



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2020; 9(3): 48-51

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www.thepharmajournal.com

Received: 10-01-2020

Accepted: 12-02-2020

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Japanese encephalitis: A persistent threat

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Abstract

JE is a vector (mosquito) borne viral zoonotic disease, caused by flavivirus belonging to family *Flaviviridae*. The virus has five genotypes which mainly affect the central nervous system. Previously genotype III was the most widely distributed genotype in Asian countries including India with the prototype of Nakayama strain but in recent years genotype I is largely replacing genotype III (Karthikeyan *et al.*, 2017). The World Health Organization (WHO) has attributed JE to be the most important cause of mosquito borne viral encephalitis in endemic Asian countries especially in the pediatric age group (0–14 years), where 75% of the cases occur. An estimated 67,900 cases of JE are reported annually, with approximately 13,600–20,400 deaths, while 2 billion people are at risk located in 24 WHO member countries. India and China experience 95% of the reported disease burden of JE. Two thirds of the at risk population for JE is in China and India only (Rustagi *et al.*, 2019). Japanese encephalitis (JE) is a major threat with case fatality rate up to 30%. It causes severe neuro-psychiatric sequelae that necessitates lifelong support amounting towards considerable socioeconomic burden. The natural maintenance reservoir for JE virus are birds of the family *Ardeidae* (herons and egrets). Pigs act as important amplifiers of the virus producing high viraemias which infect mosquito vectors (OIE, 2019). The infection causes a spectrum of clinical illness that begins with flu-like symptoms, neck stiffness, disorientation, coma, seizures, spastic paralysis and eventually death. The ideal method for laboratory confirmation of JEV is testing cerebrospinal fluid (CSF) or serum for JEV-specific IgM antibody (Kulkarni *et al.*, 2018). Currently, there is no cure for JEV, and treatment is mainly supportive. National Vector Borne Disease Control Programme (NVBDCP), Govt. of India, works towards prevention and control of six important vector borne diseases and Japanese encephalitis is among one of those diseases. The effectiveness of vector control strategies is limited due to the complex eco-epidemiology of the virus. Vaccination is the most effective means of prevention, where JEV is a major public health problem.

Keywords: Japanese encephalitis, structure of je virus, genotypes, vector, transmission, Indian scenario, global scenario, clinical signs, diagnosis, prevention and control

Introduction

JE is the most important cause of mosquito borne viral encephalitis in endemic Asian countries especially in the pediatric age group (0–14 years), where 75% of the cases occur. An estimated 67,900 cases of JE are reported annually, with approximately 13,600–20,400 deaths, while 2 billion people are at risk located in 24 WHO member countries. India and China experience 95% of the reported disease burden of JE. Two thirds of the risk population for JE is in China and India only (Rustagi *et al.*, 2019) [14]. Japanese encephalitis (JE) is a major threat with case fatality rate up to 30%. It causes severe neuro-psychiatric sequelae that necessitates lifelong support amounting towards considerable socioeconomic burden. JE is a vector (mosquito) borne viral zoonotic disease, caused by flavivirus belonging to family *Flaviviridae*. The virus has five genotypes which mainly affect the central nervous system. Previously genotype III was the most widely distributed genotype in Asian countries including India with the prototype of Nakayama strain but in recent years genotype I is largely replacing genotype III.

Structure and genome of the virus

JEV is spherical in shape (40-50 nm in diameter), comprises of a lipid envelope decorated with glycoproteins containing an isometric nucleocapsid consists of single-stranded positive-sense RNA genome and core protein. The genome contains single open reading frame (ORF) of about 11 kb size in which 5' end is capped and 3' end is not poly-adenylated with the potential to encode a large polyprotein of 3432 amino acids (Nain *et al.*, 2017) [10]. This ORF carries genes for three structural proteins and seven non-structural proteins. The structural proteins are

nucleocapsid or core protein (C), glycosylated envelope protein (E) and non-glycosylated membrane protein (M). Further, non-structural proteins are abbreviated as NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The structural component of the nucleocapsid is formed by C protein. The M protein serves as a transmission anchor which has hydrophobic domains. The M protein is initially synthesized as a precursor glycoprotein (prM) and cleaved to mature M protein by a furin-like cellular protease. Incompletely cleaved prM act as an additional target on virions for neutralizing antibodies. The envelope protein comprises of 3 domains (I, II and III) that helps in the penetration of the virion into host-cell, virulence, stimulation of neutralizing antibody and producing a protective immune response. In addition, E protein acts as a major target for host antiviral immune

response. Non-structural proteins play a significant role in viral genome replication and expression.

Genotypes

The nucleotide sequencing studies on the partial or complete genome of JEV revealed that it has five JEV genotypes, G-I to G-V (Yun *et al.*, 2003) [19]. The G-I and G-III genotypes are present mostly in temperate epidemic areas, whereas G-II and G-IV are reported in tropical endemic regions. Previously genotype III was the most widely distributed genotype in Asian countries including India with the prototype of Nakayama strain (Mackenzie *et al.*, 2006) [8] but in recent days genotype I is largely replacing genotype III (Gao *et al.*, 2013) [5].

Table 1: Genotypes of JE Virus

Genotype	Geographical distribution
Genotype I	India, China, Cambodia, Japan, Malaysia, Korea, Taiwan, Northern Thailand, Vietnam and Northern Australia
Genotype II	Indonesia, Malaysia, Northern Australia, Papua New Guinea and Southern Thailand
Genotype III	India, China, Korea, Japan, Myanmar, Philippines, Nepal, Taiwan, Vietnam, Srilanka
Genotype IV	Indonesia
Genotype V	Muar strain in Malaysia (1952), China, South Korea.

Vectors and Reservoir Hosts

JEV virus exists in an enzootic mosquito-bird or mosquito-bird-pig transmission cycle where vertebrate hosts like pigs and water birds have a vital position in upholding and intensification of the virus, whereas invertebrate hosts like *Culex spp.* mosquitoes help in virus transmission. *C. tritaeniorhynchus* is the main vector involved in the transmission of JEV in Asian continent whereas, in Northern Australia, *C. annulirostris* is the most important vector. *C. vishnui* complex (*C. tritaeniorhynchus*, *C. vishnui* and *C. pseudovishnui*) act as a principal vector of JEV which breeds in water bodies with luxuriant vegetation i.e. paddy fields, shallow ditches and pools and these vectors spread in highest density between June and November in temperate zones. *C. tritaeniorhynchus* feed during night time and shows two peaks of biting time i.e. few hours after sunset and in midnight. *Ardeidae* family water birds (i.e. cattle egrets and pond herons) act as important natural reservoirs as well as maintenance hosts for JEV. Pigs serve as amplifying hosts for JEV and significantly contributes to the dissemination of the disease in rural settings without showing any overt clinical signs except abortion and stillbirth in infected pregnant sows. Horses and humans are considered as dead-end hosts for JE infection.

Transmission

The natural cycle of JE virus involves water birds and *Culex mosquitoes* while pigs are considered to be the most important amplifying host, providing a link to humans through their proximity to housing. In India, most common vector is *Culex tritaeniorhynchus* followed by other members of *C. vishnui* and *C. pseudovishnui*. However secondary vectors like *C. gelidus*, *C. fuscocephala*, *C. whitmorei*, *Anopheles subpictus* and *M. uniform* are also responsible for transmission of JE in India (Malhotra *et al.*, 2015) [9].

Epidemiological pattern and global scenario

Two patterns are exhibited (a) Endemic pattern which is common in tropical areas of Southern Asia, occurs sporadically throughout the year with peak after the start of

monsoon (July to September) (b) Epidemic pattern prevalent in temperate areas of Northern Asia.

An estimated 67,900 cases of JE are reported annually, with approximately 13,600–20,400 deaths, while 2 billion people are at risk located in 24 WHO member countries. India and China experience 95% of the reported disease burden of JE (Rustagi *et al.*, 2019) [14]. Two thirds of the at risk population for JE is in China and India only.

Distribution of disease in India

In India, JE is a leading pediatric health issue and epidemics have been reported from many regions since 1955. Karnataka and Andhra Pradesh experiences two epidemics every year, first from April to July that is quite severe while second from September to December being milder similar to the rest of India. The earliest evidence of JEV in India was obtained through the studies conducted in 1952. A major outbreak occurred in the Bankura district of the state of West Bengal in 1973 (Chakravarty, 1975) [2]. Since then, the virus was found active almost in every part of India and outbreaks have been reported regularly. The most affected states comprise of Andhra Pradesh, Assam, Bihar, Haryana, Karnataka, Kerala, Maharashtra, Manipur, Tamil Nadu, Orissa, Uttar Pradesh and West Bengal. The state of Uttar Pradesh has been under constant surveillance for JEV activity since 1978 (Rathi *et al.*, 1993) [13]. The longest epidemic of viral encephalitis was reported from Gorakhpur district, UP between July and November 2005. The regions of eastern UP (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 annual flooding. The virus activity was reported regularly from states of northern and northeastern parts of India. A large outbreak of JE occurred during 2012 and July, 2016 in Malkangiri and Manipur respectively (Dwivedi *et al.*, 2015) [4]. During July 2019, a severe outbreak occurred at Assam with about 637 cases and 158 deaths.

An unexplained acute neurologic illness affecting children with high case-fatality rates was reported from Muzaffarpur district of Bihar since 1995 (Kulkarni *et al.*, 2018) [7]. A hypothesis linking this disease with the cultivation of litchi

fruits was also proposed. However, the disease was ascribed to the presence of hypoglycin A or methylenecyclopropylglycine (MCPG) – present in litchi that can cause hypoglycemia and metabolic derangement.

Clinical features in human

JE is a devastating human disease that affects paediatric age group but peoples of all age may get the infection. Approximately 99 % JEV infection are asymptomatic. The occurrence of symptomatic to asymptomatic infection ratio is 1: 25-1000 (1:300 on an average). Patients with Japanese encephalitis typically present after a few days of non-specific febrile illness, which may include coryza, diarrhoea, and rigors. This is followed by headache, vomiting, reduced level of consciousness and convulsion. Convulsions occur often in Japanese encephalitis, and have been reported in up to 85% of

children and 10% of adults. The classic description of Japanese encephalitis includes a dull flat mask-like faces with wide unblinking eyes, tremor, generalized hypertonia, and cogwheel rigidity (Solomon *et al.*, 2000) [16]. These features were reported in 70%-80% of American service personnel, and 20%-40% of Indian children. Other extrapyramidal features include head nodding and pill rolling movements, bizarre facial grimacing, and lip smacking

Clinical signs in animals

Vertebrate animals including cattle, sheep, goat, dog, cat, and chicken have been reported to be infected with JEV and mostly remain asymptomatic. In pigs, viremia occurs without displaying any overt clinical signs, but infection of pregnant sows results in the mummified foetus, stillborn and weak piglets with subcutaneous oedema and hydrocephalus.

Table 2: State wise number of JE Cases and Deaths from 2013-2019(NVBDCP, 2019)

S. No.	Affected states/UT	JE													
		2013		2014		2015		2016		2017		2018		2019 (Till 30 Nov.)	
		Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death
1.	Arunachal Pradesh	0	0	32	3	32	2	0	0	5	0	0	0	0	0
2.	Assam	495	134	761	165	614	136	427	92	604	87	509	94	637	158
3.	Bihar	14	0	20	2	66	12	100	25	74	11	74	11	16	1
4.	Jharkhand	89	5	90	2	116	8	47	5	29	1	66	0	112	3
5.	Karnataka	2	0	13	0	27	1	11	0	26	2	35	5	17	1
6.	Kerala	2	0	3	2	2	1	2	0	1	0	5	2	3	0
7.	Maharashtra	0	0	0	0	7	0	12	1	27	0	6	1	34	9
8.	Manipur	0	0	1	0	6	0	47	1	186	10	57	3	346	6
9.	Meghalaya	0	0	72	3	41	8	47	4	48	4	90	6	170	9
10.	Nagaland	4	0	6	0	0	0	0	0	10	2	1	0	10	0
11.	Odisha	0	0	0	0	32	2	242	42	79	0	143	0	168	0
12.	Utter Pradesh	281	47	191	34	351	42	410	73	693	92	323	25	216	14
13.	West Bengal	140	12	415	78	342	75	174	39	165	40	140	35	48	9
Grand Total		1027	198	1604	289	1636	287	1519	282	1947	249	1449	182	1777	210

Diagnosis

Diagnosis of JE can be done by laboratory based tests like cerebrospinal fluid (CSF) or serum for JEV-specific IgM antibody (MAC-ELISA), Plaque Reduction Neutralization Test (PRNT) Virus isolation, Nucleic acid amplification. The JEV-specific IgM antibody capture ELISA (MAC-ELISA) is the first-line diagnostic assay recommended by WHO for detection of acute infections (WHO, 2007) [18].

PRNT is considered as a gold standard in flavivirus diagnosis to discriminate between potentially cross-reactive antibodies with other flaviviruses (Niedrig *et al.*, 2008) [11]. A fourfold increase in IgG titre in acute and convalescent sera is considered as a confirmatory test.

Japanese encephalitis virus can be isolated by intracerebral inoculation of clinical specimens in the suckling mouse brain. Various cell cultures that have been used more recently include primary chick, duck embryo cells, and lines of Vero, LLCMK2, C6/36, PK, and AP61 cells (Tiwari *et al.*, 2012) [17]. Clinical specimens such as blood, serum, brain, CSF and spinal cord in equines, blood and aborted foetus in case of pigs; CSF and brain tissues in humans and mosquitoes are suitable for virus isolation.

The RT-PCR tests, quantitative PCR (TaqMan), Restriction Fragment Length Polymorphism (RFLP) analysis are useful molecular assay tests as they are very specific, sensitive and can detect low viral copies in acute or early phase of infection. An RT-LAMP-LFD assay that combines reverse transcription loop-mediated isothermal amplification (RT-LAMP) with a lateral flow dipstick (LFD), is of great

importance in diagnosis of JEV infection as it is a fast, highly sensitive and specific assay (Deng *et al.*, 2015) [3].

Prevention and control strategies

JE is a leading public health problem in India due to its complex eco-epidemiology. Considering the gravity of the problem of AES & JE in the country, Government of India has formulated a multipronged strategy to reduce the disease burden as well as to prevent mortality, morbidity and disability. The strategy includes JE vaccination in affected districts and strengthening of surveillance programs. Additionally, vector control, case management, timely referral of serious and complicated cases are also done. Appropriate sanitation facilities, as well as access to safe drinking water, are also aimed at this strategy. Provision for physical, medical, neurological and social rehabilitation is included to estimate disability burden due to JE. Improvement in the nutritional status of children at risk has also been designed. Immunization of pigs as well as avoiding human exposure to the infected mosquitoes seems feasible but is a short-term solution in high-risk endemic zones.

Vaccines

Following vaccines are available for prevention of JE:

Inactivated Mouse Brain-Derived Vaccines

JE-VAX is a mouse-brain derived inactivated virus vaccine manufactured in Japan. Purified mouse brain-derived wild-type Nakayama or

Beijing-1 strains were used for vaccine preparation (Beasley *et al.*, 2008) [1]. Three doses were recommended for travellers while for the children (1-3 years) 2-doses were administered in endemic regions.

Inactivated vero cell vaccines

P3 strain of JEV grown in Primary Hamster Kidney cells was used for the preparation of inactivated JE vaccine. This vaccine was solely manufactured in China and was the principal JE vaccine till 2000.

Live attenuated vaccines

SA 14-14-2 strain propagated in Vero cells as well as in primary hamster kidney (PHK) cells were used for the manufacture of this vaccine in China.

Chimeric vaccines

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 (Huang *et al.*, 2019) [6]. These structural genes were incorporated into the backbone of attenuated strain of yellow fever (YF) virus YF 17D. It is effective in the pediatric population in endemic zones and can be integrated into the national immunization program. It is licensed in China, Thailand and India.

Jenvac

The JENVAC is an inactivated Vero cell-derived vaccine prepared from an Indian strain of the JEV. It is the first indigenously developed vaccine that is safe and highly effective against all known strains of JEV. This vaccine can elicit protective responses with either a single or two doses and is recommended for JE endemic countries (Singh *et al.*, 2015) [15].

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