CHAPTER 16

JAPANESE ENCEPHALITIS VIRUS

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INTRODUCTION

Japanese encephalitis (JE) is an acute vector born viral disease that cause encephalitis in human, equids and reproductive disease in swine. Rare clinical cases are also reported in other domesticated animals such as cattle (Katayama et al. 2013). The disease is caused by Japanese encephalitis virus that belongs to Flaviviridae. JE has high peaks in horses during late summer and autumn in temperate regions, while in tropical areas virus circulates around the year. Most of cases remain asymptomatic but clinical cases tend to manifest sever encephalitis leading to death and many survivors left with neurological disorders. The morbidity and mortality rate is high in unvaccinated population during an outbreak. The case fatality rate in horses can be as high as 5-15% but in some outbreaks it has been reported up to 30-40 % (Prow et al. 2013; Kumar et al. 2018). The transmission is mainly caused by mosquito bite and life cycle is enzootic. Pigs are amplifying host and/or reservoirs, while water birds act as carrier and humans, equids and other domesticated animals serve as dead end host. The first outbreak was reported in 19th century in Japan and then extended its geographic area to Asia and western pacific region.

Etiology

Japanese Encephalitis Virus (JEV) belongs to arbovirus within the genus Flavivirus and family Flaviviridae. Genome is RNA in nature that is single stranded, positive sense, non-segmented and length of genome is about 11kbs. As genome is positive sense it acts directly as mRNA and encode a single open reading frame. The genome is translated into a single polyprotein, precursor of 3432 amino acids that undergoes proteolytic cleavage to form three structural proteins [C (capsid), pr M (pre-membrane), E (envelope)] and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). To date there is only one serotype and five genotypes [GI (GI-a, GI-b), GII, GIII, GIV, GV] of JEV are reported (Figure 1) (Gao et al. 2017; Yun et al. 2018). Genotyping is based on envelope (E) protein of virion and is considered as potential antigenic part, involved in the binding of virus to the cell receptors, virion assembly and fusion activity. Amino acid substitution in E protein plays key role in determining the neurovirulence and/or neuro invasiveness. The JEV is closely related to other arthropod borne viruses' i.e West nile virus and Murray valley encephalitis virus (Rice et al. 1986).

Host and Transmission

Disease mainly occurs in equids i.e., horses, donkeys and pigs. Domesticated and feral pigs and ardeid water birds (e.g., herons, bitterns and egrets) serve as main amplifying hosts in JEV endemic areas, as they develop high viremia and involve in disease transmission through mosquito vectors. Asymptomatic infection have also been reported in other domestic and wild mammals (cows, goats, and dogs), reptiles (snakes) and amphibians (frogs) and considered as dead-end host because they do not contribute in spread of infection as they develop low viremia that is not capable of transmitting to mosquitoes. Humans are also susceptible to the disease and considered as dead-end host (Kobayashi et al. 1948; Soman et al. 1977; OIE 2019).

The virus is transmitted through mosquitoes, the genus Culex, several species are reported but Culex (C.) tritaeniorhynchus and C.annulirostris are primary vector species involved in transmission of virus. *C. tritaeniorhynchus* is important in spreading of virus to humans and domesticated animals around the globe, while *C. annulirostris* are important vectors in Australia. *C. tritaeniorhynchus* breeds in rice fields, wells, fish ponds, connecting canals and also has been reported in urban areas close to human proximity such as water storage tanks in houses (Scherer et al. 1959; Hanna et al. 1996; Su et al. 2014). The JEV is mainly transmitted through mosquito bites but can also through mucous membrane, inhalation of aerosols or acquired through needle injuries. Human and most of domesticated animals are incidental host and are not considered important in virus transmission. Although birds, suids and pigs are amplifying host and involve in disease transmission (Figure 2). Boars are reported to transmit the virus through semen. The JE virus does not survive well outside a living host and is sensitive to ultraviolet and gamma rays. Virus can be destroyed by heating at 56°C for 30 minutes, inactivated at low pH (3) and can be killed by organic and lipid solvents like iodine, 70% alcohol, 3% formaldehyde and 1% sodium hypochlorite (OIE 2019).

Incubation Period and Clinical Signs

In experimentally infected horses' incubation period is approximately 4-14 days, while in pigs clinical signs appear after 3 days but high t'rature and viremia is detected within 24 hours. Infection ranges from subclinical to symptomatic in horses and disease severity varies. Three syndromic manifestations have been described in horses:

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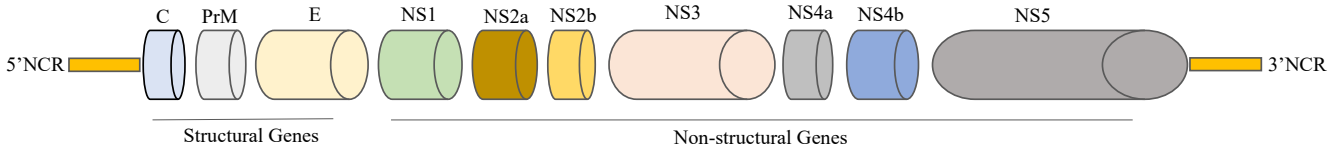

Fig. 1: Genome organization of Japanese encephalitis virus. 5'NCR (Non coding region) followed Single open reading frame and 3'NCR (Non coding region).

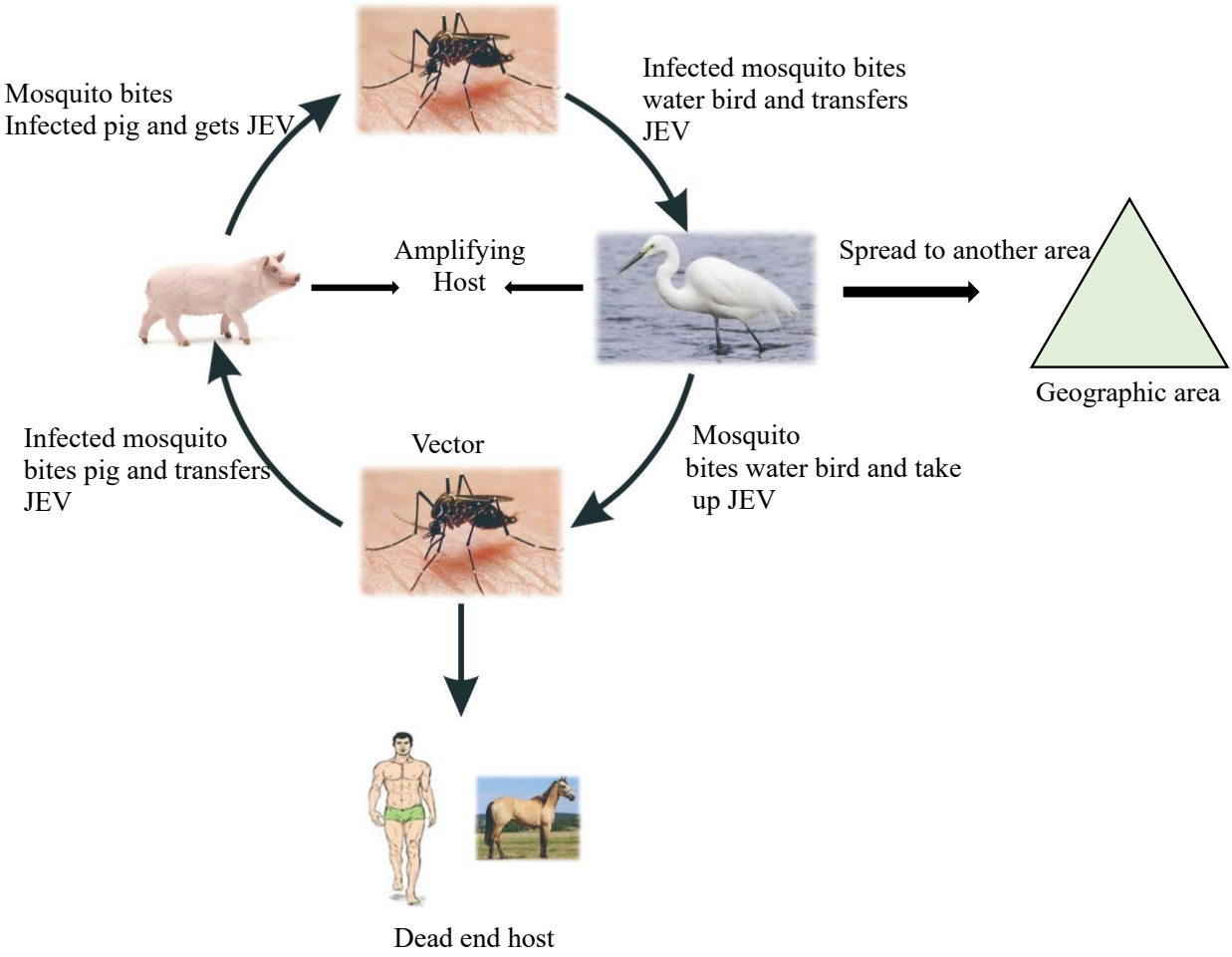

Fig. 2: Transmission cycle of Japanese encephalitis virus.

1) Transitory type syndrome: Infected horses develop mild fever lasting for 2-4 days along with loss of appetite, immobility and congested mucosa.
2) Lethargic type syndrome: Infected horses develop moderate fever, difficulty in swallowing, chewing motion, incoordination, compromised vision and paralysis. Recovery usually occurs within one week.
3) Hyperexcitable taype syndrome: Horses develop high grade fever accompanied with profuse sweating, muscle tremors, loss of vision, coma and death (Van den Hurk 2009; Go et al. 2014; OIE 2019). Mortality rate is up to 30%. Risk of abortion, still birth and congenital deformities is higher in pigs (Li et al. 2010). JEV infection in cattle is also reported but cases are very rare. Clinical signs observed in cattle include depression, loss of appetite, circling movement and inability to walk. Most JEV infections are asymptomatic or cause mild flue like disease in human that lasts for 5-15 days but some infections result in encephalitis accompanied with headache, fever, hemorrhagic lesions in brain, inflammation of meninges followed by neck stiffness, coma, paralysis of upper limbs and ultimately death. Mortality rate in human can be as high as 30% and recovered patients may have lifelong physical and mental impairments such as epileptic seizures. Miscarriages have been reported in pregnant women infected first time during pregnancy (Solomon and Vaughn 2002; Hollidge et al. 2010).

Pathogenesis and Gross Lesions

After transmission through infected mosquito bite, replication of virus occur in skin dendritic cells (Langerhans cells) and gets transported into the lymphatic and peripheral tissues resulting in viremia. Then lymphocytes harboring virus cross the blood brain barrier and enter into endothelial cells of central nervous system. The JE virus replicates in neurons which results in neural cell's death (Li et al. 2015). Although viral entry into brain is not well understood but once the virus enters brain cells, JEV is detected in cerebrospinal fluid and nervous tissues. The JEV can cross the placental barrier and cause abnormal neural development in fetus (Mathur and Chaturved 1982). Postmortem lesions in infected horses and cattle show diffuse

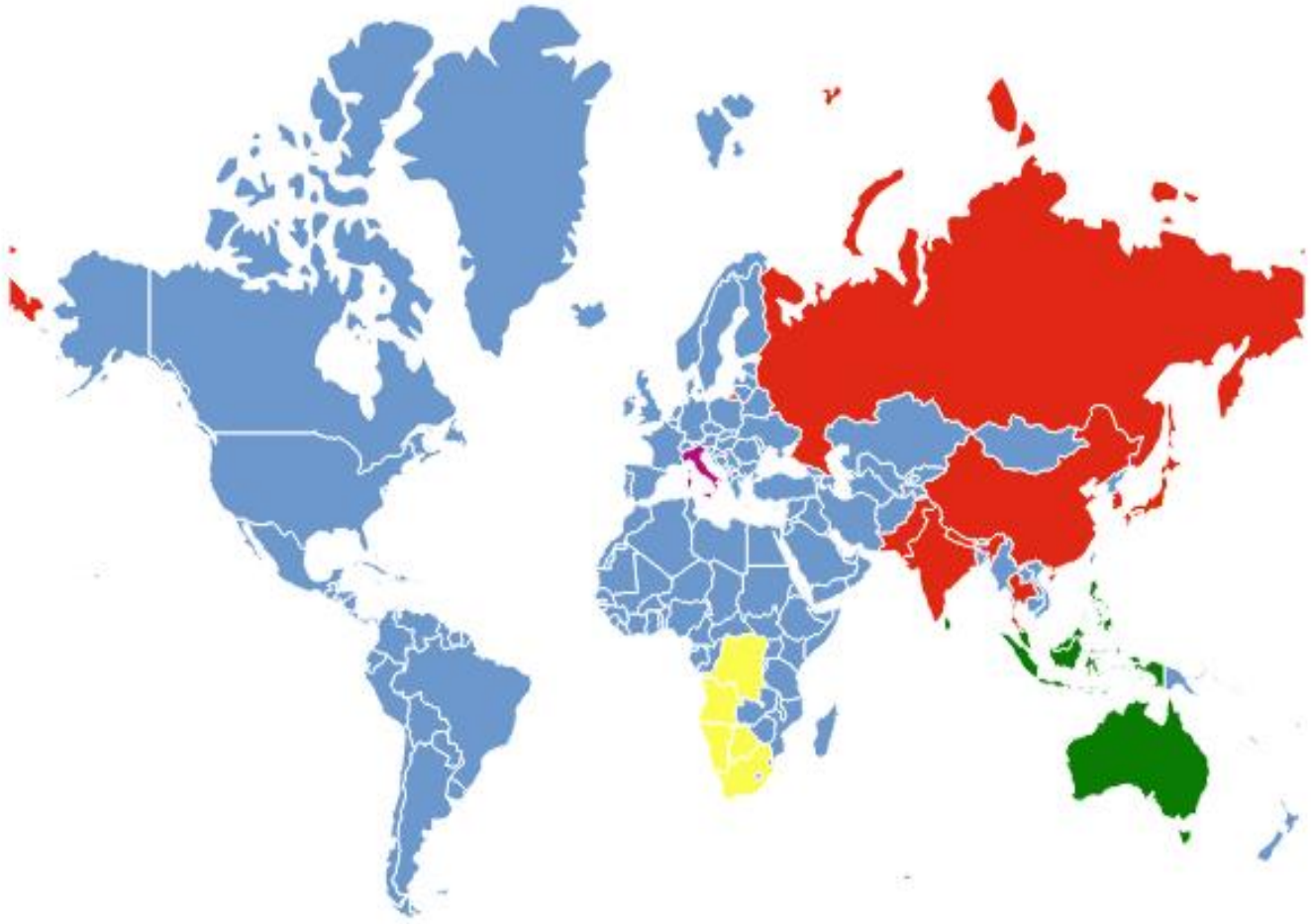

Fig. 3: Geographic distribution of Japanese Encephalitis Virus. Red color highlights countries where disease is epidemic (China, northern India, Japan, Pakistan, Korea, Nepal, Thailand and Russia). Green color highlights countries with endemic status (Australia, Indonesia, Malaysia, Singapore, Philippines, Sri Lanka and southern India.). Yellow color highlights African countries. Purple color highlights European countries**.**

non-suppurative encephalomyelitis that is characteristic microscopic lesion along with hemorrhages and congestion (Katayama et al. 2013; Kako et al. 2014). Congenital neurological defects including hydrocephalus, cerebellar hypoplasia and spinal hypomyelinogenesis has also been observed in mummified or still born fetuses from infected sows (Desingu et al. 2016).

Epidemiology and Geographic Distribution

Japanese encephalitis virus, a serious vector based viral disease is found worldwide especially in Asia, Western Pacific region and Northern Australia (OIE 2019). JEV has also been isolated from mosquitoes and birds in Italy, where human cases are not reported (Preziuso et al. 2018). A mixed infection with yellow fever has also been reported in African countries. Presence of virus in other regions is still unclear due to lack of surveillance and/or cross reactivity with other Flaviviruses. In Asia, JEV is wide spread in temperate and tropical regions especially in areas of rice farming and pig farming. Mainly two epidemiological patterns of this virus have been recognized. Epidemic pattern has been reported in China, northern India, Japan, Pakistan, Korea, Nepal, Thailand and Russia. While endemic pattern has been reported from Australia, Indonesia, Malaysia, Singapore, Philippines, Sirilanka and southern India (Figure 3). In northern region, GI and GIII are most prevalent genotypes, while in southern region GII and GIV are reported and GV is putative (Ladreyt et al. 2019).

Diagnosis

Asymptomatic infection, acute nature and cross reactivity with other viruses made difficulties in diagnosing Japanese encephalitis virus (JEV) in the past. But now with the combination of classical and advanced diagnostic techniques, it became easier to diagnose the virus (Mansfield et al. 2017).

Virus Isolation

Virus can be isolated from the CNS of diseased or dead animal. In vivo and in vitro techniques are employed for this purpose. Virus is collected from different parts of the brain including corpus striatum, cortex, or thalamus. It can also be isolated from spinal cord and blood. In vivo isolation of virus is done in mice by inoculating affected tissue intracerebrally. Mice will develop symptoms during the incubation period. In vitro primary cell cultures prepared from minced chick brain, chick embryoblasts, porcine or hamster kidney cells, or with established cell lines such as Vero (African green monkey kidney), BHK (baby hamster kidney) or C6/36 (mosquito – Aedes albopictus) are used. Discrete plaques are visualized after dyeing with crystal violet (Stear 2005, Mansfield et al. 2017).

Serology

Several serological techniques are used to detect JEV in the cerebrospinal fluid (CSF) or blood of the animal. These include

ELISA (enzyme linked immunosorbent assay), virus neutralization test (VNT), hemagglutination inhibition tests (HI) and complement fixation tests (CFT). Determination of IgM against surface proteins through ELISA is the readily available marker for current infection, within a week of the start of infection, and IgG to track previous infection. This test is also handy and suitable to perform field survey for epidemiological investigations. Drawback of this test is cross reactivity with the other flaviviruses. To overcome this issue, plaque reduction neutralization test (a variant of VNT) is an alternative which gives a reliable result and considered as gold standard for the detection of JEV. Commercially available indirect immunofluorescence test (IIFT) is also available to detect IgM and IgG using the immunofluorescence and is more sensitive. CFT is also a cheap source of identification which comes with good sensitivity. HI is also used for the detection of JEV, but it has low specificity and sensitivity that makes it a bad choice (Cardosa et al. 2002, Feng et al. 2018).

Molecular Methods

Now a days molecular methods are the advantageous over conventional methods due to number of reasons, including high specificity, sensitivity, rapid and convenience. There are five genotypes of JEV based on the viral envelop E gene, each of which can be individually detected in a single setting of assay. Genotype GI (GI-a, GI-b) is most prevalent among all. Molecular methods are based on the specific primers which amplify the nucleic acid and yield a qualitative and quantitative measure of the virus. RT PCR (Reverse transcriptase polymerase chain reaction) and multiplexed PCR techniques are used to amplify RNA of JEV. This assay can amplify genome present in samples, like serum, CSF, tissue, or any other body fluid. Primers for these assays are reported by Mansfield et al. (2017).

Prevention and Control

JEV infection can be prevented if strict measures should be taken in time. Though vaccine development against in JEV in human is under process but it is available in market for the livestock and pets. Live attenuated and killed vaccines are recommended by The World Organization for Animal Health. Vaccine is highly effective in preventing infection in livestock and rare animals like horses. This has a huge impact in the revenue generation from the production of livestock. Other than vaccination, strict mitigation of vectors is highly desirable to prevent the spread of infection in animals and human. Isolation of infected animals is also a strategy to control the spread of the virus to healthy animals. Availability of rapid, cheap, easy to handle and convenient diagnostic techniques in endemic areas can mainly help in prevention and control of the infection which can save huge losses (Fischer et al. 2008).

Biosafety

JEV has the potential biosafety threat to handlers of the infected animals, as virus can be transmitted while in contact with blood, cerebrospinal fluid, tissues, infected arthropods, needle and inhalation. So, standard biosafety measures should be taken while handling the infected material either in or outside the laboratory. These include the use of PPE and BLS level3 laboratories while culturing the virus, proper disposal of the waste and incarnation of infected material (Artika et al. 2017).

Treatment

No specific antiviral therapy is available for treating human and animals. Disease can be prevented though management and vaccination (Zhang et al. 2014).

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