

AN OVERVIEW OF JAPANESE ENCEPHALITIS

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ABSTRACT

INTRODUCTION

Japanese encephalitis virus (JEV) is a flavivirus, is the main cause viral encephalitis, endemic in South-East Asia, and Western Pacific regions and has become a major public health problem worldwide. JEV is transmitted to humans through the bite of an infected mosquito, mainly *Culex tritaeniorhynchus* that breeds in flooded rice fields, marshes, standing water around planted fields. Reservoir hosts are Adreid birds (herons and egrets), and pigs serve as amplifier hosts. Though infections are usually asymptomatic, the symptomatic Japanese encephalitis has a case fatality rate as high as 30%. Permanent neurologic or psychiatric sequelae can occur in 30-50% of those with encephalitis. There is no antiviral drug available. Treatment is mainly supportive and symptomatic. Safe and effective vaccines are available to prevent Japanese encephalitis. This article reviews recent advancements in diagnostic methods, prophylactics as well as therapeutic options and challenges to combat this zoonotic emerging and remerging viral encephalitis.

KEYWORDS

Culex mosquito, Epidemics, Japanese encephalitis virus.

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INTRODUCTION

Japanese encephalitis (JE) is the leading cause of viral encephalitis in Asia, including India. The JE virus was first isolated in Tokyo, Japan “summer encephalitis epidemics” (1871). However, it is now uncommon in Japan. Japanese encephalitis was named Japanese B encephalitis to distinguish this summer epidemic from encephalitis lethargica/von Economo’s lethargic/sleepy sickness, commonly known as Japanese A encephalitis, which was prevalent at that time. Nowadays it is named Japanese encephalitis.¹

THE VIRUS STRUCTURE

Japanese encephalitis virus (JEV) belongs to family Flaviviridae (Flavi = yellow), and to the same genus Flavivirus, as dengue, yellow fever and west Nile viruses. It is spherical in shape with a diameter of 45-60 nm. It is an enveloped virus having ssRNA (+ve sense) packaged in the capsid, which is formed by the capsid proteins (capsomers). The virus particle contains three structural proteins: nucleocapsid or core protein (C), nonglycosylated membrane protein (M), and glycosylated envelope protein (E) and is the protective antigen. It aids in entry of the virus into the inside of the host cell. The genome also encodes several nonstructural proteins (NS1, NS2a, NS2b, NS3, N4a, NS4b, and NS5). Replication occurs in cytoplasm of host cell. Assembly takes place in ER of host cell and release of progeny is by budding through host cell membrane.²

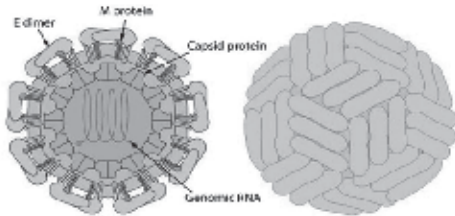


Fig 1. JEV structure²

GENOTYPES OF JEV

Five genotypes of JE have been identified based on the nucleotide sequencing of JEV genome, designated G-I to G-V.³ All genotypes of JEV form a single serotype.⁴ The genotype G-I is replacing G-III as the dominant genotype in Indian subcontinent.⁵ The genotype G-I includes isolates from Northern Thailand, Korera, Cambodia; while G-II includes isolates from Southern Thailand, Indonesia, Malaysia, Northern Australia; G-II includes isolates are mostly from temperate regions of Asia (China, Taiwan, Japan, and Philippines); G-IV is mainly from Indonesia. In 1952, a distinct strain of JEV was isolated from Muar, Malaysia (Muar strain) that has been considered as G-V genotype, based on phylogenetic analysis and cross-neutralization. All five genotypes are found in Indonesia-Malaysia region.⁶

EPIDEMIOLOGY

Natural infections of Japanese encephalitis (JE) occur in Adreid birds (herons and egrets). Virus spreads from bird to bird through *Culex tritaeniorhyncus* mosquito. *Culex vishnui*, *Culex gelidus*, *Culex whitmorei*, *Culex epidesmus* act as next common vectors in India. Human infection occurs from these reservoir birds by several species of Culicine mosquitoes. JE virus cannot transmit from one person to another. JEV infection is more common in children below 15 years. Most adults in endemic countries have natural immunity after childhood infection, but persons of any age may be affected. In South-East Asia JEV transmission intensifies during the rainy season, during which mosquito population increases.⁷



Fig 2. a) Herons and b) Egret birds⁷

TRANSMISSION

JE virus has a complex life cycle. Herons and egrets (water birds) are reservoir hosts. Pigs are amplifier hosts. JEV is transmitted to humans through bites from infected *Culex* mosquitoes. *Culex tritaeniorhynchus*, a blood sucking mosquito that breeds in flooded rice fields, marshes, standing water around planted fields is the vector. Other birds (ducks, pigeons and sparrows) may also be involved. Vertebrate hosts may include horses, cattle and buffaloes, besides pigs. The virus exists in a transmission cycle between mosquitoes, pigs and/or water birds (enzootic cycle). The disease is predominantly found in rural and periurban settings, where humans live in closer proximity to these vertebrate hosts.^{7,8}

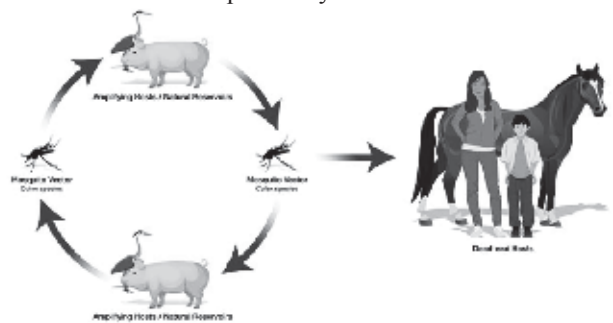


Fig 3. Transmission of JEV^{7,8}

JAPANESE ENCEPHALITIS IN INDIA

JE is a leading public health problem in India due to its complex eco-epidemiology, and epidemics have been reported in many regions since 1955, being endemic in 15 states. Gorakhpur in UP accounting for the largest burden followed by Assam, West Bengal, Bihar, Tamil Nadu and Karnataka. In India it is a major public health problem of national importance.⁹ A major outbreak was reported in the Bankura district of West Bengal in 1973 with a case fatality rate of 42.6%.¹⁰ Since then outbreaks have been reported regularly different parts of the country.¹¹ In 1978, a major outbreak occurred in Gorakhpur, Uttar Pradesh with 1,002 cases and 297 deaths being reported.¹¹ Since 1978 to 2005 in Uttar Pradesh state this JE encephalitis has taken more than 10,000 lives.¹¹ The most devastating epidemic occurred in seven districts of Eastern UP, between July and November 2005 with 6061 cases and 1,500 deaths. Another outbreak occurred in 2006, with 2,320 cases and 528 deaths.¹¹ The regions of Eastern UP, Particularly Gorakhpur and Basti divisions are conducive for the spread of the virus due to the abundance of paddy fields, a bowl shaped terrain and are also prone to flooding every year.¹¹ Recently India witnessed another large outbreak in Malkangiri¹² during 2012 and Manipur¹³ in July 2016. It is possible that many cases are unreported and the actual magnitude of the threat of JE may be higher. JE is declared as a notifiable disease in India due to its expanding geographical distribution, which facilitates effective implementation of preventive measures and case management.¹⁴

JAPANESE ENCEPHALITIS IN NEPAL

The epidemic activity of JEV has increased since the early 1970s in Northern India, Central India, and Nepal. JE is endemic in the Terai, with maximum number of cases occurring in the Western districts of Banke, Kanchanpur and Kailali.¹⁵ The disease was first recorded in Nepal in 1978 as an epidemic in Rupandehi district of the Western Development Region and Morang of the Eastern Region, and at present the disease is endemic in 24 districts.¹⁶ In the 1990s the virus spread to Kathmandu valley of Nepal.¹⁷ There is a strong seasonal pattern of JE occurrence in Nepal which peaked in August and declined by October each year, which corresponds to the monsoon season.¹⁷

PATHOGENESIS AND CLINICAL MANIFESTATIONS

JE is the most common cause of epidemic encephalitis. Cases are much less and it has been estimated that 500-1000 Subclinical infections occur for one case of clinical disease. Most of JEV infections are subclinical and asymptomatic or mild.¹⁸ After the bite of an infected mosquito, the JEV is inoculated into skin, and attaches to the host cell membranes, initially propagating at the site of bite in the skin in langerhans dendritic cells and/or in keratinocytes, then carried to the nearby lymph nodes, where further replication takes place, with subsequent viremia resulting in subclinical disease.¹⁹ If the virus is transmitted to the brain hematogenously with the invasion blood-brain barrier, encephalitis develops.^{20,21}

Incubation period varies from 5-15 days. There is often a prodromal phase of non-specific symptoms such as fever, headache, vomiting, diarrhea, and myalgia. Severe disease is

characterized by rapid onset of high fever, headache, neck stiffness, disorientation, coma, seizures, status changes, spastic paralysis and ultimately death. The case fatality rate can be as high as 30-40%. Of those, who survive, 20-30% suffer permanent intellectual, behavioral, neurological sequelae such as paralysis, recurrent seizures or the inability to speak.²²

There are many differential diagnoses of JE: West Nile virus encephalitis and other arboviral encephalitis, Herpes simplex encephalitis, fungal meningitis, Dengue fever, brain abscess, neurocysticercosis, primary amoebic meningoencephalitis, and CNS tumor.^{18,22}

LABORATORY DIAGNOSIS

Diagnosis of JE can be made by demonstration of antibodies, antigen detection, molecular methods, and virus isolation. MRI or CT scan may show bilateral thalamic edema, lesions or hemorrhage.²²

1. Demonstration of antibodies: Several serological tests have been developed for detection of antibodies such as plaque reduction neutralization test, ELISA, indirect immunofluorescence, and hemagglutination inhibition tests. IgM-capture ELISA is recommended by WHO and most widely used diagnostic method to test JEV-specific IgM antibody in a single sample of cerebrospinal fluid (CSF) or serum. Testing of CSF sample is preferred to reduce false-positivity rates from previous infection or vaccination.²³ The plaque reduction neutralization test (PRNT) is the gold standard test for measuring virus-neutralizing and protective antibodies against JEV and other flaviviruses. It is the test of choice to differentiate between cross-reactive antibodies with other flaviviruses.²⁴

2. Detection of virus antigen: In blood, NS1 antigen (non-structural antigen-1) can be detected by ELISA, IF, immunochromatography, staphylococcal coagglutination tests. In fixed tissues, group-specific antigens (*Flavivirus* antigens) can be detected in peripheral blood leucocytes, liver, lung at autopsy, and less often in the lymph nodes, spleen, bone marrow etc.^{25,26}

3. Molecular methods: RT-PCR can be used to amplify viral RNA from blood or other clinical samples.²⁷ Restriction fragment length polymorphism (RFLP) analysis is also useful molecular assay test.²⁸ An RT-LAMP-LFD assay that combines reverse transcription loop-mediated isothermal amplification (RT-LAMP) with lateral flow dipstick (LFD) is an important molecular diagnostic test as it is a fast, highly sensitive and specific assay.²⁹

4. Virus isolation: JEV can be isolated from blood, CSF, or tissue homogenates by several methods. Adult or larval mosquito inoculation is the most sensitive technique. Several mosquitoes are used such as *Toxorhynchites*, *Aedes aegypti*, and *Aedes albopictus*. Mosquito cell lines, such as C6/36 and AP61 cell lines, mammalian cell lines such as Vero, and LLC-MK2 cell lines, and yolk sac of embryonated eggs can be used. Intra-cerebral inoculation into suckling mice may lead to fatal encephalitis, but not a method of choice for diagnosis because of low level of viremia. The viral growth can be detected by identifying viral antigens in mosquito head squashes, infected cells, infected cell culture fluids, or

mouse-brain touch preparations by direct-IF using specific monoclonal antibodies.^{18,22}

TREATMENT

Treatment is limited to supportive care with intravenous fluids and antipyretics to relieve symptoms and stabilize the patient, and no effective antiviral therapy is available.³⁰ N-methylisatin-beta-thiosemicarbazone derivative (SCH 16) has shown complete inhibition of JEV replication *in vitro* and *in vivo*.³¹

PREVENTION AND CONTROL

The prevention of JE is mainly by mosquito control and active immunization. Persons travelling to JE-endemic areas should take precautions to avoid mosquito bites to reduce the risk of JE, and are recommended to get vaccinated before travel. There are 4 main types of JE vaccines available: inactivated mouse brain vaccine, inactivated Vero cell vaccine, live attenuated vaccine, and live recombinant (chimeric) vaccines.^{32,33}

Formalin inactivated mouse brain vaccine (JE-VAX) using Nakayama strain has been used for human immunization in Japan and India. It is prepared at Central Research Institute, Kasauli (India). Two doses of vaccine administered at 2 weeks interval, followed by a booster 6-12 months later. Immunity produced is short-lived. Vaccine is also useful for pigs to prevent epidemics.³³ Following reports of fatal cases of acute disseminated encephalomyelitis infrequently in children the use of this vaccine was stopped.³⁴

Live attenuated vaccine, prepared from SA 14-14-2 strain of JE virus in primary hamster kidney cell lines is widely used. The vaccine is administered subcutaneously in 2 doses, one year apart. It is very effective in preventing clinical disease. Under Universal Immunization Program, it is given to children (1-15 years) in endemic areas in India, South Korea, Sri Lanka and Nepal. It is strongly immunogenic and elicits broad protective immunity and is effective as well as safe for children.^{32,33}

Inactivated Vero cell vaccine, prepared from an Indian strain of the JEV known as JENVAC. It is the first indigenously developed vaccine that is safe and highly effective against all known strains of JEV. This vaccine can elicit protective response either with single or two doses, and is found safe and efficacious for the age group between 1-50 years.³⁵ Inactivated Vero cell culture-derived Japanese encephalitis (JE) vaccine (manufactured as IXIARO) is the only JE vaccine licensed and available in the United States. IXIARO is given as a two-dose series, with the doses spaced 28 days apart.³⁶

Live recombinant (chimeric) vaccine (ChimeriVax™-JE) is a single dose lyophilized formulation of a recombinant, attenuated, chimeric virus that consists of structural genes (Pre-membrane and E) from SA 14-14-2 strain, incorporated into the attenuated yellow fever virus YF 17D strain.³⁷

CONCLUSION

Japanese encephalitis (JE) is a significant cause of morbidity and mortality in South-East Asia, and has become a public health problem for the entire world. Due to international travel, rapid urbanization, and climate change, the virus is rapidly spreading across the world. Due to lack of an effective antiviral drug, accurate diagnosis and prevention by active immunization and vector control must be the top most priorities to reduce the disease burden. An effective, safe and readily available drug is the need of the hour to control the mortality rate in endemic areas.

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