

***Japanese  
Encephalitis (JE)***

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## General

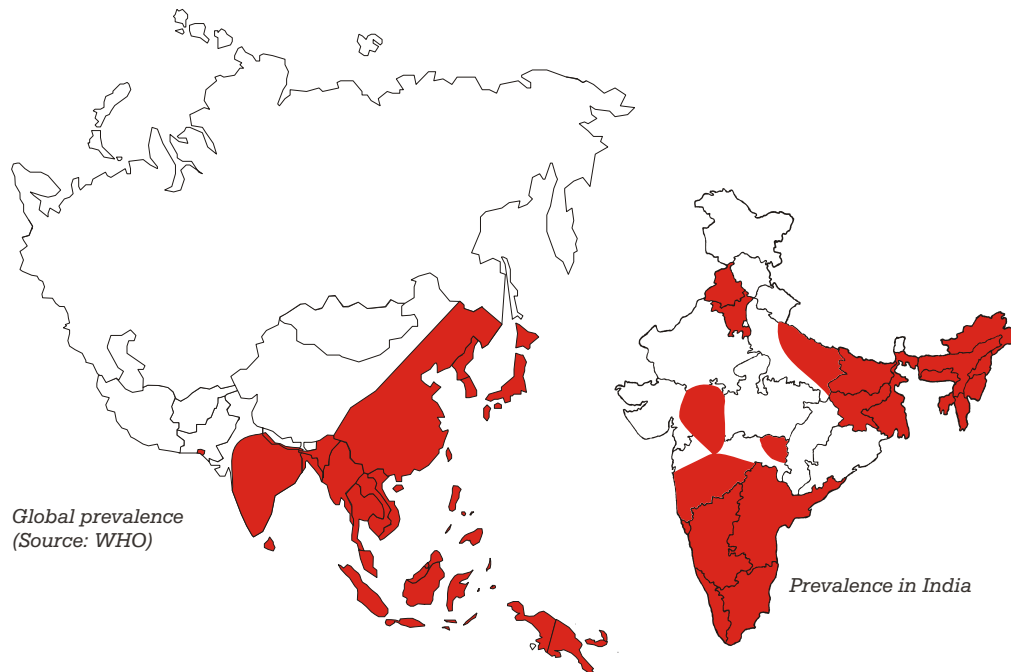
JE is an important mosquito-borne viral disease and one of the leading causes of viral encephalitis and neurological infections in Asia. Although severely under-reported, 50,000 cases are annually recorded throughout Asia, with 15,000 deaths (5-35% case fatality rate) and a 75% JE-related disability rate (767,000 DALYs, WHO, 2002).

The disease was first recognized in India in 1955, when cases of encephalitis from North Arcot district of Tamil Nadu and neighboring districts of Andhra Pradesh, admitted to Christian Medical College Hospital, Vellore, were serologically diagnosed as Japanese Encephalitis. JE virus was isolated from wild caught mosquitoes in the same year, followed by isolations from patients from the same area in 1958. JE continues to be endemic in these states.

Since 1972, JE has spread to newer areas and epidemics / outbreaks have been reported from West Bengal, Uttar Pradesh, Assam, Manipur, Bihar, Andhra Pradesh, Pondicherry, Karnataka, Goa and recently from Kerala and Maharashtra. The disease in southern India affects children below 15 years, while in north India all age groups are affected. In most of the epidemics, the incidence has been higher in males than in females (M : F= 1.2 -1.5:1).

## Distribution

Based on endemicity, epidemics, isolations of virus from patients and vectors and general serological surveys, the virus has been shown to be widely prevalent in most parts of south central, northern and northeast states of India. Apparently, part of Maharashtra and states like Gujarat, Rajasthan and Madhya Pradesh are free from JE.

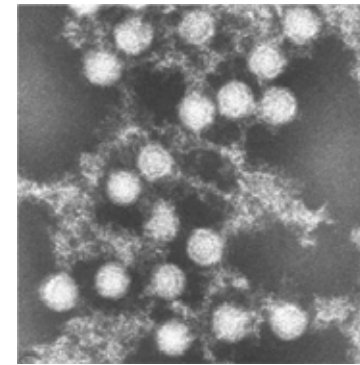


## Clinical features

The clinical disease can be divided into three stages - a prodromal febrile stage, an acute encephalitic stage marked by CNS involvement and continuing fever and a late stage marked by either recovery or persistence of symptoms of irreversible neuron injury leading to transient / permanent sequelae. Subclinical infections are relatively high; sub clinical to clinical infection ratio being 125-500:1.

## The virus

An enveloped virus, belongs to the family *Flaviviridae*. The virus is spherical and has diameter of 40-50 nm.



## Genome

Single stranded positive sense RNA of ~11000 nucleotides. The virus has three structural and 7 non-structural (NS) proteins that are translated as a single ORF and co-or post-translationally cleaved. The virus replicates exclusively in cytoplasm. The outer protein (envelope) has domains responsible for cell attachment, haemagglutination and neutralization.



## Diagnosis

The institute has a strong infrastructure and the following facilities are established, standardized and regularly used for providing diagnosis.

### Virus isolations & propagation

- Tissue culture
- Infant mouse inoculation
- Mosquito inoculation

### Antigen detection

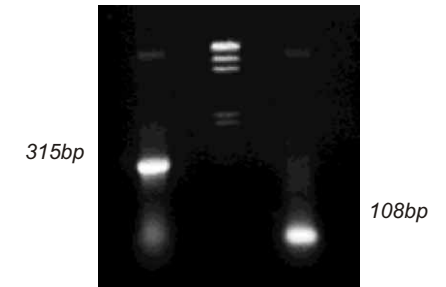
- Antigen capture ELISA
- Immunofluorescence test

### Serological tests

- Haemagglutination Inhibition (HI)
- ELISA (IgM, IgG)
- Neutralization

### Genome detection

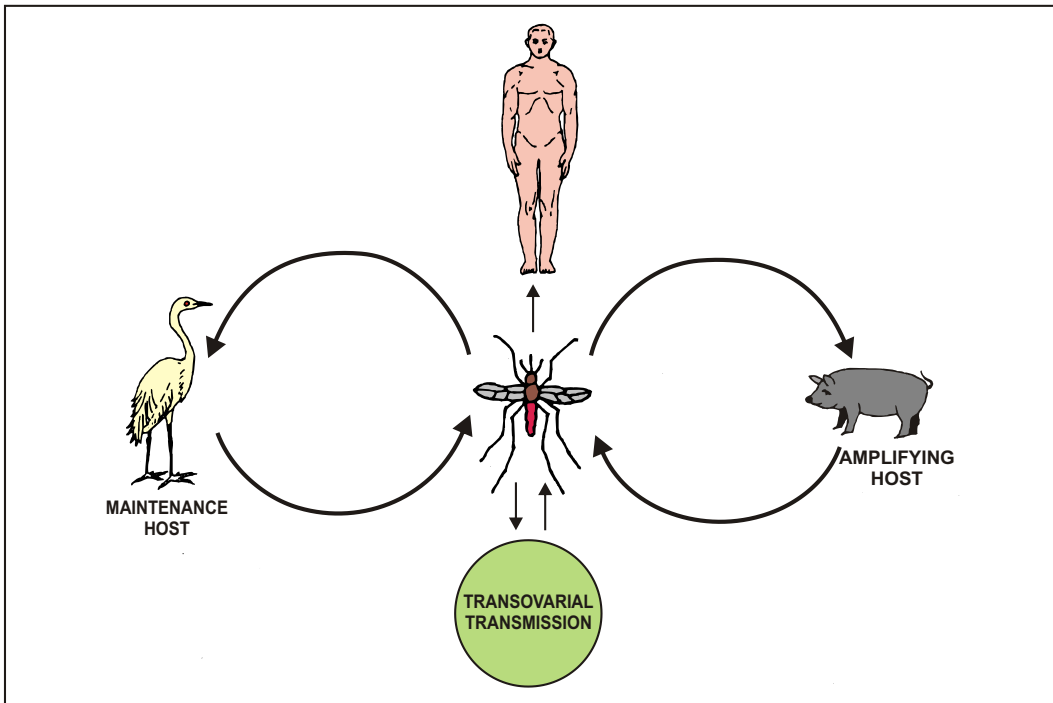
- PCR



Diagnostic PCR using 2 different regions

**The institute has developed highly reliable IgM capture ELISA. (MAC-ELISA) for rapid diagnosis. NIV supplies a limited number of kits on commercial basis.**





### Situations under which mosquitogenic conditions occur are

- Water accumulation and paddy cultivation during monsoon season
- Paddy cultivation associated with irrigation systems during non-monsoon seasons eg. Mandya district, Karnataka
- Water logging due to flooding of rivers eg. Brahmaputra river basin, Assam
- Changing of agricultural practices such as dryland wheat cultivation to paddy cultivation using ground and/or canal water eg. Uttar Pradesh.



Mosquito collection at dusk

Vector mosquitoes rest outdoors and are predominantly zoophilic in nature. Only <2% of the females feed on human blood. Therefore, very high vector density is a prerequisite for active transmission to humans. Strong association has been demonstrated between JE cases and vector densities / longevity of mosquitoes.



A blood fed female of a Culex mosquito

### Transmission of the virus

Experimental studies were conducted to determine susceptibility and transmission ability of culicine mosquitoes. *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. gelidus*, *Cx. bitaeniorhynchus*, *Cx. quinquefaciatus* were capable of transmitting the virus to susceptible hosts. Mosquitoes belonging to *Cx. bitaeniorhynchus* and *Cx. vishnui* group also transmit the virus transovarially.



Mosquito larvae being collected from paddy field

Several strains of the virus have been isolated from males and adults reared from field collected immature stages. This suggests transovarial transmission as an important mechanism for the maintenance of the virus in nature, particularly in inter-epidemic periods.

### Epidemiology, ecology and natural cycle

As in other countries of Southeast Asia, the infection is maintained in enzootic cycle involving vertebrate hosts like pigs and ardeid birds and mosquitoes. Man is considered as dead end in the natural cycle because viremia is short and most of the vectors do not feed on humans by preference. Man to man transmission has not been documented.

### Vectors

In India, the virus has been isolated from more than 15 species of mosquitoes belonging to genera *Culex*, *Aedes* and *Anopheles*. *Cx. tritaeniorhynchus* and *Cx. vishnui* however, are considered as the main vectors.

Vector mosquitoes proliferate in stagnant water, paddy fields, ditches, pools and puddles.



A typical breeding habitat for mosquitoes



## Vertebrate hosts

**Pigs:** Pigs are key hosts due to high viremia, their large turnover and close association with people belonging to low socioeconomic group. Monitoring of antibodies in sentinel pigs in Kolar and Mandya districts of Karnataka has demonstrated transmission of virus and presence of enzootic cycle in pigs almost throughout the year. Virus was also isolated from naturally infected pigs. Seroconversion in pigs generally precedes human cases.

**Goat :** The scientists of CRME, Madurai, have found goat as a suitable host for sentinel surveillance.

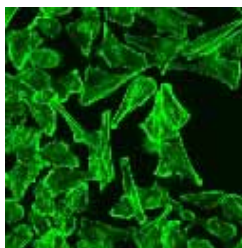
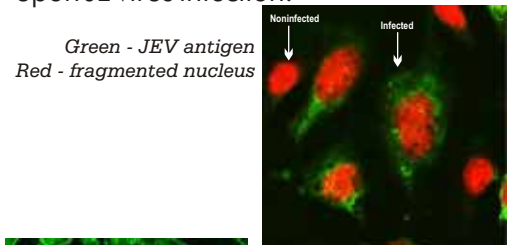
**Cattle :** Cattle do not develop enough viremia to infect mosquitoes in experimental condition. As cattle are the most important host for vector mosquitoes perhaps they act as damper to infection in nature and reduce risk to humans.



**Birds:** Demonstration of high prevalence of antibodies in field collected ardeid birds in Andhra Pradesh and Karnataka and ability of birds to circulate virus and infect mosquitoes in laboratory have provided enough evidence to consider bird as an important host in enzootic cycle.

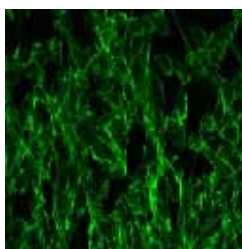
## Cellular changes

Cells infected with JE virus showed nuclear damage and changes in cytoskeleton as shown by staining of actin filaments. Mammalian cells (PS) infected with JE virus and those persistently infected with JE virus showed distinct changes in distribution of cellular actin. Mosquito cells (C6/36) also showed significant loss of actin upon JE virus infection.

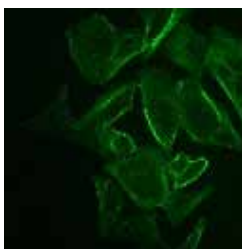


**Actin staining**

◀ Uninfected PS cells

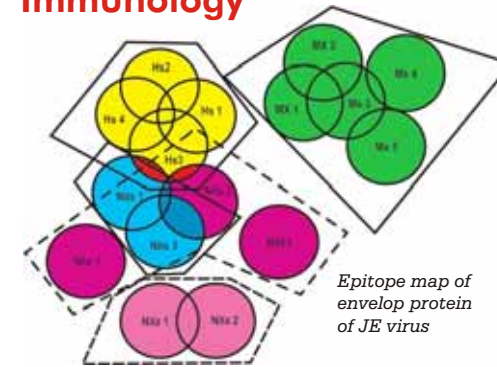


JEV infected PS cells



PS cells persistently infected with JEV

## Immunology



Epitope map of envelope protein of JE virus

A panel of 17 monoclonal antibodies (MAbs) to envelope protein of JE virus has been generated. These are used for epitope mapping, strain variation studies, generation of anti-idiotypic polyclonal antibodies, diagnostic kit development and for delineation of neutralization escape variant viruses.

Two MAbs were found to be reactive with histones. Some sera from JE patients have been shown to have anti-histone activity. Myelin basic protein (MBP) and anti-MBP antibodies were detected in CSF of JE patients and had a negative prognostic value. This indicates role of autoimmune mechanism in the pathogenesis of JE.

Determination of IgG/albumin ratio in paired CSF and serum samples has indicated that blood brain barrier gets damaged in early acute phase of the disease. Damage was observed to be a reversible phenomenon. Damage in the late phase of the disease might be due to immune complex mediated reactions.

Intrathecal synthesis of anti-JEV IgM antibodies leading to its high concentration in CSF has been demonstrated.

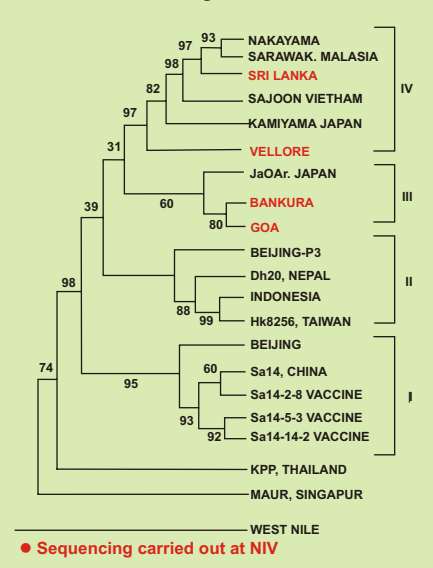
Adult Swiss albino mice are refractory to peripheral infection with JE virus. Athymic nude mice have been shown to be susceptible to the virus by subcutaneous and intraperitoneal route. These have been used for studying immunoprotective mechanism. Monocyte and mitogen stimulated B and T lymphocytes support the growth of JE virus. Phytohemagglutinin stimulated PBMC co-cultivation system has been developed for the isolation of the virus from patient's blood.

Protective role of interferon treatment and interferon inducers in JE was demonstrated in monkey model. Pretreatment of monkeys with interferon inducer (6-MFA), protected them from lethal intra-nasal challenge with JE virus.

Peptide epitopes on envelope glycoprotein were predicted using bioinformatic tools. Neutralizing antibody response could be induced in mice immunized with synthetic peptides. Similarly T helper epitopes from structural and non structural proteins of JE virus were delineated.

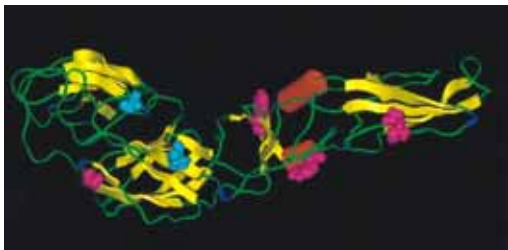
## Genome analysis

The complete envelope gene sequence and the deduced amino acid sequence of four strains of JE virus from the Indian subcontinent and sequences of other 16 strains analyzed by pairwise comparisons indicated an overall sequence conservation. Phylogenetic analysis of the E gene sequences by a variety of tree-building methods identified four clusters.



### Sequencing the envelope gene of neutralization escape mutants revealed the following

- Substitutions of G by W (Vellore) and E by to K (Bankura) are the sites involved in loss of neutralization and loss of virulence.
- Changing the substrate for growing virus from mouse brain to tissue culture and sequencing the envelope gene revealed that mutations at position 84, 184, 211, 219 and 490 are responsible for change in tropism from mouse to tissue culture (PS cells).
- All the mutations are in the domain I and II, involved in dimerization of the monomers, important for cell interaction and virus entry.



Three dimensional structure of JE virus envelope protein showing locations of peptide epitopes

## Vaccine development

### Challenge protocol

Challenge of mice with intraperitoneal virus followed by intracerebral inoculation of 1% starch has been studied as challenge protocol to determine the efficacy of vaccine. This takes into account the role of CMI response in addition to antibody response in protection.

### Attenuated vaccine

Partial attenuation of JE virus was obtained by passages in chick embryo culture. However, no stable attenuated strain could be obtained.

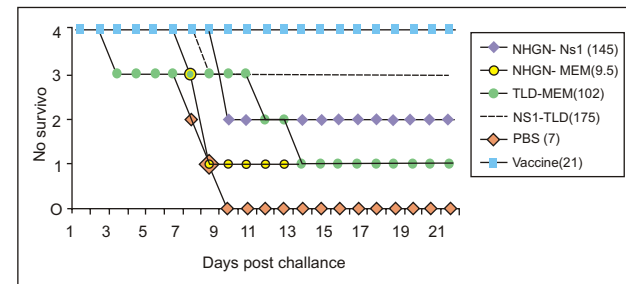
One attenuated *ts* mutant obtained from persistently infected cells showed loss of virulence at passage level of 20 by the i.p. route. This mutant could protect mice against challenge with 2.5 logs of virus and thus has the potential to be developed as an attenuated vaccine.

### Tissue culture vaccine

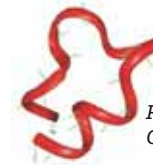
A chick embryo culture derived inactivated unpurified vaccine candidate was protective in mice.

### Peptide vaccine

Using chimeric peptides incorporating sequences of T helper and B cell epitopes, N. antibody induction and partial protection of mice from lethal challenge with JE virus has been demonstrated. Studies on increasing the protective ability of these chimeric peptides using antigen delivery systems and adjuvant are planned. These chimeric peptide epitopes are being used for development of polytope DNA vaccine.



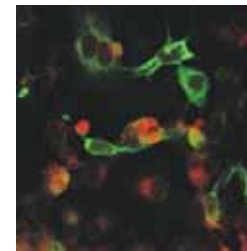
Protection of mice immunized with chimeric peptide from lethal challenge of JE virus



Predicted structure of Chimeric peptide

### DNA vaccine

The precursor M-envelope (truncated) and Ns1 genes were cloned into a mammalian expression vector to develop candidate DNA vaccine. The recombinant plasmids expressed antigen in transfected cells. Mice immunized with recombinant plasmid showed the presence of neutralizing antibodies.



Expression of JE virus protein in COS-7 cells

### Vaccine trials

NIV has conducted two JE vaccine trials using Nakayama mouse brain derived killed vaccine.

#### 1. Trial on 120 laboratory volunteers (adults)

The sero-conversion to neutralizing antibody response was detected in only

50% of subjects after two doses (0 and 7-17 days) and in ~60% of subjects after the third dose given after 13-17 months.

#### 2. Trial on 113 school children in Andhra Pradesh

Seventy-three percent of the subjects sero-converted after 2 doses (day 0 and 14). A booster dose administered one year later resulted in sero-conversion in 88% of the subjects. However, it was noted that 79% had shown significant drop in anti-JEV antibodies in pre-booster samples.

Both these studies indicated that minimum of three doses of vaccine are necessary.

### Prevention and control strategies

Data collected on population dynamics of vectors have helped in evolving mosquito control programs. Insecticide susceptibility tests were also performed on field collected mosquitoes. Control programs are implemented by State Governments under the guidance and supervision of National Malaria Eradication Program.

Central Research Institute, Kasauli, manufactures mouse brain derived Nakayama strain killed vaccine. Production is limited and endemic states occasionally vaccinate children. There is strong need to produce better and cost effective vaccine for mass immunization.

**JE is a priority disease for NIV. The main thrust areas include rapid diagnosis, molecular epidemiology, immunology, vaccine development and vaccine trials.**