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Flavivirus Encephalitis in Equines with an emphasis on the Japanese Encephalitis and West Nile Viruses

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Abstract

JEV and WNV are mosquito-borne flaviviruses that associated with both human and equine encephalitis worldwide. They cause similar symptoms ranging from asymptomatic to lethal encephalitis. JEV and WNV can emerge and reemerge in many regions due to climate change and increased travel. Serological methods can be used to detect antibody titers of these viruses but cross-reactivity with other flaviviruses often occurred. It is critical to distinguish between JEV and WNV in areas where both viruses are endemic. Molecular diagnostic techniques such as RT-PCR and real-time RT-PCR have been developed to differentiate JEV and WNV in various specimens. Prevention and protection using vaccines are available.

INTRODUCTION

The family *Flaviviridae* contains three genera including *Hepacivirus*, *Pestivirus*, and *Flavivirus*. The *Flavivirus* genus is classified into 4 groups: the Japanese encephalitis virus complex, the tick-borne encephalitis virus complexes, the Dengue virus, and the yellow fever virus. The Japanese encephalitis virus complex includes Japanese encephalitis virus (JEV), West Nile virus (WNV), St. Louis encephalitis virus, and Murray Valley encephalitis virus (MEV). The Japanese encephalitis virus complex and the tick-borne encephalitis virus complexes are neurovirulent and can cause encephalitis in humans and animals. In contrast, Dengue and Yellow fever viruses are more viscerotropic and cause hemorrhagic fever. Among the mosquito-borne flaviviruses, JEV, WNV, Dengue virus and Yellow fever virus are current major global concerns [1,2]. Flaviviral encephalitis has been occurred mainly via hematogenous and the virus spreads to the central nervous system (CNS). After injection of virus-containing saliva from mosquito, the virus replicates in local tissues and regional lymph nodes. This results in a primary viremia and subsequent infection of extraneural tissues, eg. reticuloendothelial system, where further replication leads to a more sustained secondary viremia, which causes CNS infection. The diseases spread to a number of different hosts such as humans, birds, sheep, pigs, and horses. However, flaviviruses in equines including horses, donkeys, ponies, and mules, are predominantly associated with Japanese encephalitis virus (JEV) and West Nile virus (WNV) [3,4,5].

2. Japanese Encephalitis Virus (JEV) in Equines

Japanese Encephalitis virus (JEV) is cause of the most important veterinary flavivirus disease and is usually transmitted by mosquitoes in the genus *Culex*. The most important *Culex* is *Culex tritaeniorhynchus* which has a wide host range that includes birds, horses, swine and humans. *C. tritaeniorhynchus* breeds in flooded fields, rice paddies, connecting canals and is active at twilight. Many other species of *Culex* including *C. vishnui, C. gelidus,* and *C. pipiens* can also transmit JEV. In some regions, *Aedes* mosquitoes have been implicated in transmission. Most animals are infected when they are bitten by mosquitoes. Birds are the most important reservoir hosts, and usually maintain the virus cycler in nature. Horses, swine and humans are important amplifying host, as they are bitten by the same mosquitoes that bite birds. JEV does not persist outside a living host. In the winter climates, the virus may be maintained in mosquitoes, either by transovarial passage or during hibernation, which is similar to reptiles, amphibians, and bats [4,5,6].

2.1 Geographic distribution of JEV

The geographic distribution of JEV is mainly in Southeast Asia and South Asia and has spread to West India and to the West Pacific region including Australia. The epizootic JEV season in Asia temperate climates usually begins in May and ends around October. Equine cases peak in late summer and autumn when the virus spills over into this host. In tropical regions, JEV circulates year-round in mosquitoes and birds but there may be seasonal peaks of disease associated with rainfall, irrigation or other factors that affect the local abundance of mosquitoes. Some tropical areas, epidemics may be seen at the end of rainy season. Sporadic cases of JEV in horses have been reported in small clusters in various countries throughout the mosquito season including Japan, Hong Kong, Taiwan, Indonesia, Nepal and India. Between 1948 and 1967, the morbidity rate in Asia was estimated to be 0.045% (45 cases per 100,000 horses). Higher morbidity rates can be seen during outbreaks [5,6].

2.2 Clinical manifestation of JEV

The incubation period of horses infected with JEV is 8-10 days and may develop clinical signs after 4-14 days. In horses, most infections are subclinical. Some horses have a mild illness with transient fever, anorexia, lethargy and congested or jaundiced mucous membranes which usually lasts for 2-3 days and recover without complications. Few horses develop encephalitis with neurological signs that commonly include difficulty swallowing, incoordination, transient neck rigidity, impaired locomotion, impaired vision, paresis, and paralysis. These horses often recover within a week. However, a more severe form is characterized by high fever, aimless wandering, violent and demented behavior. These symptoms are often followed by collapse and death in 1-2 days [2,7,8].

2.3 Laboratory diagnosis of JEV

Laboratory diagnosis can be made by virus isolation in both laboratory animals and cell culture. Inoculated mice are kept under clinical observation for 14 days; clinical signs observed and brains of dead mice are collected for further passage. JEV can be also isolated in primary cultures of chicken embryo, porcine or hamster kidney cells, African green monkey kidney (Vero) cells, the MDCK cell line or mosquito cell lines. Serological diagnosis is primarily used such as hemagglutination inhibition (HI) test, the IgM capture enzyme-linked immunosorbent assay (ELISA), virus neutralization and immunofluorescence[4]. The detection of specific IgM and IgG in CSF is also good evidence of infection in horses. However, cross-reactivity with related flaviviruses, including WNV, has been observed in serology assays. Molecular diagnostic techniques are preferred, RT-PCR have been used to develop sensitive and specific assays for identification of flaviviruses. For example, the detection of viral RNA in 90 serums of asymptomatic horses has been identified using specific primer pairs for flaviviruses. The results gave negative from all horses compared among single band for yellow fever virus and doublet bands of amplified DNA for all 8 mosquito-borne flaviviruses including JEV, and dengue serotype 1 to 4 using as positive control (Data not shown). Real-time PCR such as TaqMan RT-PCR using dual-labeled fluorogenic probes or SYBR Green I-based one-step real-time RT-PCR were used to detect and quantify JEV in clinical samples. The assay gave good sensitivity and specificity and no cross-reactions were detected with non-JE reference viruses [1,9,10,11]. In addition, multiplex real-time RT-PCR for detecting 8 medically important flaviviruses including JEV, WNV, Dengue virus serotype 1 to 4, Yellow fever, and St. Louis encephalitis virus were also developed in mosquitoes as an early warning system of widespread flaviviruses [12,13].

2.4 Prevention and control of JEV

JEV vaccines are available in two types, modified live and inactivated, and can prevent disease in horses and pigs. Stabling horses in screened barns can be partially protective, particularly during outbreaks. Peak mosquito biting activity is usually from dusk to dawn. Barn fans are helpful, as mosquitoes do not fly ell in strong winds. The walls may also be sprayed with insecticides. Insect repellents can help protect individual animals. In some climates, horses may be rugged and hooded in lightweight permethrin-treated material [2].

3. West Nile Virus (WNV) in Equines

WNV is transmitted by a number of mosquito species of which *C. tarsalis*, *C. pipiens*, *C. restuans,* and *C.quinque-fasciatus*. Research has shown that *C. tarsalis* is the most efficient of the *Culex* species in the spread of WNV [2]. WNV is naturally maintained in a cycle between bird feeding mosquitoes and wild animals, mainly birds. Thus, infected birds serve as the reservoir host of the WNV which is similar to JEV. These birds develop viremia which a large number of virus particles in their bloodstream. Mosquitoes become infected after taking a blood meal from an infected bird. They in turn infect other animals such as humans, horses, cats, bats, and skunks by biting them [2,4,14,15].

3.1 Geographic distribution of WNV

West Nile virus (WNV) was first isolated in Uganda in 1937 and began to appear in the United States after 1999. The WNV outbreak in the United States (1999-2010) highlighted that importation and establishment of vector-borne pathogens outside their current habitat represent a serious danger of the world. Outbreak sites are on major birds migratory routes. In 2002, more than 14,000 cases of WNV were reported in horses in the United States. WNV quickly became a health concern in several areas including Canada, Africa, West and Middle East Asia, South Europe and Oceania. According to a statement from the British Equine Veterinary Association (BEVA), WNV often identified in African, East European and West Asian horses but never been found in the U. K[8,14,16].

3.2 Clinical manifestation of WNV

The incubation period between exposure to the WNV and appearance of the first signs of disease is estimated to be 3-15 days. Clinical signs of WNV in horses may never show or may come slowly over time, and as with many other illness symptoms of WNV may vary in severity. Typical signs are weak limbs, stumbling, general clumsiness, fatigue and listlessness. Other common symptoms are loss of appetite, difficulty swallowing, walking in circles, and hyperexictability. More serious signs generally involve facial paralysis or paralysis of the hind limbs, inability to get up, fever that may cause blindness, trembling, seizures and coma. Horses with severe clinical signs can die or are euthanized due to secondary complications. It is important not to presume that horses with clinical signs of encephalitis have WNV infection. A definitive diagnosis requires ruling out other diseases with similar neurological signs. Rabies, botulism, JEV, equine protozoal myeloencephalitis (EPM), Eastern (EEE), Western (WEE), and Venezuelan (VEE) equine encephalitis are examples of other diseases with neurological signs that may be confused with WN encephalitis[2,5,14,16].

3.3 Laboratory diagnosis of WNV

Laboratory diagnosis of WNV is predominantly serological, although caution is advised because of the high degree of cross-reactivity among flaviviruses especially JEV which produces similar symptoms. Therefore, it is critical to distinguish between JEV and WNV in areas where both viruses are endemic [6,15,16]. Nested RT-PCR and multiplex RT-PCR have often been used to differentiate WNV and JEV from other flaviviruses using specific primer pairs in tissue, blood (monocytes and lymphocytes), or cerebrospinal fluid (CSF). These techniques are equally sensitive and specific when compared with quantitative (TaqMan) RT-PCR amplification assay[17,18]. Some researches[17,19] recommended using nested RT-PCR during the viremic phase of infection before clinical symptoms develop and WNV antibody could be detected. However, viruses in cell culture or live-attenuated vaccine could be used as a positive control when all horses were tested negative [19,20,21,22,23]. Genetic diagnostic method such as RT-PCR restriction fragment length polymorphism (RFLP) has also been studied to discriminate WNV and JEV by detecting point mutations in genes [9,12,24,25]. Meanwhile, WNV antibody may persist in the serum for many months after infection. Serological methods, plaque reduction neutralization test (PRNT) and IgM-capture enzyme-linked immunosorbent assay (ELISA), can be used to detect antibody titers in CSF or serum sample [26,27,28]. Assays for neutralizing antibodies are more specific than ELISA but can be performed only in specialized laboratories that can grow dangerous viruses [29,30].

3.4 Prevention and control of WNV

No WNV vaccine is available for human use although the disease is now worldwide. Two WNV vaccines are licensed and commercially available to vaccinate horses by the United States.

One of these vaccines is prepared from an inactivated whole-cell preparation of the virus while the other is produced from a chimeric DNA preparation with gene encoding protein prM and protein E spliced into a Canarypox vector [2,31,32]. When the horse receives the first time vaccine, a series of two doses should be given about 3-6 weeks apart. It appears that horses will not develop high, presumably protective, antibody levels until they receive the booster or second vaccination in the series. Optimal protection occurs 2 weeks after the second dose. Thus, this second dose should be given at least 4 weeks before mosquitoes bite and infect horses. These vaccines require an annual booster which depending on the time of year the horse is vaccinated and the regional presence of the disease[29,33,34]. In addition to the vaccine for horses, simple insect control measures should be utilized such as insect repellents, place horses in barns/stables under fans during dusk, dawn, and other times when mosquitoes are present. Clearing and eliminating containers with small pools of water which might lead to mosquito breeding[30,35,36].

CONCLUSION

JEV and WNV belong to the genus *Flavivirus* of the family *Flaviviridae*. They cause similar disease syndromes in humans and horses ranging from asymptomatic to clinical encephalitis. They are mainly transmitted by mosquitoes and ticks and distributed around the world. They are considered to be emerging infectious diseases in that they have recently shown an increase in incidence, impact or geographical range. Total eradication of flaviviral encephalitis is impossible because of natural reservoirs. Prevention and protection using vaccines should be ensured to deliver among susceptible population.

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