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Chapter 1

Japanese Encephalitis: A Neglected Viral Disease and Its Impact on Global Health

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Additional information is available at the end of the chapter

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1. Introduction

Japanese encephalitis (JE) is among the most significant viral encephalitis in Asia, particularly in rural and suburban areas where rice culture and pig farming coexist. It has also occurred rarely and occasionally in northern Australia and some parts of the western pacific. JE is caused due to infection with the JE virus (JEV), a mosquito borne flavivrus. The main JEV transmission cycle involves *Culex tritaeniorhynchus* mosquitoes and similar species that lay eggs in rice paddies and other open water resources, with pigs and aquatic birds as principal vertebrate amplifying hosts [Han *et al*., 2012]. Humans are generally considered as dead-end JEV hosts i.e. they rarely develop enough viremia to infect feeding mosquitoes. Nearly 20-30% of JE cases are fatal and 30-50% of survivors have major neurological disorders [Bhattacharyya *et al*., 2013]. JE is mostly a disease of children but other age groups may be affected [Kundu *et al*., 2013; Griffiths *et al*., 2013; Larena *et al*., 2013]. In most temperate areas of Asia, JEV is transmitted mainly during summer season, when large epidemics can occur. In the tropics and subtropics region, transmission can occur throughout the year but often increases during the rainy season [Campbell *et al*., 2011]. The first epidemic of JE was recorded in Japan in 1871. Major outbursts have been seen in nearly every 10 years. In 1924 more than 6,000 cases were reported in a major outbreak in Japan [Solomon *et al*., 2000]. The disorders caused by JEV began from Southeast Asia and now it's affecting people worldwide [Liu *et al*., 2013; Li *et al*., 2013]. Nearly 30 million people are at danger of JEV infection [Saxena *et al*., 2003]. Though intensive care and support are able to lower the death rate but patients continue to suffer from this disease for a long period of time. Some effects such as learning difficulties and behavioral problems can remain masked for several years.

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2. Epidemiological features

JE is believed to be originated in Indonesia and Malaysia long back in mid 1500s [Weaver *et al*., 1999; Sinniah *et al*., 1989]. JEV leads to major outbreaks in both temperate regions of Asia like China, Japan, Korea, Philippines, Taiwan and tropical regions like Bangladesh, India, Sri Lanka and Nepal [Bista *et al*., 2005]. The cases of JE are also reported in newer geographical regions in the Torres Strait islands of Australia and in Papua New Guinea [Fig. 1]. The reason for this wide spread of JE is unknown but it may be due to population shift or variations in agricultural practices, animal husbandry, climate, ecology or migratory birds patterns. In India the first case of JE was seen in 1955. JE is reported to be endemic in many parts such as Assam, Bihar, Madhya Pradesh, Tamil Nadu, Uttar Pradesh and West Bengal.

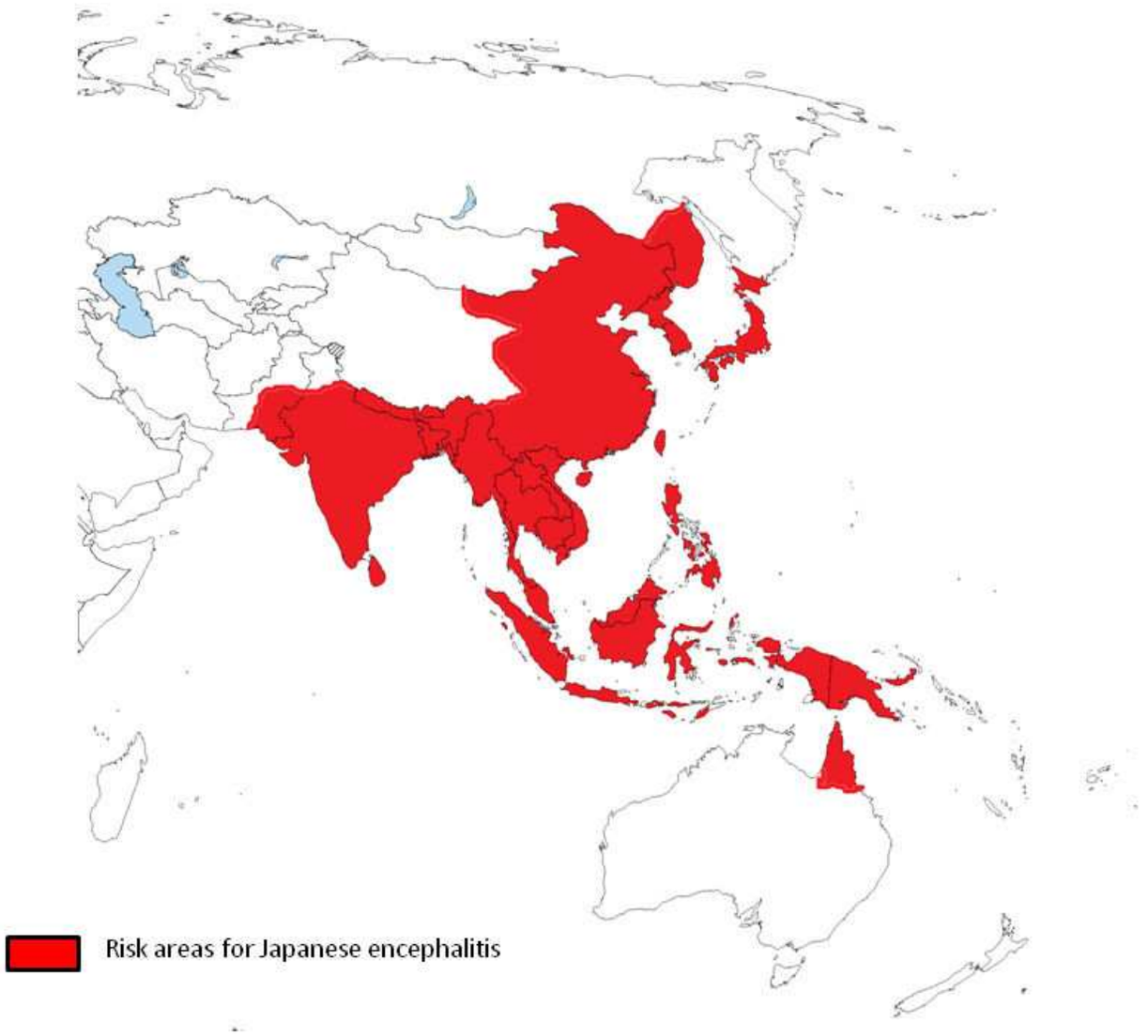


Figure 1. Epidemiology of JE globally. The areas highlighted in red display the endemic regions affected by JE.

3. Viral replication and morphogenesis

JEV virion comprises of a single strand of positive-sense RNA of around 11kb, enclosed in a nucleocapsid and surrounded by envelope made up of glycoproteins [Agrawal *et al*., 2013; Ye *et al*., 2012]. The RNA consists of a short 5′ untranslated region (UTR), a longer 3′ UTR and a single open reading frame between them. It encodes 3432 aminoacid polyprotein, which is translationally and post translationally cleaved by viral and host proteases into three structural proteins (core-C, pre-membrane-PrM and envelope-E) and seven nonstructural (NS) proteins (NS1,NS2A,NS2B,NS3,NS4A,NS4B and NS5) [Fig 2][Yang *et al*., 2013].

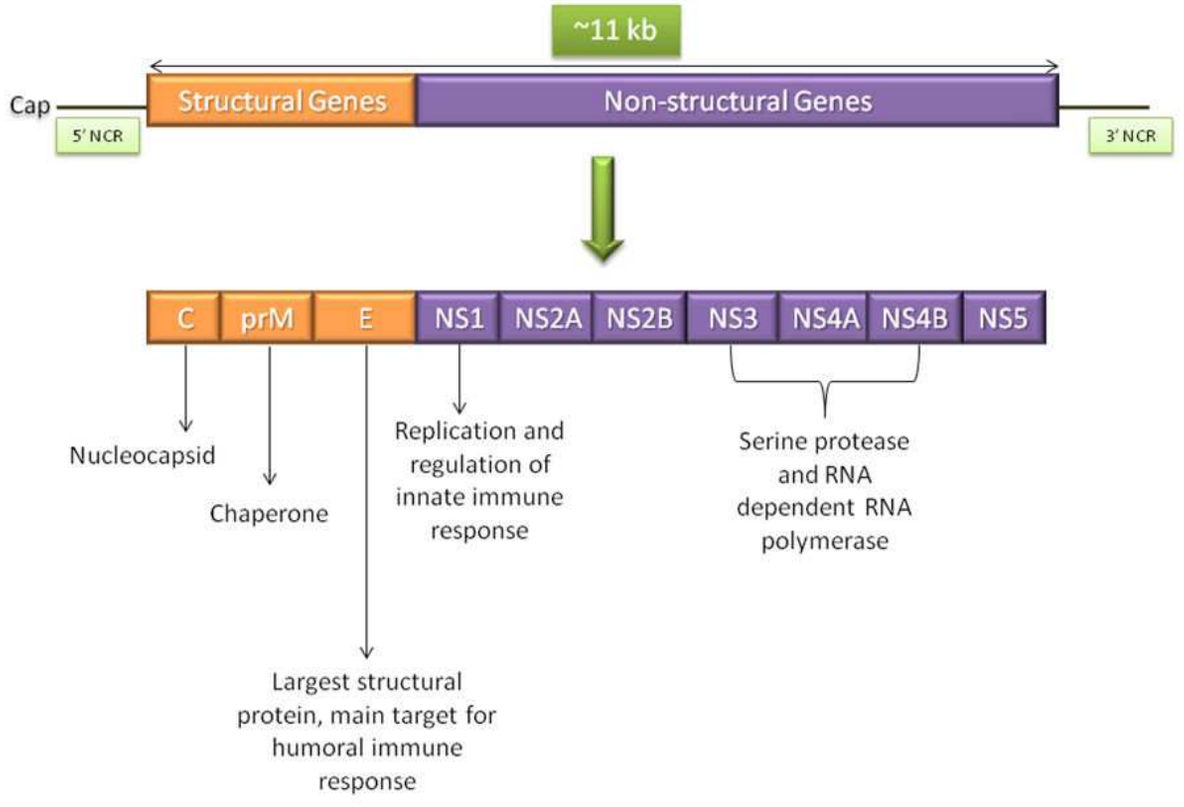


Figure 2. Organization of the JEV genome

The C protein of 12-14 kDa in size is highly basic and fuses with the RNA to form the nucleocapsid. The PrM is in close proximity with the E protein, forming a heterodimer and is believed to act as a 'chaperone' to it, hindering its function until after virion release. Just before the virion release, the PrM protein is cleaved by a protease to its mature M protein form. This alteration contributes for the formation and activation of E protein homodimers. Researchers suggested that the extremely conserved N glycosylation motif N^{15}-X^{16}-T^{17} in JEV PrM and its N-glycan substituents are essential for several stages of JEV biology: PrM biogenesis, virus release and pathogenesis. The E protein is the largest structural protein, comprising of approximately 500 aminoacids, with up to two potential glycosylation sites. It is the main target

for the humoral immune response and is believed to play a vital role in viral entry into host cells [Solomon *et al*., 2003]. The xlink protein is involved in virus replication and regulation of the innate immune response [Li *et al*., 2012; Zhang *et al*., 2012]. The functions of NS3 and NS4 are prominent, they code for serine protease and RNA dependent RNA polymerase (RdRp) [Lu *et al*., 2013]. There is a high rate of mutation in JEV because RdRp is expected to have some error which leads to vast alterations in genomic sequences of JEV worldwide [Saxena *et al*., 2008]. Since all flaviviral NS proteins are essential for viral replication, any one of them can be selected as target for selective inhibitors of viral replication for therapeutic intervention [Anantpadma *et al*., 2011; Mastrangelo *et al*., 2012].

4. Pathogenesis

The development of JEV infection, beginning from its entry till reaching its site of action, the central nervous system (CNS) is not well understood. Studies with other flaviviruses have brought us to the belief that upon entry through mosquito bite, the virus infects dendritic cells in the skin and is carried to the nearest draining lymph nodes, thereby initiating a round of early immune response. But this response is not enough to counter the virus. Meanwhile the virus spreads to secondary lymphoid organs before entering the blood circulation through the efferent lymphatic system. During the subsequent transient viremia, peripheral organs such as kidney, liver and spleen are known to be infected first, after which the neurotropic virus spreads to the CNS. It is still not clear how JEV is able to escape the host's peripheral immune response. After the virus escapes the immune system, it crosses the Blood Brain Barrier to enter the CNS. JEV may cross the BBB by passive transport across the endothelium, by active replication in endothelial cells or by a 'Trojan Horse' mechanism in which the virus is carried into the brain by infected inflammatory cells. Monocytes and macrophages are considered to be the feasible carriers of the virus in the CNS as the virus can survive for a prolonged time and effectively replicate within these cells. During the entry of infected monocytes and macrophages through the BBB, change in the structural and functional integrity of the BBB, leads to production of matrix metalloproteases released by endothelial cells of BBB. This further result in deterioration of BBB stability. BBB stability. Due to the compromised functioning of the BBB, peripheral inflammatory cells are recruited to the infected brain that extends the neuronal impairment.

JEV causes neuronal damage in the brain. However in several cases, JEV is possibly not directly involved in the destruction of brain tissue but it may activate microglia and trigger cell-mediated immune response. Microglial cells are the resident immune cells of the CNS and have a crucial role in host defense against invading microorganisms. Microglial activation is considered as an adaptive response whereby microglia release neuroprotective factors to ease the recovery of injured neurons. They also perform phagocytosis for dying or damaged neurons, before they lyse and release toxic agents into surrounding areas. JEV infection activates microglia both morphologically and functionally, *in vivo,* which causes rise in the level of pro-inflammatory mediators, such as IL-6, TNF-α, RANTES and MCP-1 [Thongtan *et al*., 2012]. These proinflammatory mediators and cytotoxins released from activated microglia

are involved in inducing neuronal death that complements JE. Neuronal death by secreted TNF is mediated by the TNF receptor-associated death domain protein (TRADD), which there upon regulates a downstream apoptotic cascade, in neurons [Swarup *et al*., 2007]. During JEV infection, nitric oxide (NO) is released by macrophages and plays a significant role in inflammation, even though NO itself is a strong antimicrobial agent, researchers have suggested that it strongly inhibits synthesis of viral RNA, protein accumulation and virus release from infected cells. NO production is higher in the JEV infected brain, and plays a crucial role in the innate immunity of the host and its ability to restrict the initial stages of JEV infection in the CNS [Saxena *et al*., 2000].

In addition to neuronal and microglial cells, astrocytes are also infected by JEV. Astrocytes are known to maintain homeostasis in the CNS and support the survival and information processing function of neurons. They respond fast to CNS infection and help regulate neuroinflammation. JEV infection results in astrocyte activation, but the infection overpowers the capacity of activated astrocytes to maintain metabolic homeostasis, resulting in an over accumulation of toxic byproducts of metabolism that are injurious to neuronal viability. However JEV infection triggers metabolic reprogramming by upregulating the expression of many proteins such as IP-10, ceruloplasmin and glutamine synthase by astrocytes, involved in the metabolic pathways vital for maintaining neuronal health. This increase is deficient to meet the increased demand that accompanies JEV infection. Astrocytes help in the transmission of JEV from peripheral tissues to the cerebrospinal fluid.

Disease course	Incubation period	Signs and symptoms
Prodromal stage	1-6 days	Fever, muscle pain, headache along with vomiting. In children gastrointestinal symptoms like nausea, vomiting, diarrhea and abdominal pain may be prominent.
Acute encephalitic stage	7-13 days	Photophobia, hyperexcitability, focal and neurological signs, muscular rigidity, dull, mask like face with wide unblinking eyes, tremor, widespread hyertonia, cogwheel rigidity, other irregularities in movement, upper motor neuron signs, cerebellar signs and cranial nerve paralysis sometimes leading to coma
Late convalescent stage	14-15 day onwards	Fever subsides, neurological signs may improve, and eventually either death may occur, or a long term psychoneurological condition may persist, if patient survives.

Table 1. Duration, signs and symptoms of Japanese encephalitis

JE usually develops in patients after an incubation period of 5-15 days [Table 1]. In humans, most JEV infections are asymptomatic, with about 1 in 300 JEV infections resulting in symptoms ranging from non-specific mild fever to severe meningoencephalitis categorized by fever, lessened consciousness, seizures and focal neurological signs. At later stages, poliomyelitis-like flaccid paralysis and parkinsonian syndrome develop, which exhibit the standard description of JE like dull, flat and mask-like face with wide, unblinking eyes, tremor, wide-

spread hypertonia, cogwheel rigidity and other irregularities in movement [Dutta *et al*., 2010]. Paralysis of the upper body is more common than that of legs. Nearly 30% of survivors have genuine persistent motor deficits and approximately 20% have severe cognitive and language impairment [Mackenzie *et al*., 2004].

5. Transmission

The JE virus exists in a zoonotic transmission cycle between mosquitoes and pigs and/or water birds; humans get infected only accidentally when bitten by an infected mosquito [Fig 3] and are a dead-end host [Gould *et al*., 2008]. JEV has been isolated from many mosquito species in field studies, and even though the major mosquito vectors differ in diverse geographical regions, the most important is *Culex tritaeniorhynchus*. For Eastern Asia, Southern Asia and Southeastern Asia, the chief vector is *C. tritaeniorhynchus* [Rao *et al*., 2001]. For Northern Australia, the chief vector is *C. annulirostris.* From India's outlook, there are several other secondary vectors such as *Anopheles peditaeniatus, A. subpictus, C. epidesmus, C. gelidus, C. pseudovishnui, C. whitemorei, Mansonia uniform* and *M. Indiana* [Borah *et al*., 2013]. Pigs are the key component in the transmission cycle with respect to human infection, while egrets, herons and other ardeid birds are significant maintenance hosts [Hecker *et al*., 2013; Sarkar *et al*., 2013]. Of other vertebrates, horses can develop CNS infection but are a dead-end host; Other domestic animals may also get infected, but do not show any evidence of viremia. Rodents are refractory to infection; and amphibians, bats and reptiles can be infected experimentally and virus can persist, but the role of these species in hibernating and maintaining the virus in the environment is undisclosed.

There are two epidemiological forms of transmission: an endemic form in tropical areas with virus circulation almost throughout the year, but with a wide seasonal peak probably due to irrigation practices; and an epidemic form in more temperate areas with clear summer seasonality [Schuh *et al*., 2013; Gao *et al*., 2013]. Subsequently, JE is mainly a rural disease, where *Cx. tritaeniorhynchus* mosquitoes breed in rice paddies and pigs provide the main source of blood meals, with the consequence of transmission cycles in close proximity to human habitation.

6. Diagnosis

Patients with JE present many signs of acute encephalitic syndrome. There are various possible causes of acute encephalitic syndrome; therefore laboratory confirmation is crucial for the accurate diagnosis of JE [Table 2], which is a tough task because of very low viremia. Reverse passive hemagglutination, immunofluorescence and staphylococcal coagglutination tests using polyclonal or monoclonal antibodies have proved the value of antigen detection in Cerebrospinal fluid (CSF) for rapid diagnosis of JE. Advanced methods like Immunogold silver staining (IGSS), have been effectually used in the detection of antigen in mononuclear cells of peripheral blood and CSF of patients. Immunohistochemistry has been used to detect viral

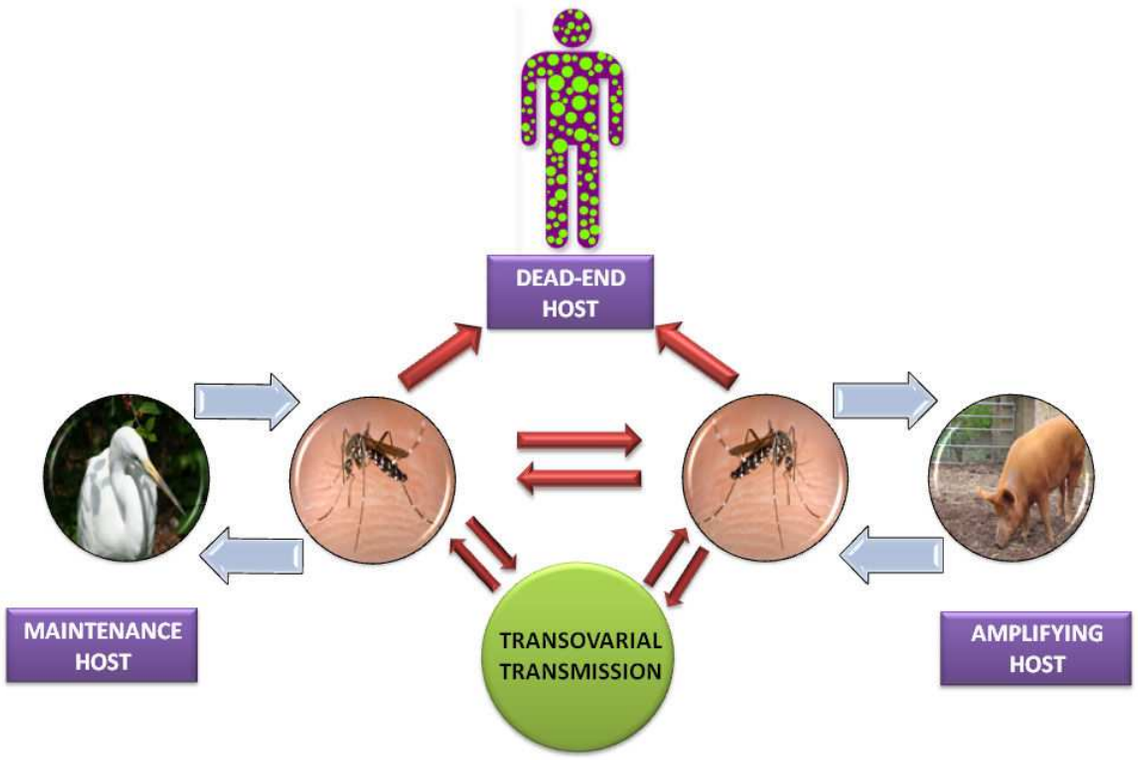


Figure 3. Transmission cycle of Japanese encephalitis virus. Infected Culex mosquitoes (vectors) play the role in the spread of JEV. Pigs are the amplifying hosts and birds (egrets) are the maintenance hosts while humans are the dead-end hosts.

antigens in the CNS. Histopathology inspection is also very obliging for clinical association and diagnosis of JEV. Diagnosis is accordingly targeted towards the detection of antibodies in serum and cerebrospinal fluid. Cases like cross-reactivity of antibodies to other flaviviruses cause confusion in the diagnosis of JEV. IgM capture ELISA has been the most extensively used diagnostic method for JE detection [Hobson-Peters *et al*., 2012; Palani *et al*., 2013; Borthakur *et al*., 2013]. Currently, dipstick method, JEV-CheX and reverse transcriptase PCR are some of the methods which are used for the early detection of JEV [Yang *et al*., 2013; Seo *et al*., 2013; Zheng *et al*., 2013].

Diagnostic tool	Detects
Immunogold Silver Staining (IGSS)	Antigens in mononuclear cells peripheral blood and CSF
Hemagglutination test	Antigens in CSF
MAC-ELISA	IgM antibodies
Dipstick method, JEVCheX	Antibodies
RT-PCR, RT-LAMP	Universal oligonucleotide primers

Table 2. Laboratory diagnostic tools for Japanese encephalitis

7. Treatment

Currently there is no therapy for JE [Saxena *et al*., 2009]. Presently, chemotherapy during JE is mainly supportive and not targeted towards JEV specifically. Interferon therapy has not proved to be a great success [Tiwari *et al*., 2012]. Naturally occurring compounds such as arctigenin, a phenylpropanoid and rosmarinic acid, which is a phenolic compound found in many *Labiatae* provide protection to mice against JEV GP78 by noticeably decreasing JEV induced neuronal apoptosis, activation of microglial cells, active caspase activity and induction of proinflammatory mediators in the brains of the infected animals.

An *in vivo* study has shown that minocycline reduces neuronal apoptosis, activation of microglial cells, active caspase activity, proinflammatory mediators and viral titer on later stages after infection. Another compound, N-methylisatin-β-thiosemicarbazone derivative is known to inhibit JEV replication completely *in vitro*. Glucosidase inhibitors of the endoplasmic reticulum such as N-nonyl-deoxynojirimycin, which block the trimming step of N-linked glycosylation, have been shown to eliminate the production of many endoplasmic reticulum-budding viruses, including dengue type-II and JEV. Another recent study carried out in mice using RNA interference showed that a single intracranial organization of lentiviruses delivering short hairpin RNA or lipid-complexed small interfering RNA (siRNA) either before or after the viral challenge was sufficient to provide protection against lethal encephalitis. From the study it was clear that by precise drug design of the conserved site, a single siRNA treatment could suppress viral infection across species, thus enhancing the treatment of acute viral infections with overlapping clinical symptoms [Ghosh *et al*., 2009].

8. Protection

The control of JE is based mainly on three measures: mosquito control, avoiding human exposure and immunization. Mosquito control has failed to be an effective measure and suffers from the lack of research into new pesticides. Avoiding complete exposure from infected mosquitoes is not practically possible. Accordingly, immunization is the only effective method for long-term protection. To prevent JE, it is crucial to apply a large scale vaccination for the human population in JE prone areas [Tiwari *et al*., 2012]. There are many groups of vaccines [Table 3] which are presently in use such as purified, formalin-inactivated mouse brain derived and cell culture derived live attenuated vaccine [Lin *et al*., 2013]. Several vaccines are still in different stages of development such as DNA vaccines, recombinant virus based/chimeric vaccines [Li *et al*., 2013]. In most countries, the currently available vaccine for use is an inactivated vaccine derived from mouse brain, which is manufactured in many regional countries, but it is costly, involves three doses, needs boosting at quite frequent intervals, may be less effective due to antigenic variation and gives rise to a number of vaccine related adverse reactions [Yun *et al*., 2013].

Vaccine	Source	Characteristics
(i) Formalin-inactivated mouse brain derived	Nakayama strain	Costly, less effective and side effects are seen
(ii) Inactivated hamster kidney cell vaccine	Beijing strain	Very less side effects are seen
(iii) Live attenuated hamster kidney cell line vaccine	SA14-14-2 strain, China	Costly but effective and very less side effects are seen

Table 3. Comparison of vaccine for Japanese encephalitis

There are several side effects of JE vaccination. Side effects which are mainly seen after vaccination are redness, swelling and tenderness. Rarely systematic adverse reactions are also seen after vaccination like headache, myalgia, abdominal pain and skin rash. Some recipients of the vaccine had very rare major neurological side effects [Sohn *et al*., 2000]. To avoid some of the adverse reactions, vero cell grown inactivated vaccines are being examined and some of them are currently in clinical trials. Live attenuated vaccines seem to offer good hope; some of the advantages are that these provide long lasting immunity and are very sensitive. Currently, the only potential vaccine is the Chinese SA 14-14-2 strain [Verma *et al.,* 2012]. Protein-protein interaction is essential for various cellular functions and impeding such interactions using synthetic composites is a very noteworthy idea for formulation of new pharmaceuticals [Haridas *et al*., 2013].

9. Conclusion

JE is a neurological disease caused by a mosquito-borne JEV. Unlike smallpox and polio, JEV cannot be completely eradicated because of its enzootic nature of transmission. Ever since its discovery, JEV has continued to expand its activity into new regions, while many JE vaccines have been made commercially available in different parts of the world. Concern about its spread has been emphasized by the recent emergence and spread of JEV in northern Australia, making it a major concern for global public health. Currently, prevention of infection with most arboviruses relies primarily on efforts to control vector populations by spraying repellents, wearing protective clothing and reducing breeding places.

One of the most important research areas is the development of an ideal JE vaccine: one that is nontoxic, less expensive and more effective and that provides life-long protection with a single dose. The development of such a vaccine will be greatly aided by a expanding our knowledge of JEV replication and pathogenesis at the molecular level, which has now become technically possible with the use of infectious JEV SA14-14-2 cDNA technology. This technology also has huge potential for developing JEV SA14-14-2 as a vaccine vector to deliver foreign gene(s), as has already been accomplished with infectious YFV 17D cDNA technology.

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