhalari VDC. The community is located about 26 km east from the district headquarter and 6 km north from the Jhalari town of Mahendra highway. There are 440 household, 2,044 total population (1,336 males and 908 females) and is inhabited by Tharu and Sarki.



Map 5: Map of Nepal Showing Study Area



Map 6: Kanchanpur District Showing Study Areas

❖ Study Design

The design of the study was cross-sectional descriptive epidemiology.

❖ Serum Sample Collection, Storage, Transportation

A total of 25 human serum samples were collected from the JE suspected cases in the Mahakali Zonal Hospital of Kanchanpur district during outbreak season.

• Protocol for Serum Sample Collection, Storage and Transportation

About 5ml of venous blood specimen was collected aseptically, from each of the JE suspected cases in the acute phase (3-4 days after the onset of the disease) after being admitted to the hospital. The collected blood in each of the case was kept at room temperature for about 15 minutes to clot and was then centrifuged. The serum was separated and kept in a highly stoppered sterile eppendorf tube (with patients' identification number and date of collection). Thereafter, the serum was placed in a deepfreezer at -20°C in the laboratory of Mahakali Zonal Hospital prior to transportation to the reference laboratory. The transportation of specimens to the reference laboratory 'National Public Health Laboratory, Teku' was carried out under cold chain maintenance.

❖ Serum Test (Sample Processing)

Anti-Japanese encephalitis IgM capture enzyme linked immunosorbent assay (ELISA) test was done in the above serum samples for the confirmatory diagnosis.

• Anti -Japanese Encephalitis IgM Capture ELISA Protocol

Goat anti-human IgM 1:1400 was diluted with carbonate buffer PH 9.0 to make "plate coat", i.e. 7.014 ml of 'plate coat' was dispensed in each well of microtiter plate and the plate was incubated at 4^0C for 24 hours. The sensitized plate was washed with phosphate buffer solution-Tween(PBS-T) 6 times and dried by tapping (over blotting paper).

Plate:5
Mahakali Zonal Hospital: a Serum Sample Collected Area

Plate:6
Taking Interview with Guardian of Suspected JE Patient at Mahakali Zonal Hospital

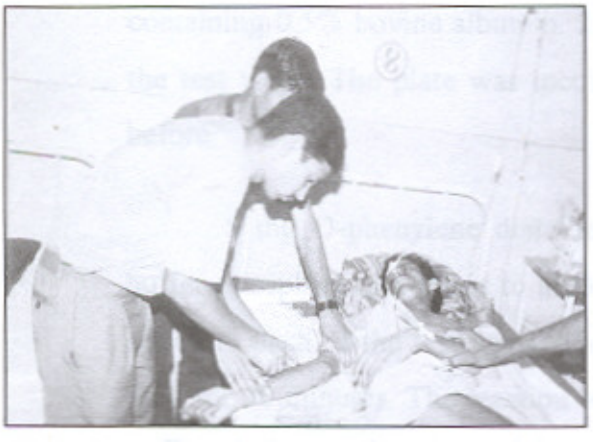


Plate:7
Collecting Blood Sample from Suspected JE Patient at Mahakali Zonal Hospital

Plate:8
Pipetting Serum Sample in the Laboratory of Mahakali Zonal Hospital

Plate:9
Storaging Serum Sample in the Laboratory of Mahakali Zonal Hospital

Plate:10
An Overview of Questionnaire Surveyed Area (Jhalari-5, 'Freed Kamaiya Community')

Normal control serum, weak positive control and test sera were diluted to 1:100 with phosphate buffer solution (PBS). 50µl of diluted control and test specimens were put into the appropriate duplicate wells of the plate and incubate at 4^0C overnight. The plate was washed with PBS-T, 6 times as before, avoiding cross contamination of wells.

JEV antigen was diluted to 1:100 by adding 50 µl of JE antigen to 5ml of 13.5% acetone extracted normal human serum (in PBS). 50 µl of JE antigen solution was put into each of test well. The plate was again incubated at 4^0C overnight and washed as before, 6 times.

Anti-flavivirus HRP was diluted to 1:350 in 13.5% acetone extracted NHS containing 0.5% bovine albumin. 25 µl of this conjugate solution was put in each of the test wells. The plate was incubated at 37^0C for 1 hour and washed 6 times as before.

5 mg O-phenylene diamine (OPD) powder was dissolved in 10 ml citrate buffer phosphate and added to it 33 µl of fresh 3% H_2O_2. 100 µl of this solution was added to each of the test wells. The plate was incubated at room temperature in the dark for 30 minutes. The reaction was stopped by adding 50ml of 4M H_2SO_4 to each well and the result was read by spectrophotometer (ELISA plate reader) at wave length 492nm.

❖ Calculations

Binding Index(BI) was calculated by using the formula

$$BI = \frac{OD\ (test\ sample) - OD\ (negative\ control)}{OD\ (WPC) - OD\ (negative\ control)}$$

$$units = BI \times 100$$

Interpretation:

The weak positive control (WPC) is defined as 100 units.

A value of anti-JE IgM ≥ 40 units is positive.

Plate:11a
Housing Condition of Questionnaire Area with Pig Reared in Open Coop

Plate:11b
Housing Condition of Questionnaire Area with Paddy Fields.

Plate:12a
Taking Interview in Questionnaire Area

Plate:12b
Taking Interview in Questionnaire Area

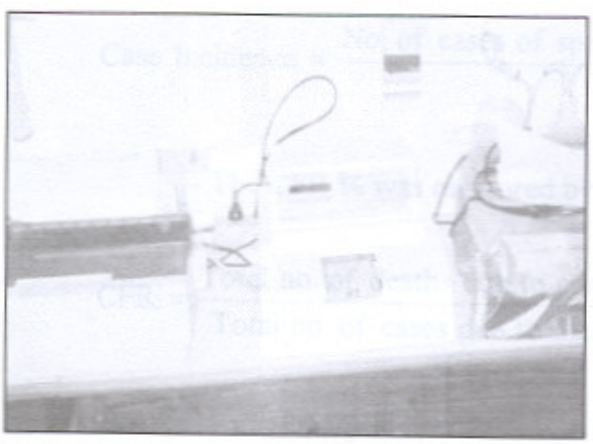


Plate:13
Spectrophotometer (ELISA Reader) with Loading Samples

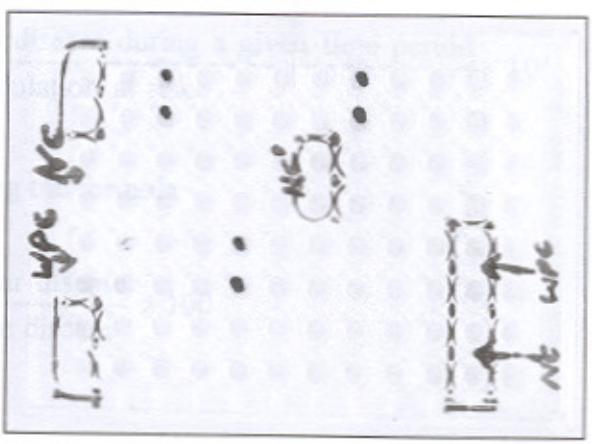


Plate:13
Microtitre ELISA Plate Showing Positive Samples

❖ Interviews Through Questionnaire

A total of 90 respondents were interviewed through structured questionnaire (Annex-6). Among them, 25 were JE suspected patients able to respond or the closer guardians of the JE suspected patients at Mahakali Zonal Hospital, and 65 were elder members of sample households in the 'Freed Kamaiya' community of ward no. 5 of Jhalari VDC. The area (Freed Kamaiya community) was selected by the hospitalization record with highest percentage of JE suspected cases (relative to population size of that area and cases deaths). Out of 440 households, 65 houses were selected in and around 2 km radius from the case clustered house by simple random sampling.

❖ Secondary Data

Record of total JE cases in Nepal, and previous years cases in Kanchanpur district were obtained from the secondary data records of Epidemiology and Disease Control Division, MoH. Vaccinated children population under 10 years of age in the district was obtained from District Public Health Office, Kanchanpur.

❖ Analysis of Data

- The case incidence was determined by using the formula

$$\text{Case Incidence} = \frac{\text{No. of cases of specific disease during a given time period}}{\text{population at risk}} \times 10^5$$

- The CFR % was measured by using the formula

$$\text{CFR} = \frac{\text{Total no. of deaths due to particular disease}}{\text{Total no. of cases due to the same disease}} \times 100$$

(Source: Park, 2002)

- The statistical analysis of data were done by using chi-square (χ^2) test

$$\chi^2 = \Sigma \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$, with n-1 df. at 95% confidence limit

Where O_{ij} = observed frequency of the cell in i^{th} row and j^{th} column

E_{ij} = expected frequency of the cell in i^{th} row and j^{th} column

(Source: Kothari, 2002)

- Effect of JE vaccination was assessed by comparing the total JE cases of age group below 14 years with vaccination history and laboratory result.

❖ Limitation of the Study

Research studies face one or more than one constraints. The present study had following limitations:

- Virus isolation from the vector, and serum collection and test of amplifying hosts viz. pigs and ducks were not carried out due to financial constraints and lack of laboratory facilities.

VI
RESULTS

The present study has been carried out in 3 different stages viz. Questionnaire survey, Clinical aspect and Laboratory diagnosis.

❖ Questionnaire Survey

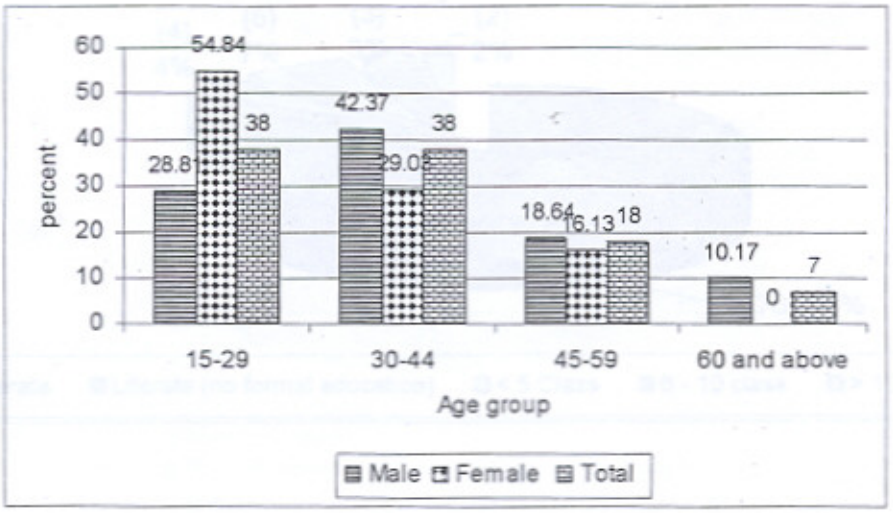
• Age and Sex of the Interviewed Respondents

A total of 90 respondents were interviewed through structured questionnaire during the study period. Among the total respondents, 38% (34) belonged to age group 15-29 years, 38% (34) to 30-44 years, 18% (16) to 45-59 years and 7% (6) to 60 and above age group. Male respondents were 66% (59) and female were 34% (31) (table no.1).

Table No. 1: Interviewed respondents: Age and Sex wise

Age groups (yrs)	Sex				Total Population	Percentage (%)
	Males		Females			
	No.	%	No.	%		
15-29	17	28.81	17	54.84	34	38
30-44	25	42.37	9	29.03	34	38
45-59	11	18.64	5	16.13	16	18
60 and above	6	10.17	0	0.00	6	7
Total	59	100.00	31	100.00	90	100

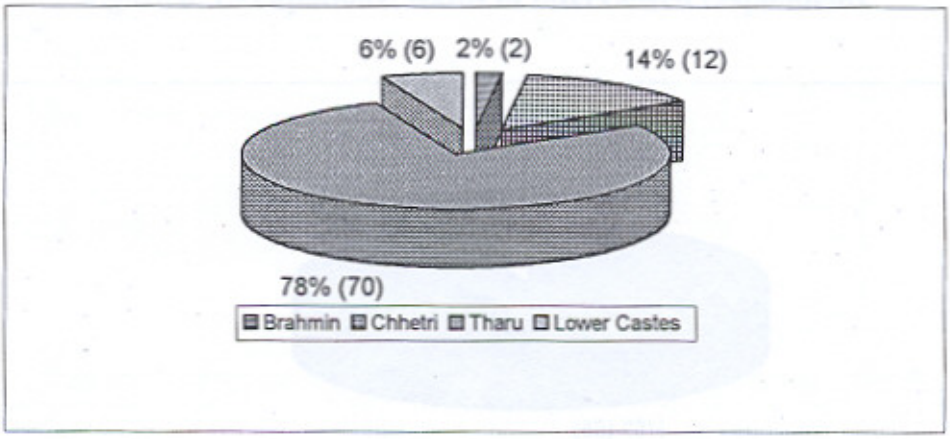
Figure 3: Interviewed respondents: Age and Sex wise



- **Caste/Ethnic Groups of the Interviewed Respondents**

The majority of the interviewed respondents were Tharu (78%), followed by Chhetri (14%), lower caste group (Sunar/Sarki/Okheda/Oad/Nepali) 6% and Brahmin were 2% as shown in figure 4.

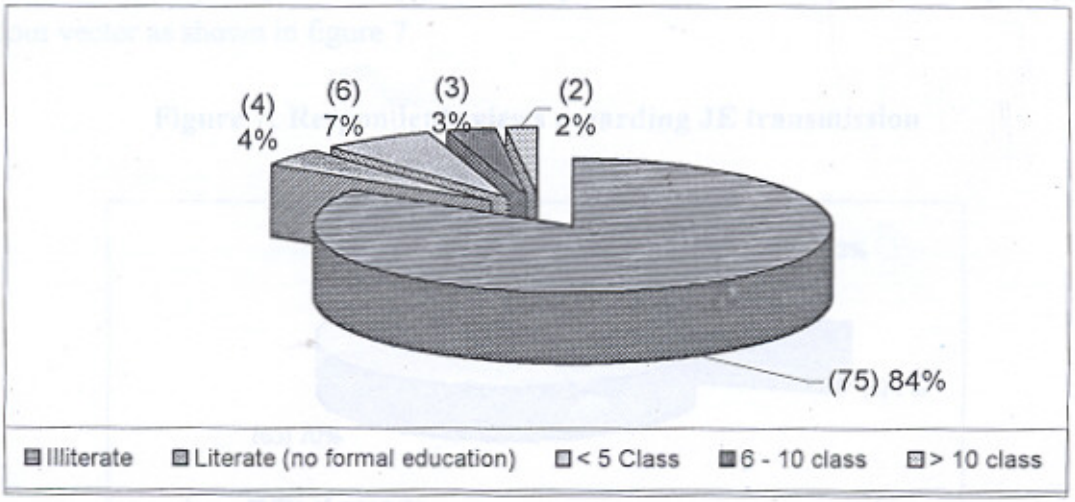
Figure 4:Interviewed respondents: Caste/Ethnic group wise



- **Educational Status of Interviewed Respondents**

Among the total interviewed respondents, 84% (75) were illiterate, 4% (4) although had no formal education were literate, 7% (6) had class ≤ 5 level of formal education, 3% (3) had 6-10 class of education and 2 % (2) had > 10 class level of formal education as shown in figure 5.

Figure 5: Educational status of interviewed respondents

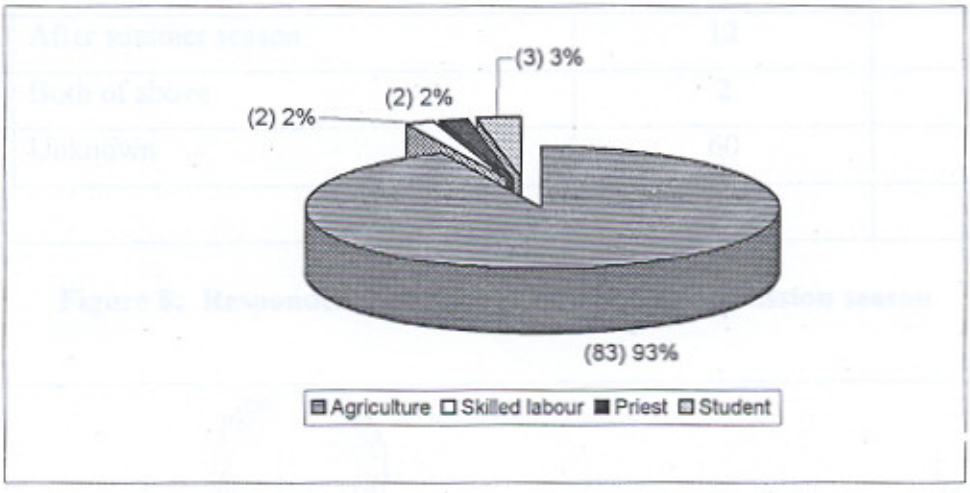


- **Occupational Status of Interviewed Respondents**

93 % (83 respondents) of the interviewed respondent's occupation was related to agriculture (farming and live stock), while 2% (2) were skilled labour, 2% (2) priest and 3% (3) were students as shown in the figure 6.

Among the total respondents, 12% reared pigs in open coop. Out of which, 2% reared their pigs without vaccination and 10% with vaccination.

Figure 6: Occupational status of interviewed respondents

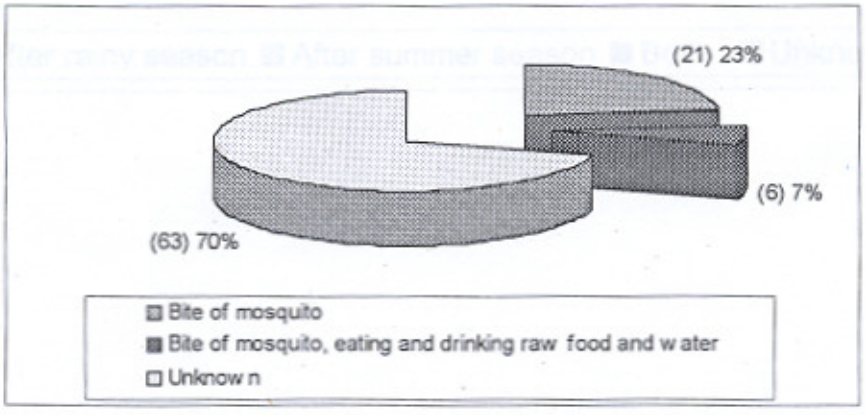


- **Knowledge of Interviewed Respondents Regarding JE**

☞ **Knowledge about JE vector**

The study showed that 70% (63) of the respondents were unaware about the vectors of JE, while only 23% (21) of respondents were aware about transmission of JE due to bite of mosquito. 7% (6) of the respondents did not have correct knowledge about vector as shown in figure 7.

Figure 7: Respondents views regarding JE transmission



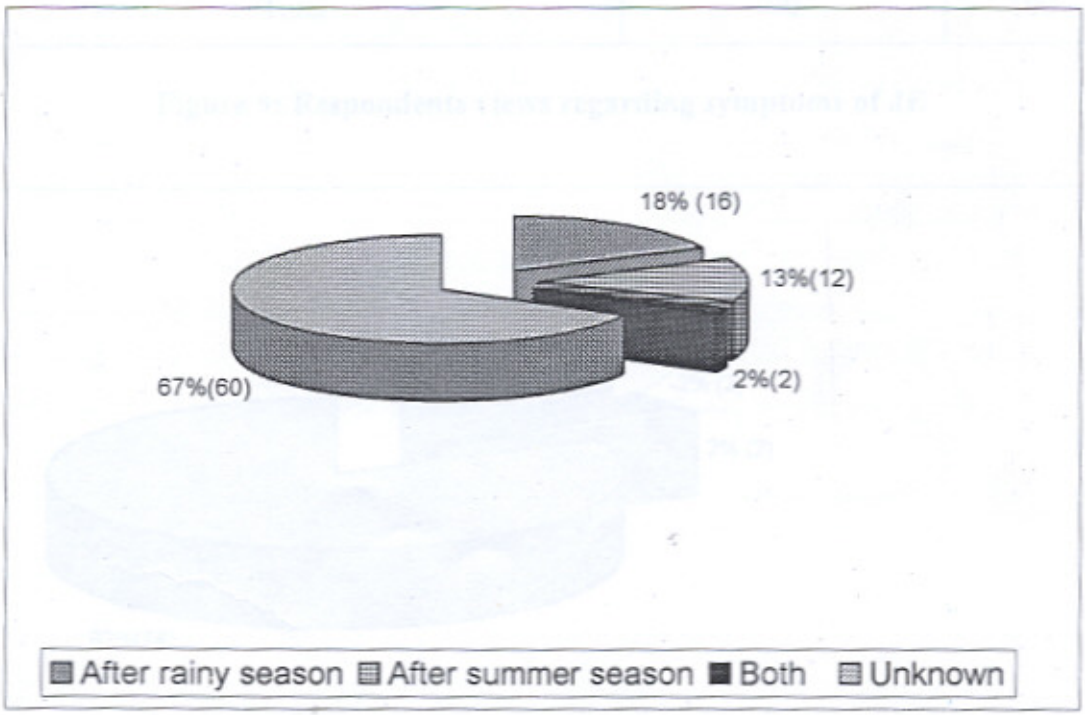
☞ **Knowledge about transmission season**

Only 18% of respondents had correct knowledge about the transmission season as shown in table no. 2.

Table No. 2: Respondents views regarding JE transmission season

S.N.	Transmission Seasons	No. of respondents	Percentage (%)
1.	After rainy season	16	18
2.	After summer season	12	13
3.	Both of above	2	2
4.	Unknown	60	67
Total		90	100

Figure 8: Respondents views regarding JE transmission season



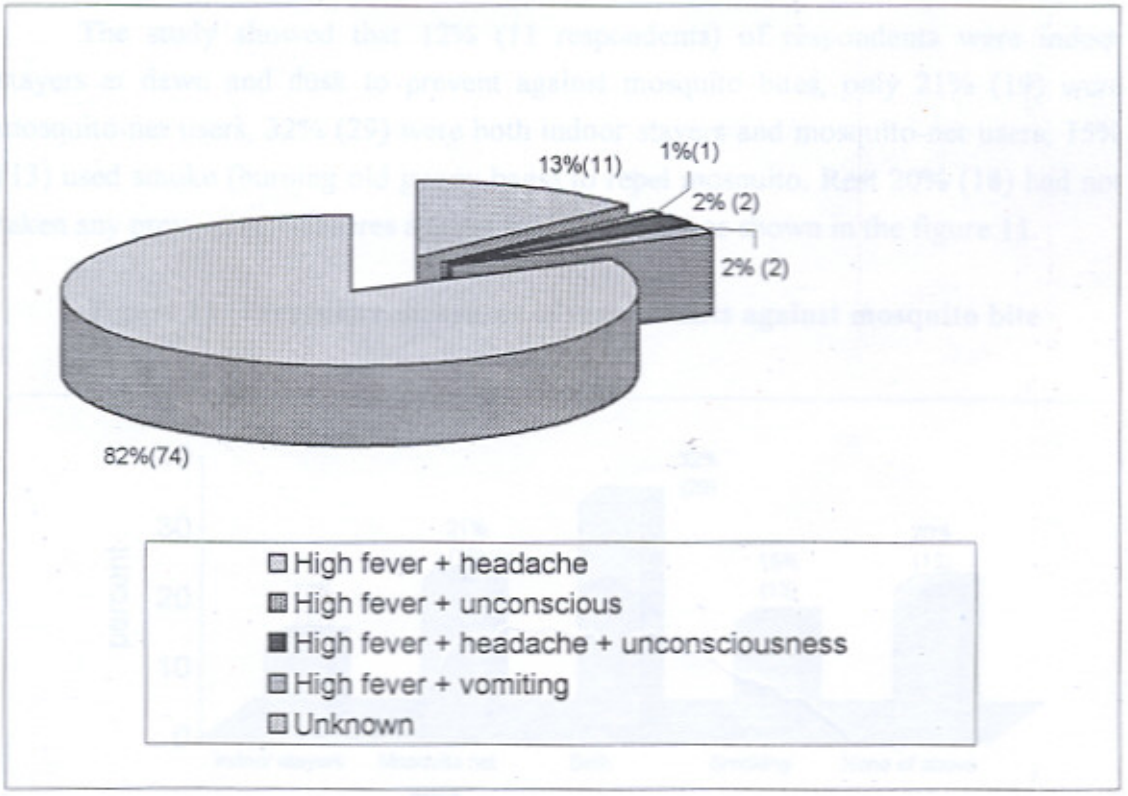
☞ **Knowledge about JE symptoms**

Among the total respondents only 18% had knowledge regarding symptoms of JE and 82% were unknown about symptoms as shown in the table no. 3.

Table No. 3: Respondents views regarding symptoms of JE

S.N.	Symptoms	No. of respondents	Percentage (%)
1.	High fever + headache	11	13
2.	High fever + unconscious	1	1
3.	High fever + headache + unconsciousness	2	2
4.	High fever + vomiting	2	2
5.	Unknown	74	82
Total		90	100

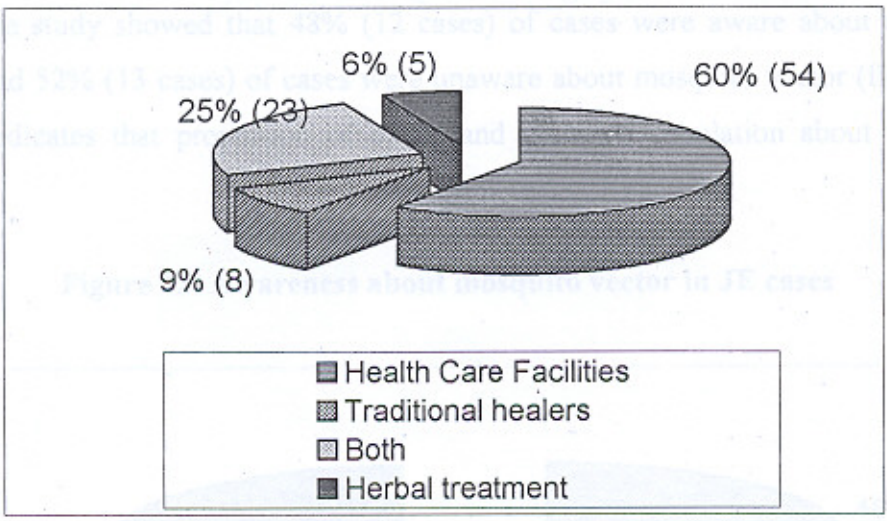
Figure 9: Respondents views regarding symptoms of JE



- **Treatment Approach**

The study showed that 60% (54 respondents) of the respondents visited the health care facilities for medical treatment, while 9% (8), 25%(23) and 6%(5) were dependent upon traditional healers (eg. dhami, guruwa), medical treatment plus traditional healers, and herbal treatment respectively as shown in the Figure 10.

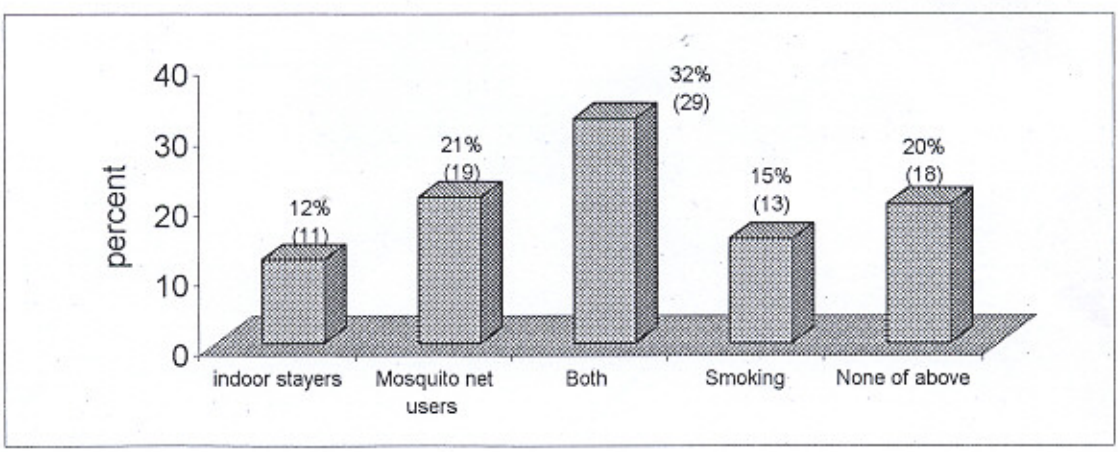
Figure 10: Treatment approaches of respondents



- **Preventive Measures Adopted by Respondents Against Mosquito Bite**

The study showed that 12% (11 respondents) of respondents were indoor stayers at dawn and dusk to prevent against mosquito bites, only 21% (19) were mosquito-net users, 32% (29) were both indoor stayers and mosquito-net users, 15% (13) used smoke (burning old gunny bags) to repel mosquito. Rest 20% (18) had not taken any preventive measures against mosquito bites as shown in the figure 11.

Figure 11: Preventive measures of respondents against mosquito bite



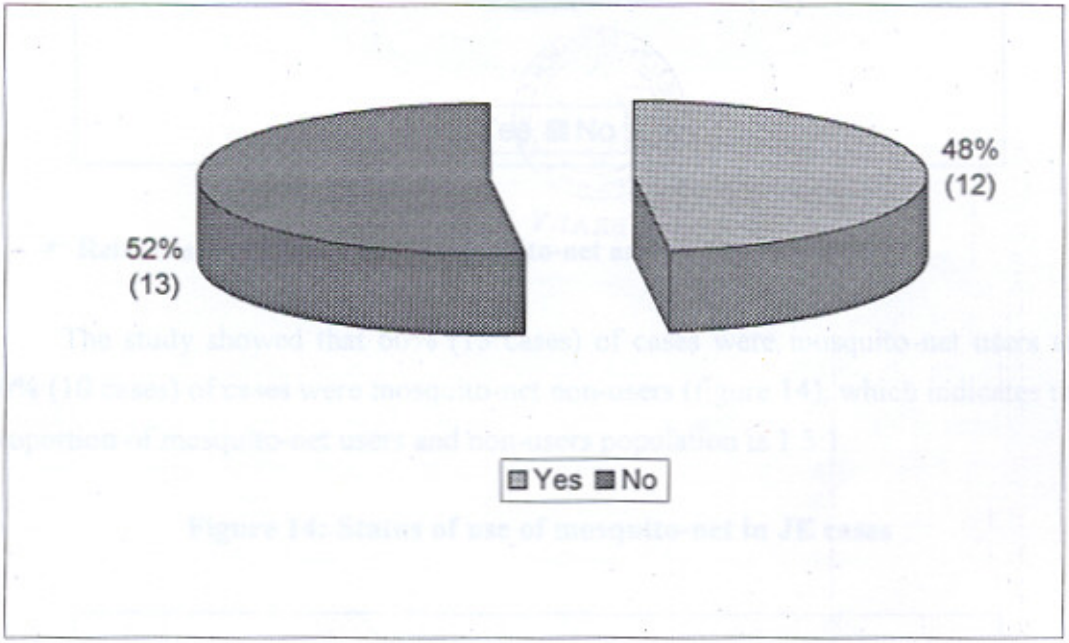
- **Correlation of JE with KAP of Respondents**

The study showed that most of the respondents were unaware about the JE and its preventive measures, but did take measures to be prevented from mosquito bites.

☞ **Relationship between vector awareness and occurrence of JE**

The study showed that 48% (12 cases) of cases were aware about mosquito vector and 52% (13 cases) of cases were unaware about mosquito vector (figure 12), which indicates that proportion of aware and unaware population about vector is 1:1.08.

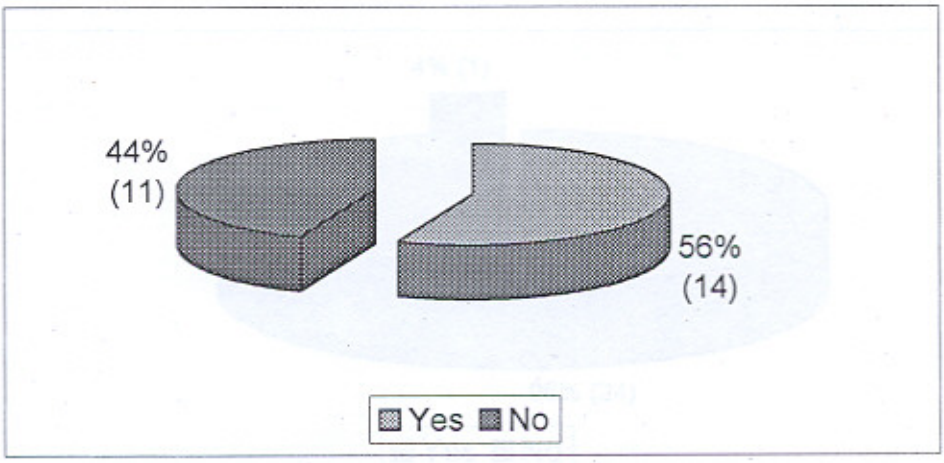
Figure 12: Awareness about mosquito vector in JE cases



☞ **Relationship between outdoor activity at dawn and dusk and occurrence of JE**

The study showed that, 56% (14 cases) of cases did outdoor activity at dawn and dusk and 44% (11 cases) of cases indoor activity at dawn and dusk (figure 13), which indicates that proportion of outdoor activity and indoor activity at dawn and dusk of population is 1.27:1.

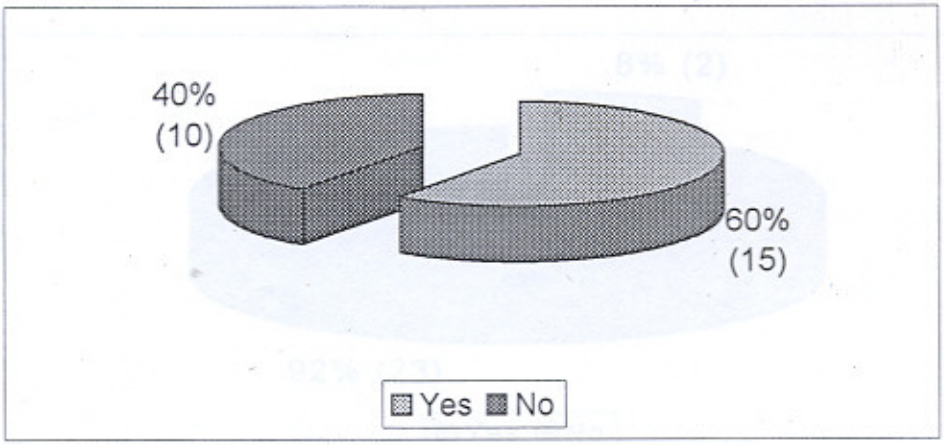
Figure 13: Outdoor activity at dawn and dusk in JE cases



☞ **Relationship between use of mosquito-net and occurrence of JE**

The study showed that 60% (15 cases) of cases were mosquito-net users and 40% (10 cases) of cases were mosquito-net non-users (figure 14), which indicates that proportion of mosquito-net users and non-users population is 1.5:1.

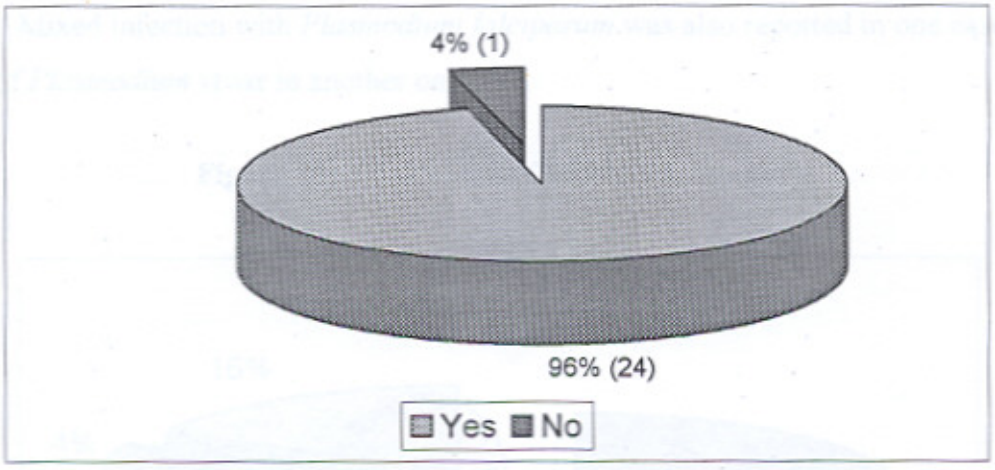
Figure 14: Status of use of mosquito-net in JE cases



☞ **Relationship between paddy cultivation around house and occurrence of JE**

The study showed that 96% (24 cases) of cases had paddy cultivation around houses during rainy season and 4% (1 case) did not have (figure 15), which indicates that proportion of paddy cultivators and non paddy cultivators around house during rainy season is 24:1.

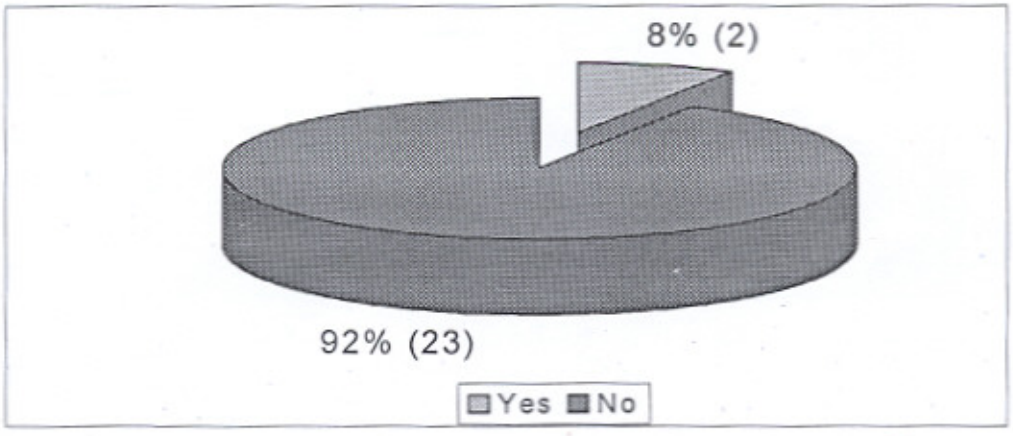
Figure 15: Presence of paddy cultivation around houses in JE cases



☞ **Relationship between presence of household pigs and occurrence of JE**

The study showed that 8% (2 cases) of cases had reared pigs in open coop by traditional method, and 92% (23 cases) did not have reared pigs in their houses (figure 16), which indicates that proportion of pig owners and no piggery is 1:11.5.

Figure 16: Presence of household pigs in JE cases

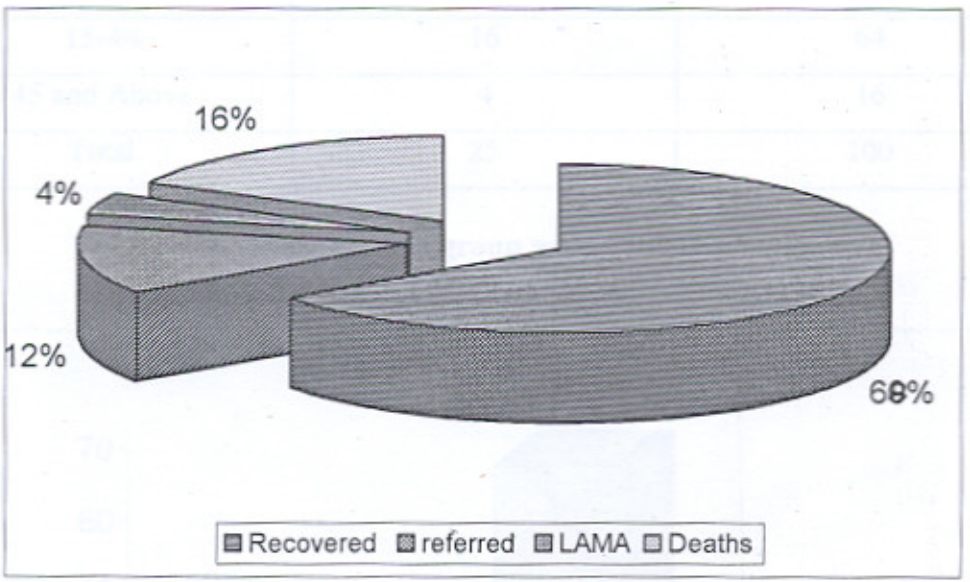


❖ Distribution of JE Cases and Deaths in Kanchanpur District, 2003

During the study period (April, 2003-November, 2003) a total of 25 cases of JE were recorded from the Mahakali Zonal Hospital of Kanchanpur district. Among them, 68% (17 cases) of cases were recovered, 12% (3 cases) referred, 4% (1 case) LAMA (Left Again Medical Advice) and 16% (4 cases) died (figure 17).

Mixed infection with *Plasmodium falciparum* was also reported in one case and that of *Plasmodium vivax* in another one case.

Figure 17: JE situation, 2003 (Kanchanpur)



- **JE Cases Distribution in Kanchanpur District, 2003**

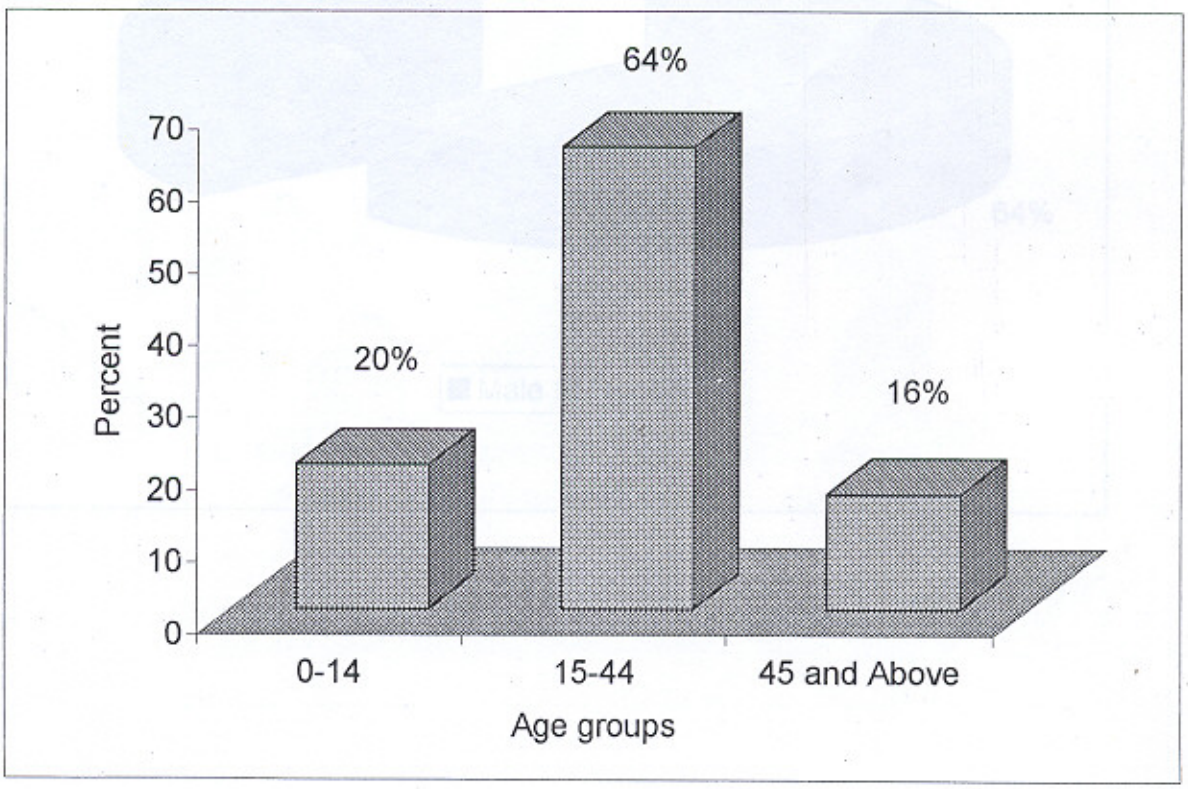
☞ **Age group wise JE case distribution**

Among the 25 cases, 20% (5 cases) of cases were belonged to age group 0-14 years, 64% (16 cases) to 15-44 years and 16% (4 cases) to 45 and above years age group as shown in the table no. 4. The result, thus, indicated that people of age group 15-44 years are at greater risk of JE infection. Statistically, the difference was found to be significant (χ^2 =10.64, $p < 0.05$) .

Table No. 4: JE cases: Age group wise, 2003 (Kanchanpur)

Age group (yrs)	Cases	Percentage (%)
0-14	5	20
15-44	16	64
45 and Above	4	16
Total	25	100

Figure 18: JE cases: Age group wise, 2003 (Kanchanpur)



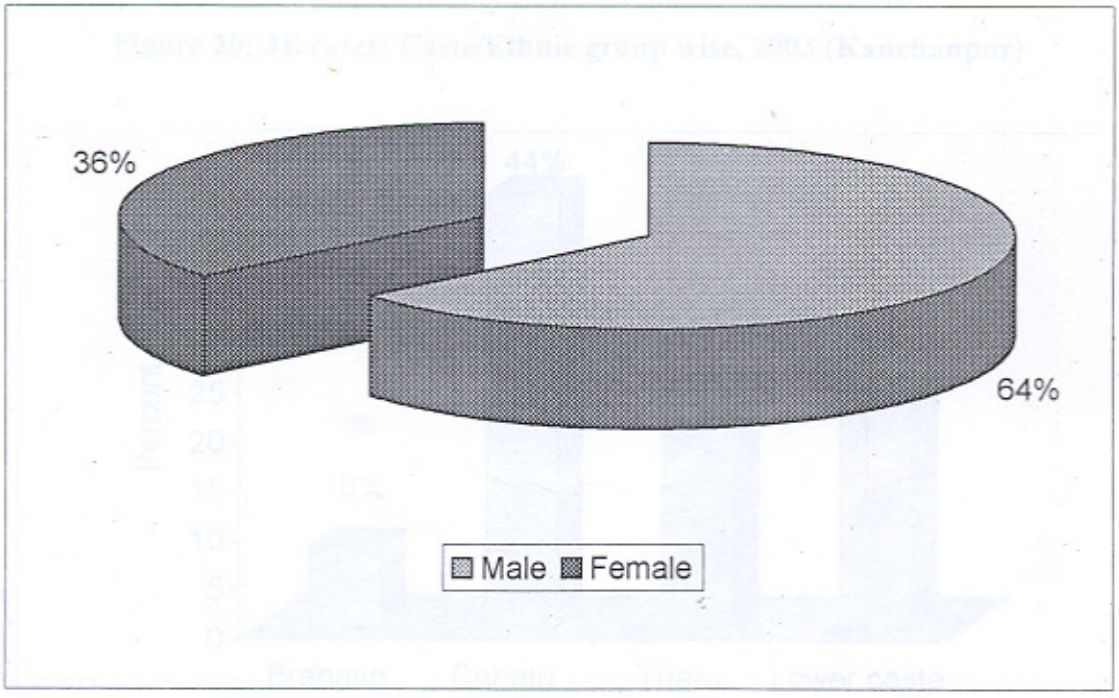
☞ **Sex wise JE case distribution**

Among 25 cases, 64% (16 cases) of cases were male and 36% (9 cases) of cases were female (table no. 5). Statistically, the difference was not found to be significant (χ^2=1.96, p>0.05). The result, thus, indicated that though males and females are in risk of JE infection but males are at greater risk.

Table No. 5: JE cases: Sex wise, 2003 (Kanchanpur)

Sex	Cases	Percentage (%)
Male	16	64
Female	9	36
Total	25	100

Figure 19: JE cases: Sex wise, 2003 (Kanchanpur)



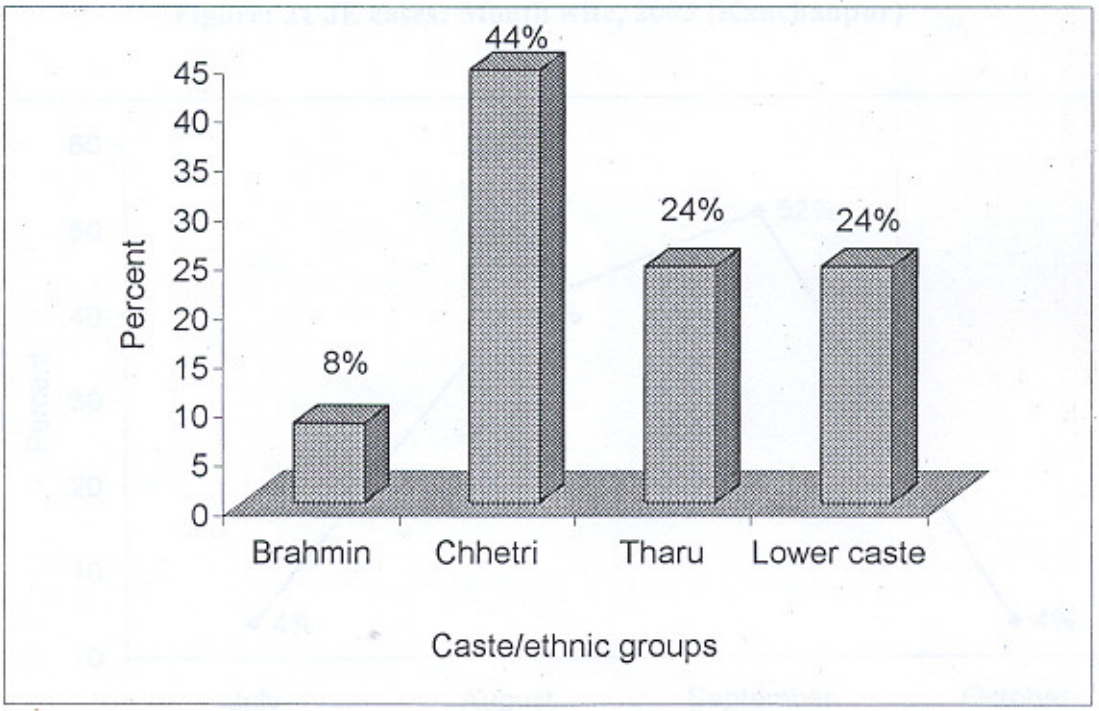
☞ **Caste/Ethnic group wise JE case distribution**

The study showed that among the 25 cases, 8% (2 cases) of cases were belonged to Brahmin, 44% (11 cases) to Chhetri, 24% (6 cases) to Tharu and 24% (6 cases) to lower caste/ethnic group (Sunar/Sarki/Okheda/Oad/Nepali) (table no. 6). Statistically, the difference was not found to be significant ($\chi^2 = 6.52$, $p > 0.05$).

Table No. 6: JE cases: Caste/Ethnic group wise, 2003 (Kanchanpur)

Caste/ethnic group	Cases	Percentage (%)
Brahmin	2	8
Chhetri	11	44
Tharu	6	24
Lower caste	6	24
Total	25	100

Figure 20: JE cases: Caste/Ethnic group wise, 2003 (Kanchanpur)



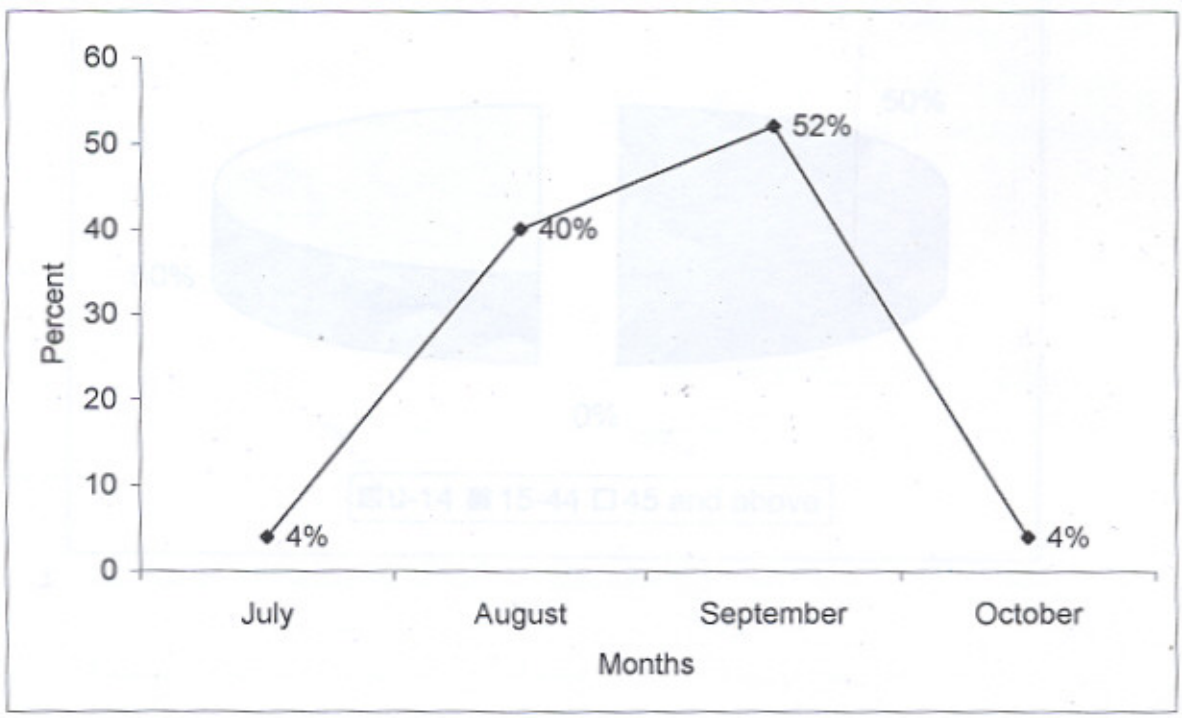
☞ **Month wise JE case distribution**

During the study period, 4% (1 case) of cases were recorded in the month of July, 40% (10 cases) in the month of August, 52% (13 cases) in the month of September, and 4% (1 case) in the month of October as shown in the table no. 7. The study showed that JE cases started in Kanchanpur district from July, reached its peak in the month of September, declined following and null off after October. Statistically, the difference was found to be significant (χ^2 =18.36, $p < 0.05$).

Table No. 7: JE cases: Month wise, 2003 (Kanchanpur)

Month	Cases	Percentage (%)
July	1	4
August	10	40
September	13	52
October	1	4
Total	25	100

Figure: 21 JE cases: Month wise, 2003 (Kanchanpur)



- **Deaths due to JE in Kanchanpur District, 2003**

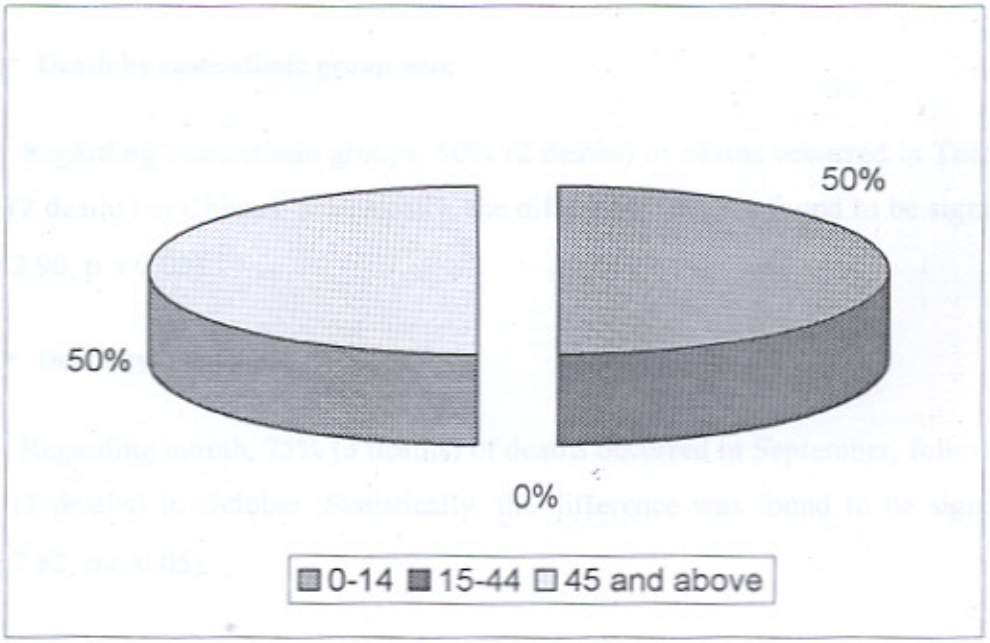
☞ **Death by age group**

Among total 4 deaths, 50% (2 deaths) of the deaths due to JE were in the age group 0-14 years, and 50% (2 deaths) in 45 and above years age group. No death occurred in 15-44 years age group (table no. 8). Statistically, the difference was found to be significance ($\chi^2 = 8.75$, $p < 0.05$).

Table No. 8: Deaths: Age group wise, 2003 (Kanchanpur)

Age groups (yrs)	Death	Percentage (%)
0-14	2	50
15-44	0	0
45 and above	2	50
Total	4	100

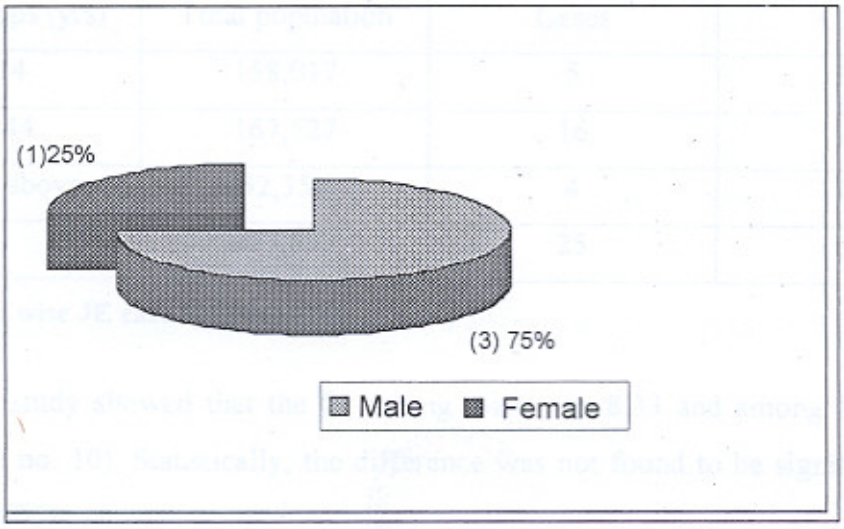
Figure 22: Deaths: Age group wise, 2003 (Kanchanpur)



☞ **Deaths by sex wise**

Regarding sex, 75% (3 deaths) of the deaths due to JE were in male sex group and 25% (1 death) in female sex group (figure 23). Statistically, the difference was not found to be significant ($\chi^2 = 1.14$, $p > 0.05$).

Figure 23: Deaths: Sex wise, 2003 (Kanchanpur)



☞ **Death by caste/ethnic group wise**

Regarding caste/ethnic groups, 50% (2 deaths) of deaths occurred in Tharu and 50% (2 deaths) in Chhetri. Statistically, the difference was not found to be significant ($\chi^2 = 2.90$, $p > 0.05$).

☞ **Death by month wise**

Regarding month, 75% (3 deaths) of deaths occurred in September, followed by 25% (1 deaths) in October. Statistically, the difference was found to be significant ($\chi^2 = 7.82$, $p < 0.05$).

- **JE Case Incidence (CI/ 10^5) in Kanchanpur District, 2003**

The study showed that the CI/10^5 due to JE in Kanchanpur district, 2003 was 6.61.

☞ **Age group wise JE case incidence/10^5**

The CI were 3.16, 9.55 and 7.64 among the age groups 0-14 years, 15-44 years and 45 and above years respectively as shown in the table no. 9. Statistically, the difference was not found to be significant ($\chi^2 = 3.17$, $p > 0.05$).

Table No. 9: JE CI/10^5: Age group wise, 2003 (Kanchanpur)

Age groups (yrs)	Total population	Cases	CI/10^5
0-14	158,017	5	3.16
15-44	167,527	16	9.55
45 and above	52,355	4	7.64
Total	377,899	25	6.61

☞ **Sex wise JE case incidence/10^5**

The study showed that the CI among male was 8.33 and among female was 4.83 (table no. 10). Statistically, the difference was not found to be significant ($\chi^2 = 0.93$, $p > 0.05$).

Table No. 10: JE CI/10^5: Sex wise, 2003 (Kanchanpur)

Sex	Total population	Cases	CI/10^5
Male	191,910	16	8.33
Female	185,989	9	4.83
Total	377,899	25	6.61

- **Case Fatality Rate (CFR%) due to JE in Kanchanpur, 2003**

The study showed that CFR% in the Kanchanpur district in 2003, was 16%.

☞ **Age group wise CFR% due to JE**

The CFR% were 40%, 0% an 50% in the age groups 0-14 years, 15-44 years, and 45 and above years, respectively (table no. 11).

Table No. 11: JE CFR%: Age group wise, 2003 (Kanchanpur)

Age groups (yrs)	Total cases	Deaths	CFR%
0-14	5	2	40
15-44	16	0	0
45 and above	4	2	50
Total	25	4	16

☞ **Sex wise CFR% due to JE**

The study showed that CFR% was 18.75% in male sex group and 11.11% in female sex group (table no. 12).

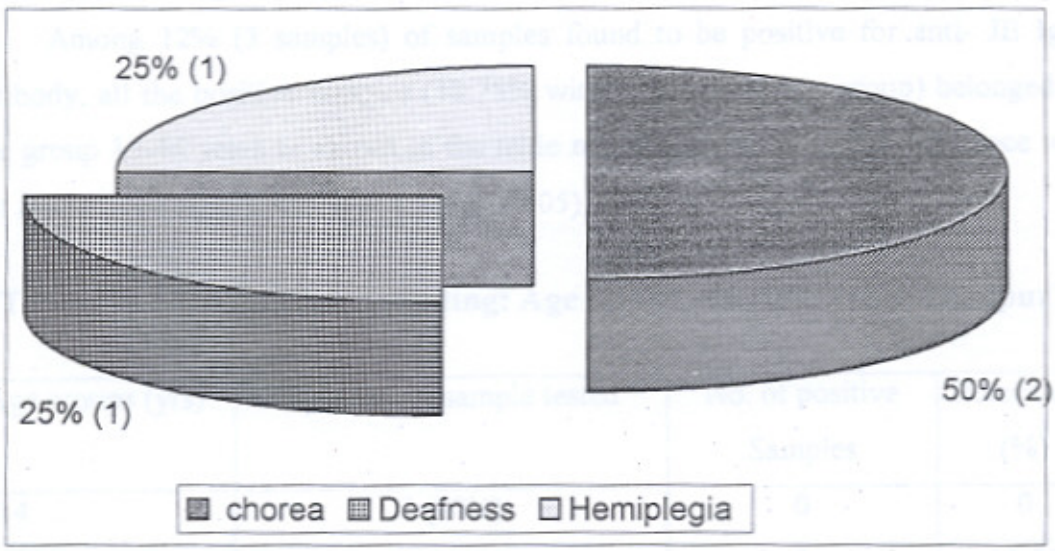
Table No. 12: JE CFR%: Sex wise, 2003 (Kanchanpur)

Sex	Total cases	Deaths	CFR%
Male	16	3	18.75
Female	9	1	11.11
Total	25	4	16

◆ **Post JE Sequelae**

Among the 17 recovered cases of 25, post JE sequelae was developed only in 24% (4 people). Among them JE Chorea was persisted in 50% (2 people) of the post sequelae developed people, followed by deafness in 25% (1) people and left side hemiplegia in 25% (1) people (figure 24).

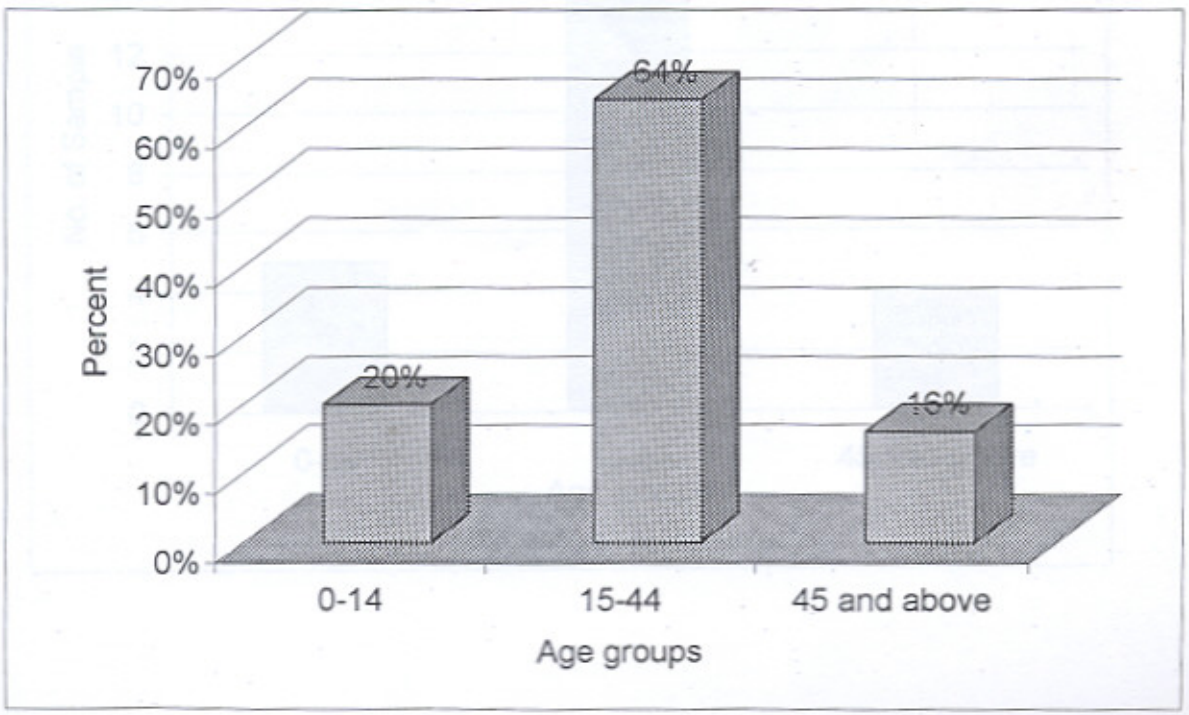
Figure 24: Post JE sequelae



❖ Laboratory Result

Among 25 serum samples collected from all the JE suspected cases presented at the Mahakali Zonal Hospital (figure 25), 12% (3 samples) of samples were found to be positive for anti- JE IgM antibody.

Figure 25: Serum sample collected: Age group wise, 2003 (Kanchanpur)



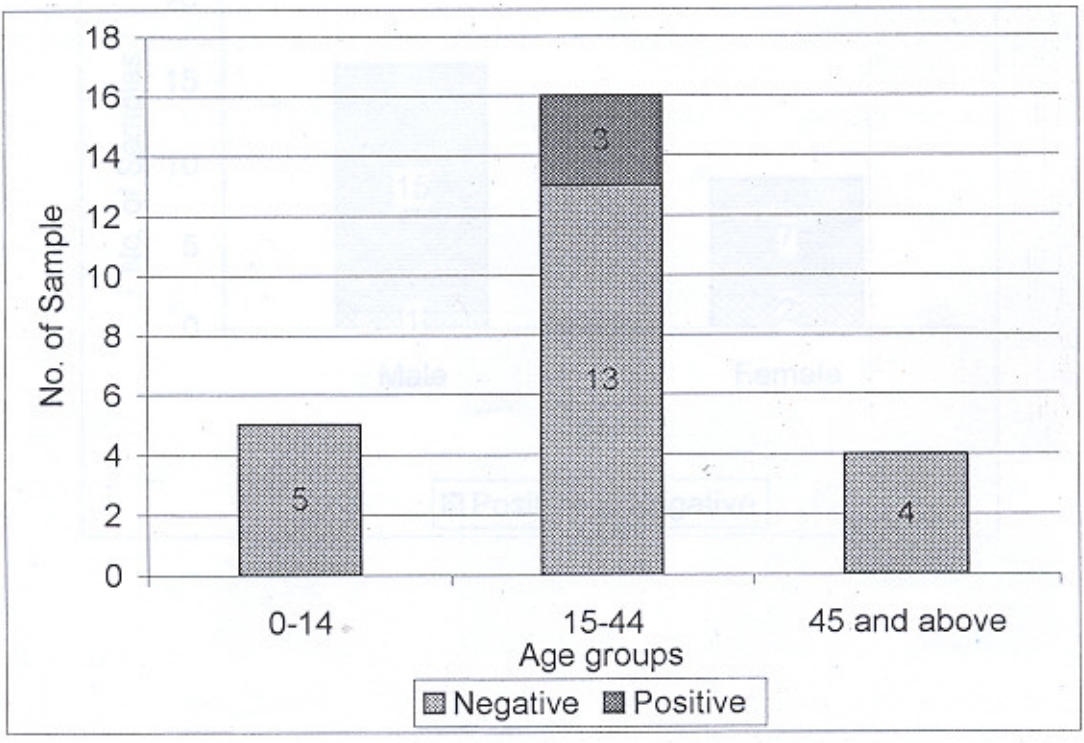
- **Age Group Wise Laboratory Result**

Among 12% (3 samples) of samples found to be positive for anti- JE IgM antibody, all the positive samples (18.75% with in the same age group) belonged to age group 15-44 years as shown in the table no. 13. Statistically, the difference was not found to be significant ($\chi^2 = 1.91$, $p > 0.05$).

Table No. 13: Laboratory finding: Age group wise, 2003 (Kanchanpur)

Age groups (yrs)	Total no. of sample tested	No. of positive Samples	Percentage (%)
0-14	5 (20%)	0	0
15-44	16 (64%)	3	18.75
45 and above	4 (16%)	0	0
Total	25	3	12

Figure 26: Laboratory finding: Age group wise, 2003 (Kanchanpur)



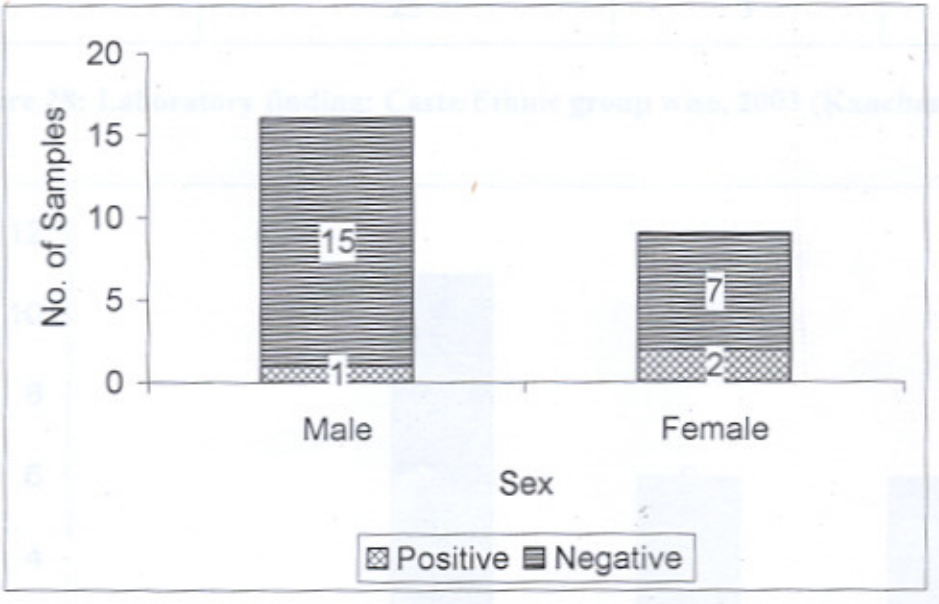
- **Sex Wise Laboratory Result**

Among the 3 (25%) samples found to be positive, 1 (6.25% within the same sex) sample was found to be positive in male sex group and 2 (22.22% within the same sex) samples in female sex group (table no. 14). Statistically, the difference was not found to be significant ($\chi^2 = 3.31$, $p > 0.05$).

Table No. 14: Laboratory finding: Sex wise, 2003 (Kanchanpur)

Sex	Total no. of sample tested	No. of Positive Samples	Percentage (%)
Male	16 (64%)	1	6.25
Female	9 (36%)	2	22.22
Total	25	3	12

Figure 27: Laboratory finding: Sex wise, 2003 (Kanchanpur)



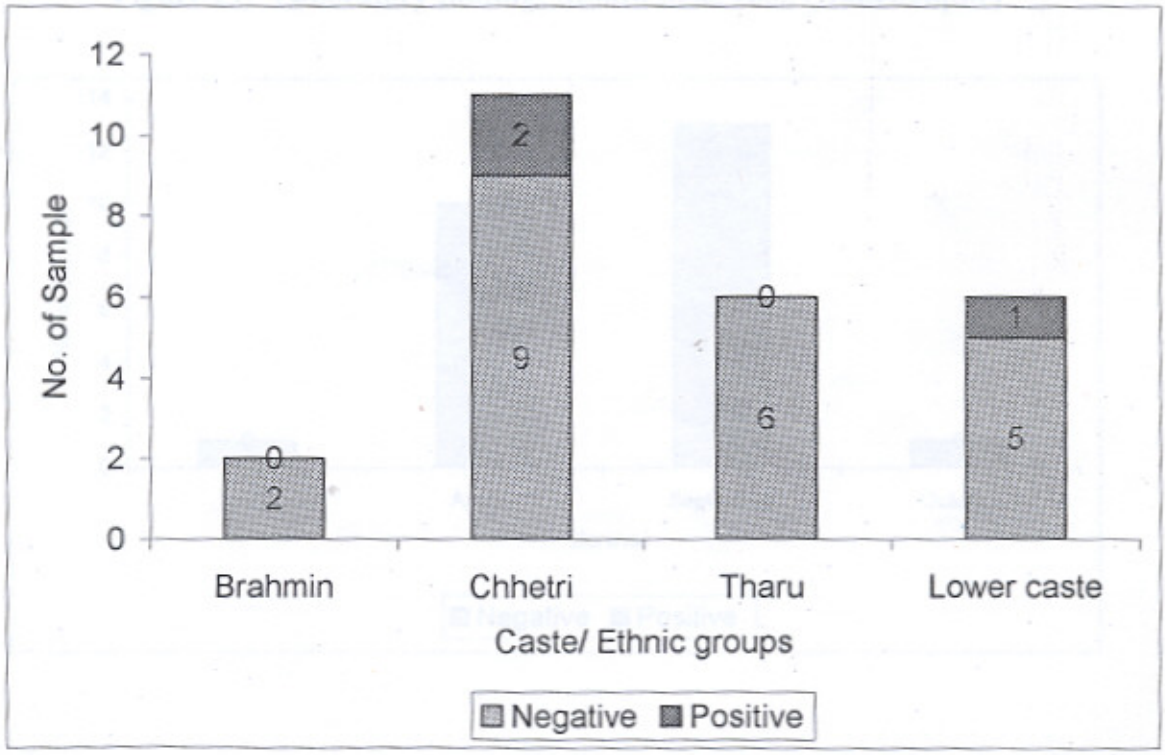
- **Caste/Ethnic Group Wise Laboratory Result**

Among 3 positive samples, 2 (18.19% with in the same caste) samples were found to be positive in Chhetri and 1 (16.7% within the same caste) among the lower caste group (Sunar/Sarki/Okheda/Oad/Nepali) as shown in the table no. 15. Statistically, the difference was not found to be significant ($\chi^2 = 1.61$, $p > 0.05$).

Table No. 15: Laboratory finding: Caste/Ethnic group wise, 2003 (Kanchanpur)

Caste/ethnic group	Total no. of sample tested	No. of positive Samples	Percentage (%)
Brahmin	2(8%)	0	0
Chhetri	11(44%)	2	18.19
Tharu	6(24%)	0	0
Lower caste	6(24%)	1	16.7
Total	25	3	12

Figure 28: Laboratory finding: Caste/Ethnic group wise, 2003 (Kanchanpur)



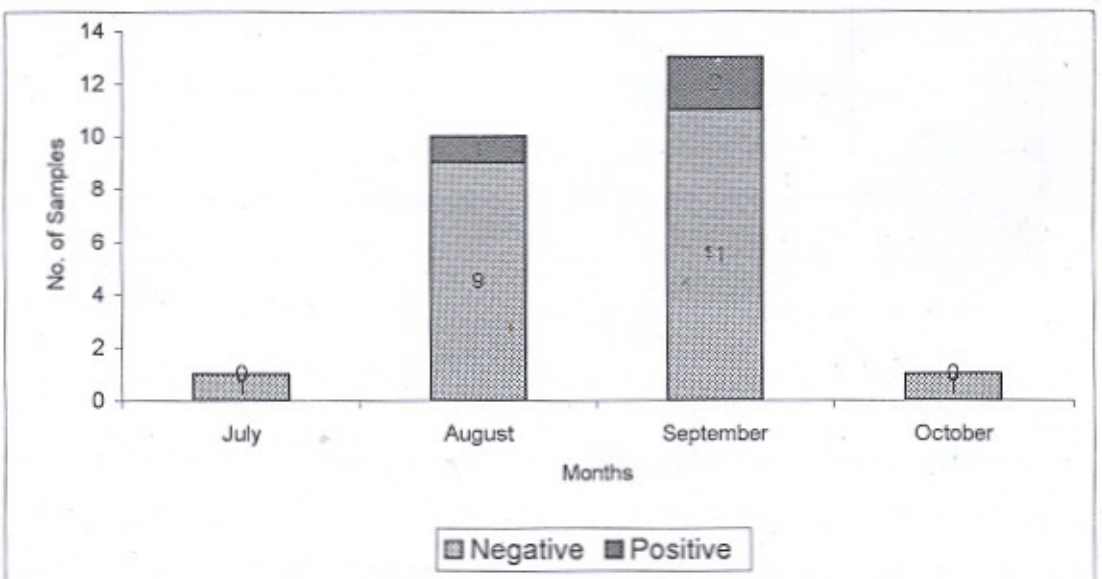
- **Month Wise Laboratory Result**

Out of 23 suspected patient samples obtained during month of August and September (2003), 3 (12%) samples were found to be serologically positive. Among 3 (12%) positive samples, 1 (10% within the same month) positive sample was found in August and 2 (15% within the same month) in the month of September (table no. 16). Statistically, the difference was not found to be significant ($\chi^2 = 0.45$, $p > 0.05$).

Table No. 16: Laboratory finding: Month wise, 2003 (Kanchanpur)

Month	Total no. of sample tested	No. of the positive samples	Percentage (%)
July	1(4%)	0	0
August	10(40%)	1	10
September	13(52%)	2	15
October	1(4%)	0	0
Total	25	3	12

Figure 29: Laboratory finding: Month wise, 2003 (Kanchanpur)



❖ Effect of JE Vaccination in Kanchanpur District

During the study period, 20% (5 cases) of cases were recorded below the age group 14 years, but all were non-vaccinated (according to guardians history). In the laboratory report, all the samples were found negative for anti-JE IgM antibody (table no. 17).

Table No. 17: Immunization status of below 14 years JE cases, 2003 (Kanchanpur)

Immunization Status	Cases	Lab result
Non-vaccinated	5(20%)	-ve for anti JE IgM antibody
Vaccinated	0	-
Total	5	-

VII

DISCUSSION AND CONCLUSION

Japanese encephalitis (JE) is one of the most serious public health problem in Nepal since 1978, because of the severity of the disease and high mortality rate. Therefore, it is important to investigate the actual circumstances of the prevalence of JE virus infection in Nepal.

The present study was conducted from April, 2003 to November, 2003 in Kanahcnpur district of the country. In this study, an attempt was made to determine the case incidence (CI) and case fatality rate (CFR%) due to JE on the basis of provisional diagnosis (clinical symptoms) and laboratory result (confirmed cases); to know the public awareness about JE and to assess the effect of JE vaccination.

A total of 25 JE suspected cases were recorded in the study period and their serum samples were collected during acute phase of illness at Mahakali Zonal Hospital and tested by anti-JE IgM capture ELISA test. The present recorded cases stand 2.89% of national figure and lower than the previous year (2002) which was 4.03% (EDCD). The district has tropical an subtropical type of climate conditions but the low morbidity of the disease may be due to little awareness about the disease and its control methods among the residents leading to lower attendance of patients to the hospital. Another reason for the lower attendance of JE patients to the hospital may be due to their believe in dhami/ guruwa and also because of their economic problems. Further more beliefs of residents on foreign (India) hospitals rather than national hospitals and consequently visiting there for the treatment. Moreover, low population of amplifying hosts (pigs) which is 2.21% and 1.57% of national figure in the year 2000 and 2001 respectively and mass vaccination in the district may be another factors of low morbidity in the district.

Among the total clinical cases, 68% (17 cases) of cases were recovered, 12% (3 cases) referred, 4% (1 cases) Left Again Medical Advice (LAMA) and 16% (4 cases) died. Mixed infection with *plasmodium falciparum* was also reported in 4% of cases and that of *P. vivax* in another 4% of cases. None of the cases were vaccinated. Among the 17 recovered clinical cases, JE sequlae was developed only in 24% (4

cases) of cases. Among them, JE chorea was persisted in 50% (2 cases) of the post sequelae developed cases, followed by deafness in 25% (1 cases) of cases and left side hemiplegia in another 25 % (1 case) of cases. The possibility in the development of JE sequelae may be due to delayed hospitalization.

Ziemmerman *et al.*, (1997) reported 15 patients with meningoencephalitis in Kathmandu valley first time during September and October, 1995.

Saito *et al.*, (1999) reported three JE cases in US Marines Stationed on Okinawa island, Japan, 1991.

Victor *et al.*, (2000) reported three cases of JE for the first time from two villages of Dharmapuri district (Krishnagari Health Unit) in Tamil Nadu during November, 1999. Two children died and one developed neurological sequelae.

Rao *et al.*, (2000) reported a total of 873 cases with 178 deaths in the Andra Pradesh JE epidemic during October-November, 1999.

Thakare *et al.*, (2002) reported 7 cases of JE during the study on prevalence of West Nile virus infection in India.

Ayukawa *et al.*, (2004) reported 6 patients from early August to mid September, 2002 in the Chugoku district of Japan. Five Patients, except for one without sequelae, had a severe outcome, including one death.

The laboratory results of present study revealed that only 12% (3 cases) of cases were found to be serologically positive for anti-JE IgM antibody. The study demonstrated that JE was less prevalent in the district. Akiba *et al.*, (2001) showed that of 53 blood samples collected from suspected JE cases during the epidemic period in 1998, anti-JE IgM antibody were serologically confirmed in an average 78% of patients in three collaborating hospitals of south-western part of Nepal. Their studies demonstrated that JE was highly prevalent in the area and clinical diagnoses were reliable.

Rai *et al.*, (1987) reported 4 confirmed JEV infection cases out of 154 serum samples collected from the staff and students of the Tribhuvan University, Teaching Hospital (TUTH), Kathmandu by haemagglutination inhibition (HI) and neutralization (N) tests. All were from the terai.

Kubo *et al.*, (1996) observed JEV seropositivity in 15.4% (98/638) of the study population by neutralization method in individuals visiting TUTH, Kathmandu.

In the present study, among 3 seropositive laboratory confirmed cases, mixed infection with *P. falciparum* was reported in a 17 years old female and JE sequale (left side hemiplegia) was developed in 38 years old male who was admitted to the Mahakali Zonal Hospital in unconsciousness state from the India-Nepal border street after 3 days.

Neogi *et al.*, (1998) studied on HIV seropositivity during epidemic outbreak of JE during mid 1995 in Manipur. Of the 16 serum samples from patients with history of febrile headache, convulsions, mental confusion, neck rigidity, etc., 12 (75%) showed HIV antibody titre between 1:40 and 1:60. Out of these 12 sera showing HIV antibody, 8 (66.6%) showed IgM antibody, living the presumptive diagnosis of recent JEV infection. Five of these 16 sera showed HIV seropositivity (31.25%). Concomitant JEV and HIV infection could be detected in 3 cases. However in 2 sera HIV titre were less than 1:20.

Yoshida *et al.*, (1999) reported 2 serologically confirmed JE cases out of 12 patients, by using IgM- capture ELISA both on serum and CSF of the patient in Bali, Indonesia.

Rao *et al.*, (2000) reported that out of 873 JE cases 93.75% of serum samples showed the evidence of JE virus infection in the Andra Pradesh JE epidemics during October-November, 1999.

Johansen *et al.*, (2001) reported two confirmed human JE cases in the Torres Strait, Australia and Cape York Peninsula, in northern Queensland, 1998, during entomological investigations.

Watt and Jongsakul (2003) reported 22 (14%) of confirmed JE cases in a cohort of 156 adults presenting to a hospital in Chaingmai, Thailand.

In the present study, none of the clinical JE cases of questionnaire carried out in the community (i.e. 'free kamaiya community' of ward no. 5 Jhalari VDC) were serologically confirmed. This may reflect to immunization of pigs in the month of June 2003 in collaboration with National Labour Association (NLA).

In the present study, the suspected JE cases were categorized into three age groups, viz., 0-14 years (children), 15-14 years (economically active age group), and 45 and above years (elder age group). Among these age groups, the highest clinical cases (64%) were recorded in the 15-44 years age group, followed by 0-14 years (20%), and 45 and above years (16%). Statistically, the difference was also found to be significant (χ^2=10.64, p< 0.05).

The possible factors related to age difference in JE cases may be as follows: those of 15-44 years age group are considered to be the most physically fit to do laborious jobs (i.e. economically active age group) and hence spend most of their time outdoor activities and thus have a higher chance of being bitten by mosquitoes. The susceptiability of children of 0-14 years age group may reflect more outdoor play activity, particularly at dusk, when *Culex tritaeniorhynchus* feeds most actively and the fact that the overall level of anti-JE immunity is still relatively low in this age group, but comparatively low cases in the present study may be due to mass vaccination carried out in the district during 2001 and 2002 and its effectiveness. The susceptibility in the elderly people i.e. 45 and above age group consistent with waning immunity with increasing age, but relative low cases in the present study may be due to staying of most of time indoor during dusk and dawn thus have a less chance of being bitten by JE vector.

Joshi *et al.*, (1995) recorded 68.5% (1,058) of children cases out of 1,545 total JE cases from 1990 to 1993 during the epidemiological survey of JE in all endemic areas of Nepal.

Solomon *et al.*, (1998) reported acute JEV infection in 12 (55%) of the 22 children with acute flaccid paralysis, compared with only one (1%) of 88 age matched

hospital controls to a referral centre in Ho Chi Minh city, Vietnam. This study is different from the present study.

Lowry *et al.*, (1998) reported acute JE in 31 (67%) of the 46 pediatrics acute encephalitis syndrome (AES), compared with only two (6%) of 33 adult AES in the patients admitted to the neurology service of Bach Mai Hospital, Hanoi, Vietnam between June 5 and August 3, 1995.

Victor *et al.* (2000) reported HI antibodies to JEV is 8.9% children and IgM antibodies to JEV in 3 children out of 146 sera samples from children aged below 15 years in two villages of Dharmapuri district, Tamil Nadu, during November 1999.

Vijayarani and Gajanana (2000) reported low rate of JE infection, 1.8% and 5.1% in two consecutive transmission season of 1991-92 and 1992-93, among rural children aged 5-12 years in Thanjavur district (Tamil Nadu), an area with extensive paddy cultivation.

Rao *et al.*, (2000) reported that age groups 1-14 years including infants had been affected but nearly 86.8% of cases had been among 1-9 years age group out of 873 cases in Andra Pradesh JE epidemic during October-November, 1999.

Chokephaibulkit *et al.* (2001) reported JE in 6 children out of the viral agents identifiable in 26 (65%) of 40 children during a prospective study of childhood encephalitis in Bangkok from 1996 to 1998.

The overall present clinical case incidence (CI) was 6.61 cases per 100,000 population, which stands 0.21% of national figure and lower than the previous (2002) year (8.98 cases per 100,000 population) in the district. It may reflect to mass vaccination in the district and consequently low children cases. At present the highest CI (9.55 cases per 100,000 population) was in the 15-44 years age group, followed by 45 and above years age group (7.64 cases per 100,000) and 0-14 years (3.16 cases per 100,000). However, statistically, the difference was not found to be significant (χ^2=3.17, $p > 0.05$).

In the present study, all the 20% (5 cases) of cases of under 14 years of age were unvaccinated against JE (according to guardians history). All the cases were

serologically negative for anti-JE IgM antibody, which indicates highly effectiveness of past years (2001 and 2002) JE vaccination in the Kanchanpur district.

Bista *et al.*, (2001) found that of several cases of JE admitted to Bheri Zonal Hospital, Nepalgunj from early August, 1999, 20 children aged 1-15 years were resided in the villages, which had JE vaccination campaign. None of 20 JE cases had received JE vaccine compared with 326 of 557 age sex matched village controls.

Konishi and Suzuki (2002) reported that the ratio of subclinical to clinical infections in vaccinated population was estimated to be 2000,000:1, which was 2,000-80,000 times higher than the ratio previously reported for unvaccinated populations, during their study in Japan.

All the serolgically confirmed 12% (3 cases) of cases of the present study belonged to age group 15-44 years. However, none of the cases of the age groups, 0-14 years, and 45 and above years, were found to be serologically positive for anti-JE IgM antibody. But, statistically, the difference was not found to be significant (χ^2=1.91, p>0.05), for which we do not have explanation and this needs to be investigated further with larger sample sizes.

In the present study, clinical cases were recorded more in the male (64%) than in females (36%). The result, thus, indicated that though males and females are in risk of JE infection but males are at greater risk. The proportion of male to female cases was 1.8: 1. The CI was found higher (8.33 cases per 100,000) in males than in female (4.83 cases per 100,000). This may due to more outdoor activities of males leading to more chances of mosquito bites. Moreover, females have less approach to health care facilities in comparison to males. But, statistically, the difference was not found to be significant (χ^2=0.93, p> 0.05).

Parajuli *et al.* (1992) reported that the male were affected about 1.5 times more than females in all age groups (except infants) in the epidemiological study of JE in all epidemic districts of Nepal during the year 1989. This study findings is matched with the present study.

Chattopadhay (2001) diagnosed 162 cases as JE with predominance in male sex and lower age group in the hospitals of Arunachal Pradesh from 1986 to 1995.

Of the 3 serologically confimed JE cases of the present study, one case was male and 2 were females. But, there was not significant statistical difference (χ^2=3.31, $p > 0.05$) for which we do not have explanations and this needs to be investigated further with larger sample sizes.

Thakare *et al.*, (1999) detected IgM antibodies to JE virus in the sera of 5 cases of 52 suspected viral encephalitis cases admitted to the Government Hospital, Singli district, Maharashtra from June to December, 1997. All age groups and both genders were affected.

Regarding caste/ethnic groups, the highest clinical cases of the present study were recorded in Chhetri (44%), followed by Tharu (24%) and lower caste group (24%), and Brahmin (8%). Of the 3 serologically confirmed cases, 2 cases belonged to Chhetri and 1 to lower caste/ethnic group. But statistically, the difference was not found to be significant (χ^2=1.61, $p > 0.05$). It is simply due to the higher number of cases visiting the hospital.

Among the total clinical JE cases of the present study, the highest number of the cases were recorded in he month of September (52%), followed by August (40%), July (4%) and October (4%). Statistically, the difference was also found to be significant (χ^2=18.36, $p < 0.05$). Of the 3 serologically confirmed JE cases, 2 were recorded in the month of September and one in the month of August but not statistically significant (χ^2=0.45, $p > 0.05$) The case burden in the respective months may be related to environmental factors (i.e. rainfall, temperature, paddy cultivation) and consequently JE vector density.

Parajuli *et al.*, (1992) recorded higher number of cases in June/July and peak in September/October in the epidemiological study of JE in all epidemic districts of Nepal during the year 1989.

Joshi *et al.*, (1995) observed the months from July to October as the peak period for JE cases in the epidemiological survey of JE in all endemic areas of Nepal from 1990-1993.

Chattopadhay (2001) recorded maximum JE cases between June to October in the hospitals of Arunachal Pradesh from 1986 to 1995.

In the present study, the case fatality rate (CFR%) was 16% (4 deaths out of 25) which stands 0.47% of national figure. The present CFR% was some what higher than previous (2002) years (14.70%) in the district. It may due to delays in hospitalisation of cases.

Parajuli *et al.*, (1992) carried out epidemiological study of JE in all epidemic districts of Nepal during the year 1989 and reported that an average CFR% was 26.6%, however, the area wise CFR% varied from 6.4 to 66.7%.

Shoji *et al.*, (1994) reported a high mortality due to JE, 4 of 10 patients in the Kurume region of Kyushu island, Japan from 1984-1990. A low mortality due to JE, none of 4 patients, was reported in the same region during the outbreak of 1991-1993 (Shoji *et al.*, 2002).

Zimmerman *et al.*, (1997) reported overall 53% mortality due to menigoencephalitis among 15 treated patients in Kathmandu valley, Nepal.

Rao *et al.*, (2000) reported that the overall CFR% had been 18.4% in the Andra Pradesh JE epidemic during October-November, 1999.

In the present study, the highest mortality occurred in the 0-14 years age groups (50%), followed by 45 and above years (40%). No deaths occurred in 15-44 years age group. Statistically, the difference was also found to be significant (χ^2=8.75, $p < 0.05$).

The highest mortality in the 0-14 years age group may reflect to low immunity, low health care practices and delays in disease complaints to guardians leading to hospitalization. The mortality in the elderly age group (45 and above years) consistent with waning immunity. The null mortality in the economically active age

group 15-44 years may be due to high immunity and timingly health care facilities approaches.

Of the total deaths, the highest deaths occurred in the male sex group (75%) followed by female (25%), consequently higher CFR% was in male (18.75%) than in females (11.11%). This may reflect higher morbidity of male sex recorded in the hospital than females. But statistically, the difference was not found to significant ($\chi^2 = 1.14$, $p > 05$).

Joshi *et al.*, (1995) reported that CFR% were 36.0%, 38%, 35.2% and 31.7% in 1990, 1991, 1992 and 1993 respectively in the epidemiological survey of JE in all endemic areas of Nepal.

- Akiba *et al.*, (2001) conducted an epidemiological study of JE outbreak in the south-western part of Nepal in 1997 and reported that the case fatality rate was 13.2% and there was no difference in the fatality rate between male and female over 5 years old.

Regarding months, the highest mortality occurred in the month of September (75%) followed by October (25%) during the present study period. It may reflect to morbidity rate in the respective months. Statistically, the difference was also found to be significant ($\chi^2=7.82$, $p<0.05$).

However, the CFR% was zero among three serologically confirmed cases in Kanchanpur district during 2003.

To know the public awareness about JE, a total of 90 respondents were interviewed through structured questionnaire. Among them, 84% of respondents were illiterate, and 93% had agriculture (faming and livestock) as an occupation. About 12% of them rears pigs in open coop, out of which, 2% rears their pigs without vaccination and 10% with vaccination.

Among the interviewed respondents, only 23 % had correct knowledge about JE vector, 18% had correct knowledge regarding JE transmission season and JE symptoms. About 60% of respondents visited the health care facilities (national as well as international) for medical treatments, while 9% to traditional healers (eg.

dhami, guruwa/bharra), 25% medical treatment plus traditional healers, and 6% herbal treatment. To be prevented against mosquito bites, 12% of respondents were indoor stayers at dawn and dusk, 21% mosquito-net users, 32% both indoor stayers and mosquito-net users, 15% used smoke (burning old gunny bags) to repel mosquito and 20% had not taken any preventive measures against mosquito bites due to economic problems.

The present study, thus, revealed that most of the respondents were unaware about the JE and its preventive measures but did take measure to be prevented from mosquito bites.

The study also showed the following relationship between KAP (knowledge, attitude and practice) of cases and occurrence of JE:

The proportion of JE cases having awareness and unawareness about JE vector was 1:1.08, which indicates the risk of JE is more among those who were unaware about JE vector.

The proportion of JE cases having outdoor activity and indoor activity at dawn and dusk was 1.27:1, which revealed that the risk of JE more among those who did outdoor activity at dawn and dusk. Gurung *et al.*, (2003) reported that 38.7% of JE cases were outdoor sleepers in Banke, Bardia and Dang districts in their study. Their study showed that outdoor sleepers are at 2.09 times greater risk of developing JE infection in comparison to indoor sleepers.

In the present study, the proportion of mosquito-net users and non-users JE cases was 1.5:1. This may reflect to lack of knowledge about proper use of mosquito-net and also use of non-insecticide impregnated bed-nets. The study agreed with the findings of Lowry *et al.*, (1998). They found that use of bed net nearly universal among their study participants in Bach Mai Hospital, Hanoi, Vietnam, however, it appeared to confer little or no protection against JE. In studies conducted in central China, bed netting without chemical impregnation did not protect children against JE, but pyrethroid treatment of bed nets in late May or June conferred protective efficacies of 28-48% (Dapeng *et al.*, 1994a and 1994 b). It has been well established

that the mosquito vector of JE, *Cx. tritaeniorhynchus*, tends to bite much more frequently outdoors than indoors, which would explain why such household protection measures as bed netting, window coverings or DDT residual indoor spraying are likely to have only limited impact (Dapeng *et al.*, 1994a; Mitchell *et al.*, 1973).

Gurung *et al.*, (2003) found that 60.6% of JE cases were mosquito-net non-users in their study on Banke, Bardia and Dang districts. Their study showed that mosquito-net non-users are at 2.6 times greater risk of developing JE in comparison to mosquito-net users. This result does not agree with the present study.

In the present study, the proportion of JE cases having paddy cultivation and no paddy cultivation around house during summer-rainy season was 24:1, which revealed that paddy cultivation increase the risk of JE by favoring vector breeding. Service (1991) suggested that during peak population densities of mosquito vector, *Cx. tritaeniorhynchus*, it has been estimated that a single paddy field can produce more than 30,000 adult mosquitoes in one day in subtropical areas of South East Asia such as northern Vietnam. Sherer *et al.*, (1959) and Paul *et al.*, (1993) did not identified paddy cultivation as specific risk factor because in their case control studies in Japan, rice farming and living in close proximity to a paddy were common place among case and control-patients.

In the present study, the proportion of JE cases having household pigs and not having pigs was 1:11.5. This appears to be attributive to plenty of rice (paddy) cultivation and other suitable environmental conditions for the breeding of mosquitoes of genus *Culex* that acts as vector for JEV. Further, this is probably due to the very wide distribution of *Cx. tritaeniorhynchus*. However, Tsai (1994) and Baily *et al.*, (1975) suggested that centralization of pig husbandry has had an effect on reducing JE cases in Korea and Japan. Further, the present study does not agree with the study of Gurung *et al.*, (2003). They reported pigs in households of 45.1% cases in their study period on Banke, Bardia and Dang districts and suggested that households having pigs

are at 1.51 times higher risk of acquiring JE infection in comparison to subjects not having household pigs.

The observed data suggest that the JE problem in Nepal will be progressively worse if effective intervention strategies are not implemented. Unfortunately, before effective prevention and control can be implemented, more information must be obtained on the epidemiology of JE virus in Nepal including Kanchanpur district.

VIII

RECOMMENDATION

1. Mass vaccination of non-vaccinated children is strongly recommended thereby reducing population at risk to reduce morbidity and mortality greatly.
2. Immunization of amplifying hosts of JE (like pigs) is suggested as a control measure for reducing the risk of JE. Moreover, there should be restriction on free movement of pigs to be controlled by local authority and pig shed should be wire mesh screened. In country like Nepal, prevention of JE in reservoir may be regarded as the best intervention approach to control the disease.
3. To reduce the vector contact, use of insecticide impregnated mosquito (bed) nets is strongly suggested. Moreover, reduction of outdoor activities, discouraging to sleep outside the house during summer and rainy season, and unexposing body parts by wearing long sleeves clothes should be highlighted to protect the people from mosquito bites.
4. Intermittent irrigation and periodic flushing of rice fields during rice cultivation should be followed.
5. Use of biological control methods viz., microbial agents like *Bacillus thuringiensis* and *B. Sphaericus*, and introduction of carnivorous fishes like *Gambusia affinalis* etc are recommended to control JE vectors rather than chemical ones.
6. The upgrading of laboratory facilities at hospital as well as national level and reviewing guidelines and trainings to the clinicians regarding the diagnosis of JE is strongly recommended.
7. There is a general lack of knowledge about JE in the district. Considerable mass awareness and public health education campaigns are thus conducted to change public perception of communities and VDCs level about the disease problem, it's emergence and for its prevention and control.
8. Environmental and entomological studies in relation to the endemicity of JE particularly with respect to vector, larval habitat, temperature, humidity etc as well as amplifying hosts should be carried out.

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ANNEX-1

Table No. 1

Distribution of JE cases, Deaths and CFR% during period 1978-2003, Nepal.

Year	No. of Cases	Deaths	CFR%
1978	422	199	28.2
1979	182	49	26.92
1980	622	234	37.14
1981	54	16	29.63
1982	843	399	46.26
1983	242	36	14.88
1984	142	45	31.69
1985	629	183	29.09
1986	1615	415	27.5
1987	502	140	27.89
1988	1403	380	27.08
1989	868	227	26.15
1990	365	102	27.95
1991	650	145	22.22
1992	702	127	18.09
1993	446	108	24.22
1994	1836	385	20.86
1995	1246	257	20.63
1996	1450	263	18.14
1997	2953	407	13.78
1998	1161	149	12.83
1999	2934	434	14.84
2000	1729	169	9.77
2001	1401	155	14.5
2002	842	168	19.95
2003	865	142	16.41

Table No. 2

Distribution of JE Cases, Deaths, CI andCFR% during period 1992-2002, Kanchanpur district.

Year	No. of Cases	Deaths	CI/10^5	CFR%
1992	51	3	19.77	5.89
1993	1	0	0.39	0
1994	50	3	19.38	6
1995	43	4	16.68	9.30
1996	21	2	8.14	9.52
1997	96	7	37.22	7.29
1998	85	10	32.96	11.77
1999	138	13	53.5	9.42
2000	118	8	45.76	6.78
2001	160	22	42.33	13.75
2002	34	5	8.98	14.70

(Source: Epidemiology and Disease Control Division, MoH)

Table No. 3

Inactivated Cell culture JE vaccination Kanchanpur (0, 7, 180 days): 2001-2002

Year	Population Coverage		
	1st dose	2nd dose	3rd dose
2001	98,004	91,062	87,555
2002	15,700	12,438	11,504

(Source: District public Health Office, Kanchanpur)

ANNEX-2

Mosquito species naturally infected with JE Virus

Genus	Species	Place	Authors
Aedes	*albopictus*	Fukein, China	Wu *et al.*, 1957
	curtipes	Sarawak	Macdonald *et al.*, 1967
		Sarawak	Simpson *et al.*, 1970,1974
Aedes	*esoensis*	USSR	Chagin *et al.*, 1943
	togoi	USSR	Chagin *et al.*, 1943
	vexans	Japan	Fukumi *et al.*, 1975
		Japan	Hayashi *et al.*, 1966,1970
		Korea	Lee *et al.*, 1971
		Japan	Shichijo *et al.*, 1968,1970
Anopheles	*annularis*	Philippines	Ksiazek *et al.*, 1980
	barbirostris	India	Chakravarty *et al.*, 1975
	hyrcanus	West Bengal	Chakravarty *et al.*, 1975,1979
	(nigherrimus)	Karnataka	
	subpictus	South India	Dhanda *et al.*, 1980
Armigeres	*subalbatus*	Japan	Fukumi *et al.*, 1975
		Japan	Shichijo *et al.*, 1968
Culex	*bitaeniorhynchus*	India	Banerjee *et al.*, 1979
		Philippines	Ksiazek *et al.*, 1980
	epidesmus	West Bengal	Banerjee *et al.*, 1975
	fuscocephala	Japan	Okuno *et al.*, 1975
		Thailand	Gould *et al.*, 1974
		Thailand	Muangman *et al.*, 1972
		Thailand	Okuno *et al.*, 1975
	gelidus	Sarawak	Macdonald *et al.*, 1967
		Thailand	Gould *et al.*, 1969
	gelidus	Thailand	Simasathien *et al.*, 1972
			Simpson *et al.*, 1970,1974
	pseudovishnui		Fukumi *et al.*, 1975
	pipiens	Japan	Hayashi *et al.*, 1970
	quinquefaciatus		Chen *et al.*, 1957
		China	Chagin *et al.*, 1943
		USSR	Fukumi *et al.*, 1975
		Japan	Hayashi *et al.*, 1966
			Maedao *et al.*, 1978

		Taiwan	Lien *et al.*, 1980
		Korea	Thoa *et al.*, 1974
		China	Tasi, S.t. *et al.*, 1957
Culex	*Tritaeniorhychus*	Japan	Buescher *et al.*, 1959
			Fukumi *et al.*, 1975
		India	Carey *et al.*, 1968
		Sarawak	Simpson *et al.*, 1974
		Japan	Takahashi *et al.*, 1966
		Nepal	Leake, 1986 litt.
		Japan	Wada *et al.*, 1976
	vishnui	Japan	Ura, 1977.
		Japan	Hayashi *et al.*, 1965,1968
		Taiwan	Lien *et al.*, 1976,1986
	whitmorei	Andrapradesh	Rodrigues *et al.*, 1980
Mansonia	*annulifera*	India	Chakraborty *et al.*, 1980
		Sarawak	Simpson *et al.*, 1964
		Sarawak	Simpson *et al.*, 1974

(Source: Pradhan *et al.*, 1991)

ANNEX-3

Potential reservoir population in Nepal, 2000/2001.

	Pig population	Duck Population
National (Grand total)	912,350	411,410
In 24 endemic district	358,850	331,646
Percentage in endemic districts	39%	81%

(Source: Statistical information on Nepalese agriculture, 2000/2001)

ANNEX-4

Risk of Japanese encephalitis, by country, region, and season

Country	Affected Areas	Transmission Season	Comments
Australia	Islands of Torres Strait	Probably year-round transmission risk	Localized outbreak in Torres Strait in 1995 and sporadic cases in 1998 in Torres Strait and on mainland Australia at Cape York Peninsula
Bangladesh	Little data, but probably widespread	Possibly July to December, as in northern India	Outbreak reported from Tangail District, Dacca Division; sporadic cases in Rajshahi Division
Bhutan	No data	No data	No comments
Brunei	Presumed to be sporadic-endemic, as in Malaysia	Presumed year-round transmission	No comments
Burma (Myanmar)	Presumed to be endemic-hyperendemic countrywide	Presumed to be May to October	Repeated outbreaks in Shan State in Chiang Mai valley
Cambodia	Presumed to be endemic-hyperendemic countrywide	Presumed to be May to October	Cases reported from refugee camps on Thai border
India	Reported cases from all states except Arunachal, Dadra, Daman, Diu, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Orissa, Punjab, Rajasthan, and Sikkim	South India: May to October in Goa; October to January in Tamil Nadu; and August to December in Karnataka. Second peak, April to June in Mandya District Andrha Pradesh: September to December North India: July to December	Outbreaks in West Bengal, Bihar, Karnataka, Tamil Nadu, Andrha Pradesh, Assam, Uttar Pradesh, Manipur, and Goa. Urban cases reported (e.g., in Luchnow)
Indonesia	Kalimantan, Bali, Nusa, Tenggara, Sulawesi, Mollucas, and Irian Jaya (Papua), and Lombok	Probably year-round risk; varies by island; peak risks associated with rainfall, rice cultivation, and presence of pigs Peak periods of risk: November to March; June and July in some years	Human cases recognized on Bali, Java, and possibly in Lombok
Japan*	Rare-sporadic cases on all	June to September,	Vaccine not routinely

	islands except Hokkaido	except April to December on Ryuku Islands (Okinawa)	recommended for travel to Tokyo and other major cities. Enzootic transmission without human cases observed on Hokkaido
Korea	North Korea: No data. South Korea: Sporadic-endemic with occasional outbreaks	July to October	Last major outbreaks in 1982 and 1983. Sporadic cases reported in 1994 and 1998.
Laos	Presumed to be endemic-hyperendemic countrywide	Presumed to be May to October	No comments
Malaysia	Sporadic-endemic in all states of Peninsula, Sarawak, and probably Sabah	Year-round transmission	Most cases from Penang, Perak, Salangor, Johore, and Sarawak
Nepal	Hyperendemic in southern lowlands (Terai)	July to December	Vaccine not recommended for travelers visiting only high-altitude areas
Pakistan	May be transmitted in central deltas	Presumed to be June to January	Cases reported near Karachi; endemic areas overlap those for West Nile virus. Lower Indus Valley might be an endemic area.
Papua New Guinea	Normanby Islands and Western Province	Probably year-round risk	Localized sporadic cases
People's Republic of China	Cases in all provinces except Xizang (Tibet), Xinjiang, Qinghai. Hyperendemic in southern China. Endemic–periodically epidemic in temperate areas. Hong Kong: Rare cases in new territories Taiwan: Endemic, sporadic cases islandwide*	Northern China: May to September Southern China: April to October (Guangxi, Yunnan, Guangdong, and Southern Fujian, Sichuan, Guizhou, Hunan, and Jiangxi provinces) Hong Kong: April to October Taiwan: April to October, with a June peak*	Vaccine not routinely recommended for travelers to urban areas only Taiwan: Cases reported in and around Taipei and the Kao-hsiung–Pingtung river basins*
Philippines	Presumed to be endemic on all islands	Uncertain; speculations based on locations and agroecosystems. West Luzon, Mindoro, Negros, Palawan: April to November Elsewhere: year-round,	Outbreaks described in Nueva Ecija, Luzon, and Manila

		with greatest risk April to January	
Russia	Far Eastern maritime areas south of Khabarousk	Peak period July to September	First human cases in 30 years recently reported
Singapore	Rare cases	Year-round transmission, with April peak	Vaccine not routinely recommended
Sri Lanka	Endemic in all but mountainous areas Periodically epidemic in northern and central provinces	October to January; secondary peak of enzootic transmission May to June	Recent outbreaks in central (Anuradhapura) and northwestern provinces
Thailand	Hyperendemic in north; sporadic-endemic in south	May to October	Annual outbreaks in Chiang Mai Valley; sporadic cases in Bangkok suburbs
Vietnam	Endemic-hyperendemic in all provinces	May to October	Highest rates in and near Hanoi
Western Pacific	Two epidemics reported in Guam & Saipan since 1947	Uncertain; possibly September to January	Enzootic cycle might not be sustainable; epidemics might follow introductions of virus.
Local JE incidence rates might not accurately reflect risks to nonimmune visitors because of high immunization rates in local populations. Humans are incidental to the transmission cycle. High levels of viral transmission can occur in the absence of human disease.			

(Source: http://www.cdc.gov/travel/disease/jenceph.htm)

ANNEX-5

Sequelae in patients recovery from Japanese encephalitis

Motor sequelae: Limb paralysis 34-44% Fine motor deficits 72% Abnormal movements 5.5-8%	Behavioral sequelae: Aggressiveness 72% Depression 38% Attention deficit 55%
Other neurologic sequelae: Epilepsy 16-20% Memory deficit- 46% Cranial nerve paralysis 16% Blindness 2%	Intellectual sequelae: Abnormal intelligence 42-72% Borderline intelligence 33% Mild mental retardation 11% Moderate mental retardation 11%

(Source: Kalyanarooj, 1995).

ANNEX-6

(A) QUESTIONNARIRE FOR DATA COLLECTION

Name:

Age: Sex:

Address: Religion:

Education: Occupation:

1. Do you know about JE? Yes No

1.1. If, yes,

1.1.1. What are the sings and symptoms of JE?

i) Fever ii) Vomiting iii) Neck rigidity iv) Convulsion

v) Unconsciousness. vi) Paralysis vii) Others .....

1.1.2. Where do you go for Medication/treatment?

i) Hospital ii) Traditional Healers/Dhami/Jhankri iii)Others.....

2. Do you know about transmission of JE? Yes No

2.1. If yes,

2.1.1. What is the vector of JE?

i) .Mosquito ii)House-fly iii) Dirty water/Raw food materials

iv) Others.....

2.1.2. Where does the vector breed?

i) Irrigated rice field ii)Open coops of pigs/ducks iii) Bushes iv) Stored polluted water v) Others.....

2.1.3 How JE is transmitted?

i.) By biting of mosquito ii) By defecation of house-fly iii) By ingestion of dirty water& raw food materials iv) Due to unhygienic personal life v) Others.....

3. When JE Spreads?

i.) Summer season ii) Rainy season iii) Winter season iv) Spring season v) Others.....

4. Do you know about preventive measures of JE? Yes No

4.1. If yes,

4.1.1. What measures to be adopted to prevent from JE?

i) In-door staying at dawn and dusk. ii) Use of mosquito repellent cream while staying outdoor at dawn and dusk. iii) Wearing long sleeve clothes while staying outdoor at dawn and dusk. iv) Use of mosquito net while sleeping outdoor. v) Not rearing pigs and ducks in open coops near house. vi) Houses far from paddy fields vii) Filling clear water colleting sites near house. viii) Others..... ix) None.

4.1.2. What preventive measures do you have adopted?

i) In-door staying at dawn and dusk. ii) Use of mosquito repellent cream while staying out-door at dawn and dusk. iii) Wearing long sleeve clothes while staying out-door at dawn and dusk. iv) Use of mosquito net while sleeping out-door v) Not rearing pigs and ducks in open coops near house. vi) Houses far from paddy fields vii) Not clear water colleting sites near house. viii) Others..... ix) None.

(B) HOSPITAL RECORDING OF JE SUSPECTED CASES AT M.Z.H.

S.N.	NAME OF PATIENT	AGE	SEX	ADDRESS	EDUCATION	OCCUPATION	RELIGION	OPD NO.	BED NO.	DATE OF HOSPITALISA-TION	DATE OF SERUM SAMPLE COLLETION	DATE OF DISCHARGE	OUT-COME				IF UNDER 15 YRS AGED	
													CURED	DEATH	LAMA	REFE-RRED	JE VACCIN-ATED	NOT VACCIN-ATED

(C) LABORATORY RESULTS

S.N.	NAME OF PATIENT	AGE	SEX	ADDRESS	EDUCATION	OCCUPATION	RELIGION	OPD NO.	BED NO.	DATE OF SERUM SAMPLE COLLECTION	DATE OF ELISA TEST	ANTI JE-IgM TITER	RESULTS

ANNEX-7
HOSPITAL RECORDING AND LABORATORY RESULTS OF JE SUSPECTED CASES FROM MZH

S.N.	NAME OF PATIENTS	AGE (YRS)	SEX	ADDRESS	OCCUPATION	DATE OF HOSPITALIZATION	BED NO.	DATE OF SERUM SAMPLE COLLECTION	DATA OF DISCHARGE	DATE OF ELISA TEST	ANTI JE-IGM TITER	RESULTS	REMARKS
1.	Jay Bahadur Kunwar	40	M	Belauri-1	Agriculture	060-4-13	1	060-4-13	060-4-19	060-7-19	16.7	-ve	
2.	Ram Lal Chaudhary	30	M	Pipaladi-9	Agriculture	060-4-15	2	060-4-16	06-4-25	060-7-19	0	-ve	Deafness
3.	Doctor Chaudhary	16	M	Suda-7	Agriculture	060-4-17	3	060-4-19	060-4-23	060-7-19	0	-ve	JE chorea
4.	Geeta Nepali	22	F	Laxmipur-6	Agriculture	060-4-18	24	060-4-26	060-4-30	060-7-19	0.9	-ve	
5.	Kalawati Sarki	26	F	Jhalari-5	Agriculture	060-4-28	18	060-4-30	060-5-2	060-7-19	12.3	-ve	JE chorea
6.	Deepa pal	18	F	MNNP-3	Study	060-4-29	21	060-4-30	060-5-2	060-7-19	0	-ve	*Pv* +ve
7.	Jayanti Bohara	10	F	Pipaladi-4	Study	060-5-3	9	060-5-5	060-5-8	060-7-19	13.2	-ve	
8.	**Kalu Khatri**	**30**	**M**	**Daijee-4**	**Agriculture**	**060-5-8**	**4**	**060-5-8**	**060-5-24**	**060-7-19**	**40**	**+ve**	**Lt. Side hemiplegia**
9.	Bhatu Ram Chaudhary	40	M	MNNP-2	Agriculture	060-5-10	1	060-5-11	060-5-13	060-7-19	2	-ve	
10.	Laxmi Bhakta Joshi	18	M	MNNP-4	Study	060-5-11	2	060-5-14	060-5-14	060-7-19	35	-ve	Referred
11.	Bishal Rana	5	M	Daijee-9	-	060-5-13	9	060-5-14	060-5-18	060-7-19	2	-ve	
12.	Amar Okhaeda	12	M	Rauteli Bichuwa-9	Study	060-5-14	15	060-5-16	060-5-19	060-7-19	16.7	-ve	
13.	Rajendra Bhandari	17	M	Belauri-4	Study	060-5-19	15	060-5-19	060-5-21	060-7-19	0	-ve	Referred
14.	**Dambar Sunar**	**29**	**F**	**Dodhara chandani-8**	**Agriculture**	**060-5-21**	**22**	**060-5-22**	**060-5-28**	**060-7-19**	**51**	**+ve**	
15.	Suresh Bharati	22	M	Belauri-7	Study	060-5-21	7	060-5-22	060-5-23	060-7-19	9	-ve	
16.	Man Bahadur Chaudhary	11	M	Jhalari -5	Study	060-5-23	14	060-5-23	060-5-24	060-7-19	26.3	-ve	Death
17.	Dhan Bahadur Chaudhary	49	M	MNNP-18	Agriculture	060-5-25	7	060-5-26	060-6-2	060-7-19	1	-ve	LAMA
18.	Priya Dagaura	50	F	Jhalari-5	Agriculture	060-5-26	20	060-5-28	060-5-28	060-7-19	12.3	-ve	Death
19.	Ganga Dhami	49	M	MNNP-10	Agriculture	060-5-29	80	060-5-30	060-6-2	060-7-19	11	-ve	Death
20.	Rajendra Rawat	21	M	Krishnapur-9	Agriculture	060-5-30	3	060-5-31	060-6-4	060-7-19	3	-ve	
21.	Devaki Bista	40	F	MNNP-10	Agriculture	060-6-2	19	060-6-2	060-6-14	060-7-19	18	-ve	
22.	Janaki Joshi	45	F	Daijee-4	Agriculture	060-6-2	20	060-6-2	060-6-8	060-7-19	5	-ve	
23	Ram Oad	17	M	Pipaladi-9	Agriculture	060-6-2	6	060-6-6	060-6-8	060-7-19	32.5	-ve	
24.	**Sarswati Karki**	**17**	**F**	**MNNP-10**	**Study**	**060-6-8**	**14**	**060-6-13**	**060-6-6**	**060-7-19**	**88**	**+ve**	***Pf*+ve**
25.	Som Raj Thakulla	4	M	Daijee-1	Study	060-6-28	9	060-629	060-6-30	060-7-19	5	-ve	Death

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फोन नं. २६२२३८
२६१४३६

पत्र संख्या :- ८५४।०८८
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☎ २६२२३८
२६१४३६

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