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REVIEW

Japanese Encephalitis: A Persistent Threat

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Abstract Japanese encephalitis virus (JEV) belongs to arthropod-borne virus family, which are infectious agents transmitted by blood-sucking arthropods. They multiply in the tissues of the arthropod without evidence of disease or damage. The principal vector is *Culex* mosquito, most important being *C. tritaenorhynchus*, which is a rural mosquito, breeding extensively in rice fields and bites large domestic animals. In temperate zones, this vector is present in greatest density from June to November. The natural cycle of JEV consists of pig–mosquito–pig or bird–mosquito–bird cycle. Pigs serve as most important biological amplifiers and reservoirs. JEV is transmitted to humans through the bites of infected mosquitoes. Humans are accidental, dead-end hosts as they do not develop a level of viraemia sufficient to infect mosquitoes. The infection with JEV ranges from nonspecific febrile illness to a severe meningoencephalomyelitis illness. The risk for Japanese encephalitis varies by local ecology and season. In temperate areas of Asia, human disease usually peaks in summer and falls down after that and these seasonal epidemics can be explosive with thousands of cases occurring over a period of several months; whereas, in the subtropics and tropics, transmission can occur throughout the year, with disease at a peak during rainy season. A detailed study has been done on geographic distribution of JEV and incidence and burden of disease in India. Vaccination proves to be the best measure to protect the individual against any disease. So, large scale immunization of susceptible human population is highly important to prevent this deadly infection. In India, vaccinations against Japanese encephalitis are administered in areas where the disease is hyperendemic.

Keywords Japanese encephalitis · Encephalopathy · Mosquito-borne infection

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Introduction

Japanese encephalitis (JE), one of the most common cause of acute encephalitis in tropics [1, 2], has generated considerable public anxiety in India. Caused by a neurovirulent RNA flavivirus, JEV and transmitted by mosquitoes, the occurrence of this disease highlights the inter relationship between man and animals, birds and the environment. Among an estimated 35,000–50,000 cases reported annually, approximately 20–30% of patients die and 30–50% of survivors suffer from neurological sequelae [3, 4]. The infection with JEV ranges from nonspecific febrile illness to a severe meningoencephalomyelitis illness [5]. The symptoms are serious and no definite treatment is available. JE is mainly a disease of children, but it can occur among persons of any age which is mostly travel related and is generally very rare [6–8]. Travellers living for prolonged periods in rural areas where JE is endemic or epidemic are at greater risk than short term and urban travellers.

Vaccination proves to be the best measure to protect the individual against any disease. Hence, large scale immunization of susceptible human population is highly important

to prevent this deadly infection. In India, vaccinations against Japanese encephalitis are administered in areas where the disease is hyperendemic, like Gorakhpur region of Uttar Pradesh [9]. In the case of JE, it is essential to immunize the pigs (amplifying host) also to interrupt the transmission of disease [10]. Thus, control of vectors and intense immunization in endemic regions are the only effective preventive measure. The vaccines available for JE these days are of three types—(i) A Mouse-brain derived inactivated vaccine (only vaccine which is WHO approved), (ii) cell-culture derived inactivated JE vaccine (which was developed in China), and (iii) cell-culture derived live attenuated JE vaccine (which is the most cost-effective vaccine [11]). Apart from them, recombinant protein based vaccine and DNA vaccines are still in development process.

Historical Background

Japanese encephalitis was first recognised as a clinical entity in Japan in 1871; but the causative agent (JEV) was later isolated from a fatal human case in 1934 in Japan and was serologically and virologically established as a prototype (Nakayama) strain [12]. The Nakayama strain was later used in the development of mouse brain inactivated virus vaccine. It took almost 25 years after its first isolation, to elucidate the mode of transmission by mosquito vector. In last three decades, the focus of viral epidemics has changed from temperate zone of Asia to South and Southeast Asia [13]. In India, acute viral encephalitis was first detected in 1955 and since then, it has taken away thousands of lives. Several encephalitis outbreaks reported in the later years remained undiagnosed.

Ecology and JEV Transmission

JEV belongs to arthropod-borne virus family (arboviruses), which are a group of infectious agents that are transmitted by blood-sucking arthropods. They multiply in the tissues of the arthropod without evidence of disease or damage. The principal vector of this zoonotic disease is *Culex* mosquito, most important being *C. tritaenorhynchus*, others are *C. vishnui* and *C. pseudo-vishnui* [14, 15], which are primarily rural mosquitoes breeding extensively in rice fields and preferentially bite large domestic animals like pigs and wading birds [16]. In temperate zones, this vector is present in greatest density from June to November. The natural cycle of JEV consists of pig–mosquito–pig or bird–mosquito–bird cycle (Fig. 1). Other minor hosts are cattle, buffaloes, goats, sheep, horses, rodents, monkeys, dogs and bats [17].

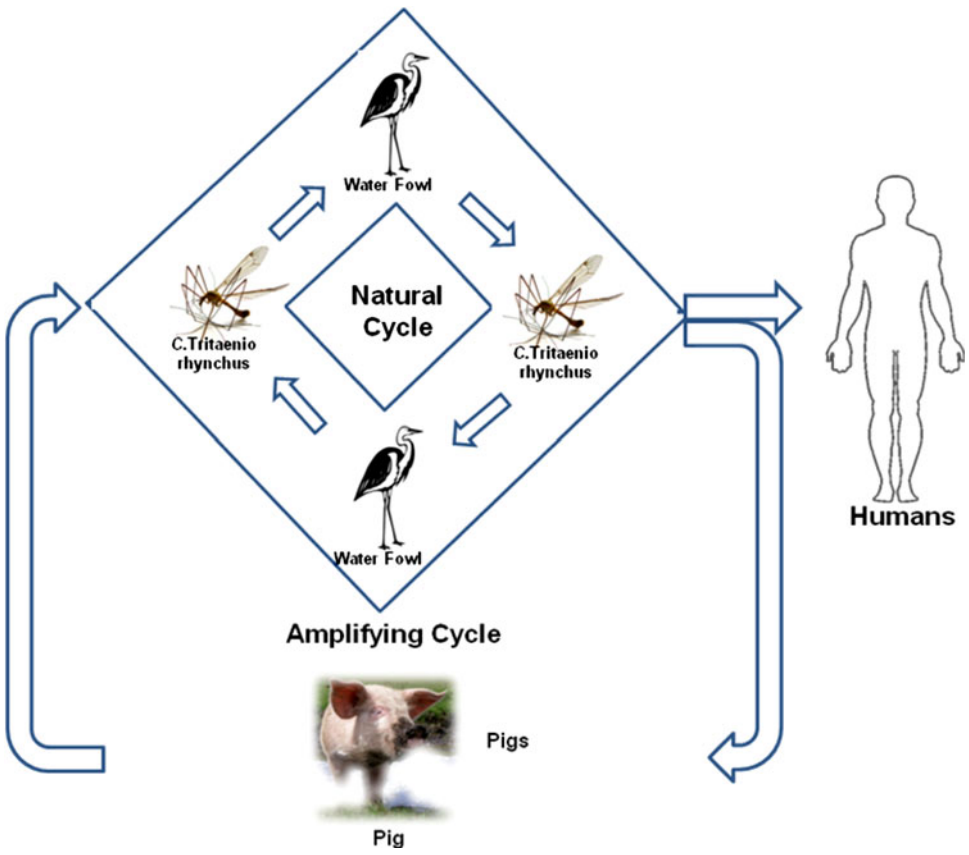

Fig. 1 Transmission cycle of Japanese encephalitis virus (JEV). JEV is transmitted in an enzootic cycle between *Culex* mosquitoes and amplifying vertebrate hosts, primarily pigs and wading birds like water fowls and egrets. Human beings serve as a dead-end host in the JEV transmission cycle with low levels of viremia. Humans play no role in the maintenance or amplification of JEV, and the virus is not transmitted directly from person to person

Pigs are the most important biological amplifiers and reservoirs [18]. Countries, like Pakistan, that do not rear pigs experience rare JE cases [19]. In the study done on rural children in Thanjavur district (Tamil Nadu), an area with extensive paddy cultivation, JE occurrence was found to be very low and without epidemics. The reason could possibly be high cattle to pig ratio (400:1) [20]. JEV is transmitted to humans through the bites of infected mosquitoes. Humans usually do not develop a level of viraemia sufficient to infect mosquitoes [21]. Therefore, humans are accidental, dead-end hosts, and human JE cases imported into non-endemic areas represent a minimal risk for subsequent transmission of the virus [22]. Direct person-to-person spread of JEV does not generally occur until and rarely it is through intrauterine transmission [23, 24]. Blood transfusion and organ transplantation may also serve as potential mode of JEV transmission [25]. The risk for JE varies by local ecology and season. In temperate areas of Asia, human disease usually peaks in summer and falls down after that [26–28] and these seasonal epidemics can be explosive with thousands of cases occurring over a period of several months; whereas, in the subtropics and tropics, transmission can occur throughout the year, with disease at a peak during rainy season. A recent study in China has correlated the different weather variables like temperature, relative humidity as possible predictors of Japanese encephalitis incidence for regions with similar geographic, weather and socio-economic conditions [29]. However in endemic areas (e.g., southern India) sporadic cases may occur throughout the year due to congenial climatic conditions [30].

Geographic Distribution of JE

Japanese encephalitis occurs throughout Asia and parts of the Western Pacific areas (Fig. 2). The disease was recognized in the first half of 20th century in the temperate areas of Asia, mainly Japan, Korea, Taiwan and mainland China [31–33]; but over the past few decades, the disease appears to have spread to Southeast Asia, India, Bangladesh, Sri Lanka and Nepal [34–37]. By 1990s, JEV had spread to non-Asian regions, Saipan and Australia, where it was reported first from Torres Strait islands and then from northern mainland [38, 39]. The reasons for this wide distribution can be population shifts or changes in climate, ecology, agricultural practices, animal husbandry or migrating bird patterns [40]. Though exact cause is uncertain, these factors could very well contribute to further spread, potentially beyond Asia and Western Pacific.

Incidence and Burden of Disease in India

In India, the disease has been recognized since 1945 and though virus causes epidemics, it is endemic in several parts of the country [41–44]. The earliest evidence of the prevalence of JE virus was cited through serological

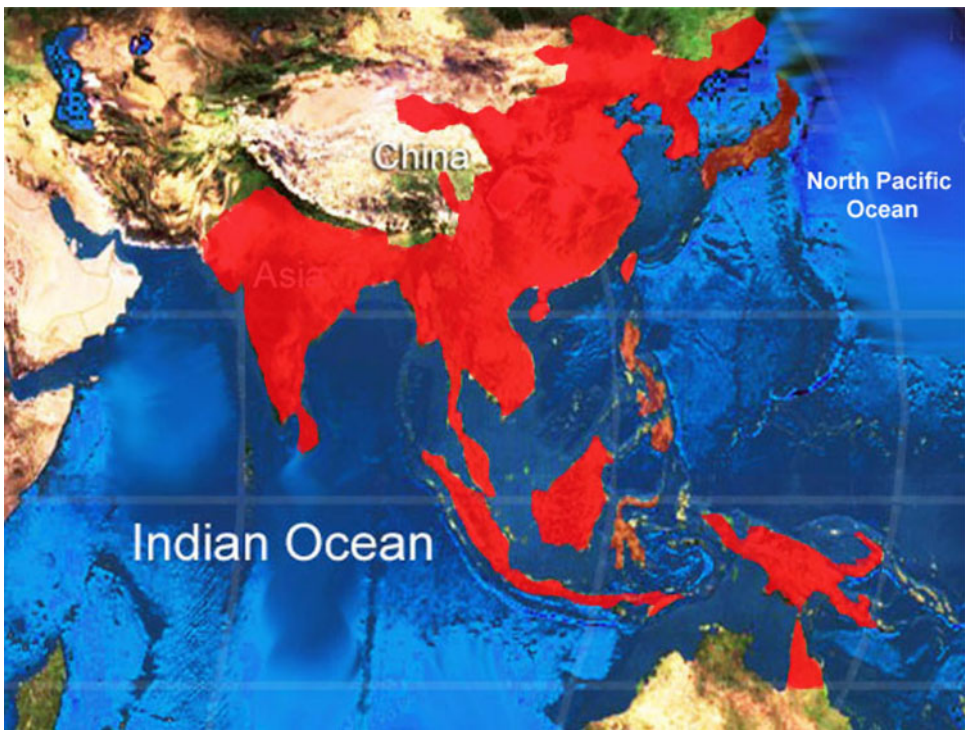

Fig. 2 Approximate geographic range (epidemiology) of Japanese encephalitis highlighting the areas which are engulfed by Japanese encephalitis virus

studies carried out at the Virus Research Centre, Pune in 1952 and then in 1956, the isolation of JE virus was made from the wild caught mosquitoes, establishing its presence in India. However, the virus could not be recovered from human until 1958, when three isolations were made from the brain tissue of encephalitis cases in Tamil Nadu [45]. The geographic area affected by JEV has expanded in the last 60 years with higher epidemic activity in North India and Central India (Fig. 3). Since the confirmation of first clinical case of JE from Vellore in 1955 [46], there had been many major JE outbreaks reported from Bihar, Uttar Pradesh, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa and Union Territories of Goa and Pondicherry [47, 48].

Carey et al. [49] had reported almost 65 JE cases from South India from 1955 to 1966 by demonstrating specific neutralizing antibodies to JE. In its first major outbreak in West Bengal, JE had taken about 300 lives from the two districts, Burdwan and Bankura in 1973 [50]. During 1978, several outbreaks of JE occurred in different parts of India viz. Kolar district of Karnataka, Tirunelvelli, Benpura, Burdwan and adjoining areas of West Bengal, Dhanbad district of Bihar, Dibrugarh district in Assam, Goa on the west coast [42] and eastern districts of Uttar Pradesh [41], which was one of the worst JEV outbreaks. From this outbreak, a successful isolation and identification of JE virus as the causative agent was made from the brain tissue of a fatal case of encephalitis from Gorakhpur district for the first time by Asha Mathur and her team at the King Georges' Medical College, Lucknow [41]. Outbreak of JE was again reported in 1981 from South Arcot district [46] and from Gorakhpur in 1988 with as much as around 875 cases reported [51]. Eastern paddy growing districts of Haryana suffered an epidemic of encephalitis in 1990 [43] and Muzaffarpur district of Bihar in 1995. JE had emerged as a major public health problem in naive non-endemic area like Kerala [52], where a large outbreak of JE occurred in Kuttanad area of Allepey district [53]. In a severe epidemic of JE during October–November 1999 in

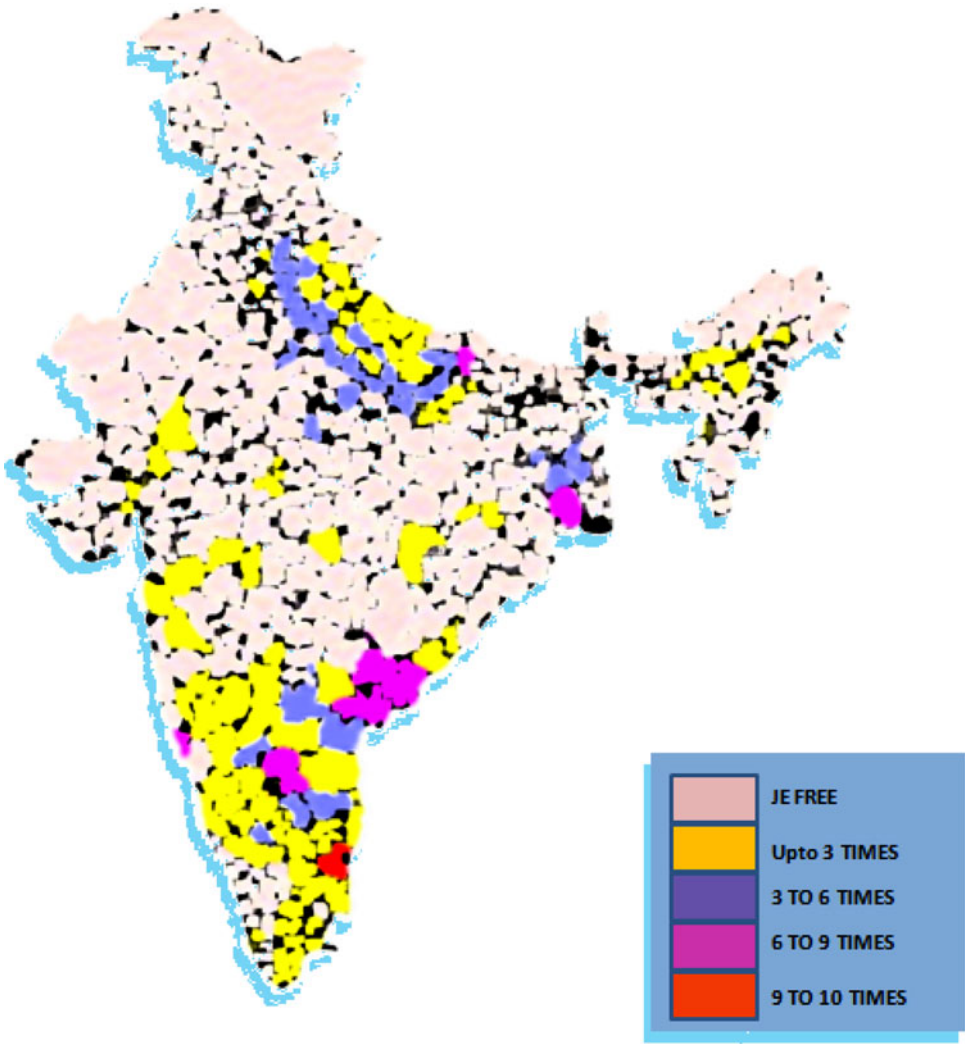

Fig. 3 Approximate display of JE occurrence areas in India. Different colours in the map highlight the intensity by which JEV occurs in different areas

Andhra Pradesh, out of 23 districts, fifteen districts were affected with 873 cases and about 20% mortality [54]. Another outbreak occurred in July 2003 in Warangal and Karim Nagar district of Andhra Pradesh [55]. From north-east regions, JE outbreaks which were reported from Assam during July–August 1989, had affected a population of ~36,000 with 50% case fatality rate [56]. After that, several other outbreaks have been reported from Assam between 2000 and 2002 [57].

Thus, encephalitis by JEV that was known to cause epidemics in several parts of the country now has become endemic in many parts of India, especially in South India [58] (including Tamil Nadu [47]) and terrain belt of Uttar Pradesh [59], which is a matter of serious public health problem. In Uttar Pradesh, the most severely JE affected region is Gorakhpur and the longest epidemic in three decades, had been reported from this district from July to November, 2005, in which 5,737 people were affected and approximately 1,344 died [60]. More recent studies on phylogeny of virus revealed that the epidemic was caused by JEV GP78 [61]. Gorakhpur and surrounding region in Uttar Pradesh still gets consistent outbreaks of fatal acute encephalitis syndrome (AES), of which ~10–15% is caused by JEV [62].

Among children in endemic zone, the incidence of laboratory confirmed JE varies widely areawise, the range being 5–50 cases per 100,000 children/year [28, 63]. The number of affected persons is magnified also because of many non-JE cases with unidentified etiologies. So, now the cases are classified as JE and non-JE and enterovirus 89 and 76 were isolated from the CSF of non-JE encephalitis cases from Gorakhpur in 2006 [11].

Japanese Encephalitis Virus

Four Flaviviral infections, of which three are encephalitis, are transmitted by mosquitoes from birds [64]. Clinically the most serious of these infections is "Japanese encephalitis", caused by Japanese encephalitis virus (JEV). The North Indian strain of Japanese encephalitis virus (JEV) is GP78, which is phylogenetically closer to the Chinese SA14 isolate. The other, Vellore P20778 isolate, was obtained from southern India in 1958 [65]. JEV is an enveloped virus and has a linear, single stranded, positive-sense ~11 kb long RNA as a genome and measures 40–50 nm in diameter with a nucleocapsid which is surrounded by a lipid envelope (Fig. 4). The genomic RNA

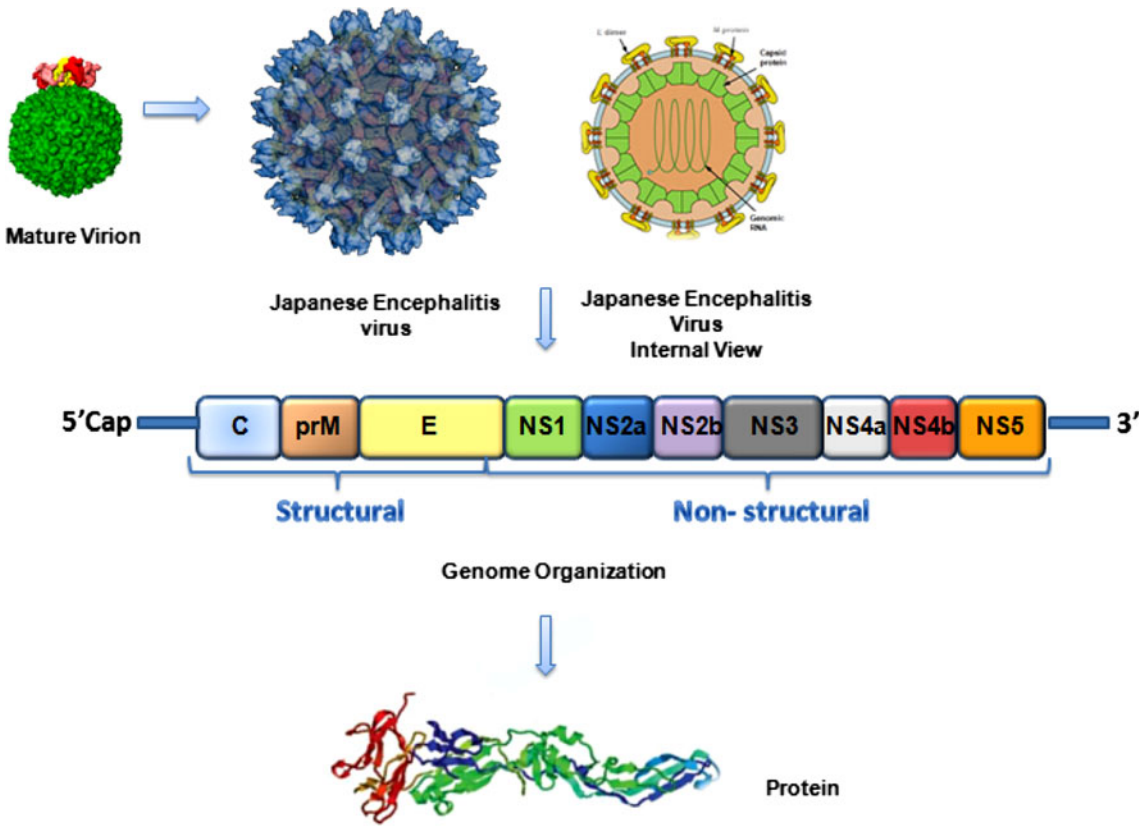

Fig. 4 Morphology of the Japanese encephalitis virus and the detailed display of the organisation of viral genome highlighting the structural and non-structural genes and also the structure of protein extracted

contains a single open reading frame (ORF) and codes for a polyprotein of ~3,400 amino acids. This polyprotein is cleaved by viral and host proteases into ten proteins: three structural proteins (capsid, M and E proteins) and seven non-structural proteins [66] (NS1, 2A, 2B, 3, 4A, 4B & 5). Envelope or E defines the type specificity. The viral RNA has non-coding regions of 95 and 585 bases at 5′ and 3′ ends which interact with viral or host proteins which are important for virus replication [67]. Looking at the regular epidemics of JE, studies are being conducted to look for any mutation with the viral genome and as demonstrated by Pujhari et al. [68] a novel mutation was detected in domain II of the envelop gene of JEV circulating in North India. More studies are required to ascertain its role in JE pathogenesis.

Genotypes of Japanese Encephalitis Virus

Epidemics of encephalitis were described in Japan from 1870s onwards and have subsequently been found across most of Asia [3]. For most of the major human pathogens, we have little idea about where they have originated. Because JEV has so rapidly spread to new areas, tracing its geographical origin has been somewhat possible [69] and phylogenetic comparison with other flaviviruses suggest that it evolved from an ancestral virus a few centuries ago. Five genotypes of JEV have been identified based on nucleotide sequences of C/PrM and E genes: Genotype 1 to 5 [70]. Among the five, GIV and GV appeared rarely. Genotype 1 (GI) is found to be circulating in northern Thailand, Cambodia and Korea. It has been less associated with human disease than genotype 3 in China; but a recent study reported isolation of genotype 1 (GI) from a vaccinated boy who developed JE [71]. Genotype 2 (GII) includes isolates from southern Thailand, Malaysia, Indonesia and Northern Australia. Isolates from temperate regions of Asia i.e. Japan, China, Taiwan, Philippines, Vietnam, Sri Lanka, Nepal and India has been put in Genotype 3 (GIII), whereas GIV includes isolates from Indonesia only [72]. GV included only a single strain isolated from a patient from Mummer, Malaysia in 1952 [73]. Since all five genotypes have been found in the Indonesia Malaysia region, it is likely that these current five genotypes evolved around the same region (Fig. 5). GI and GIII occur in epidemic regions, whereas GII and GIV are associated with endemic disease [3].

JEV is naturally transmitted between vertebrate hosts by *Culex* and other mosquitoes. The reasons for its spread are uncertain but many include changing agricultural practices, such as increasing irrigation and animal husbandry [74]. JEV continues to spread with recent outbreaks in India, Nepal and Australia. GIII is most widely distributed and was the only genotype found in India till 2007 [62]. However, in a recent study done by Fulmali and his co-workers demonstrated simultaneous detection and isolation of GI and GIII JEV strains from 66 acute encephalitis syndrome (AES) patients. Clinical syndrome for the two strains was indistinguishable. Thus GI and GIII detection indicates their co-circulation and association with human infections in Gorakhpur region [9]. GI isolate from India has been found to share close relationship with GI strain from Japan and Korea. GIII was the major genotype circulating in Japan, Korea and Vietnam until the early 1990s. However, by mid 1990, G III disappeared in Japan and Vietnam and GI supplanted it. This phenomenon is called "Genetic Shift" [75]. In Japan, JEV has been shown to overwinter and settle [76]. In India, the exact mode of introduction of GI JEV is not clear. It is possible that it may have been introduced into India through migratory birds like other Asian countries [77]. More studies are required to establish the role of migratory birds in JE transmission.

Pathology and Pathogenesis

A considerable degree of variation exists in neurovirulence and peripheral pathogenicity among JE virus strains and the mechanism of JEV pathogenesis is still unclear [78]. It has also been noted that typical pathological changes associated with each epidemic varied dramatically [79]. After the virus gets inoculated into the skin by the bite of an infected mosquito, it is quickly disseminated in the body and proliferates in the reticuloendothelial system. There is a transient period of viremia after which invasion of the central nervous system occurs. JEV is thought to invade brain via vascular endothelial cells by endocytosis [80], which involve clathrin and membrane cholesterol, referred to as lipid rafts acting as portals for virus entry [81]. In brain, the distribution of virus is in the thalamus, hippocampus, substantia nigra and medulla oblongata [82]. In the neurons, JEV replicates and matures in the neuronal secretary system. Neurocysticercosis (NCC) in 33% of patients dying with JE has been seen, but in only 1% of patients dying due to other causes, suggesting that NCC may somehow predispose to JE [79, 83]. A high level of tumour necrosis factor is correlated with adverse outcome in JE [84].

Clinical Spectrum

The majority of human infections with JEV are asymptomatic with proportion of apparent to inapparent JEV infection found to be 1:300 to 1:1000 during epidemics [85]. In a study of JE infection in Assam, the ratio of

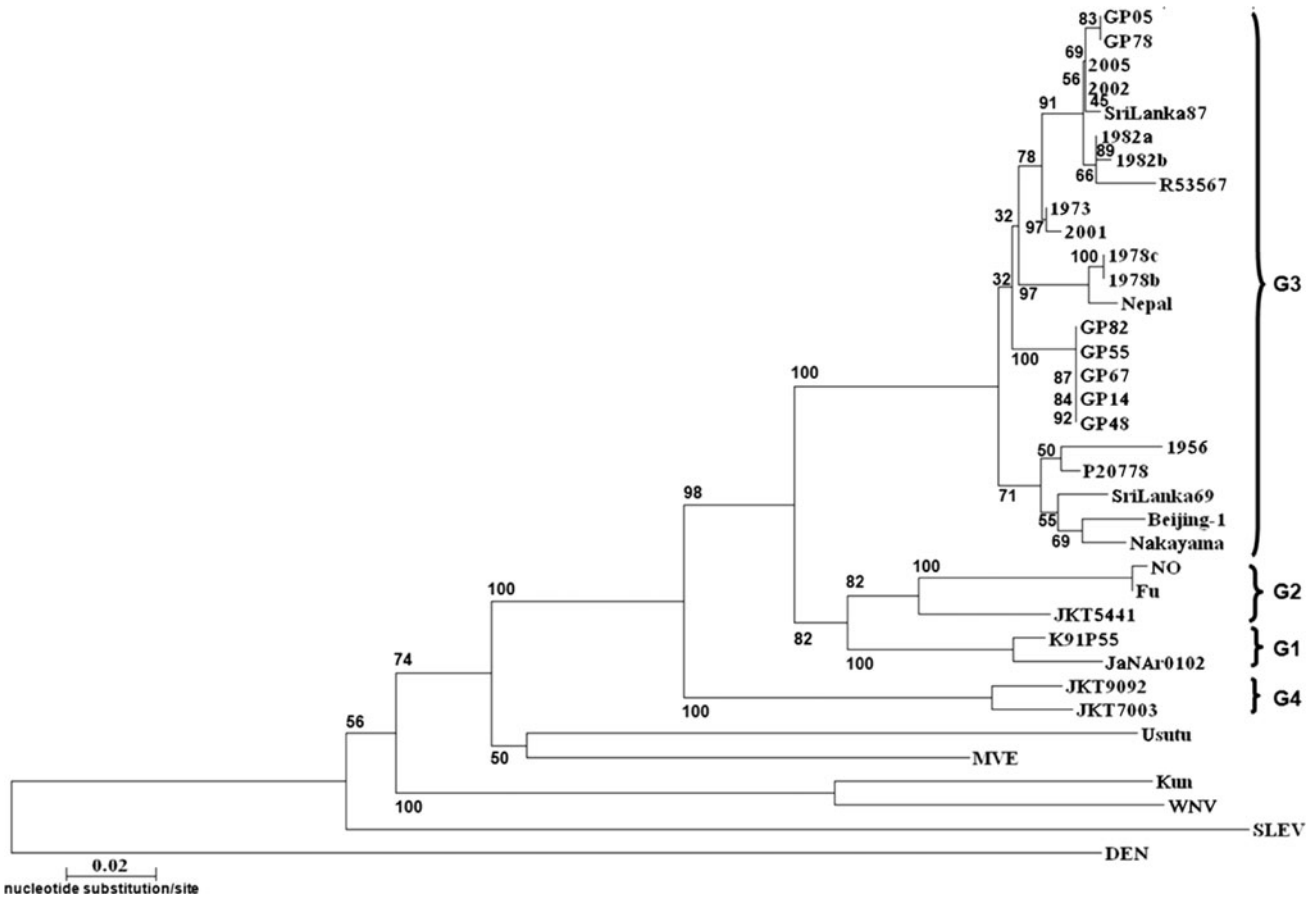

Fig. 5 Sequence phylogeny of JEV isolates. Sequence phylogeny of Japanese encephalitis virus isolates from Gorakhpur (India) 2005 epidemic, with reference to other Southeast Asian isolates and flaviviruses based on partial E gene sequence. The tree was generated by neighbor-joining method. Bootstrap values are indicated at the branch points. DEN, WNV, KUN, SLEV and MVE denotes Dengue virus, West Nile virus, Kunjin virus, Saint Louis encephalitis virus and Murray Valley encephalitis virus respectively [61]

apparent to inapparent infection was shown to be 9.1:100 [86]. Milder forms of disease (e.g. aseptic meningitis or undifferentiated febrile illness) can also occur, but have been reported more commonly among adults [87]. JE affects all age groups, with higher incidence in children aged <15 years of age [37, 88]. However, in countries (China and Japan) with childhood JE immunization programmes, the overall incidence of JE has decreased and similar numbers of cases are observed among children and adults [89, 90]. Because unvaccinated travellers from non endemic countries usually are immunologically naive, travel associated JE can occur in persons of any age [22].

Among patients, who develop clinical symptoms, the incubation period is 5–15 days. Table 1 shows the course of disease which is divided into three stages—prodromal stage, acute encephalitic stage and late convalescent stage. Fatality in JE patients is usually seen within 24–48 h of admission [91] and among the survivors; at least one in 200 affected individuals develop severe psychoneurological sequelae [92] in the form of parkinsonism [93], convulsive disorders, motor abnormalities, impaired intellect, hearing deficit, scholastic backwardness, speech disturbances [94] and other subtle neurological signs. Movement disorders have also been observed [95]. The disease can be much more difficult to detect in infants.

Immune Mechanism Against JE Infection

After the entry, JE virus is partially destroyed at the site of its entry and the remaining virus is disseminated by local and systemic extra neural replication leading to viraemia. After primary infection with JEV, presence of IgM antibodies and T-lymphocytes are seen until about 2 weeks [96, 97]. But antibodies alone are neither capable of terminating the viraemia nor preventing the subsequent infection [98]. Pregnancy is known to cause immunosuppression and persistent maternal infection or pregnancy induced reactivation of the virus may cause foetal infection. Isolation of JEV from human placenta and foetuses has been reported by Chaturvedi et al. [23], whereas Mathur et al. [24] have described an animal model of

Table 1 Clinical picture of JE

Disease course	Signs and symptoms
Prodromal stage	General malaise, fever, anorexia, headache, vomiting. Abdominal pain & diarrhoea common in children
Acute encephalitic stage	Continuous high fever, nuchal rigidity, seizures, muscle weakness, speech, hearing & vision problems, partial paralysis, altered sensorium leading to coma
Late convalescent stage	Complete recovery in mild cases, but neurological sequelae in severe cases

Table 2 Vaccines available against Japanese encephalitis virus

Three types produced and used worldwide:
Formalin-inactivated mouse brain derived—produced from Nakayama strain, safe and effective, most widely used. But expensive, complicated dosing schedule and side effects reported
Inactivated hamster kidney cell vaccine—developed from Beijing P3 strain in China, has efficacy of ~75–90% with few side effects.
Live attenuated hamster kidney cell vaccine—developed from SA 14-14-2 strain by China, has shown high efficacy with single dose and is cost effective. Being successfully used in India, Japan, Korea, Thailand etc.

congenital infection with JEV, showing an active replication of virus in placenta. They have also shown transplacental transmission of virus during consecutive pregnancies of mice. JEV can establish latency within different organs despite the presence of antiviral antibodies [99]. Immunological and virological evidence for persistence of JEV have been described in human nervous system also [100]. Latent and recurrent infection as a consequence of activation of latent JEV has been reported in humans [101].

A significant decrease in serum iron levels, a frequent feature of microbial invasion is observed during JE infection [102, 103]. An early influx of macrophages followed by neutrophils at the site of injury in different organs in humans [104] and mice [105] has been reported, which is correlated with the production of a neutrophil chemotactic macrophage derived factor, MDF [106], with development of hypoglycaemia [107, 108]. This chemotactic protein (MDF) has been shown to play a protective role in the host defence against JEV, through production of reactive oxygen intermediates [109] in neutrophils and reactive nitrogen oxide species [110] degrading the virus protein and RNA. In other studies, mechanism of proinflammatory cytokine [111] and cytokine secretion at molecular level has been studied and the involvement of monocyte and macrophage receptor, CLEC5A, in severe inflammatory response in JEV infection of brain is reported [112]. Misra et al. [113] have found increased levels of proinflammatory and anti-inflammatory cytokines and monocyte chemoattractant protein-1 in the serum of rats after JEV infection. The mechanism of JEV pathogenesis is still unclear [78]. For the development of appropriate and effective therapy there is an immediate requirement to understand the role of host factors in JEV-induced neuropathogenesis [114].

Laboratory Diagnosis

Clinical findings with JE are nonspecific and might include moderately elevated white blood cell count, mild anaemia and hyponatremia [22, 115]. The clinical suspicion of encephalitis can be supported with CSF analysis [30, 115]. Usefulness of various conventional magnetic resonance imaging (MRI) of the brain is better than computed tomography for detecting JEV associated changes [116]. Recently the utility and usefulness of various conventional MRI sequences in the diagnosis of acute viral encephalitis has been evaluated. Viral encephalitis patients were assessed by clinical evaluation and confirmation was done by analyzing serum or CSF for dengue, JE, herpes, measles, echo, coxsackie and polio viruses using ELISA or PCR. It was found that the MRI changes are correlated with type of encephalitis and duration of illness [117].

In India, the actual JE burden could be estimated only by strengthening diagnostic facilities for JE confirmation in hospitals [10]. The laboratory diagnosis of a confirmed case of Japanese encephalitis is based on either isolation of virus from specimen or by antibody detection. Because humans have low or undetectable levels of viremia by the time distinctive clinical symptoms are recognized, virus isolation and nucleic acid amplification tests (NAAT) are insensitive and should not be used for ruling out a diagnosis of JE [22]. For rapid diagnosis of JE, detection of antigen in CSF using immunofluorescence [118] and reverse passive haemagglutination [119] using poly or monoclonal antibodies has been successful. The reverse transcriptase polymerase chain reaction amplification of viral RNA may help in rapid detection of JEV in samples [13].

Acute phase specimens are tested for JEV-specific IgM antibodies using a capture enzyme-linked immunosorbent assay (MAC-ELISA) [22]. Many modifications of MAC-ELISA such as nitrocellulose membrane based IgM capture dot enzyme immunoassay (Mac DOT), Avidin biotin system (ABC Mac ELISA), biotin labelled immunosorbent assay to

sandwich ELISA and antibody capture radioimmunoassay (ACRIA) have been successfully tested for antibody detection [13]. Therefore, a positive IgM test has good diagnostic predictive value, but cross-reaction with arboviruses from same family can occur. The performance of three commercial JEV IgM antibody capture ELISA (MAC ELISA) kits has been evaluated with acute encephalitis specimens from India and Bangladesh and were found to be having high specificity (95–99.5%), but low sensitivities (15–57%) [120]. The two commercially available kits in India: Japanese encephalitis—Dengue IgM combo ELISA (Panbio Diagnostics) and JEV-Chex IgM capture ELISA (Cyton Diagnostics Limited) for the diagnosis of Japanese encephalitis in field samples have also been compared recently, in which the sensitivity and specificity were found to be 63.6 and 98.4% respectively for XCyton ELISA and 75 and 97.7% for Panbio ELISA [121].

Treatment and Management

There is no specific treatment or specific antiviral agent or other medication to mitigate the effects of JEV infection [122]. Thus it becomes highly important that the infection be managed carefully. Only one in every six deaths is directly due to JE virus and five out of six are preventable with prompt and early management, thus bringing down the case fatality rate of JE from 35–50% to less than 6% [30]. In different studies, monoclonal antibodies [13], corticosteroids, interferon alpha-2a or ribavirin have not been shown to improve clinical outcome [22]. Swarup et al. [123] have shown the effect of rosamarinic acid (RA) as an efficient antiviral agent that reduced JE viral load along with proinflammatory cytokines in experimental animals. Therapeutic role of diethyldithiocarbamate (Anti JEd) have been demonstrated during JEV infection [124]. Nazmi et al. [125] have demonstrated an effective way to counter the virus through inhibiting viral replication by using anti-sense molecules (Vivo-Morpholino) directed against the viral genome. Mycophenolic acid has been found to inhibit the replication of JEV in vitro and protected mice in vivo [126].

Outcome and Sequelae

JE has a case-fatality ratio of approximately 20–30% [22, 37, 123] and although some motor deficits improve after acute illness, 30–50% survivors have neurologic or psychiatric sequelae for years [123, 127]. Drug therapy and physiotherapy are necessary for survivors with sequelae like seizures, paralysis, movement disorders, Parkinsonism and psychiatric problems [30]. Also recurrence of symptoms after recovery with fresh neurologic deficits can occur as the virus remains latent in peripheral lymphocytes, and can later cause recurrence [99].

Prevention and Control

Preventive measures are very important for minimizing JE infection. Vector control should include measures such as eliminating potential mosquito breeding areas, shifting pigsties and environmental sanitation. Reduction of breeding source for larvae can be done by water management system with intermittent irrigation system, which is nothing but a strategy of alternate drying and wetting water management system in the rice fields. Equine and swine in affected areas should be vaccinated [10]. For humans in endemic areas, vaccination should be implemented, as well as personal protective measures. Vector control alone cannot be relied upon to prevent JE. Rapid identification and diagnosis is important for protecting the public and our livestock from this disease. For the disinfection, Biosafety level three precautions and practices are recommended for investigators working with this virus [128].

Vaccination

In the last five decades, JE has been effectively controlled and virtually eliminated in Japan, Taiwan, China and Korea after implementation of childhood immunization programs using inactivated mouse brain derived vaccine [129]. In Cambodia, JE surveillance commenced in 2006, and an immunization program has been planned [130]. Therefore, vaccination of the population at risk against JE, appears to be the most important control measure now, and in the future. At present, three types of vaccines are in use worldwide (Table 2).

(i) Formalin-inactivated mouse brain derived vaccine is the only WHO approved vaccine. Asian field trials have shown that two doses (1–2 weeks apart) provide an efficacy of 91% [131]. However, three doses (0, 1 and 6 months) are being followed to immunize children in endemic zone. The vaccine is contraindicated for women who are pregnant and those who are immunocompromised. It is also being produced from Beijing-1 strain due to its high cross-reactivity among the JEV strain. The vaccine is being manufactured by Research Institute, Kasauli in India [132]. Vaccine has some common allergic reactions like fever, headache, malaise, dizziness, erythema, swelling and tenderness [133]. The mucocutaneous and neurologic allergic reactions reported after JE vaccination, may vary in frequency, and therefore these should be evaluated

very carefully yearly [133]. Though safe and effective, the vaccine has limitations in terms of cost, availability and lack of long term immunity.

(ii) Inactivated primary hamster kidney cell derived vaccine has been developed in China. The efficacy was found to be 76–90% in a field trial in China [134] and there were fewer side effects. A vero-cell culture derived inactivated vaccine from attenuated SA 14-14-2 strain has been developed, which induced high titers of neutralizing antibodies in experimental animals [135]. Another vero cell-culture derived inactivated JE vaccine using Indian isolate (P 20778) has been developed. It generated high titers of anti-JEV antibodies in mice [136].

(iii) A cell-culture derived live, attenuated SA 14-14-2 vaccine from China has recently become internationally available and is increasingly replacing the inactivated, mouse brain-derived vaccine in Asia. A single dose of the vaccine demonstrated 96% efficacy after 5 years [137]. The vaccine is very cost-effective and appears to be safe and neuroattenuation is stable [13]. The government of India introduced the SA 14-14-2 vaccine in 2006, and nearly 50 million children 1–15 years of age have been reached through vaccination campaigns and routine immunization [138].

JE vaccine candidates in late-stage of development include a live, attenuated chimeric virus vaccine (IMOJEV®) and an inactivated Vero cell-derived vaccine; each based on the SA 14-14-2 strain. IMOJEV® (previously known as ChimeriVax™—JE), is a novel recombinant chimeric vaccine, developed using the yellow fever virus (YFV) vaccine vector as its backbone. It is found to be safe, highly immunogenic and capable of inducing long-lasting immunity in both preclinical and clinical trials [139]. Additionally, 2 inactivated, Vero cell-derived vaccines based on the Beijing-1 strain are being developed in Japan [140]. Second generation recombinant vaccines are also being developed, where signal sequences of PrM with genes encoding PrM and E proteins are packaged into vectors, e.g. *E. coli* and vaccinia [141]. Studies on plasmid DNA-based JEV vaccine are also giving promising results on experimental animals [142] and in one of the study; the JEV candidate DNA vaccine was capable of generating protective levels of neutralizing antibodies in rhesus monkeys [143]. DNAzymes (Dzs) that cleave the RNA sequence of the 3′-NCR of JEV genome in vitro, on intra-cerebral administration in JEV-infected mice led to more than 99.99% inhibition of virus replication in brain, resulting in a dose-dependent extended life-span or complete recovery of the infected animals [144]. New vaccine development, along with progress in surveillance and immunization, offers promise for sustainable control of clinical JE. To achieve this, global partners are working together to develop a strategic plan for JE control by 2015 [145].

Future Challenges

Currently in India, since the risk of JE is limited to focal areas, its immunization is not included in the National Immunization Program. It is recommended for active immunization only in JE prone areas [30]. There is a need for a proper pilot trial on JE vaccination involving hyperendemic districts for making a policy on mass JE vaccination in the community. JE vaccination should be considered in travelers, who are likely to spend >30 days in a JE-endemic country or travelers on brief trips if they have extensive outdoor or night time exposure in rural areas during periods of active transmission [22, 146]. It is also been reported that routine availability of vaccine and information, education and communication strategies play important roles in improving knowledge and achieving high vaccination rates [147]. For the development of effective therapy, there is an immediate requirement to understand the host factors in JEV-induced neuropathogenesis [109]. Because the live attenuated JE vaccine used in India is derived from GIII strain SA14-14-2, vaccine's efficacy to protect against GI must be evaluated. Also the expansion of GI JEV into other parts of India should be continuously tracked [9]. It is also necessary to elucidate the ecology of migrating reservoir animals. Genotyping of both the mosquitoes and JEV should be done concurrently at many locations in temperate and tropical regions simultaneously. Any distinct biological markers, like susceptibility of replaced strains in mosquitoes, birds etc. should be elucidated.

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