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JAPANESE ENCEPHALITIS VIRUS: AN EMERGING PATHOGEN

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ABSTRACT

Japanese Encephalitis Virus (JEV) is a *flavivirus* maintained in a zoonotic cycle which involves pigs, birds and *Culex* species of mosquitoes causing fatal encephalitis endemic most of Asia and as far as Australia from its putative origin in Indonesia and Malaysia. The principle vector is *Culex* mosquito, most important being *Culex tritaenorhynchus*, present in greatest density in rainy season (June to November) Humans are accidental dead-end-hosts as they do not develop a level of viraemia sufficient to infect mosquitoes. The natural cycle of JEV consists of pig-mosquito-pig or bird-mosquito-bird and pigs serve as a biological amplifiers and reservoirs. The risk for Japanese encephalitis varies by appropriate ecological conditions and season to cause epidemics and epizootics. Disease control by vaccination is considered to be most effective. The Envelope (E) protein is dominant antigen including immunologic responses in infected host and eliciting virus neutralizing antibodies. Large scale immunization of susceptible human population is highly important to prevent this deadly infection. Attempts are being made to develop enhanced vaccines using the recombinant DNA technology. Since the existing inactivated, live attenuated or killed vaccines have side effects such as neurological disorders and systemic hypersensitivity, DNA based vaccines might aid the purpose of combating against JEV which are presently under clinical trials. Protection at personal level would help to reduce the incidence of the disease. In India vaccination against Japanese encephalitis are administered in areas where the disease is hyper-endemic.

Keywords: Japanese Encephalitis (JE), Epidemiology, *Flavivirus*, Transmission, Pathogenesis, Vaccination, Diagnostic Tools, Personal Hygiene, Large Scale Immunization

1. INTRODUCTION

Japanese encephalitis is mainly a pediatric disease (CDC, 2009) causing acute infection and inflammation of the brain. It is caused by Japanese encephalitis virus which belongs to arthropod-borne virus family and it is transmitted through *Culex* mosquito. JE was first recognized as a clinical entity in Japan in 1817, but the causative agent (JEV) was later isolated from a fetal human case in 1934 (Erlanger *et al*., 2009). JE was first reported in India in 1955 since than it has taken away thousands of lives. The total numbers of cases reported annually are about 35,000-50,000 (Zheng *et al*., 2012). Out of them ~30-50 % patients gets affected with neurological sequelae and 20-40 % die (Singh *et al*., 2009; Nett *et al*., 2009). The natural cycle of JEV consists of pig-mosquito-pig or bird-mosquito-bird (Hurk *et al*., 2009) circulation of virus. When an infected mosquito bites a healthy individual, it may lead to a nonspecific febrile illness or a severe meningoencephalomyelities illness (Nemeth *et al*., 2010). In rainy season the incidences of the disease increases (Saxena *et al*., 2008). Hence high level of immunization is needed to prevent the wide spread of this disease amongst human population (Weaver and Reisen, 2009).

1.1. Genome

Japanese encephalitis virus is an RNA virus of *Flaviviridae* family. It measures around 40-50 µm in diameter and structurally it is spheroidal and of cubic symmetry. It is an enveloped virus having single stranded RNA as a genome which is infectious. The

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genome is of ~11kb with positive polarity and a 5' cap but it lacks a 3' poly tail. The genome can be divided into two parts: structural and Nonstructural (NS) genes. Structural genes are three in number and are involved in antigenicity since they are are expressed on the virus coded by capsid protein and involved in capsid formation: Core (C), pre Membrane (prM) and Envelope (E). Among all three the E gene is the most important and is the most studied one. There are seven NS genes: NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5 and these are involved in virus replication (**Fig. 1**). The viral RNA has noncoding regions of 95 and 585 bases at 5' and 3'ends which interacts with viral or host proteins which are important in virus replication (Vashist *et al*., 2011). A novel mutation in domain II of the envelop gene of JEV circulating in North India has been reported (Pujhari *et al*., 2011). The high rate of mutation in JEV is due to RNA dependent RNA polymerase (RdRp) coded by NS5 (Neyts *et al*., 1999). JEV replicates exclusively in the cytoplasm of infected cells, in a perinuclear location and matures on intracellular membranes.

1.2. Epidemiology

JE originated reportedly in Indonesia and Malaysia long back (Weaver *et al*., 1999; Sinniah, 1989). JE has spread extensively to several countries in Asia including both temperate- Japan, Korea, Taiwan, China and tropical countries like India, Sri Lanka, Bangladesh and Nepal (Bista and Shrestha, 2005). The cases of JE were also reported in newer geographical areas in the Torres Strait Islands of Australia (Kaur and Vrati, 2003) and in Papua New Guinea (Mackenzie *et al*., 2002). The reasons for this wide distribution is unclear but can be due to population shift or changes in climate, ecology, agricultural practices, animal husbandry or migratory birds patterns (Mackenzie *et al*., 2005; Saxena *et al*., 2011; Oya and Kurane, 2007).

In India, JE was first detected in India in 1955 and it is endemic in several parts such as Bihar, Uttar Pradesh, Assam, Manipur andhra Pradesh, Karnataka, Madhya Pradesh, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa, Union territories of Goa and Pondicherry (Kabilan *et al*., 2004). The geographical area affected by JEV has expanded in the last 60 years with higher epidemic activity in North India and Central India (Ghosh and Basu, 2009). Almost 65 JE cases from South India from 1956-1966 were reported by demonstrating specific neutralizing antibodies to JE. During 1978, several outbreaks of JE occurred in different parts of India viz. Burdwan and Bankura and adjoining areas of Bengal, Kolar district of Karnataka, Dhanbad district of Bihar, Dibrugarh district in Assam, Goa on the west coast and eastern districts of Uttar Pradesh, which was one of the worst JEV outbreaks. A successful isolation and identification of JE virus (GP78) as the causative agent was made from the brain tissue of a fatal case of encephalitis from Gorakhpur district for the first time in Lucknow (Mathur *et al*., 1982). Recently, another novel strain of JEV (GP05: NCBI accession no. FJ979830) was isolated during 2005 encephalitis outbreak in India (Saxena *et al*., 2009). Around 38 patient samples were tested to study the trend of the viral infection in north India (Saxena *et al*., 2009). Out breaks of JE was reported several times in many places of India including Haryana, Kerala, Bihar and several districts of Andhra Pradesh from 1988-2003.

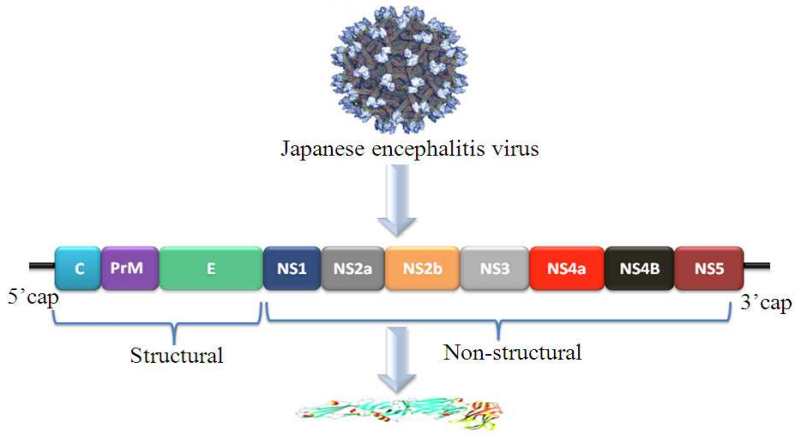

Fig. 1. Japanese Encephalitis Virus (JEV) morphology and a detailed display of the organisation of the viral genome – structural and non-structural genes and also the structure of the protein

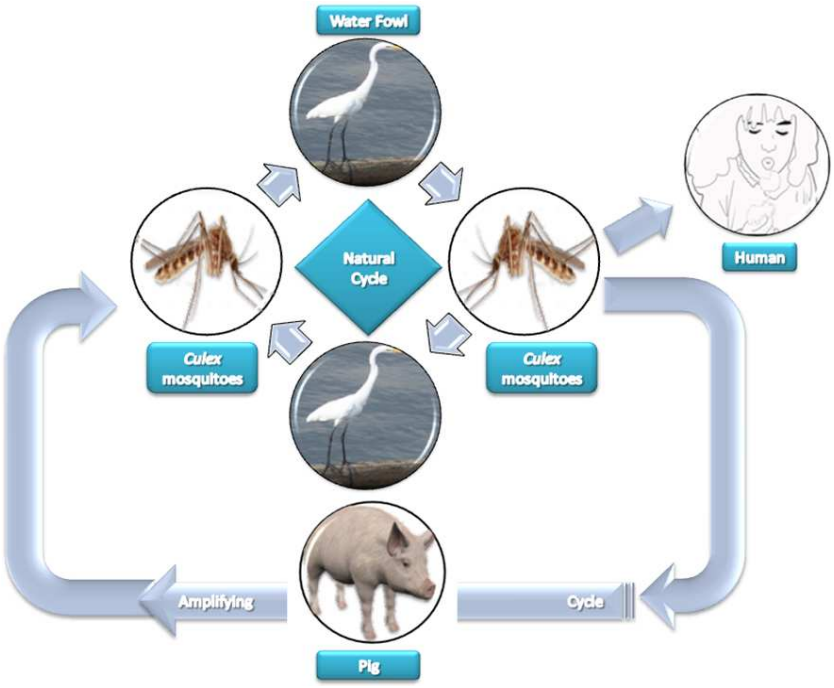

Fig. 2. Transmission cycle of Japanese encephalitis virus. JEV is transmitted in an enzootic cycle between *Culex* mosquitoes and amplifying vertebrate hosts, primarily pigs and wading birds like water fowls and egrets. Human beings serve as a dead-end-host in the JEV transmission cycle with low levels of viremia. Virus is not transmitted directly from human to human

In India the longest epidemic in three decades, had been reported during July to November 2005, in which more than 6,097 people were affected and approximately 1,398 died. Till date in Gorakhpur and around (in North India) several outbreaks of fatal Acute Encephalitis Syndrome (AES) were reported and approximately >10-15% were caused by JEV.

1.3. Transmission Cycle

Japanese encephalitis virus is maintained in enzootic forms and appears as focal outbreaks under specific ecological conditions. They multiply in the tissues of arthropods without evidence of disease and damage. Man is an accidental, dead-end host fir JE. The principal vector species is *Culex tritaeniorhynchus* (Sucharit *et al*., 1989), which is a rural mosquito, present in great density in rainy season in both tropical and temperate regions. The other minor hosts are cattle, buffaloes, goats, sheep, horses, rodents, monkeys, dogs and bats. It has an extrinsic incubation period of 10-12 days. The natural cycle of JEV consists of pig-mosquito-pig or bird-mosquito-bird cycles (**Fig. 2**). GIII was the only widely distributed genotype found in India until till when GI JEV strains were detected and isolated from 66 Acute Encephalitis Syndrome (AES) patients along with GIII strains (Fulmali *et al*., 2011). This detection indicates their co-circulation and association with humans. In the mid 1990's genetic shift (Nabeshima *et al*., 2009) had occurred in Japan, Korea and Vietnam that lead to disappearance of GIII and then progressively GI supplanted it (Zhang *et al*., 2011a). In India exact mode of introduction of GI is not clear, but it is possible that it may have been introduced through migratory birds (Huang *et al*., 2010). Pigs are the most important biological amplifiers and reservoirs. Generally direct person to person spread of JEV does not or rarely occurs until it is through intrauterine transmission (Guy *et al*., 2010). Blood and organ transplantation also serve as a mode of transmission (Plesner, 2004). The risk for JE is more in rainy season both in temperate and tropical regions (Singh *et al*., 2012).

1.4. Maternal to Foetal Transmission

JEV infection transmits from mother to fetus through vertical mode of transmission. This may be due to persistent maternal infection or due to pregnancy induced reactivation of virus. An animal model of congenital infection with JEV has been described and

transplacental transmission of the virus during consecutive pregnancies in mice has been shown experimentally (Mathur *et al*., 1982).

1.5. Pathogenesis

Japanese Encephalitis (JE) is now the foremost cause of viral CNS infection. JEV pathogenesis is still unclear (Yang *et al*., 2011). Since the variation exists in neuro-virulence and peripheral pathogenicity among JE virus strains. After the infected mosquito bite, the virus enters into the reticulo-endothelial system and invades the central nervous system after the transient period of viremia. It distributes itself in hypothalamus, hippocampus, substantia nigra and medulla oblongata regions of brain via vascular endothelial cells by the mechanism of endocytosis which involves cholesterol and clathrin mediated pathways, referred to as lipid rafts acting as portals for virus entry (Das *et al*., 2010). The virus replicates in neurons and matures in the neuronal secretary system. Nearly 33% of JE infected patients die due to neurocysticercosis (NCC), suggesting that it may somehow predispose to JE (Desai *et al*., 1997). During acute stages congestion, edema, hemorrhagic symptoms are found in brain. Pathological changes in the neural tissues have also been reported in lymphoid organs and immune cells such as spleen and kupffer cells respectively.

1.6. Host Immune Responses

The virus enters the neuro-parenchyma by crossing capillary walls in the brain and distributes itself in various parts of brain. Initially JE virus is partially destroyed at its site of entry and the remaining virus is disseminated by local and systemic extra neural replication leading to viremia. After primary infection with JEV, presence of IgM antibodies and T-lymphocytes are seen until 2 weeks approximately. But antibodies alone are neither capable of terminating the viremia nor preventing the subsequent infection. Pregnancy is known to cause immunosuppression and persistent maternal infection or pregnancy induced reactivation of the virus which causes foetal infection. Isolation of JEV from human placenta and foetuses has been reported. JEV can establish latency within different organs despite the presence of antiviral antibodies. A significant decrease in serum iron levels, a frequent feature of microbial invasion is observed during JE infection. An early influx of macrophages followed by neutrophils at the site of injury in different organs of humans and mice has been reported, which is correlated with the production of a neutrophil chemotactic macrophage derived factor MDF, with development of hypoglycemia. This chemotactic protein (MDF) has been shown to play a protective role in the host defense against JEV, through production of reactive oxygen intermediates in neutrophils and reactive nitrogen oxide species degrading the virus protein and RNA.

1.7. Signs and Symptoms

The disease affects all the age groups, predominantly in children under 15 years of age (CDC, 2011). Sex distribution of a patient shows slight male predominance with the male: female ratio being approximately ~1.5-1 to 2-1. Death in JE patients is usually seen within 24-48 h of admission. Among survivors, one in 200 affected individuals develop severe psycho neurological sequelae (Solomon and Winter, 2004) in the form of parkinsonism, convulsive disorders, motor abnormalities, impaired intellect, hearing deficit, scholastic backwardness, speech disturbances, other subtle neurological signs and movement disorders. There is alteration in plasma glucose levels found in the JEV positive patients (Tandon *et al*., 2002). Incubation period is 6-16 days among effected patients and symptoms have been given in **Table 1**.

1.8. Diagnosis

With the advent of monoclonal antibodies as potential diagnostic tool (Chavez *et al*., 2010), the rapid detection of JE antigen in cerebrospinal fluid has become possible. The different diagnostic tests have been given in **Table 2**. However, the most rapid and potential diagnostic tool (Ishida *et al*., 2002*)* for JE diagnosis have been shown to be MAC-ELISA (Robinson *et al*., 2010) and indirect fluorescent antibody. MRI of the brain can also be used in diagnosis. MRI changes can be co-related (Misra and Kalita, 2010) with the type of encephalitis and duration of illness.

1.9. Treatment and Prevention

There is no specific treatment or anti-viral agent for JEV infection, it is proving to be a persistent threat. Monoclonal antibodies (Yamanaka *et al*., 2010), corticosteroids, interferonα-2a or ribavirin were not that effective in clinical outcome. The effect of Rosamarinic Acid (RA) has been shown as an effective anti-viral agent that reduces JE viral load along with proinflammatory cytokines in experimental animal (Swarup *et al*., 2007). Neutrophils have been also shown to have degradative effect on JEV (Srivastava *et al*., 1999). Usage of anti-sense molecules (vivo-morpholino) directed against the viral genome, in combating the virus through inhibiting viral replication has been demonstrated (Nazmi *et al*., 2010). Mycophenolic acid (Sebastian *et al*., 2011) inhibits JE virus by inhibiting its replication. List of different available vaccines (Lewthwaite *et al*., 2010) have been given in **Table 3**.

Table 1. Duration, Signs and Symptoms of Japanese encephalitis

Disease course	Incubation duration	Signs and symptoms
Prodromal stage	1-6 days	General malaise, anorexia, headache, fever, vomiting. In children diarrhoea and abdominal pain may be prominent.
Acute encephalitic stage	7-13days	Photophobia, hyperexcitability, focal and neurological signs, muscular rigidity, dull, mask-like-face, tremulous eye movements, cranial nerve palsies, loss of co-ordination, pathological reflexes and in severe cases leads to coma.
Late convalescent stage	14, 15th day	Fever subsides, neurological signs tend to improve, temperature rises to 42^{o}C and eventually death occurs. If no death, it leads to long term psychoneurological sequelae

Table 2. Laboratory Diagnostic tools for Japanese encephalitis

Diagnostic tool	Detects
MAC-ELISA	IgM antibodies
Reverse passive hemagglutination test	Soluble JEV antigen in CSF
Indirect immune fluorescence test	Cell bound JEV antigen
Rapid micro neutralization test	Neutralizing antibodies to JEV in CSF
Reverse transcriptase PCR	Universal oligonucleotide primers

Table 3. Comparison of vaccines for Japanese encephalitis

Vaccine	Source	Side effects
Formalin-inactivated mouse brain derived	Nakayama strain	Expensive and side effects reported.
Inactivated hamster kidney cell vaccine	Beijing strain	Very low side effects reported.
Live attenuated hamster kidney cell line vaccine	SA14-14-2 strain, China	Expensive with very low side effects reported

Prevention methods are very important for minimizing JE infection (Saxena *et al*., 2006). Childhood Immunization is done by using inactivated mouse brain-derived vaccine which is based on either the Nakayama or Beijing strains of the JE virus, the cell culture derived, inactivated JE vaccine based on the Beijing P-3 strain; and the cell cultures derived, live-attenuated vaccine based on the SA 14-14-2 strain (Halstead and Thomas, 2011) of the JE virus. Recombinant poxvirus vectors expressing the E and NS1 proteins of the JEV boosting a good immune response in mice models can be used as a vaccine. The prevention of vector-man contact is very good preventive method this can be done by eliminating potential mosquito breeding areas, environmental sanitation, waste water management by treating the water with larvicide either by *Gambusa* (larva-eating fish), drying and wetting of rice fields, frequent vaccination should be implemented and as well as personal protective measures. Reports have shown that induction of nitric oxide synthase plays a protective role against JEV (Saxena *et al*., 2001; 2000). Diethyldithiocarbamate has been also experimentally shown to inhibit JEV infection (Saxena *et al*., 2003). Future predictions of the disease and drug designing can be enhanced by computer aided design databases, which can design *in silico* the most efficient drugs which can be tested experimentally and then can be clinically tried. For the development of appropriate and effective therapy there is an immediate need to understand host factors role in JEV-induced neuropathogenesis (Gupta *et al*., 2010).

1.10. Vaccination

Through vaccination in the last five year, JE has been effectively controlled and eliminated in China, Japan, Taiwan and Korea (Chung *et al*., 2007; Takahashi *et al*., 2000; Jelinek, 2009). Second generation recombinant vaccines (Nalca *et al*., 2003) are also being developed, where genes encoding Prm and E proteins are packed into vectors. DNA based JEV vaccines which may be very efficient against the virus (Stephenson, 1998) are under clinical trials. DNAzymes (DZs) that cleave the RNA sequence of the 3'-NCR of JEV genome in vitro, on intra-cerebral administration in JE infected mice almost completely inhibit virus replication in the brain. Use of neutralizing bodies for vaccine designing may also serve the process (Markoff, 2000).

2. CONCLUSION

Viral encephalitis is the most common CNS infection, causing acute infection of the brain especially in children less than 15 years of age. It has proved to be a massive disaster globally taking several thousands of lives. Intense research for the knowabouts of the virus is carried in several countries, devising strategies to fight with the virus. In the last four decades, JE has been virtually eliminated in most of the countries after the immunization with inactivated mouse brain-derived vaccine. Unfortunately, there is no treatment for JE. Protection at the personal level would help to reduce the incidence of disease. Development of specific antivirals and vaccine should be taken up at a higher priority.

Mosquito control is the sole available preventive measure for JEV transmission.

2.1. Future Implications

As JE is proving as a huge disastrous disease, research on JEV should be initiated at much wider scale, which should include development of effective anti-viral agents and vaccine strategies (Zhang *et al*., 2011b). Immunization is needed in JE prone areas (Rao, 2001). The virus is needed to be studied carefully, so that effective antivirals can be developed to target one of the stages in its life cycle. Over use of the vaccines should be avoided otherwise the virus might develop resistance against drugs which are administered frequently. It is also necessary to elucidate the ecology of migrating reservoir animals. Quarantine checks should be done at international immigration and emigration points, to keep a check on the spread of virus via foreign travelers. Vector control program should be effective enough to combat the risk. General awareness camps would be a good option to spread alertness in the local population level, to keep personal as well as surrounding areas and neighborhood clean and hygienic. Awareness campaign can educate people about the hygienic management and preventive measures which would immensely help in reducing disease incidences. Systematic approach is the need of hour, with the joint efforts of scientists, molecular biologists, doctors, drug developers, policy makers and local population to combat against the virus. A high sense of urgency is required to address this matter.

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