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ADULT IMMUNIZATION

Editors

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12 Japanese Encephalitis

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Japanese encephalitis (JE) is the most important form of viral encephalitis in the world. This disease is so named because it was originally detected in Japan. The disease has now spread to most parts of South-East Asia, China, India, and even to far-off places like Australia. JE virus (JEV), responsible for causing JE is an arthropod-borne virus belonging to the family *Flaviviridae* and genus *Flavivirus*. It is spread by the bite of infected Culicine mosquitoes, predominantly *Culex tritaeniorhynchus* and *Culex vishnui*. The major amplifying vertebrate hosts are domestic pigs. Wading and migratory birds are also involved in the transmission cycle. Man is an incidental host and viral titers are so low that further transmission does not occur. For this reason, humans are regarded as dead-end hosts.

This is predominantly a disease of children living in rural areas, although people residing in suburban areas may also be afflicted. Approximately 50,000 cases of JE are reported annually, of which 10,000 prove to be fatal. Those who survive the disease are left with lifelong neurological and psychiatric problems. Therefore, JE, is a serious public health problem, even though its severity is often underestimated in literature. The control measures for JE may be two-pronged, namely vector control and prophylactic vaccination. Since the former has its limitations, it is the latter that has to be relied upon to keep the disease at bay. It should, however, be noted that unlike smallpox and polio, for which humans are the only host, JE is a zoonotic disease and hence cannot be eradicated from the face of earth. Moreover, since no specific antiviral therapy is currently available, vaccination is the mainstay for controlling this disease (Table 1).

Table 1: Japanese encephalitis: Historical timeline

1870's	–	First JE outbreak in Japan
1924	–	Isolation of JEV virus from a fatal case of encephalitis in monkeys
1935	–	Isolation of Nakayama strain from a fatal human case
1950	–	Elucidation of route of virus transmission
1955	–	First Indian JE cases in Vellore
1973	–	First Indian JE epidemic in Bankura
1990	–	JE spreads to Western and North Western India
2006	–	India imports SA 14-14-2 vaccine from China

JE = Japanese encephalitis

The Pathogen

The disease causing agent Japanese encephalitis virus is a Flavivirus that is antigenically related to several other flaviviruses prevalent in Asia, including Dengue and West Nile viruses. It is spherical and is approximately 50 nm in diameter. Its nucleocapsid core is surrounded by an envelope. Its single-stranded, positive sense RNA genome codes for a single polyprotein 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. The envelope is the most important viral protein from vaccine development standpoint as it possesses most of the neutralizing epitopes that are targets for neutralizing antibodies forming the primary mediators of immunity. The JEV has five genotypes that have different

geographical distribution, but all belong to the same serotype and are similar in terms of virulence and host preference.

Epidemiology

Two major epidemiological patterns of disease are observed. These can be classified as either endemic cases or epidemic cases confined to specific geographical areas. In areas of northern hemisphere, such as northern Vietnam, northern Thailand, Korea, Japan, Taiwan, China, Nepal, and northern India, JE occurs in the form of epidemics during the summer and monsoon months. The southern hemisphere areas, such as southern Vietnam, southern Thailand, Indonesia, Malaysia, Philippines, Sri Lanka and southern India are endemic for JE and cases occur sporadically throughout the year with a peak after the onset of the monsoon season. The Indian scenario reveals that JE is progressively moving westwards and it would not be very surprising if it soon spreads all across India (Table 2).

Table 2: Japanese encephalitis: Fact sheet

Geographic range	India, Pakistan, Nepal, China, South Asia, South-East Asia, Oceania, Northern Australia
Major vectors	*Cx. tritaeniorhynchus, Cx. vishnui, Cx. pipiens, Cx. gelidus, Cx. fuscocephala*
Major vertebrate hosts	Domestic pigs, migratory birds, ardeid (wading) birds
High risk groups	Infants and children below 10 years, elderly, non- immune adults, immunocompromised individuals
Annual incidence	~50,000
Annual mortality	~10,000
Annual morbidity	~15,000

Pathogenesis

Japanese encephalitis is spread by the bite of infected Culicine mosquitoes. After penetrating the skin by mosquito bite, the virus multiplies in the Langerhans type, dendritic cells in regional lymph nodes. After a transient phase of viremia, invasion of the central nervous system (CNS) takes place by the haematophagus way or via the endothelial system. Unknown factors allow the breakdown of the blood-brain barrier (BBB), but it could be facilitated by the neurotransmitters. CNS lesions are predominantly seen in the thalamus and the cerebral peduncles; however, lesions can also be found in the substantia nigra, the cerebral and the cerebellar cortex as well as in the anterior horn cells of the spinal cord. The JEV has also been reported to infect the developing fetus transplacentally and cause abortions.[1]

Clinical Features

JE is most often asymptomatic. According to the World Health Organization (WHO), approximately 1 in 300 cases of JE result in symptomatic disease. This, by and large depends on four major factors; (1) route of entry (2) titer of virus (3) neurovirulence of virus and (4) host factors, such as age, health, genetic make-up and pre-existing immunity. The first signs of infection appear after an incubation period of 5 to 15 days. It usually starts with a fever above 38°C, chills, myalgia, and meningitis-type headaches accompanied by nausea, vomiting and abdominal pain similar to those found in an acute abdominal syndrome. These non-specific signs may continue for 2 to 4 days. Thereafter,, the patient's condition may deteriorate rapidly with a progressive decline in sensorium eventually leading to coma. Convulsions are experienced in 85% of patients. The meningeal syndrome predominates with painful neck stiffness. Motor paralysis including hemiplegia and quadriplegia may also be present. The signs of extrapyramidal involvement, including tremor, rigidity and abnormal movements are observed in around 30% of patients. Upper and lower motor neuron disorders as well as poliomyelitis-like acute flaccid paralysis have also been observed. The disease is fatal in 20 to 30% of cases. The terminal event includes acute cerebral edema or noncardiogenic pulmonary edema. The majority of patients who recover also have serious behavioural and neurological sequelae, most notably

persistently altered sensorium, epileptic seizures and severe mental retardation in children. The patients with neurological deficits are very often in their early childhood and have to live with these complications for the rest of their lives. Moreover, even those who have a good recovery, often have minor sequelae like learning and behavioural problems. Hence, the human face of the disease burden is enormously more than what is revealed by statistics (Table 3).

Table 3: Japanese encephalitis: Classical clinical signs

Dull flat mask-like facies with wide unblinking eyes
Tremor
Generalized hypotonia
Cogwheel rigidity

Diagnosis

The diagnosis of JE viral infection should be made keeping the epidemiological considerations in mind. Because of clinical, biological and epidemiological similarities, three other viral diseases should be considered in the differential diagnosis . 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 differential diagnosis like bacterial and fungal meningitis, tuberculosis, cerebral malaria, leptospirosis, tetanus and typhoid encephalopathy. Some non-infectious diseases that exhibit CNS manifestations include tumors, cerebrovascular accidents, Reye's syndrome, toxic and alcoholic encephalopathies and epilepsy. However, in case of epidemics, occurring in JE endemic areas, it is often easier to rule out the other causes.

The confirmation of the diagnosis of JE must be based on multiple criteria including clinical, biological, neurophysiologic and cerebral imaging findings. JE should be suspected if biochemical investigations reveal the following: (i) high CSF opening pressure (> 250 mmHg); (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. The aetiological diagnosis of JE is mainly based on serology using immunoglobulin M (IgM)-capture enzyme linked immunosorbent assay (ELISA), which detects specific IgM in the CSF or in the blood of almost all patients within 7 days of onset of disease. After the first few days of illness, the presence of anti-JEV IgM in the CSF has a sensitivity and specificity of more than 95%. A membrane-based immunoglobulin M (IgM) capture dot enzyme immunoassay (MAC DOT) assay is available that is useful in field settings.[2] A viral genome amplification reverse transcriptase-polymerase chain reaction technique allows rapid detection of RNA in the CSF but is not yet routinely used.[3]

Computed tomography (CT) as well as magnetic resonance imaging (MRI) have proved useful adjuncts in the diagnosis of JE. The presence of thalamic hypodensities, both in T1- and T2-weighted images extending to the base of brain and often to other regions such as the lenticular nucleus, caudate nucleus and frontal lobe white matter are pointers to the diagnosis of JE on neuroimaging. The JEV shows a fairly strong tropism for the thalamus and thalamic damage can be bilateral.[4] In a JE-endemic region, bilateral hemorrhagic lesions of the thalamus are characteristic of JE. It has been observed that another new technology, namely single-photon emission CT (SPECT) is useful in differentiating JE from Herpes Simplex encephalitis and other types of encephalitis. Moreover, SPECT may be useful as a diagnostic tool in the early stages of JE.[5]

Treatment

There is currently no specific treatment for JE. The management is mainly supportive and nursing care is of utmost importance, particularly to prevent decubitus ulcers, infections and phlebitis. The fever should be treated with antipyretics like paracetamol. Other measures include maintenance of nutrition, fluid and electrolyte balance and judicious use of antibiotics. Seizures can be controlled with diazepam, clonazepam or phenytoin. Abnormal movements can be treated using haloperidol. Intracranial hypertension, possibly associated with herniation of cerebral peduncles, can be controlled by hyperventilation ($PaCO_2$ target between 23-25 mm Hg) and mannitol (0.25 mg/kg for 3 days). It

should be noted that intubation and protection of the airways reduce the risk of aspiration pneumonia and subsequent hypoxia and the head should be kept elevated to reduce raised intracranial pressure.[6]

Corticosteroids are ineffective as highlighted in a double-blind randomized placebo controlled trial of dexamethasone, which failed to show any benefit.[7] Furanonaphthoquinone derivatives have been found to be effective *in vitro*, while rosmarinic acid and monoclonal antibodies have been reported to be effective in murine models of JE.[8-10] Recombinant interferon-α (IFN-αA) has been given in open trials to a few patients with encouraging results. However, a subsequent randomized double-blind placebo-controlled trial using IFN-α 2A did not show any difference between the treatment and placebo groups.[11] Diuretics have also not shown definitive benefit.

Prevention

There are two broad avenues of prevention: Vector control and vaccination of susceptible populations. Vector control on a large-scale has its limitations, both economically and logistically. However, vaccination is a feasible preventative measure available to control JE especially in endemic areas.

Who Should be Vaccinated?

Japanese encephalitis vaccine is recommended for native and expatriate residents of endemic areas, laboratory workers potentially exposed to the virus, and for travellers spending 30 days or more in endemic areas. It should be borne in mind that vaccination against JE in endemic areas will be a continuous process as approximately 3 billion people currently live in JE-endemic regions worldwide, with 70 million children being born each year. It should be noted that given the JE occurs very rarely in early infancy and the likely interference with passively acquired maternal antibodies during the first months of life, vaccination is not recommended for children below the age of 6 months.

Vaccines Against Japanese Encephalitis

There are three types of JE vaccines: (i) mouse brain-derived inactivated vaccine; (ii) cell culture-derived inactivated vaccine; and (iii) cell culture-derived live-attenuated vaccine.

Mouse brain-derived inactivated vaccine

The mouse brain-derived inactivated vaccine is produced by growing the virus by inoculating the brain of suckling mice pups and subsequent virus purification and inactivation with formaldehyde. Both the Nakayama and Beijing-1 strains have been used to manufacture the vaccine, though studies have shown that the latter strain produces a higher antigenic yield in cultured mouse brain tissue.

The primary vaccination is done between the ages of 1 and 3 years at doses of 0.5 ml subcutaneously. The dose regimen consists of administering one injection on day 0, day 7 and day 30 with a booster after 1 year and thereafter every 3 years until the child attains 10 years of age. The protective efficacy is above 90%.[12] The only contraindication of the mouse brain-derived inactivated JE vaccine is a history of hypersensitivity reactions to a previous dose. However, pregnant women should be vaccinated only when at high risk of exposure to the infection. This vaccine has been given safely in various sates of immunodeficiency, including HIV infection.[13] However, since 1989, new adverse events has been recognized among European, American and Australian vaccine recipients. These include itching, urticaria, and occasionally angio-oedema of the face – sometimes requiring admission to hospital and corticosteroid therapy. This vaccine used to be manufactured by Biken (Japan), but due to safety concerns, particularly the possibility of occurrence of acute disseminated encephalomyelitis (ADEM) temporally linked to the vaccine, production has been discontinued. Moreover, the Central Research Institute, Kasauli, who was the sole manufacturer of this vaccine in India, has also ceased its production.[14]

Cell culture-derived inactivated vaccine

A cell culture-derived inactivated vaccine, manufactured by propagating the Beijing P-3 strain in primary hamster kidney (PHK) cells is available exclusively in China. However, since PHK cells are not approved by WHO as a vaccine-production substrate, this Chinese vaccine has not received international acceptance. Other cell culture-derived inactivated JE vaccines that have been accepted internationally involve the Vero cell platform.[15] These are being manufactured in accordance with international norms following good manufacturing practices (GMP) and good clinical practice (GCP) criteria and are in various stages of development.[16] One such vaccine, named IC51, is being produced by the Austrian company Intercell and is currently undergoing pre-licensure Phase 3 clinical trials with promising results.[17]

Cell culture-derived live-attenuated vaccine

Cell culture-derived live-attenuated vaccine vaccine is based on the genetically stable, neuro-attenuated SA 14-14-2 strain of the JEV that is derived from serial passage of the SA 14 strain of the virus in primary hamster kidney (PHK) cells. It elicits broad immunity against heterologous JEV strains. The reversion to neurovirulence is considered extremely unlikely. As the vaccine is produced on primary cells, the manufacturing process includes detailed screening for endogenous and adventitious viruses. It has been licensed for use in China since 1988, where over 200 million children have been successfully immunized so far, with an excellent safety record. The Republic of Korea subsequently acquired a commercial licence to manufacture this vaccine. This vaccine is also widely used in Nepal, Sri Lanka and India. A case-control study conducted in Nepal indicated that five years after administration of a single dose of SA 14-14-2 provided a protective efficacy of 96% and the persistence of neutralizing antibody titer was 63.8%.[18] In the Indian context, 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. An expert committee reported that the 65 serious adverse events that were reported, including 22 fatalities, were not related to the vaccine.[19]

The contraindications of this vaccine are as follows: (i) a severe reaction (anaphylaxis) to a prior dose of SA 14-14-2 vaccine; (ii) pregnant women (no data is available in this group); (iii) reaction to a vaccine component (gelatin, gentamicin, kanamycin); (iv) immunosuppressed individuals such as drug-induced immunosuppression or human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) (no data is currently available in this category of patients); and (v) acute infectious disease, renal, hepatic or cardiac disease, active tuberculosis, otitis media and epilepsy.

A recent study in the Philippines has revealed that the SA 14-14-2 vaccine can be safely administered along with measles vaccine in 9-month-old infants without any loss of immunogenicity in either of the vaccines.[20] Combined administration with other vaccines currently used in the national immunization program need to be carried out so that SA 14-14-2 vaccine can, in the future, be incorporated into the routine childhood immunization program. A major advantage of this vaccine is that it is relatively inexpensive and hence would be affordable by developing nations.

Genetically Engineered Vaccines

Quite a few innovative genetically engineered third generation vaccines against JE are in various stages of development. The most promising is the Chimeri-Vax-JE(TM) developed by Acambis, UK. This recombinant DNA vaccine is based on the yellow fever (YF) 17D vaccine strain as a backbone, but with the envelope and pre-membrane protein genes of YF virus replaced by those of JEV. Clinical trials are currently ongoing for this vaccine. The results in terms of immunological response and side-effects have thus far been very favourable. However, it remains to be seen whether this vaccine can cost-wise compete with the SA 14-14-2 vaccine. The currently available vaccines against JE are listed in Table 4.

Table 4: Vaccines against Japanese encephalitis

Vaccine	Type	Virus used	Manufacturer	Dosing	Status
Biken/JE-Vax(TM)	Mouse brain-derived; inactivated	Nakayama	Biken, Japan; Sanofi Pasteur, USA; Green Cross, South Korea; CRI, India	Three doses at 0, 7, 30 days. Boosting after 1 year and subsequently every 3 years till child is 10 years of age	JE vaccine production stopped at CRI
SA 14-14-2	PHK cell-cultured; live-attenuated	SA 14-14-2	Chengdu Institute of Biological Products, China	Single dose	Currently in use in China, India, Korea, Sri Lanka and Nepal
IC-51	Vero cell-cultured; inactivated	SA 14-14-2	Intercell, Austria	Two doses at 0 and 30 days	Phase 3 trial completed; licensing expected in 2008
Chimeri-Vax-JE(TM)	Infectious clone; live-attenuated	JEV SA 14-14-2 and YF 17D chimeric infectious clone	Acambis, UK	Single dose	Phase 3 trial in USA, Australia and Asia

CRI - Central Research Institute; YF = Yellow Fever; USA = United States of America

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Conflict of Interest : None

Key Points

- JE, caused by the flavivirus JE virus, is the most serious form of viral encephalitis and is responsible for high mortality and morbidity
- The disease is prevalent in South Asia, India, and China but has the potential to spread to hitherto unaffected areas such as northern Australia and Pakistan
- A mouse brain-derived inactivated vaccine was available until recently, but has been discontinued due to safety concerns
- The live-attenuated SA 14-14-2 vaccine, manufactured in China and administered in some Asian countries including India, is not manufactured according to WHO-approved international norms
- Several candidate JE vaccines are in various stages of research and development. These include the Vero cell culture-derived inactivated JE vaccine, the chimeric yellow fever-JE vaccine and DNA vaccines
- JE cannot be completely eradicated, as it is a zoonotic disease. However, mass-scale vaccination of human population, coupled with disease surveillance and vector control, can bring down the disease burden significantly.

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