Chapter 10

Japanese Encephalitis Virus: The Complex Biology of an Emerging Pathogen

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1. Introduction

Japanese encephalitis virus (JEV) is a flavivirus, which is an emerging threat globally, majorly being southern and Southeast Asia and Australia. Even though most JEV infections are asymptomatic, it is estimated that only 0.3% leads to disease causing and results in over 35,000 cases including 10,000 deaths annually worldwide, and remaining cases which somehow escape death produce permanent sequelae, proving to be as a persistent threat (Singh et al., 2012). The human infections caused by encephalitic flaviviruses are more often asymptomatic or they cause mild febrile illness but sometimes this low percentage of mild infection turns into a dangerous and life-threatening encephalitis. The conditions which support viral survival are concerned to both viral and host factors that allow virus entry from the blood into the central nervous system (CNS). Host factors play important role in disease susceptibility. Japanese encephalitis, caused by JEV which belongs to arthropod-borne virus family and transmitted through *Culex* mosquito, is centrally a pediatric disease which causes acute infection and inflammation of the brain. Historically, in 1817 JE was first identified in Japan, but the causative agent (JEV) was later isolated from a fetal human case in 1934 (Erlanger *et al.*, 2009). First report of JE in India was in 1955, and since then this deadly virus has engulfed thousands of lives and has shaken several economies. The total numbers of cases reported annually are about 35,000-50,000 (Zheng *et al.*, 2012). Out of these reported cases ~30-50 % patients suffer from neurological sequelae and ~20-40 % cases turn to be fatal (Nett *et al*., 2009). The actual counts are still higher than reported due to lack of reach of technology and surveillance towards extreme rural areas, which contain more vulnerable and needy population. The natural cycle of JEV consists of pig-mosquito-pig or bird-mosquito-bird (van den Hurk *et al.*, 2009) circulation of virus. When an infected mosquito bites a healthy

individual, it may lead to febrile illness or a severe meningoencephalomyelities illness which is life taking. The incidence of the disease intensifies in rainy season as the environment supports the viral growth because of temperature, moistness and dampness which are plus factors letting the virus to bloom and flourish (Saxena *et al*., 2008) (Fig.1). Today the need is to fight against this reemerging virus by the aid of high level of immunization and therapeutic and preventive measures to slow down the spread of the disease amongst human population.

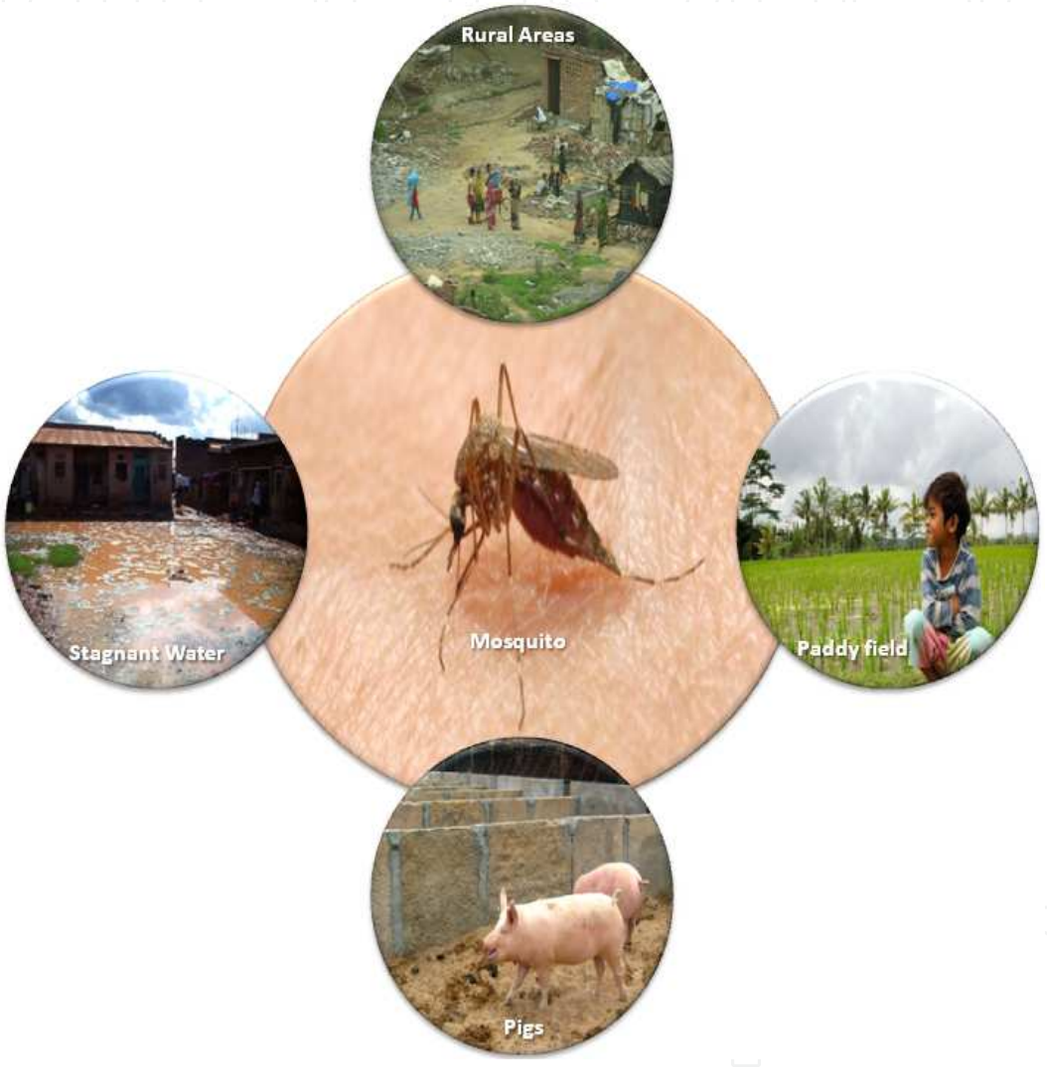

Figure 1. Displaying the contributing factors, which are responsible for the emergence and reemergence of JEV.

2. Genome of the virus

Japanese encephalitis virus belongs to the *Flaviviridae* family, it is an RNA virus measuring ~ 40-50 nm in diameter and structurally it is a spheroid having cubical symmetry. It is an en-

veloped virus having single stranded RNA as a genome which is infectious. The genome is of ~11kb with positive sense and a 5′ cap but it lacks a 3′ poly tail (Vashist et al., 2011). It contains nucleocapsid which is surrounded by a lipid envelope. The genomic RNA contains a single open reading frame (ORF) and codes for a polyprotein of ~3400 amino acids. This polyprotein is cleaved by viral and host proteases into 10 proteins. Structural genes are three in number and are involved in antigenicity since they 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 non structural genes: NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5 (Fig.2) and these are involved in virus replication (Saxena 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.

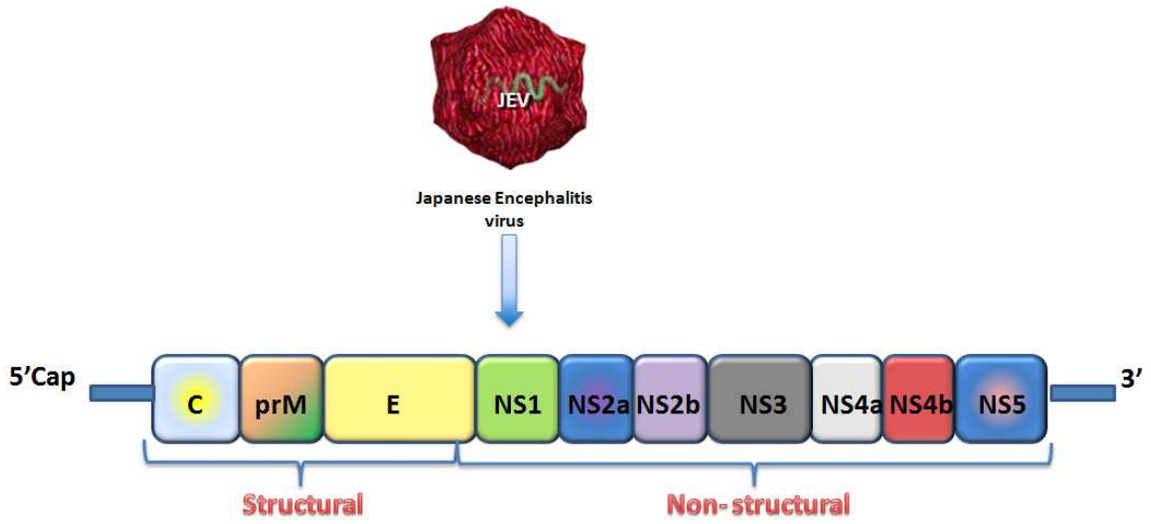

Figure 2. The genome of Japanese Encephalitis Virus, constituting the 3 Structural genes: C, prM, E and 7 Non-structural genes: NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5.

Japanese encephalitis (JE), caused by Japanese encephalitis virus (JEV), is the most important form of viral encephalitis in Asia. The epidemiology of JE has changed in the past 50 years and the area affected by JEV has expanded to India, China, Southeast Asia and Western Pacific regions. About 50, 000 cases and 10 000 deaths are reported in JEV-endemic areas among a population of 3 billion people. However, the true number is unknown, because most areas where JEV occurs lack diagnostic facilities. Most JEV infections are subclinical. JEV is a member of the JEV serological complex, which causes significant morbidity and mortality. 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. JEV infection transmits from mother to foetus through vertical mode of trans-

mission (Mathur et al., 1982). Symptomatic infections are usually present in the form of non-specific febrile illness, including diarrhoea and rigors followed by headache, vomiting and reduced levels of consciousness and aseptic meningitis or encephalitis. The incubation period after JEV exposure varies from 6 to 16 days (Saxena et al., 2009). One in 200/800 infected people develop clinical signs like high fever and nausea. A quarter of patients with symptoms die; a third of survivors suffer brain damage.

3. Epidemiology

Encephalitis outbreaks have been recorded since early 19th century from countries like Southeast Asia including Japan, Vietnam, Cambodia, Myanmar, India, Nepal, Malaysia, China, Korea, Taiwan, Thailand and reached to the West including Pakistan and the northeast and southwest of India, also the East (New Guinea), the South (Northern Australia Archipelago) and it is estimated theoretically to spread further West (Afghanistan). Between 1978 and 1992, 24 imported cases were reported in above regions due to high transmission. Since the 1990s, the JE is variedly transmitting in humans and is reaching new extended regions encompassing new geographic limits. History tells that a woman in France suffered from JE after she reached Thailand, in 1938. Also JE epidemic was reported in restricted seacoast of the 'USSR' in 1939. Also in 1946 major outbreaks of JE were recorded in Korea, both in civil in 1949 and in American military personnel in 1950.

Sequencing analysis divides JEV into five genotypes (GI–V) rising from ancestor viruses from Indonesia–Malaysia region. These ancestors have evolved into five genotypes; GI, GII, GIII, GIV and GV, out of them GIV and GV are the most divergent. They always remained confined to their origin region of Indonesia–Malaysia. However GI, GII and GIII are the most recent genotypes which have spread across Asia. All phylogenetic characterization and studies done on a large scale highlights GIII as a predominant genotype of JEV in Japan and Korea since 1935 (Schuh et al., 2009). GI was been isolated in Cambodia and then in China in 1979 and GIII was isolated before the 1970s and then in Vietnam and Japan during 1986 and 1990. Later even GI was reported there in 1995 and 2002 proving that all the strains isolated before 1991 were GIII, and after 1994 were GI. The natural cycle of JEV consists of pig-mosquito-pig or bird-mosquito-bird cycles. 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., 2011). 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).

JEV is basically transmitted by *Culex spp* mosquitoes. JEV is distributed in temperate and tropical areas of eastern and southern Asia. Its range has extended from eastern Asia (China, Japan, Korea, maritime Siberia, Taiwan, the Philippines, and Vietnam), to South-

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east Asia and northern Australasia (Cambodia, Indonesia, Laos, Malaysia, Papua New Guinea, Thailand, and the Torres Strait islands of northern Australia), and to southern Asia (Bangladesh, Bhutan, India, Myanmar, Nepal, and Sri Lanka) (Fig. 3). Evidences have also been seen for Pakistan. JE is largely a disease of rural areas, especially associated with irrigated rice agriculture. During endemics, no seasonal pattern exists and sporadic cases of encephalitis occur throughout the year, most often in infants and young children. There is a peak in vector density and virus activity during October-December in endemic zones. However, epidemic activity in temperate and subtropical areas occurs most commonly in summer and early autumn (van den et al., 2009).

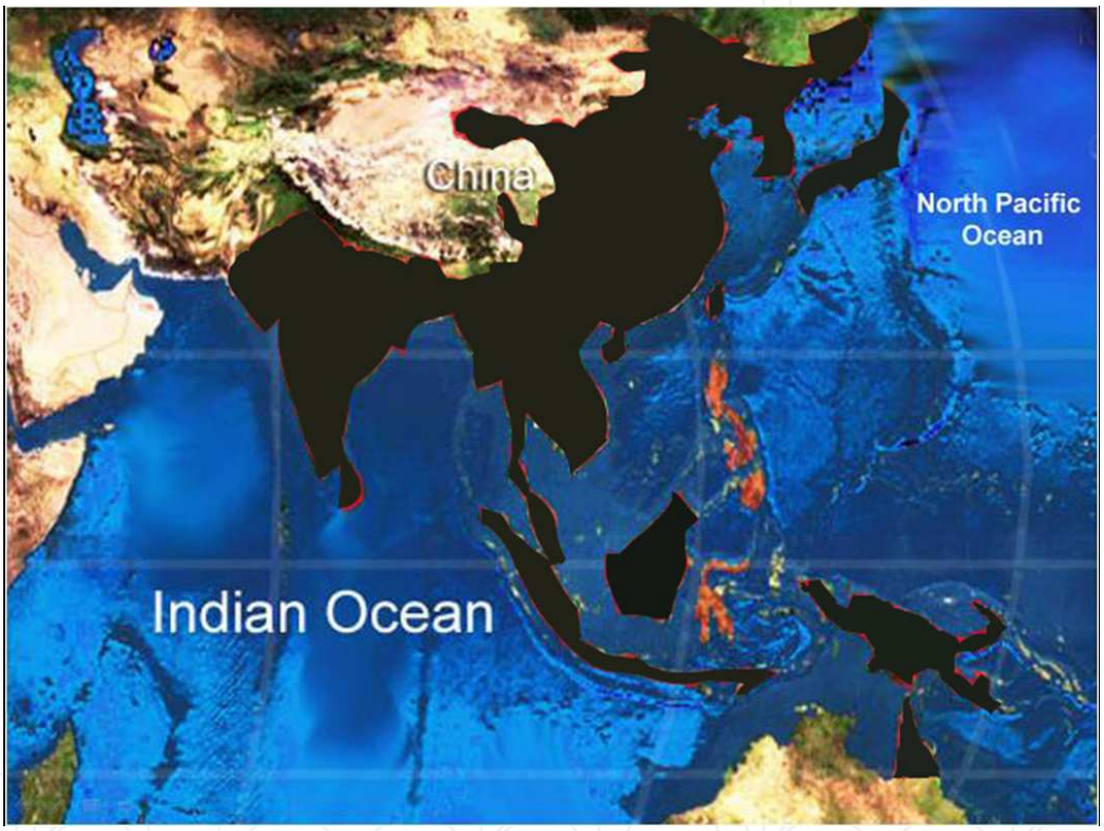

Figure 3. Epidemiology of JE globally. The areas highlighted in black display the regions under the attack of JEV infections and are high infection prone areas.

From past 75 years the major focus of JE coverage was on China and Southeast Asia, but now it has extended its horizons to westward towards India and Pakistan, northern to eastern Russia, eastward towards Philippines and southward to Australia. Occurrence of JE is more closely related to temperature and humidity in the atmosphere (Misra and Kalita, 2010). JEV is also engulfing new geographical regions which are shown by JEV sequencing analysis and results exhibit that JEV is expanding alarmingly to the new regions of Papua New Guinea and Australia. GI strains are often isolated from Northern Thailand, Cambodia, Korea, China, Japan, Vietnam, Taiwan and Australia between 1967 and the present. GII are isolated from Southern Thailand, Malaysia, Indonesia, Papua

New Guinea and Northern Australia. GIII are isolated from temperate regions of Asia. GIV are isolated in Indonesia and GV is isolated in Singapore. Sequence analyses of viral genes are further showing that a "genotype shift" from III to I has occurred in Japan since early 1990s, reasons for which remains unclear (Shimojima et al., 2011). Considering India, rapid spread of Japanese encephalitis (JE) towards the newer areas of northern states of India is also reported (Saxena et al., 2006, 2009).

4. JEV: Infectious agents

The genomic RNA of JEV is ~11 kb in length encoding three structural proteins and seven non-structural proteins. The RNA genome of the virus is infectious which can spread the horizon of disease. The virus genome contains C proteins complexed with the genomic RNA present in a nucleocapsid, and this whole complex is surrounded by enveloped lipid bilayer containing E and prM/M proteins, which is derived from the infected host cells. The prM proteins present in the immature particles, cleave to mature into M proteins. The E protein is the major infectious part which covers the entire surface of the mature virion, and it is the antigen majorly recognized by virus neutralizing antibodies. Further, subviral particles (SVPs), containing prM/M and E proteins enclosed in lipid layers but not surrounded by nucleocapsid, are secreted from flavivirus infected cells, proving these SVPs to be as excellent immunogens. Along with infective E protein, even NS1 protein is also considered as quite infective which may cause lethal effects to hosts when produced and expressed in large quantities. If antiNS1 immunity steps are taken and cytolytic antibodies against NS1 are administered, it would contribute in the reduction of the release of progeny viruses from infected cells. Hence a drug with a mixture of antiE and antiNS1 immunity would definitely pose as a potent fighter against flavivirus infection (Ishikawa et al., 2011).

5. Transmission of disease

JE virus undergoes zoonotic cycles which involve mosquitoes and several vertebrate species as hosts and human beings as dead end hosts. *Culex tritaeniorhyncus* and *Culex gelidus* are reported as principal vectors. These vectors breed in rice fields, irrigation canals and water pools filled with stagnant water and in standing puddles, open sewers, fish ponds etc. These infected mosquitoes (~3%) bite domestic animals and birds, but sometimes they may bite a healthy host (human), which are accidental hosts, facilitating the transmission of the virus to man. Pigs and birds serve as reservoirs and amplifying hosts. Man is an incidental host of the JEV (Fig. 4). In humans, after a bite of infected mosquito, initial viral replication occurs in local and regional lymph nodes. Viral invasion of the central nervous system occurs probably via blood causing infection and subsequent illness.

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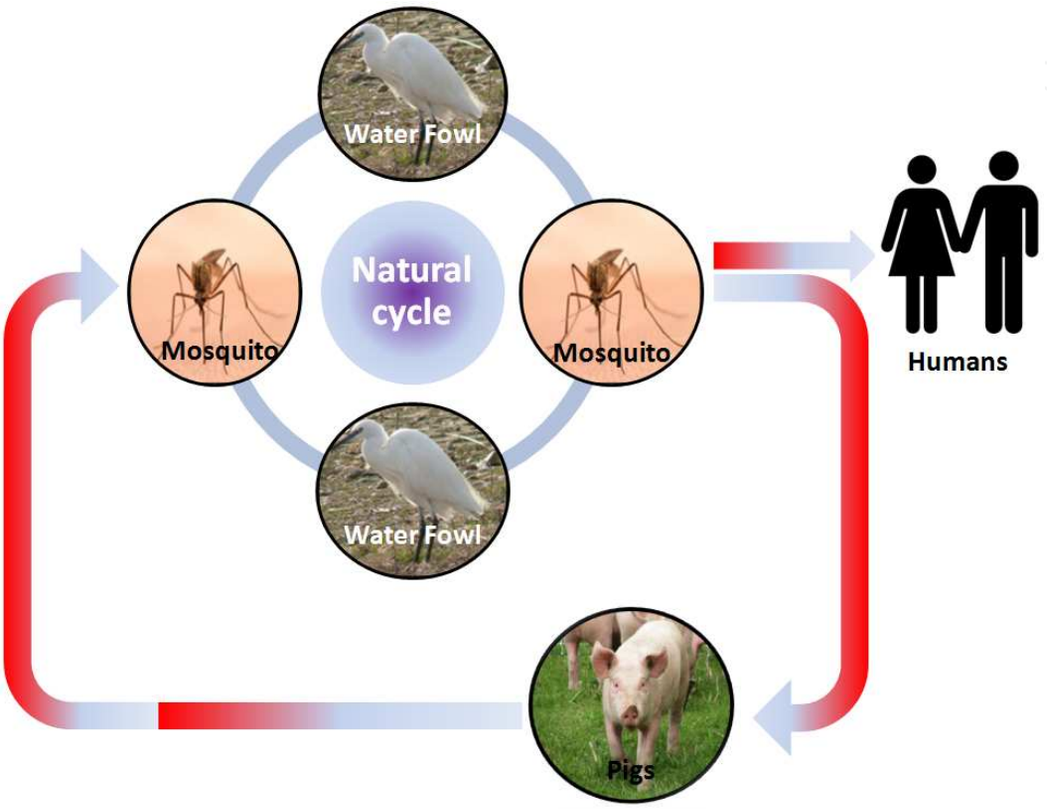

Figure 4. Life cycle of JEV crossing through important vectors and intermediate hosts and finally to dead end reservoirs human hosts.

6. Pathogenesis and pathology

The majority of human infections with encephalitic flaviviruses are asymptomatic or give rise to only a mild febrile illness. However, in a small percentage of infected individuals the mild infection turns into life-threatening encephalitis. Thus, a key question in the pathogenesis of encephalitic flaviviral disease concerns the conditions that allow virus entry from the blood into the central nervous system (CNS). Hypertension, diabetes mellitus, and coinfection with other virus may further deteriorate the infection and can worsen the condition of infected persons by increasing neurological complications which may happen due to facili-

tation of virus across the blood–brain barrier (Singh et al., 2009). Histological examination shows that virus can affect neurons present in thalamus and brain. Viral antigen is later gets cleared from there due to the induction of adaptive immune. A strong virus-specific antibody response, in CNS may act for recovery from encephalitic infection. Clinical infections with the mosquito-borne encephalitic flaviviruses in humans mostly occur in the absence of detectable viremia consistent with the notion that humans are dead-end hosts in the natural transmission cycle (Müllbacher et al., 2003).

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.

7. Immune response

The pathogenesis of the neurotropic flaviviruses like JEV, involves both virus-mediated damage and the host immune responses. After the mosquito bite, when the virus is inoculated in the host, it replicates in skin dendritic cells, and then is transported to lymph nodes, from where it spreads to peripheral organs, enhancing the viremia. Entry into CNS is an important event which aids viral encephalitis [9]. Roles of both the innate and adaptive immune responses in controlling flaviviral infection are important. IgM and IgG are involved in preventing viral dissemination to the CNS, however CD8+ T cells are important for recovery and immunopathological phases of viral infection.

Infection with flavivirus triggers the host's innate immunity, resulting in signalling pathways and production of interferons (IFN) which are secretory cytokines produced as a response against viral infection. When these IFN bind to the cell-surface receptors, Jak–Stat signaling pathway is activated which in turn induces the transcription of interferon-stimulated genes (ISGs), and the resulting products have potent antiviral, antitumor, and immunomodulatory effects. To win against the IFN defence system, viruses encode viral proteins which are potent enough to block IFN signaling, via various mechanisms (Sen 2001, Weber et al., 2004) like blocking IFN action by preventing Tyk-2 phosphorylation by the production of NS5 protein of JEV (Lin et al., 2004; Lin et al., 2006;Liang et al., 2009).

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8. 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 (Tiwari et al., 2012).

The earliest host response to viral infection is the induction of IFN. Type I IFNs, IFN-α and β are produced by leukocytes and fibroblasts, respectively, in response to infection and activate the transcription of a host of IFN inducible genes that leads to the induction of antiviral pathways. IFN-α has important immunoregulatory functions including the activation of monocytes, enhancement of chemokine expression and MHC class I and II induction. Most of the antiviral activity of IFN-α is mediated by NO radicals synthesized by monocytic phagocytes, mortality in JEV-infected mice increased when the activity of NO synthase was inhibited (Saxena et al., 2000, 2001) as NO blocks mechanism of viral RNA and protein synthesis (Müllbacher et al., 2003). Also natural killer (NK) cells are important part of the innate immune response which is activated at the viral invasion which helps in early defence as NK cells synthesize and regulate cytokines, necessary for adaptive immune response.

9. Adaptive immune response

The importance of humoral response in recovery from encephalitis is demonstrated by several studies showing that administration of antibody during early infection can protect against JE. Studies of the entry process of JEV using electron and confocal microscopy techniques showed that neutralizing mAb strongly inhibits JEV-induced fusion and internalization into cells, but not binding of virus to cells. T cells are of crucial importance for the recovery from most virus infections and individuals deficient in T cells are unable to control virus infections. T cells are necessary for recovery and protection after

JEV infection and also their depletion affects the humoral and cellular, immune defence against flavivirus infections (Müllbacher et al., 2003).

10. RNAi effect on JE

Inhibitory effect of RNAi on JEV replication has been thoroughly studied *in vitro* and *in vivo* (Murakami ET AL., 2005). It is also reported that defective interfering (DI) RNA aids in the persistence of JEV (Yoon et al., 2006). Effectiveness of using siRNA expression based vectors targeting the JEV NS5 gene to inhibit JEV replication, viral protein expression, and RNA levels of JEV E-protein is hot topic of research nowadays. Several studies demonstrate that shRNAs targeting the NS5 gene could specifically and efficiently inhibit JEV replication. Many researchers have shown that siRNA/shRNAs targeting the RdRP coding gene could efficiently inhibit viral replication; the inhibition of viral replication triggered by siRNA/shRNA targeting of the RdRP gene are reported to be more efficient compared to other genes from the genome (Neyts et al., 1999). Therefore, the NS5 gene which is highly conserved among different strains is often employed as an RNAi target for different studies. shRNAs targeting NS5 gene in the JEV genome are shown to be capable of interfering with JEV replication with very high specificity and efficiency. Hence shRNAs could be used as a potential tool against JEV replication *in vitro*. More research investigating RNAi methodologies to prevent infection or reduce viremia is necessary which may lead to the development of antiviral compounds that are efficacious and inexpensive against Japanese encephalitis infections (Qi et al., 2008).

11. JAK-STAT pathway for JE

IFN-α and IFN-β, play important role in recovery from flaviviral infections. However, they fail sometimes due to ability of JEV to inhibit the JAK-STAT (Janus kinase signal transducer and activator of transcription) pathway (Lin et al., 2006). Studies of DEN-2 antagonism of STAT1 phosphorylation have revealed NS4B as the primary and important antagonist. Still the exact mechanism of IFN antagonism is under study. The specific receptor complex for each IFN-α and IFN-β is composed of two major subunits and several JAK tyrosine kinases constitutively associated with the receptor. Jak1 and Jak2 are required for IFN-α/β signaling. Following binding of the receptor subunits by IFN, the JAKs trans-phosphorylate each other and then phosphorylate critical tyrosine residues within the intracellular domains of the receptor subunits (Lin et al., 2004). These phosphorylated residues serve as recruitment sites for STAT proteins, which bind the activated receptor and are in turn phosphorylated by the JAKs. The phosphorylated STAT proteins then form homodimers, or heterodimers, with other STAT proteins and translocate to the nucleus, where they bind specific DNA sequences within the promoter regions of IFN-stimulated genes (ISGs) (Fig. 5). ISG expression induces an antiviral state within the cell, can modulate cell proliferation and cell death, and modulates immune re-

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sponses via its roles in activation and maturation of antigen-presenting cells. The ability of the individual non-structural proteins to antagonize JAK-STAT signaling has been studied and results indicated that NS5 blocked STAT1 phosphorylation in response to either IFN-α or IFN-β which highlights the function of NS5 to have a critical role in virus pathogenesis (Best et al., 2005).

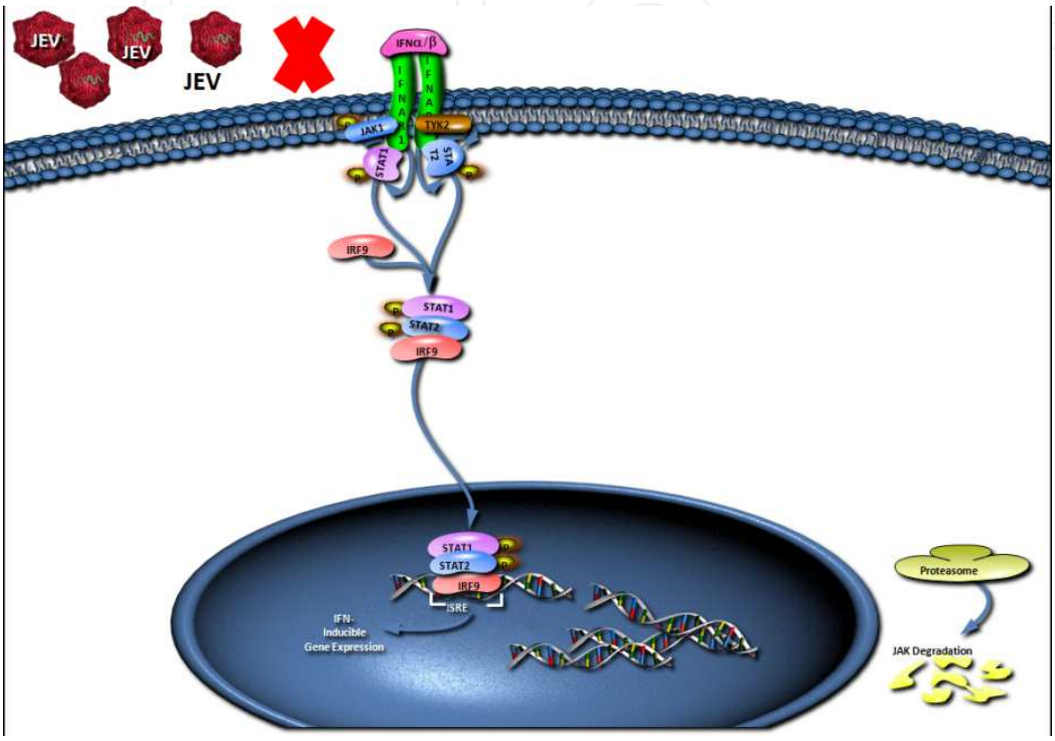

Figure 5. Displaying the JAK STAT Pathway. JAK STAT signaling pathway is important for transduction of information between cells carrying information for cellular differentiation and homeostasis. Cytokines and their receptors are the major activator of JAK/STAT pathway. IFN are antiviral cytokines produced by cells as soon as the onset of viral infection. IFN-α and IFN-β bind to different receptors and, play important role in recovery from flaviviral infections. However, they fail sometimes due to ability of JEV to inhibit the JAK-STAT pathway.

12. Diagnosis

Serology is an important tool for the diagnosis of JE since the virus is difficult to isolate from clinical samples. The hemagglutination inhibition assay also is used but it has practical limitations as it requires paired serum samples from the acute and convalescent phases. The IgM antibody capture ELISA for CSF and serum samples is currently the standard test for diagnosis of JE but still has the drawback of not being able to diagnose about the infection in early stage. Molecular methods using reverse-transcriptase (RT) - PCR techniques have proved to be highly effective for diagnosing infection by RNA viruses. JE viral genome sequences have been detected by RT-PCR in CSF from acute encephalitis cases from several places around the globes. The conventional RT-PCR has

shown good specificity in the diagnosis of JEV in both blood and CSF samples but it has poor sensitivity as the virus is often cleared from the peripheral circulation/CSF by the time the test is performed. With the advent of monoclonal antibodies as potential diagnostic tool (Chávez *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 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 *et al.,* 2011) with the type of encephalitis and duration of illness.

13. Vaccines: Immunization against JE

Immunization against JE is cost effective strategy for control and prevention of JE. It has been reported globally that there is a decrease in incidence rates of JE in endemic areas which are administered with high immunization. The 3 most important types of JE vaccines, administered in current era are: the mouse brain derived, purified, inactivated vaccine based on either the Nakayama or Beijing strains of the JE virus; the cell culture derived inactivated JE vaccine based on the viral Beijing P3 strain and the cell culture derived live attenuated JE vaccine based on the SA 14-14-2 strain of the JE virus. In JEV infection, the immunity against prM, E and NS1 proteins is more effective than that of other viral proteins in host defense. (Gao et al., 2010). Currently available vaccines against JE include chemically inactivated vaccines (INV) and a live attenuated vaccine (LAV). Although a mouse brain derived INV produced by BIKEN had been the only internationally approved vaccine and has been used worldwide since the 1960s. But it had a drawback as there were reports of severe adverse events including acute disseminated encephalomyelitis (ADEM) in people vaccinated with it. In early 2009, Vero cell derived INVs produced by Intercell (Austria) and BIKEN (Japan) were licensed. Although these INVs are useful in developed markets, INVs are not ideally suited for nationwide vaccination programs for many endemic countries, since INVs require multiple doses to induce long lasting immunity. LAVs are thus a useful alternative and have been used for decades in China, and other Asian countries, but their substrate and the production methods have still not been approved in other markets, which serve as a drawback to this vaccine. (Ishikawa et al., 2011). Live-attenuated virus vaccines (LAVs) and inactivated virus vaccines (INVs) serve against flaviviral disease, they are potent and economical but do not suit immunocompromised patients. INVs are safer, but are more expensive to produce and less potent. Hence there is an immense need of devicing new and improved products.

Type I IFNs are critical for controlling pathogenic virus infections and can enhance immune responses. Hence their impact on the effectiveness of live-attenuated vaccines involves a balance between limiting viral antigen expression and enhancing the development of adaptive immune responses. The influence of type I IFNs on these parameters has been examined following immunization with RepliVAX WN, a single-cycle flavivirus vaccine (SCFV). Repli-

VAX WN-immunized mice produced IFN-α and displayed increased IFN-stimulated gene transcription (Winkelmann et al., 2012). Multiple vaccines exist to control Japanese encephalitis (JE), but all suffer from problems but this new flavivirus vaccine, a pseudoinfectious virus (RepliVAX WN) that expresses the JE virus (JEV) prM and E proteins prevents flaviviral disease. Engineered second-generation RepliVAX (RepliVAX JE.2) elicited neutralizing antibodies in experimental mice and provided 100% protection from a lethal challenge with JEV (Ishikawa et al., 2008).

Although a licensed vaccine has been available to prevent JE for over 40 years, approximately 20,000 cases are reported annually with 6000 resulting in death. Unfortunately, due to gaps in surveillance, the incidence of JE is also likely to be much higher than reported. A number of different vaccines are available to prevent JE and these have demonstrated an excellent record of efficacy throughout their history. The vaccine that has been in use the longest is the INV prepared from JEV infected mouse brains. This vaccine has been used extensively in East Asia since the 1960s to control JE, and is widely used throughout the world to immunize travellers who visit endemic areas, its protective efficacy is reported to be 80–90% in JEV endemic regions. But still it has a drawback that the product requires a three dose vaccination schedule in order to induce protective immunity and this, along with the recommendation of boosters every 2–3 years which poses as quite expensive for nominal patients from low income group countries and time-consuming too. Furthermore they are also reported for causing allergic reactions and more dangerous side effects like complications including severe neurological disorders such as acute disseminated encephalomyelitis, etc in people being administered by the vaccine (Widman et al., 2008).

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 et al., 2009). Second generation recombinant vaccines 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 are under clinical trials. DNAzymes cleave the RNA sequence of the 3'-NCR of JEV genome *in vitro*, on intra-cerebral administration in JE infected mice almost completely (Appaiahgari et al., 2007) and inhibit virus replication in the brain. Use of neutralizing bodies for vaccine designing may also serve the process.

14. Treatment, prevention and control

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. Neutrophils have been also shown to have degradative effect on JEV. Usage of anti-sense molecules (vivo-morpholino) directed against the viral genome, in combating the virus through inhibiting viral replication has been dem-

onstrated (Nazmi et al., 2010). Mycophenolic acid (Sebastian et al., 2011) inhibits JE virus by inhibiting its replication.

Prevention methods are very important for minimizing JE infection (Saxena *et al.,* 2008). 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 *et al.,* 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). Diethyldithiocarbamate has been also experimentally shown to inhibit JEV infection. 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.,* 2011). Effective anti-viral drugs have yet to be found. Medicines are given mainly to relieve symptoms. Vaccination for people at risk, eliminate mosquito breeding grounds, improve drainage, maintain clean piggeries, use insect repellent and mosquito nets are some of the preventive measures which should be commonly undertaken.

The strategy for prevention and control of JE should include major components such as awareness among general public on the prevention and control of the disease, vector control and immunization. Environmental control is also one important factor as it has been studied that there is a positive impact of urbanization and economic development in the reduction of JE transmission, as clean and sanitized economies will not support environment necessary for mosquito breeding. Land areas under cultivation with impact of agrochemicals may work for reduction of vector density. Along with good environmental strategies vector control is also an important aspect. Maintenance of low vector densities is the need of hour.

Spraying, larviciding and aerial application are the method used for reduction of vector densities. However, alternatives to aerial application like spraying/fogging/Ultra Low Volume (ULV) application are also under consideration. Along with this long term i.e. non-chemical vector control such as water management is also helpful. Use of agrochemicals to control pests may have had indirect effect on vector control. Also, use of larvivorous fish may also be applicable in permanent water bodies. Personal protection is very important as vectors can feed on humans in outdoors, hence over vegetation and shaded humid places should be bit ignored. Minimizing outdoor activity for reducing the exposure time to mosquitoes and wearing long sleeved clothes are some habits needed to be undertaken consideration along with public information and awareness.

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15. Conclusion and future implications

Viral encephalitis has proved to be a huge disaster globally, which has engulfed several lives and has shattered various economies. It has been a hot topic amongst the researchers today globally and various ways necessary to combat against the virus are on the way. Intense research for the knowabouts of the virus is carried in several countries, devising strategies to fight with the virus. As a result of severe efforts, JE has been virtually eliminated in most of the countries after the immunization with inactivated mouse brain-derived vaccine, during last four decades. Because of absence of treatment strategies personal protection is the only apt way to reduce disease incidence. Mosquito control is the sole available preventive measure for JEV transmission. Research on JEV needs to be initiated at much wider scale, which should include development of effective anti-viral agents and vaccine strategies. Immunization is needed in JE prone areas. Over use of the vaccines should be avoided otherwise the virus might develop resistance against drugs which are administered frequently. 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 designed in a way that they can control the risk of vectors in an efficient way. General awareness camps should be organized in rural areas to spread alertness in the local population and confronting them with hygiene management and preventive measures. Systematic and combinatorial approach with the joint efforts of scientists, molecular biologists, doctors, drug developers, policy makers and local population is the need of hour. A high sense of urgency is required to address this matter.

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References

[1] Appaiahgari, M.B. and Vrati, S. (2007). "DNAzyme-mediated inhibition of Japanese encephalitis virus replication in mouse brain." *Mol Ther*. 15(9): 1593-1599.

[2] Best, S.M., Morris, K.L., Shannon, J.G., Robertson, S.J., Mitzel D.N., Park, G.S., Boe,r E., Wolfinbarger, J.B. and Bloom, M.E. (2005). "Inhibition of interferon-stimulated JAK-STAT signaling by a tick-borne flavivirus and identification of NS5 as an interferon antagonist." *J Virol.* 79(20): 12828-12839.

[3] Chávez, J.H., Silva, J.R., Amarilla, A.A. and Moraes Figueiredo, L.T. (2010). "Domain III peptides from flavivirus envelope protein are useful antigens for serologic diagnosis and targets for immunization." *Biologicals.* 38(6): 613-618.

[4] Chung, C.C., Lee, S.S., Chen, Y.S., Tsai, H.C., Wann, S.R., Kao, C.H. and Liu, Y.C. (2007). "Acute flaccid paralysis as an unusual presenting symptom of Japanese encephalitis: a case report and review of the literature." *Infection.* 35(1): 30-32.

[5] Das, S., Chakraborty, S. and Basu, A. (2010). "Critical role of lipid rafts in virus entry and activation of phosphoinositide 3' kinase/Akt signaling during early stages of Japanese encephalitis virus infection in neural stem/progenitor cells." *J. Neurochem.* 115(2): 537-549.

[6] Desai, A., S.K. Shankar, Jayakumar P.N., Chandramuki A., Gourie-Devi, M., Ravikumar, B.V. and Ravi, V. (1997). "Co-existence of cerebral cysticercosis with Japanese encephalitis: a prognostic modulator." *Epidemiol Infect.* 118(2):165-71.

[7] Erlanger, T.E., Weiss, S., Keiser, J., Utzinger, J. and Wiedenmayer, K. (2009). "Past, present, and future of Japanese encephalitis." *Emerg Infect Dis.* 15(1): 1-7.

[8] Weber F., Kochs, G. and Haller, O (2004). "Inverse interference: how viruses fight the interferon system." *Viral Immunol.* 17(4): 498–515.

[9] Fulmali, P.V., Sapkal, G.N., Athawale, S., Gore, M.M., Mishra, A.C. and Bondre, V.P. (2011). "Introduction of Japanese encephalitis virus genotype I, India." *Emerg. Infect. Dis.* 17(2): 319-321.

[10] Sen, G.C. (2001). "Viruses and interferons." *Annu Rev Microbiol.* 55: 255–281

[11] Gao, N., Chen, W., Zheng, Q., Fan, D.Y., Zhang, J.L.., Chen, H, Gao, G.F., Zhou, D.S. and An, J. (2010). "Co-expression of Japanese encephalitis virus prM-E-NS1 antigen with granulocyte-macrophage colony-stimulating factor enhances humoral and antivirus immunity after DNA vaccination". *Immunol Lett.* 129(1): 23-31.

[12] Gupta, N., Lomash, V. and Rao, P.V. (2010). "Expression profile of Japanese encephalitis virus induced neuroinflammation and its implication in disease severity." *J. Clin. Virol.* 49(1): 4–10.

[13] Guy, B., Guirakhoo, F., Barban, V., Higgs, S., Monath, T.P. and Lang, J. (2010). "Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue, West Nile and Japanese encephalitis viruses." *Vaccine.* 28(3): 632-649.

[14] Halstead, S.B. and Thomas, S.J. (2011). "New Japanese encephalitis vaccines: alternatives to production in mouse brain. Expert Rev." *Vaccines*. 10(3): 355-364.

[15] Huang, J.H., Lin T.H., Teng, H.J., Su, C.L., Tsai, K.H., Lu, L.C., Lin, C., Yang, C.F., Chang, S.F., Liao, T.L., Yu, S.K., Cheng, C.H., Chang, M.C., Hu, H.C. and Shu, P.Y. (2010). "Molecular epidemiology of Japanese encephalitis virus, Taiwan." *Emerg. Infect. Dis.* 16(5): 876-878.

[16] Ishikawa, T., Wang, G., Widman, D.G., Infante, E., Winkelmann, E.R., Bourne, N. and Mason, PW. (2011). "Enhancing the utility of a prM/E-expressing chimeric vaccine for Japanese encephalitis by addition of the JEV NS1 gene." *Vaccine.* 29(43):7444-7455.

[17] Ishikawa, T., Widman, D.G., Bourne, N., Konishi, E. and Mason, P.W. (2008). "Construction and evaluation of a chimeric pseudoinfectious virus vaccine to prevent Japanese encephalitis." *Vaccine.* 26(22):2772-2781.

[18] Jelinek, T. (2009). "Ixiaro: a new vaccine against Japanese encephalitis." *Expert Rev Vaccines*. 8(11): 1501-1511.

[19] Liang, J.J., Liao, C.L., Liao, J.T., Lee, Y.L. and Lin, Y.L. (2009). "A Japanese encephalitis virus vaccine candidate strain is attenuated by decreasing its interferon antagonistic ability." *Vaccine.* 27(21): 2746-2754.

[20] Lin, R.J., Chang, B.L., Yu, H.P., Liao, C.L. and Lin, Y.L. (2006). "Blocking of interferon-induced Jak-Stat signaling by Japanese encephalitis virus NS5 through a protein tyrosine phosphatase-mediated mechanism." *J Virol*. 80(12): 5908-5918.

[21] Lin, R.J., Liao, C.L., Lin, E. and Lin, Y.L.(2004). "Blocking of the alpha interferon-induced Jak-Stat signaling pathway by Japanese encephalitis virus infection." *J Virol*. 78(17): 9285-9294.

[22] Mathur, A., Chaturvedi, U.C., Tandon, H.O., Agarwal, A.K., Mathur, G.P., Nag, D., Prasad, A. and Mittal, V.P. (1982). "Japanese encephalitis epidemic in Uttar Pradesh, India during 1978." *Indian J Med Res.* 75: 161-169.

[23] Misra, U.K. and Kalita, J. (2010). "Overview: Japanese encephalitis." *Prog Neurobiol.* 91(2): 108-120.

[24] Müllbacher, A., Lobigs, M., Lee, E. (2003). "Immunobiology of mosquito-borne encephalitic flaviviruses." *Adv Virus Res.* 60: 87-120.

[25] Murakami, M., Ota, T., Nukuzuma, S. and Takegami, T. (2005), "Inhibitory effect of RNAi on Japanese encephalitis virus replication *in vitro* and *in vivo*." *Microbiol Immunol.* 49(12): 1047-1056.

[26] Nabeshima, T., Loan, H.T., Inoue, S., Sumiyoshi, M., Haruta, Y., Nga, P.T., Huoung, V.T., del Carmen Parquet, M., Hasebe, F. and Morita, K. (2009). "Evidence of fre-

quent introductions of Japanese encephalitis virus from south-east Asia and continental east Asia to Japan." *J Gen Virol.* 90(Pt 4): 827-832.

[27] Nazmi, A., Dutta, K. and Basu, A. (2010). "Antiviral and neuroprotective role of octaguanidinium dendrimer-conjugated morpholino oligomers in Japanese encephalitis." *PLoS Negl Trop Dis*. 4(11): e892.

[28] Nett, R.J., Campbell, G.L. and Reisen, W.K. (2009). "Potential for the emergence of Japanese encephalitis virus in California." *Vector Borne Zoonotic Dis.* 9(5): 511-517.

[29] Neyts, J., Leyssen, P. and De Clercq, E. (1999). "Infections with flaviviridae." *Verh K Acad Geneeskd Belg.* 61(6): 661-697.

[30] Pujhari, S.K., Prabhakar, S., Ratho, R.K., Modi, M., Sharma, M. and Mishra, B. (2011). "A novel mutation (S227T) in domain II of the envelope gene of Japanese encephalitis virus circulating in North India." *Epidemiol Infect.* 139(6): 849-856.

[31] Qi, W.B., Hua ,R.H., Yan, L.P., Tong, G.Z., Zhang, G.H., Ren, T., Wu, D.L. and Liao, M. (2008). "Effective inhibition of Japanese encephalitis virus replication by small interfering RNAs targeting the NS5 gene." *Virus Res.* 132(1-2): 145-151.

[32] Robinson, J.S., Featherstone, D., Vasanthapuram, R., Biggerstaff, B.J., Desai, A., Ramamurty, N.,. Chowdhury, A.H, Sandhu, H.S., Cavallaro, K.F. and Johnson, B.W. (2010). "Evaluation of three commercially available Japanese encephalitis virus IgM enzyme-linked immunosorbent assays." *Am J Trop Med Hyg.* 83(5): 1146-1155.

[33] Saxena, S.K. (2008). "Japanese encephalitis: perspectives and new developments." *Future Neurol*. 3(5): 515-521.

[34] Saxena, S.K., Tiwari, S., Saxena, R., Mathur, A. and Nair, M.P.N. (2011). 'Japanese Encephalitis: an Emerging and Spreading Arbovirosis: In Flavivirus Encephalitis" (Book), Daniel Ruzek (Ed.), ISBN 979-953-307-775-7, InTech, Croatia (European Union), 295-316.

[35] Saxena, S.K., Mathur, A. and Srivastava, R.C. (2001). "Induction of nitric oxide synthase during Japanese encephalitis virus infection: evidence of protective role." *Arch Biochem Biophys*. 391(1): 1-7.

[36] Saxena, S.K, Mathur, A. and Srivastava, R.C. (2003). "Inhibition of Japanese encephalitis virus infection by diethyldithiocarbamate is independent of its antioxidant potential." *Antivir Chem Chemother.* 14(2): 91-98.

[37] Saxena, S.K., Mishra, N., Saxena, R., Singh, M. and Mathur, A. (2009). "Trend of Japanese encephalitis in North India: evidence from thirty-eight acute encephalitis cases and appraisal of niceties." *J Infect Dev Ctries*. 3(7): 517-530.

[38] Saxena, S.K., Singh, A. and Mathur, A. (2000). "Antiviral effect of nitric oxide during Japanese encephalitis virus infection." *Int J Exp Pathol.* 81(2): 165-172.

[39] Saxena ,S.K., Singh, M., Pathak ,AK. and Mathur, A. (2006). "Reply to 'Encephalitis outbreak finds Indian officials unprepared." *Nat Med.* 12(3): 269-270.

[40] Saxena, V., Mishra, VK, Dhole, TN. (2009). "Evaluation of reverse-transcriptase PCR as a diagnostic tool to confirm Japanese encephalitis virus infection." *Trans R Soc Trop Med Hyg.* 103(4): 403-406.

[41] Schuh, A.J., Li, L., Tesh, R.B., Innis, B.L. and Barrett, A.D. (2010). "Genetic characterization of early isolates of Japanese encephalitis virus: genotype II has been circulating since at least 1951." *J Gen Virol*. 91(Pt 1): 95-102.

[42] Sebastian, L., Madhusudana, S.N., Ravi, V. and Desai, A. (2011). "Mycophenolic acid inhibits replication of Japanese encephalitis virus." *Chemotherapy.* 57(1): 56-61.

[43] Shimojima, M., Nagao, Y., Shimoda, H., Tamaru, S., Yamanaka, T., Matsumura, T., Kondo, T. and Maeda, K. (2011). "Full Genome Sequence and Virulence Analyses of the Recent Equine Isolate of Japanese Encephalitis Virus." *J Vet Med Sci*. 73(6): 813-816.

[44] Singh, A., Saxena, S.K., Mishra, N., and Mathur, A. (2009). "Neuromicrobiology in India." In: Dhawan BN and Seth PK, ed. 'Neurosciences in India' Published by: Indian Academy of Neurosciences (IAN) and Council of Scientific and Industrial Research (CSIR, India), 269-318.

[45] Singh, A., Saxena, S.K., Srivastava, A.K. and Mathur, A. "Japanese Encephalitis: A Persistent Threat."(2012) *Proc Natl Acad Sci.* 82(1): 55-68.

[46] Takahashi, H., Pool V., Tsai, T.F. and Chen, R.T. (2000). "Adverse events after Japanese encephalitis vaccination: review of post-marketing surveillance data from Japan and the United States. The VAERS Working Group. " *Vaccine.* 18(26): 2963-2969.

[47] Tiwari, S., Chitti ,S.V.P., Mathur, A., and Saxena, S.K. (2012). "Japanese encephalitis virus: an emerging pathogen." *American Journal of Virology*. (in press).

[48] van den Hurk, A.F.,. Ritchie, S.A and Mackenzie, J.S. (2009). "Ecology and geographical expansion of Japanese encephalitis virus." *Annu Rev Entomo.* 54: 17-35.

[49] Vashist, S., Bhullar, D. and Vrati, S. (2011). "La protein can simultaneously bind to both 30- and 50-noncoding regions of Japanese encephalitis virus genome." *DNA Cell Bio.* 30(6): 339–346.

[50] Widman, D.G., Frolov, I. and Mason, P.W. (2008). "Third-generation flavivirus vaccines based on single-cycle, encapsidation-defective viruses." *Adv Virus Res*. 72: 77-126.

[51] Winkelmann, E.R., Widman, D.G., Xia J., Ishikawa T., Miller-Kittrell, M., Nelson, M.H., Bourne, N., Scholle, F., Mason, P.W., and Milligan, G.N. (2012). "Intrinsic adjuvanting of a novel single-cycle flavivirus vaccine in the absence of type I interferon receptor signaling." *Vaccine.* 30(8): 1465-1475.

[52] Yamanaka, A., Mulyatno, K.C., Susilowati, H., Hendrianto, E., Utsumi, T. , Amin, M., Lusida, M.I., Soegijanto, S. and Konishi, E. (2010). "Prevalence of antibodies to Japa-

nese encephalitis virus among pigs in Bali and East Java, Indonesia, 2008." *Jpn J Infect Dis*. 63(1): 58-60.

[53] Yang, Y., Ye, J., Yang, X., Jiang, R., Chen, H. and Cao, S. (2011). "Japanese encephalitis virus infection induces changes of mRNA profile of mouse spleen and brain." *Virol J.* 8: 80.

[54] Yoon, S.W., Lee, S.Y., Won, S.Y., Park, S.H., Park, S.Y. and Jeong, Y.S. (2006). "Characterization of homologous defective interfering RNA during persistent infection of Vero cells with Japanese encephalitis virus." *Mol Cells*. 21(1): 112-120.

[55] Zhang, J.S., Zhao, Q.M., Guo, X.F., Zuo, S.Q., Cheng, J.X., Jia, N., Wu, C., Dai,, P.F. and Zhao, J.Y. (2011). "Isolation and genetic characteristics of human genotype 1 Japanese encephalitis virus, China, 2009." *PLoS One*. 6(1): e16418.

[56] Zheng, Y., Li, M., Wang, H. and Liang, G. (2012). "Japanese encephalitis and Japanese encephalitis virus in mainland China." *Rev Med Virol.* doi:10.1002/rmv.1710. [Epub ahead of print].