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Japanese Encephalitis

Sudhanshu Vrati and Kaushik Bharati

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I. JAPANESE ENCEPHALITIS: AN INTRODUCTION

Japanese encephalitis (JE) is so called simply because of the fact that it was first reported from Japan, and the major complication is encephalitis (inflammation of the brain). JE is the most important form of viral encephalitis commonly known as brain fever that mostly affects children and young adolescents in Asia. JE is caused by a virus called Japanese encephalitis virus (JEV). Though outbreaks of encephalitis attributed to JEV were reported in Japan as early as 1871, it was not until 1924 that JEV was isolated from a clinical case in the first recorded JE epidemic from Japan. The celebrated "Nakayama strain" was isolated in 1935 from the brain of a dying JE patient. Moreover, the mode of transmission, by Culicine mosquitoes, was not elucidated till 1950 (Table 110.1).

The disease is endemic in most parts of Southeast Asia, China, India and Oceania. The disease is continuously expanding its geographical territory. Over the last decade or so, it has spread to hitherto unaffected regions, such as the western parts of India, Karachi (Pakistan), Western provinces of Papua New Guinea and the Torres Strait islands of Northern Australia. There is a realistic possibility that JE could spread further. The changing geographical distribution pattern of JE is presented in Figure 110.1.

A. Japanese Encephalitis Virus: The Etiologic Agent of JE

Japanese encephalitis virus (JEV), which causes JE is an arthropod-borne virus (arbovirus) that belongs to the family *Flaviviridae* and genus Flavivirus. The prototype virus of this genus is the yellow fever virus (Latin flavus: yellow). The other Flaviviruses that cause human disease are dengue virus (DEN), St. Louis encephalitis virus (SLE), Murray Valley encephalitis virus (MVE), West Nile virus (WNV) and tick-borne encephalitis virus (TBE). Another flavivirus of importance in Karnataka (India) is the Kyasanur Forest disease virus (KFD), which causes a febrile illness, popularly called "monkey fever" (Table 110.2). JEV is spread by the bite of infected Culicine mosquitoes, predominantly *Culex tritaeniorhynchus*. The major amplifying vertebrate hosts are domestic pigs and ardeid wading birds, such as herons and egrets. A number of other animals become naturally infected with JEV, including donkeys, chicken, ducks, water buffalos, cattle, sheep, mice, snakes and frogs, but their role in JEV transmission is questionable. However, there is evidence that bats could play a role in overwintering of the virus, as well as viral persistence, since transplacental transmission has been demonstrated in bats. It has been suggested that bats, migratory birds as well as wind-blown mosquitoes that have been infected with JEV, may be responsible for introducing the virus to hitherto unaffected geographical regions.

TABLE 110.1 Japanese Encephalitis – Major Incidents

Year	*Incident*
1871	First recorded clinical case of JE, reported from Japan
1924	Large outbreak of JE in Japan with >6,000 cases and a fatality rate of 60%; Isolation of JEV from human brain
1933	First cases of JE reported from the Korean peninsula
1935	Isolation of Nakayama strain of JEV
1938	Isolation of JEV from *Culex tritaeniorhynchus* mosquitoes
1940	First cases of JE reported from the Chinese Mainland
1950	First cases of JE reported from the Philippines
1950s	Elucidation of transmission cycle of JEV, with pigs and ardeid birds identified as amplifying hosts and *Culex tritaeniorhynchus* as primary vector species
1955	First cases of JE reported from Vellore, India
1965	Major epidemic in northern Vietnam
1969; 1970	Major epidemic in Chiang Mai Valley, Thailand
1973	First epidemic in India, in the state of West Bengal
1978	Major epidemic in Terai region of Nepal
1983	JE reaches Pakistan, the furthest geographical extension to the West
1985-86; 1987	Major epidemics in Sri Lanka
1995	JE reaches Papua New Guinea and Torres Strait islands (Australia), the furthest geographical extension to the South
2005	Major epidemic in Gorakhpur, Uttar Pradesh state of India. 5,737 cases, with 1,344 deaths; India imports live-attenuated SA 14-14-2 vaccine from China

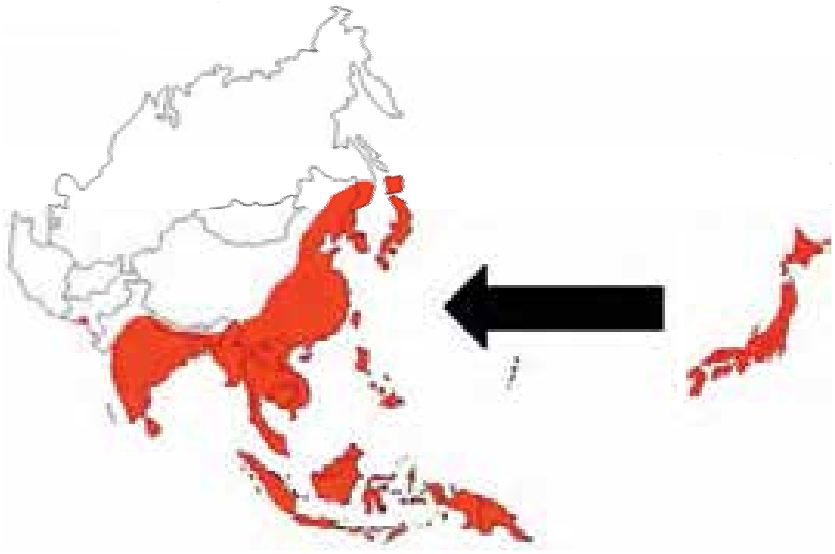

Figure 110.1 Changing geographical distribution pattern of Japanese encephalitis.

TABLE 110.2 Major Human Flaviviruses and their Endemic Areas

Virus	*Endemic areas*
Japanese encephalitis	Asia and Oceania
Yellow fever	South America and Africa
Dengue	Tropics, worldwide
West Nile	Europe, Africa, Asia and North America
St Louis encephalitis	North and South America
Murray Valley encephalitis	Australia
Tick-borne encephalitis	Europe and Asia

B. The JEV Life Cycle

The JEV life cycle normally involve pigs that act as the amplifying hosts, mosquitoes that act as the vectors and humans that act as dead-end hosts. Usually after two 4-day amplification cycles in pigs, approximately 20% of the pigs become seroconverted. Mosquitoes become infected by feeding on the viremic pigs. After this, the virus undergoes a 7–14 day extrinsic incubation period (EIP) in mosquitoes, where it multiplies in various organs and body compartments. After the EIP, mosquitoes infect other pigs, as a result of which almost 100% of the swine population becomes seroconverted. Blood meal from these highly viremic pigs is followed by another round of EIP, following which, the virus spills over to the human population and clinical cases start to appear. Humans become infected coincidentally when they encroach this enzootic cycle between mosquitoes, birds and pigs. Viral titers in humans are not high enough to cause further transmission, which is why humans are regarded as dead-end hosts. The life-cycle of JEV in nature is depicted in Figure 110.2.

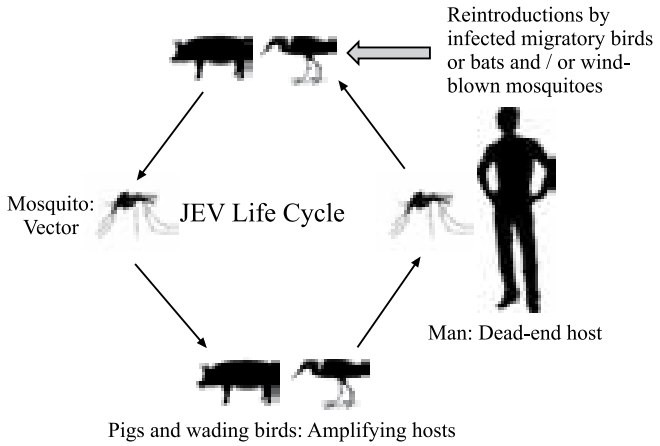

Figure 110.2 JEV Life Cycle.

C. Structural Organization of the JEV Genome

JEV is spherical in shape and is ~50 nm in diameter. Its nucleocapsid core is surrounded by an envelope. Its single-stranded, positive sense RNA genome contains a single long open reading frame (ORF) flanked by 5′- and 3′-untranslated regions (UTRs), which have secondary structures that are essential for the initiation of translation and for replication. The 5′ end of the genome has a type 1 cap, while the 3′ end lacks a poly-A tail. The viral genome is ~11 kb, and codes for a single polyprotein of ~3400 amino acids that is cleaved into 3 structural and 7 non-structural proteins. The structural proteins are capsid (C), envelope (E), and membrane (M). The non-structural proteins are NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5.

JEV structural proteins

As the name suggests, the structural proteins constitute the structural component of the virion. Importantly, the nucleocapsid is formed by the C protein. The M protein is formed by the cleavage and removal of the N-terminal segment from its precursor, the pre-membrane protein (prM). The prM helps in the proper folding of the E protein, which is a typical membrane glycoprotein, consisting of a C-terminal membrane-anchorage domain, and the remaining portion forms the outer structural protein component of the virus. The E protein is the major virion antigen responsible for a number of important processes that include virion assembly, receptor binding, and membrane fusion. The E protein is the most important viral protein from a vaccine development standpoint, as most of the neutralizing epitopes are located on its domain III.

JEV non-structural proteins

The non-structural proteins generally perform the "house-keeping functions" of the virus. The NS1 protein is a glycosylated protein that is believed to be involved in the assembly and release of virions. NS2A and NS2B are low molecular-weight proteins that are thought to be involved in the processing of other viral proteins. NS3 protein is conserved among flaviviruses and has protease and nucleotide triphosphatase / helicase activities. NS4A and NS4B are small proteins whose functions are not clear, although they may be involved in the membrane localization of NS3 and NS5 through protein-protein interactions, or in the formation of the genomic RNA replication complex. NS5 protein is the largest and most conserved protein and is the viral RNA-dependent RNA-polymerase (RdRp). The genome organization of JEV is depicted in Figure 110.3.

D. JEV Genotypes

Four distinct genetic subtypes or genotypes of JEV have been identified on the basis of nucleotide sequence data of C/PrM and E genes and phylogenetic analysis. Genotype I includes isolates from Northern Thailand, Cambodia and Korea. Genotype II includes isolates from Southern Thailand, Malaysia, Indonesia and Northern Australia. Genotype III includes isolates from mostly temperate regions of Asia, including Japan, China, Taiwan, the Philippines and the Asian subcontinent. Genotype IV includes some isolates from Indonesia. In addition, based

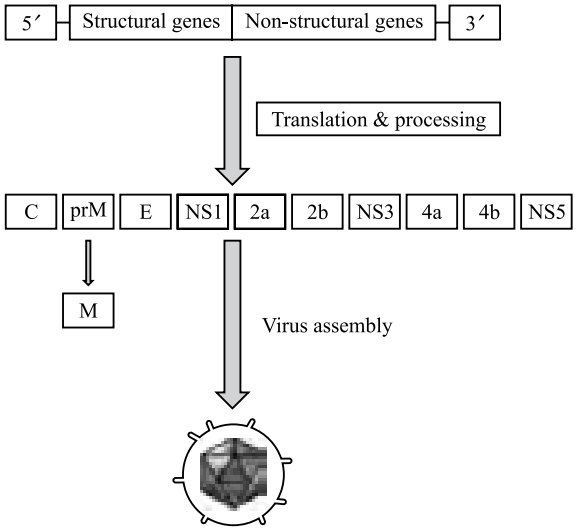

Figure 110.3 JEV genome organization. The genome is a single-stranded, plus-sense RNA molecule containing a long open reading frame (ORF) encoding the viral polyprotein with 5′ and 3′ untranslated regions (UTRs). The encoded proteins subsequently self-assemble into complete JEV particles.

on phylogenetic evidence, the Muar strain of JEV isolated in Singapore in 1952 from a patient who originated in Muar (Malaysia), may represent a fifth genotype.

All the genotypes differ from each other by about 10–20% at the nucleotide level and 2–6% at the amino acid level, and belong to the same serotype and are similar in terms of virulence and host preference. As a consequence, any JEV strain used for the development of a vaccine antigen may be expected to confer protection against all other genotypes. Current JEV vaccines are based on genotype III strains and it has been demonstrated that these vaccines confer protection against heterologous JEV strains, although the neutralizing antibody titers in these instances are lower than those against homologous strains.

II. EPIDEMIOLOGY OF JE

JEV is distributed throughout the temperate and tropical regions of Southern and Eastern Asia. Two major epidemiological patterns of disease are observed—endemic or epidemic. In the Northern temperate areas, such as Northern Vietnam, Northern Thailand, Korea, Japan, Taiwan, China, Nepal, and Northern India, JE occurs in the form of epidemics during the summer/monsoon months. In Southern tropical areas, such as Southern Vietnam, Southern Thailand, Indonesia, Malaysia, Philippines, Sri Lanka and Southern India JE is endemic. In regions where the tropical and temperate climes intermingle, the disease pattern also becomes superimposed. The disease burden is estimated to be ~175,000 cases annually, which is probably a gross under-estimate, since under-reporting often occurs, as reflected in the number of reported JE cases, which is only 50,000 annually (Table 110.3).

TABLE 110.3 Japanese Encephalitis – Facts & Figures

Causative agent	Japanese encephalitis virus (JEV): Single-stranded, positive-sense, RNA virus. Family: *Flaviviridae.* Genus: *Flavivirus.*
Geographic range	Indian subcontinent (including Pakistan), South Asia, Southeast Asia, China, Oceania, Northern Australia
Major vectors	Paddy-breeding mosquitoes of the *Culex vishnui* subgroup, particularly *Cx. tritaeniorhynchus.* Other important secondary or regional vectors include *Cx. gelidus, Cx. fuscocephala, Cx. pipiens, Cx. annulirostris*
Major vertebrate hosts	Domestic pigs (amplifying hosts); ardeid wading birds such as the black-crowned night heron (*Nycticorax nycticorax*), plumed egret (*Egretta intermedia*), and little egret (*Egretta garzetta*) (primary enzootic hosts); domestic fowls; migratory birds
High risk groups	Infants and children below 10 years in endemic areas; the elderly in endemic areas; non-immune adults (e.g. travelers from non-endemic countries visiting JE endemic areas for more than a month); immunocompromised individuals
Annual estimated cases	~175,000
Annual reported cases	~ 50,000
Annual reported morbidity	~ 15,000
Annual reported mortality	~ 10,000

JE is predominantly a disease of children and serological tests have shown that when the children reach adulthood, all have been exposed to the virus and therefore exhibit neutralizing antibody (NtAb) titers. However, non-immune adults coming from non-endemic regions of the world and staying in endemic areas for more than a month are likely to get infected. Another instance when adults become infected is when the virus spreads to new geographical locations, as has been the case in Nepal and Northern Australia. Immunocompromised adults are also susceptible to JEV infection, as are the elderly, possibly due to waning immunity.

JE is essentially a disease of rural areas, although populations residing in peri-urban areas are now-a-days also afflicted, possibly due to unplanned constructions that encroach agricultural areas, thereby disrupting the vector population dynamics, leading to human JE cases. Moreover, climate change will definitely have a drastic effect on the epidemiology of JE in the coming decades, as it is likely to alter the mosquito breeding patterns.

Of the 50,000 JE cases reported annually, 10,000 prove to be fatal. Those who survive the disease are very often left with

neurological and psychiatric problems, which can sometimes be life-long in the absence of proper medical care. JE, therefore, is a serious public health problem, even though its severity is often under-stated and under-appreciated.

A. Pathogenesis

As has been highlighted above, JEV transmission occurs by the bite of infected Culex mosquitoes. Following the mosquito bite, the virus initially replicates locally, after which it spreads to the blood stream, causing a transient viremic phase. The virus also multiplies in the regional lymph nodes. Studies indicate that in normal circumstances, the virus enters the CNS by passage across the cerebrovascular endothelium, rather than across the olfactory membrane, where the blood-brain barrier (BBB) is scanty or lacking. Moreover, a recent study indicates that the permeability of the BBB is differentially altered in response to JEV infection, leading to entry of the virus particles into the cerebrum, as the initial site of virus entry into the CNS. The olfactory route of transmission is important in case of laboratory workers, especially during administration of injections into mice with the live virus, where aerosols can easily enter the nasal cavity if appropriate protective measures are not taken. JEV has also been reported to infect the developing fetus transplacentally and cause abortions.

B. Mechanism of Neuronal Damage

Within the CNS, JEV replicates in the neurons, more specifically within their secretory system, involving the rough endoplasmic reticulum (RER) and Golgi apparatus, eventually leading to their destruction as the virions mature and the infection spreads to other neighboring neurons. Although there is no direct evidence from humans, neuronal apoptosis has been demonstrated in flavivirus disease models, both *in vitro*, as well as *in vivo*. It has been demonstrated that activation of microglial cells leads to production of proinflammatory cytokines that may elicit neuronal death. It has been suggested that an elevation in proinflammatory cytokine levels in the cerebrospinal fluid (CSF) indicates a poor prognosis. Other mechanisms of neuronal damage that have been suggested include astrocyte activation and nitric oxide (NO)-mediated damage. The latter has received particular attention due to the fact the JEV has been found to induce the expression of inducible NO synthase (iNOS), the major enzyme in NO synthesis, thought to be a key component of the host innate immune response. Importantly, the elucidation of the mechanism of neuronal damage may lead to identification of targets for drug intervention.

III. CLINICAL FEATURES OF JE

JE infections are often asymptomatic, but might follow a course resembling a mild non-specific febrile illness. Approximately 1 in 300 cases of JE result in symptomatic disease, which depends on 4 major factors; (i) route of entry, (ii) titer of virus, (iii) neurovirulence of virus, and (iv) host factors, such as age, health, genetic make-up, and pre-existing immunity. The first signs of infection appear after an incubation period of 1–6 days, but may take as long as 15 days to manifest. The onset of the disease may be acute or gradual. The disease may be crudely divided into three stages: (i) a prodromal stage, before central nervous system (CNS) involvement occurs, (ii) CNS stage, where the virus infects the CNS, and (iii) a convalescent stage, where either marked improvement occurs or the CNS symptoms may persist. The prodromal stage is marked by fever above 38°C, chills, myalgia, malaise, headaches accompanied by nausea, vomiting and abdominal pain. Besides these common symptoms, in some instances, gastric hemorrhage, thrombocytopenia and liver dysfunction have also been observed. These non-specific signs may continue for 2–4 days or the patient's condition may deteriorate rapidly. CNS involvement (day 3–5) is marked by a progressive decline in alertness, often leading to coma. CNS infection can result in encephalitis, meningitis or myelitis, or a combination of all the three, in the form of meningoencephalomyelitis.

A. Movement Disorders

A Parkinson's like syndrome, characterized by dull mask-like facies, tremors and cogwheel rigidity have been observed in JE cases. Convulsions may be experienced in up to 87% of patients. The meningeal syndrome predominates with painful neck stiffness. Motor paralysis including hemiplegia and tetraplegia may also be present. A poliomyelitis-like acute flaccid paralysis can also occur. Other movement disorders include opisthotonus (Figure 110.4), dystonia (Figure 110.5), oro-facial dyskinesias (e.g., lip-smacking), hemiparesis, choreoathetosis and gaze palsy. Abulia is another striking feature observed in JE patients.

It is important to detect early clinical signs of CNS involvement, such as abnormal oculocephalic reflexes, acute onset hemiparesis with hypertonia and decorticate

Figure 110.4 Opisthotonus in a boy with JE (Photo: Courtesy Dr. P. Nagabhushana Rao).

Figure 110.5 Dystonia in the right hand of a boy with JE (Photo: Courtesy Dr. P. Nagabhushana Rao).

and decerebrate posturing, which help in the early clinical identification of intracranial hypertension. Effective management of intracranial hypertension will dictate whether the patient survives or dies. Clinical diagnosis can be buttressed by various neuroimaging techniques.

B. Neuroimaging

Neuroimaging techniques such as Computed Tomography (CT Scan) and Magnetic Resonance Imaging (MRI) carried out in JE patients have revealed extensive bilateral thalamic lesions (Figure 110.6). In a JE-endemic region, bilateral lesions of the thalamus are indicative of JE. It has been suggested that the movement disorders are the clinical correlates of damage to the thalamus and other parts of the brain. Lesions to sites such as the former, as well as that to the lentiform nucleus and basal ganglia could result in Parkinsonism. Other complications such as abulia could be due to thalamic lesions disrupting the prefrontal cortex-caudate-pallido-thalamocortical circuit. Dystonia results from putaminal and possibly thalamic lesions. Another powerful technology is the Single-Photon Emission Computed Tomography (SPECT). SPECT may be useful as a diagnostic tool in the early stages of JE.

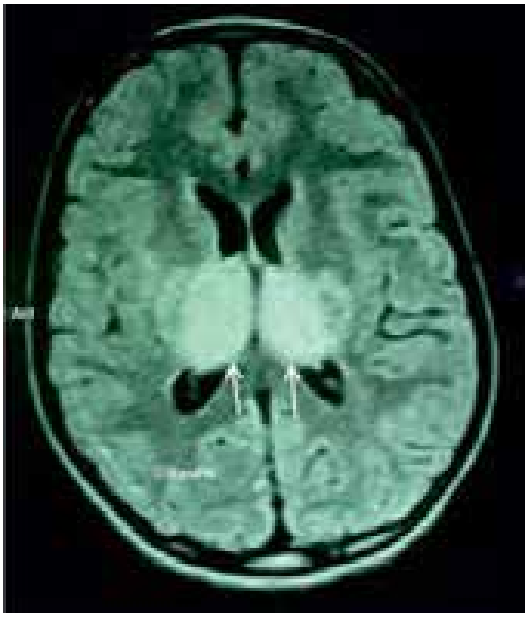

Figure 110.6 MRI scan showing hyperintense thalami (Photo: Courtesy Dr. P. Nagabhushana Rao).

C. Prognosis

Fatality is seen in 20–30% of the cases, with signs of acute cerebral edema or severe respiratory distress from pulmonary edema. Children who survive the disease usually regain neurological function over the following weeks to months. However, almost half of the survivors are left with serious neuropsychiatric sequelae. These include a persistently altered sensorium, epileptic seizures and severe mental retardation. These neurological complications are very often understated in the medical literature. One should understand that the patients with neurological deficits are very often in their early childhood and hence, have to bear with these complications for the rest of their lives, given the fact that most of them cannot afford treatment. Moreover, even those who have a good recovery, often have minor sequelae like learning and behavioral problems, giving rise to stigmatization. Hence, the human face of the disease burden is enormously more than what the mere statistics suggest.

IV. DIAGNOSIS

A. Differential Diagnosis

Diagnosis of JEV infection should be made within an epidemiological context. Because of clinical, biological and epidemiological similarities, three other viral diseases should be considered in the differential diagnosis (DD). These are (i) Herpes Simplex Virus (HSV) Encephalitis, (ii) Dengue, and (iii) West Nile Encephalitis. Moreover, other CNS infections should also be kept in the DD. These include bacterial and fungal meningitis, tuberculosis, cerebral malaria, leptospirosis, tetanus and typhoid encephalopathy. Moreover, enteroviruses, paramyxoviruses, rabies virus, Chikungunya virus and Nipah virus should also be kept in mind. Some non-infectious diseases that exhibit CNS manifestations include tumors, cerebrovascular accidents, Reye's syndrome, toxic and alcoholic encephalopathies, and epilepsy. Hence, it is of paramount importance to spend some time in taking down the case history. However, in case of epidemics, occurring in JE endemic areas, it is often easier to rule out the other causes from the DD.

B. Physical Examination

A thorough physical examination is crucial. The level of consciousness should be established with a quantitative scale such as the Glasgow Coma Scale (GCS) and any seizures

arising out of the infection should be immediately treated. Any other mental and behavioural abnormalities should be documented. The examination should search for any other causes of altered levels of consciousness.

C. Lumbar Puncture

If the condition of the patient permits, a lumbar puncture should be performed, since the initial CSF findings, such as opening pressure, cell count, glucose and protein levels are crucial for initially establishing a CNS infection. The CSF findings will demonstrate whether the infection is viral or bacterial in origin. Moreover, downstream laboratory testing, by techniques such as MAC ELISA or PCR will pin-point the incriminating organism, and thus provide a guide for management. JE should be suspected if biochemical investigations reveal: (i) high CSF opening pressure (>250 mm), (ii) moderate CSF pleocytosis (10–100 cells/mm^3), (iii) mildly increased CSF protein (50–200 mg/dL), but (iv) normal levels of CSF glucose.

D. Rapid Diagnostic Tests

Confirmation of a suspected case of JE requires laboratory diagnosis, which relies on virus isolation or demonstration of virus specific antigen or antibody in the CSF or serum. The humoral immune response to JEV infection involves early production of IgM antibodies in both serum and CSF, followed by IgG production. Hence, the IgM-capture ELISA (MAC ELISA), which detects specific IgM in CSF or serum of almost all patients within 7 days of onset of disease has become the practical standard for the diagnosis of JE. Three MAC ELISA kits are being manufactured commercially. These are the (i) JE-Dengue IgM Combo ELISA Test (Panbio Limited), (ii) JE IgM ELISA (InBios International, Inc.), and (iii) JEV CheX Kit (XCyton Diagnostics Ltd.). These tests use cell culture-derived inactivated virus or recombinant E protein as the antigen; the Panbio test also uses recombinant Dengue 1-4 antigens and can detect both JE and Dengue antibodies. The Panbio kit has shown 90% agreement to the USAMC-AFRIMS (United States Army Medical Component – Armed Forces Research Institute of Medical Sciences) standard, the internationally accepted standard diagnostic test for JE, when both JE and dengue IgM-positive samples were considered in the analysis. In this situation, sensitivity was good for the InBios and XCyton kits, but lower for the Panbio kit. However, specificity was low for both the InBios and XCyton kits as a result of cross-reactivity with dengue antibodies. When dengue cross-reactivity was eliminated, all the three kits had specificities of 96% or above. If pricing is competitive, then the Panbio kit would have a distinct advantage over the other two.

E. Molecular Diagnostics

A viral genome amplification using RT-PCR technique allows rapid detection of viral RNA in the CSF of JE patients. Another molecular technique, the reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay is a rapid real-time detection system for JEV, and the results can be obtained within 30 minutes under isothermal conditions at 63°C. This test could become useful in low resource settings as it does not require a thermocycler. However, these molecular tests are not currently used for routine diagnostic purposes.

V. TREATMENT

There is currently no specific treatment for JE. The antiviral, ribavirin has been evaluated for therapeutic applicability in JE patients in a clinical trial in children in the state of Uttar Pradesh (India).This antiviral agent did not have any effect in reducing early mortality associated with JE. Although isolated case studies have indicated that IFN-α could be an effective and promising agent for the treatment of JE, a randomized, double-blind, placebo-controlled trial of IFN-α2a did not improve the outcome of JE patients. Likewise, the efficacy of intravenous immunoglobulin for treatment of JE cases is also inconclusive.

Although specific antivirals against JEV are still lacking, a number of approaches are under laboratory scale development against this virus. It has been demonstrated that JEV, as well as other flaviviruses that share a similar genome organization are susceptible to a broad spectrum of agents, including chemical compounds, natural products, siRNAs and DNAzymes. Other efforts have included targeted drug discovery approaches that have been aimed to find specific inhibitors against JEV. These have been briefly highlighted below.

A. Targeted Drug Discovery Efforts to find Specific Inhibitors of JEV

The members of the Flavivirus family all follow a similar replication strategy. So, there is a high possibility that viral inhibitors can be discovered that have a broad-spectrum of antiviral activity. The targeted approach towards drug discovery involves, first identifying the targets, and then designing molecules that could act as inhibitors of these selected molecular targets.

Polymerase inhibitors

The polymerases are the most promising targets for development of antiviral agents. The RdRp is a multimeric complex and is essential for viral replication. Importantly, since this molecular complex is encoded by the viral genome, and has no cellular counterpart, there is no chance of cellular toxicity.

Protease inhibitors

Another attractive target for drug development is the viral protease. Viral protease inhibitors are already being used in clinical practice. The HIV-protease inhibitors are examples of this class of drugs that are components of the highly active anti-retroviral therapy (HAART). The flavivirus proteases could also be targeted in a similar fashion. However, it needs to be

investigated whether these viral serine proteases are sufficiently different from the cellular serine proteases, so that toxicity issues do not arise.

Virus entry inhibitors

Virus entry inhibitors are ideal antiviral agents. These agents are in many ways superior to the polymerase or protease inhibitors as issues of toxicity and emergence of resistant strains does not occur in this class of antiviral agents. In fact, virus entry inhibitors have been approved for use against HIV-1.

Capping inhibitors

The flaviviruses, like other RNA viruses, replicate in the cytoplasm, and have evolved their own capping enzymes that are independent of the host. Since 5′ capping is an essential component of viral replication, the methyltransferase enzyme, which is an essential component of the capping machinery, is an attractive target for antiviral drug development.

Helicase inhibitors

The NS3 is the helicase of flaviviruses. It is an essential component for viral replication. It is involved in unwinding the double-stranded RNA intermediate during genome replication and acts in a multimeric complex with NS5. This enzyme is also an attractive target for antiviral drug development.

VI. MANAGEMENT

Management essentially involves measures to control both the immediate complications of infection, including seizures and raised intracranial pressure, and the longer-term consequences of neurologic impairment, such as limb contractures and bed sores. Corticosteroids have been investigated in the treatment of JE, but they failed to show any beneficial effects in acute JE cases. Hence, management is essentially symptomatic and supportive. The major management strategies during the acute phase and convalescent or recovery phase are briefly discussed below.

A. Management during the Acute Phase

Management of patients during the acute phase of the disease is of the utmost importance because the treatment given in this period decides whether the patients live or die. Here, the knowledge, experience, skills and dexterity of the clinical staff is called into play. This period is a real testing time for the health infrastructure of the hospital where the patients are managed. Everything, from laboratory diagnostics to the actual treatment administered, has to be well coordinated for the patients' survival.

Management of fever

One of the major clinical signs of children being brought to hospital is fever. Management of fever is a clinical priority, mainly because of the fact that high fever can lead to increase in intracranial pressure by increasing cerebral metabolism and cerebral blood flow, leading to cerebral edema. Antipyretics such as paracetamol may be used along with sponging in order to lower the temperature.

Management of seizures

Seizures are very common in JE patients, particularly in children. Seizures may be generalized tonic-clonic seizures, or more subtle, such as twitching, which may occur in a digit or around the lips or eyes. Uncontrolled seizures can lead to raised intracranial pressure, leading to increased metabolism and other physiological changes that can further aggravate the intracranial hypertension, operating in a positive-feedback vicious cycle, which can lead to brain herniation. Seizures can often be managed with phenytoin and benzodiazepines. If not, intubation and artificial ventilation should be initiated so that higher doses of sedatives and anticonvulsants can be administered. The patient should be monitored by carrying out an electro-encephalogram (EEG) from time to time. EEG should be carried out after administration of anticonvulsants and should be continued until seizures subside. Coma should be monitored by the GCS score. Other parameters such as blood pressure, urine output, serum osmolality, oxygen saturation and central venous pressure should be monitored until the condition of the patient improves.

Management of raised intracranial pressure

From the foregoing discussion, it is evident that raised intracranial pressure is one the most life-threatening complications of JE infection. Management of raised intracranial pressure is crucial and it is important to keep the patient's head in a midline position, tilted at 30°. Keeping the head tilted improves the cerebral perfusion pressure, a key determinant in cerebral circulation, influenced by the intracranial pressure. Moreover, keeping the head elevated increases CSF drainage and maximizes cerebral venous return. Importantly, the head should lie on the midline in order to prevent any obstruction in the venous drainage through the jugular vein. Hyperventilation can reduce the intracranial pressure by bringing about a decrease in cerebral blood flow, which in its turn is brought about by a decrease in the CO_2 tension (pCO_2) caused by the hyperventilation itself. Intracranial pressure begins to fall within seconds to minutes, stabilizes in about 30 minutes, and returns back to the original value in about an hour. Although hyperventilation is useful for bringing down the intracranial pressure, prolonged hyperventilation can, in fact, be harmful, and may worsen the outcome. Hyperventilation should always be withdrawn gradually. Mannitol is commonly used to control raised intracranial pressure. The action of mannitol is dependent on the rate of administration – higher infusion rates lead to a rapid fall in intracranial pressure that is short-lived, while slower infusion rates bring about a sustained decrease in the intracranial pressure. However, overdosing with mannitol should be avoided at all costs, as this can aggravate cerebral edema. Loop diuretics such as frusemide can be used alone, or in combination with mannitol to lower the elevated

intracranial pressure. Frusemide alone causes slow reduction in intracranial pressure, but when combined with mannitol, the fall in pressure is rapid and much more sustained than when either drug is used alone.

A. Management during the Convalescent Phase

Management during the convalescent or recovery phase is no less important than that during the acute phase. This phase is often neglected, because the danger of death has passed. But it is in this phase that the real struggle for the surviving patients begins. It may be recalled that ~50% of the survivors, usually small children, have severe neuropsychiatric sequelae, which are likely to be life-long. A thorough neurological and neuropsychiatric examination of the patients is required, soon after discharge from hospital. This will help assess the condition of the patient for leading a normal life. Intellectual and behavioural problems are common in children recovering from JE. These conditions should be treated properly and promptly as these can lead to absenteeism from school and thus hamper normal social functioning. Hence, management of neurological deficits is a challenging task, and requires a lot of patience, encouragement and supportive care, both on the part of the healthcare provider, as well as the family of the affected child. Moreover, treatment of neurological problems is a costly affair and is particularly difficult in the resource-poor developing countries of Asia, where JE is most prevalent.

VI. JE VACCINES

Currently, there are three JE vaccines, one of which is licensed for use in China and four other Asian countries, while the other two are in late stages of development. Two of the vaccines are cell culture-based, while the third is genetically-engineered.

A. SA14-14-2

This vaccine is based on the genetically stable, neuro-attenuated SA14-14-2 strain of JEV that is derived from serial passage of the SA14 strain of the virus in primary hamster kidney (PHK) cells, and has been developed by the Chengdu Institute of Biological Products, China. As the vaccine is produced on primary cells, the manufacturing process includes detailed screening for endogenous and adventitious viruses and is currently being manufactured as per WHO Guidelines for production of live JE vaccines for human use. The vaccine elicits broad-spectrum immunity against heterologous JEV strains. Reversion to neurovirulence is considered highly unlikely. This single-dose vaccine has been licensed for use in China since 1988, where over 200 million children have been successfully immunized so far, with a brilliant safety record. Recently the SA14-14-2 vaccine has been licensed for use in India, Nepal, Sri Lanka and South Korea. In India, the imported SA 14-14-2 vaccine has been used to immunize over 9.3 million children (aged between 1 and 15 years) in the summer of 2006 in 4 states. A major advantage of this vaccine is that it is inexpensive and hence would be affordable by the economically weaker countries of Asia where JE is endemic.

B. IXIARO®

IXIARO® is a purified, formalin-inactivated, whole-virus JE vaccine, manufactured by Intercell, an Austrian company. It is based on the SA14-14-2 strain of JEV adapted to grow on Vero cells. After extensive clinical trials of IXIARO®, using a two-dose regimen, the vaccine was approved by the US Food and Drug Administration (USFDA) on March 30, 2009. It has also been approved by the European Commission and the Australian Therapeutic Goods Administration (TGA). The vaccine is currently licensed for use in USA, Australia and Europe. The vaccine is currently undergoing regulatory clearance in India, and will be available soon and will be marketed by Biological E.

C. IMOJEV®

Besides the above two JE vaccines that are commercially available, a very innovative genetically engineered third generation JE vaccine is in late stages of clinical development. This uses the proprietary Chimeri-Vax™platform, developed at the St. Louis University Health Sciences Center and by Acambis. This recombinant DNA vaccine is based on the Yellow Fever (YF) virus 17D vaccine strain as a backbone, but with the envelope and pre-membrane protein genes of YF virus replaced by those of JEV. Importantly, YF 17D has been extensively used as a live-attenuated yellow fever vaccine for over 60 years, with excellent record of safety and efficacy. Clinical trials using a single dose of IMOJEV® have shown the vaccine to be well tolerated as well as effective. Importantly, pre-existing immunity to yellow fever virus did not dampen the immune response. An update on the current vaccines against JE is given in Table 110.4.

VII. OTHER PREVENTIVE MEASURES

JE control measures may be three-pronged, namely (i) changes in pig rearing techniques, (ii) vector control, and (iii) prophylactic vaccination of susceptible human populations. Since the former two approaches have their limitations, it is the third that has to be relied upon to keep this disease at bay. However, it must be stressed that the other approaches should not be neglected altogether. In the Southeast Asian countries, where pig-rearing is widely practiced, the pigsties should be located far away from human dwellings, although this is not always practicable. Vaccination of swine has also been suggested and practiced in countries like Japan. But a universal and sustained swine vaccination effort is likely to be

TABLE 110.4 Vaccines against Japanese Encephalitis

Vaccine name	*Type*	*Virus strain*	*Manufacturer*	*Dosing*	*Status*
SA14-14-2	PHK cell-cultured; Live-attenuated	SA14-14-2	Chengdu Institute of Biological Products, China	Single dose	Licensed by respective National Regulatory Authorities for use in China, India, Nepal, Sri Lanka and South Korea; WHO approval awaited.
IXIARO®	Vero cell-cultured; Purified-inactivated	SA14-14-2	Intercell, Austria	Two doses	USFDA approved. Licensed in USA, Australia and Europe in 2009.
IMOJEV®	Infectious clone; Live-attenuated	JEV SA14-14-2 and YF 17D chimeric infectious clone	Acambis, UK; Sanofi Pasteur	Single dose	Phase III clinical trials in USA, Australia and Asia. Marketing authorization sought by Sanofi Pasteur from Australian and Thai authorities.

a costly affair, and thus, out of reach of most resource-poor JE-endemic countries. If pig-rearing is practiced in large pig-farms, located far away from human dwellings, and managed by co-operatives instead of following family-centric pig-rearing practices, then this problem could be overcome to a large extent. Mosquito control by insecticides has largely been found to be ineffective, impractical and costly. However, mosquito larva control by biological means such as keeping larvivorous fish in the paddy fields is an alternative and eco-friendly approach that may be adopted. Avoiding mosquito bites by wearing full-sleeved shirts, restricting outdoor activities at dawn and dusk, and using mosquito repellents, could dramatically reduce the incidence of JE. Hence, what is truly required for JE control is the adoption of an integrated approach, including improved agricultural practices, improved living standards, greater health awareness, as well as a sustained mass childhood vaccination program. It should, however, be noted that unlike smallpox and polio, for which humans are the only host, JE is a zoonotic disease with large animal reservoirs and hence cannot be totally eradicated.

CONCLUDING REMARKS

The JE problem, unlike many other Public Health problems, is multifaceted and complex. This complexity stems from the fact that we are still not totally aware of the true magnitude of the problem. This, in turn, stems from the lack of good quality disease burden data. In many countries, such as Bangladesh, Cambodia, Indonesia, North Korea, Laos, Myanmar, Papua New Guinea, and Pakistan, there is definite lack of data regarding the distribution and Public Health importance of JE. Until and unless solid disease burden data is available, National Regulatory Authorities in the respective JE-endemic countries cannot be convinced about the importance of implementation of a childhood immunization program for JE. Hence, much more aggressive and thorough epidemiological studies are required. This is especially true in areas like Pakistan and possibly, beyond. We know that JE cases have been documented from Karachi. But in countries like Afghanistan, where JE surveillance systems are non-existent, the virus could well be circulating since the necessary vectors are readily available. This is a challenge for the epidemiologists.

Two main prerequisites for controlling JE are (i) greater political will, and (ii) sound financial resources. With a good combination of these two factors, initiation and sustaining of childhood vaccination programs would become much easier. There is ample evidence from the wealthier JE-endemic Asian countries like Japan, South Korea and Taiwan, what a good vaccination program can achieve. This type of vaccination program is urgently required in countries like India, which has a high disease burden, and thereby contributes a large share of the JE burden to the Asian region. Vaccination programs have been initiated in two states, but a national immunization program is required. For implementation of any vaccination program, adequate trained staff, a functional cold-chain, good roads for transportation of vaccines and a good campaigning mechanism, for educating the masses, all contribute to acceptability of the vaccine.

In the near future, greater challenges are likely to occur. These may stem from newer issues of this day and age. Climate change could well be one such issue. Changing weather patterns, especially rainfall patterns, could lead to altering agricultural practices, which would lead to unpredictable changes in mosquito breeding patterns. Moreover, changing bird migration patterns brought about by climate change, could lead to introduction of JE to new geographical locations. A sustained and integrated approach would definitely lead to proper management of JE in the near future, given the fact that good quality and effective vaccines are on the horizon.

SUMMARY

The present chapter on Japanese encephalitis provides an up-to-date review of the current status of disease in the area. The chapter begins with a discussion on JEV, the etiologic agent of JE, including its life cycle, genome organization, as well as the various genotypes circulating in the region. This is followed by the epidemiological aspects, including pathogenesis as well as the mechanism of neuronal damage, which is followed by a section on clinical aspects of JE. The subsequent sections address aspects pertaining to diagnosis, treatment, as well as the current status on JE vaccines, which are the cornerstone for JE control. The chapter has been written in the light of current international knowledge on the subject.

SUGGESTIONS FOR FURTHER READING

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