18

Genetic Evolution of Japanese Encephalitis Virus

Ching-Kai Chuang and Wei-June Chen
Department of Public Health and Parasitology, Chang Gung University
Taiwan

1. Introduction

Japanese encephalitis (JE) is widely distributed in most areas of Asia, particularly in eastern, southern, and southeastern Asia (Rosen 1986). It expanded to the Torres Strait of northern Australia in 1999, and has now become widespread in Australia (Mackenzie, 1999; Mackenzie et al., 2004). Mass vaccinations were implemented in a number of Asian countries such as Japan, Korea, China, Taiwan, Thailand, and India, leading to declines in the incidences of JE (Wu et al., 1999; Monath, 2002; Erlanger et al., 2009). Generally, swine serve as an amplifying host in the transmission cycle of the JE virus (JEV) (fig. 1), giving it an important role in the maintenance of the JEV in nature (Nidaira et al., 2009). Due to changes in the socioeconomic status, households that breed swine have sharply decreased in Japan and many other countries (Yoshida et al., 2005). Despite, an estimated 3 billion persons living in countries with endemic JE, it causes about 30,000~50,000 cases per year (Solomom, 2004; van den Hurk et al., 2008); among these, there are estimated to be 10,000~15,000 human deaths yearly worldwide (Erlanger et al., 2009). The potential for the emergence of frontiers of JEV is now a new concern (Nett et al., 2009; van den Hurk et al., 2009).

The etiological agent of JE is one of about 70 members of the genus *Flavivirus* that belongs to the family Flaviviridae (Monath & Heinz, 1996). JEV frequently causes encephalitic diseases through bites by rice-field breeding *Culex* mosquitoes (van den Hurk et al., 2009; Unni et al., 2011), especially *Culex tritaeneorhynchus* which is a species that breeds in paddy fields (Lindenbach & Rice, 2001). This suggests that most species of JE vectors are rural mosquitoes. In association with seasonal fluctuations in mosquito population densities, JE cases mostly appear during the summer, particularly May to October in most endemic areas (Rosen, 1986). In spite of this, other mosquitoes classified in different genera including *Armigeres*, *Aedes*, *Anopheles*, and *Mansonia* have also been documented to be a potential vector (Chen et al., 2000; Deng et al., 2009; Weng et al., 1999).

The JE virion is about ca. 50 nm in diameter (Westaway et al., 1985); its genome contains linear, single-stranded positive-sense RNA (~11 kb in length) (Chambers et al., 1990). The genomic RNA of the JEV comprises a 5′ untranslated region (UTR), a longer 3′ UTR, and an intervening single open reading frame (ORF) (Chambers et al., 1990) that encodes 3 structural proteins in the order of the capsid (C), membrane (prM/M), and envelope (E), followed by 7 non-structural proteins (NS1~NS5) (Chambers et al., 1990; Sumiyoshi et al., 1987). The functions of some viral proteins are now clearer. Among these, protein E is the

major component of the viral envelope which binds to host cell surface receptors (Ren et al., 2007) and is also involved in membrane fusion between the virion and host late endosomes (Stiasny & Heinz, 2006). NS3 is an enzyme with both serine protease and NTPase/helicase activities (Assenberg et al., 2009), while NS5 was confirmed to be a RNA-dependent RNA polymerase as well as a methyltransferase (Lescar & Canard, 2009; Zhou et al., 2007).

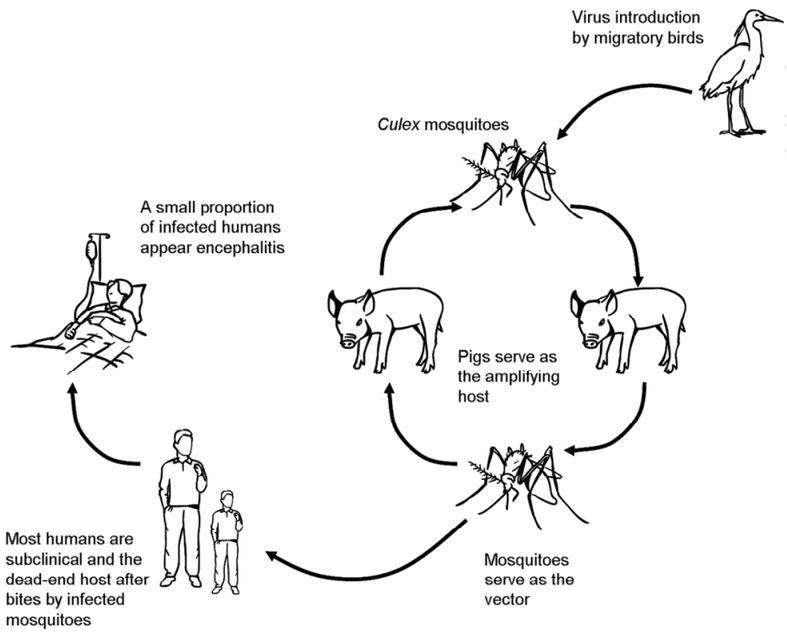


Fig. 1. Transmission cycle of Japanese encephalitis virus in nature. A proportion of infected humans exhibit encephalitis and are a dead-end host after being bitten by infected mosquitoes

In general, the JEV replicates inefficiently and thus causes delayed low-titer viremia in hosts (Shope & Sather, 1979). The central nervous system (CNS) is the principal target of infection by the JEV (Ravi et al., 1994). However, the virus is usually cleared from the CNS by an immune response before the development of encephalitis (McMinn et al., 1996). This thus leads to a relatively high number of subclinical infections in humans or those who rapidly recover from clinical symptoms, defined as a dead-end host (Mathur et al., 1987, Ravi et al., 1994). It is estimated that about 1 in 25~1000 humans with infections, depending on the endemicity, exposure to mosquitoes, preexisting antibodies to flaviviruses, and viral strain differences (Mackenzie et al., 2004; Solomon and Vaughn, 2002), will exhibit clinical symptoms (Konishi & Suzuki, 2002). Despite this, the case fatality rate of JE can be as high as 25%~30% (Burke & Leake, 1988); 15%~50% of the survivors may end up with sequelae of neurologic impairment and/or mental retardation (Monath, 2002).

2. The route through which JEV enters the CNS

The JEV tends to infect the CNS in dead-end hosts, *i.e.*, humans (Johnson et al., 1985; Johnson, 1987). However, it is not clear how the virus gains entry into the CNS from the peripheral circulation (Myint et al., 2007). Theoretically, after the bite of an infected mosquito, the JEV is amplified in dermal tissues and then lymph nodes via migration of

dendritic (Langerhans) cells before invading the CNS (Johnson et al., 2000; Solomon et al., 2000). A variety of flaviviruses were speculated to enter the CNS via the olfactory pathway (Monath et al., 1996), across cerebral capillary endothelial cells (Liou and Hsu, 1998), or virion budding on the parenchymal side after replication in endothelial cells (McMinn et al., 1996). Nevertheless, features including the diffuse infection throughout the brain and no viral antigens in the choroid plexus or ependyma indicate that viruses probably enter the CNS via a hematogenous route (Kimura-Kuroda et al., 1992), especially with a status of severe viremia (Yamada et al., 2004).

In both animals and humans, the blood-brain barrier (BBB) generally prevents viral invasion of the CNS (Ballabh et al., 2004), unless it has been disrupted, resulting in increased permeability and inflammatory cell infiltration (Muller et al., 2005). The BBB integrity was observed to evidently be weakened in the primary phase of JEV infection, showing modified distribution of occludin, a tight-junction (TJ) component protein of the BBB (Liu et al., 2008). Increased permeability of the BBB actually allows peripheral blood monocytes (PBMCs) to migrate from the peripheral blood into brain tissues (Stephens *et al*., 2003; Diamond & Klein, 2004). Normally, lymphocytes constantly enter the CNS in small numbers (Hickey et al., 1991), but their presence in the CNS may increase in response to viral infections (Griffin et al., 1992).

PBMCs isolated from inoculated mice were detected to be infected by the JEV (Chuang et al., 2003; Liu et al., 2009). Leukocytes move across endothelial cells of capillaries at sites where TJs appear to be dissociated (Liu et al., 2008), suggesting that the JEV enters brain tissues via penetration of infected PBMCs. Uniquely, this pathway of penetration may begin at the cerebrum, not in the cerebellum (Liu et al., 2008). Destruction of the BBB can be caused by apoptosis of astrocytes and/or neurons (Suri & Banerjee, 1995; Tseng et al., 2011). Viral replication in peripheral tissues may also trigger a Toll-like receptor inflammatory response that alters the BBB (Wang et al., 2004). The stimulus capable of causing changes in BBB permeability can also be derived from the effects of the chemokine, monocyte chemoattractant protein (MCP)-1 (Stamatovic et al., 2005; Yamada et al., 2004).

3. Pathogenesis of the JEV

The JEV infects a variety of brain tissues with a characteristic pattern of mixed intensity of hypodense lesions in the thalamus, basal ganglia, and midbrain (Kalita & Misra, 2000, 2002). Most JE patients present typical clinical symptoms including headaches, vomiting, an altered mental state, as well as dystonia, rigidity, and a characteristic mask-like facies (Chuang et al., 2002). Clinically, movement disorders are frequently seen in patients who survive the acute phase of JE (Misra & Kalita, 1997), implying that sensorimotor neuropathy eventually occurs. It is now known that encphealitis associated with flaviviral infections may cause Guillain-Barré-like syndrome, showing a demyelinating feature in sensorimotor tissues of the brain (Sejvar et al., 2005). This suggests that demyelination is an important step causing disruption of motor coordination during JEV infection.

The JEV has repeatedly been demonstrated to infect neurons in brains of experimental animals and humans (Johnson et al., 1985; Yamada et al., 2004) and is usually not cleared from tissues by elicited antibodies (Desai et al., 1995). Generally, encephalitic flaviviruses induce apoptosis in infected neurons and subsequently cause fatal outcomes to the host (Liu et al., 2008; Nargi-Aizenman & Griffin, 2001; Samuel et al., 2007; Xia et al, 2001). This possibly occurs by way of reactive oxygen specie-mediated induction of matrix

metalloproteinase-9 expression and subsequent activation of nuclear factor-κB (Tung et al., 2010). Neuronal death, which is widely seen in JEV-infected mouse brains, is probably important for induction of axonal injury and demyelination (Meyer et al., 2001; Tsunoda et al., 2003). Demyelination is a common feature in brains infected with encephalitic viruses (Stohlman & Hinton, 2001); this is the process by which axons lose myelin that normally serves as an insulator, resulting in loss of balance and coordination, although it may vary among patients (Sarma, 2010). In fact, demyelination is rather common in mouse brains infected with the JEV; this is supposed to be triggered by T cell-mediated autoimmunity against the myelin basic protein (MBP), a component of myelin (Grigoriadis & Hadjigeorgiou, 2006; Tseng et al., 2011). In other words, the JEV, which normally causes inflammation and neuronal degeneration in the CNS, eventually induces the proliferation of specific T cells which mediate autoimmunity to destroy components of axon-surrounding myelin such as MBP (Tseng et al., 2011).

4. Origin and genetic diversity of the JEV

The first JE epidemic in history was reported in Japan in 1871 (van den Hurk et al., 2009); however, the causal virus was not isolated until 1935 (Rosen, 1986). There are 2 epidemiological patterns of JE: one restrictedly occurs in tropical zones including South India, Indonesia, Malaysia, Singapore, and southern Thailand with no seasonal preference, and the other mainly occurs in northern parts of tropical zones, such as China, Taiwan, Japan, Korea, Nepal, and northern parts of Myanmar, India, and Viet Nam (Umenai et al., 1985). The JEV in temperate areas is supposedly re-introduced by migratory birds or bats and/or is wind-borne (Rosen, 1986; Solomon et al., 2003).

Phylogenic studies revealed that the JEV possesses genetic diversity among strains, through which the origin, evolution, and spread of the virus were theoretically determined (Gould, 2002; Gould et al., 2004). It seems that the JEV originated from an ancestral virus in the Indonesia-Malaysia region, and subsequently evolved there into the different genotypes before they spread across Asia (Solomon et al., 2003). It was estimated that the ancestor common to all genotypes of JEV arose within the last 300 years (Uchil & Satchidanandam, 2001).

Major alterations in the genome of resulting viral variants frequently occur in the envelope (E) protein (Deubel et al., 1993; Trent et al., 1983, 1987). Based on the nucleotide sequences of the C/PrM and E genes, 5 genotypes (I~V) of the JEV were identified (fig. 2); these probably originated from the ancestral virus and evolved into the different genotypes (Solomon et al., 2003). Geographically, genotype I includes isolates from Korea, India, Cambodia, Laos, and northern Thailand (Fan et al., 2010; Fulmali et al., 2011; Wang et al., 2007, 2010;); genotype II includes isolates from Malaysia, Sarawak, Indonesia, southern Thailand, and northern Australia (Pyke et al., 2001); genotype III includes isolates widely distributed in Asian countries with temperate climates, including Japan, Taiwan, China, India, Sri Lanka, Nepal, Viet Nam, and the Philippines (Chen et al., 1990; Mackenzie et al., 2004; Wang et al., 2007); and genotype IV includes isolates from Indonesia (Chen et al., 1992). The last one identified was genotype V, which includes isolates with a restricted distribution in India (Solomon et al., 2003; Uchil & Satchidanandam, 2001). Based on the composition of nucleotides and amino acids, strains belonging to genotype II are the most similar to genotype I (9.1%~10.7% difference), followed by genotype III (10.2%~11.7% difference), and genotype IV (16.2%~16.6% difference) (Schuh et al., 2011). The phylogenetic tree presented here shows

that genotype V is the most distant from genotype I. This newly identified genotype was suggested to have originated from Malaysia (Solomon et al., 2003). Nevertheless, it must be noted that the genetic similarity does not necessarily match their epidemiological distributions (Schuh et al., 2011).

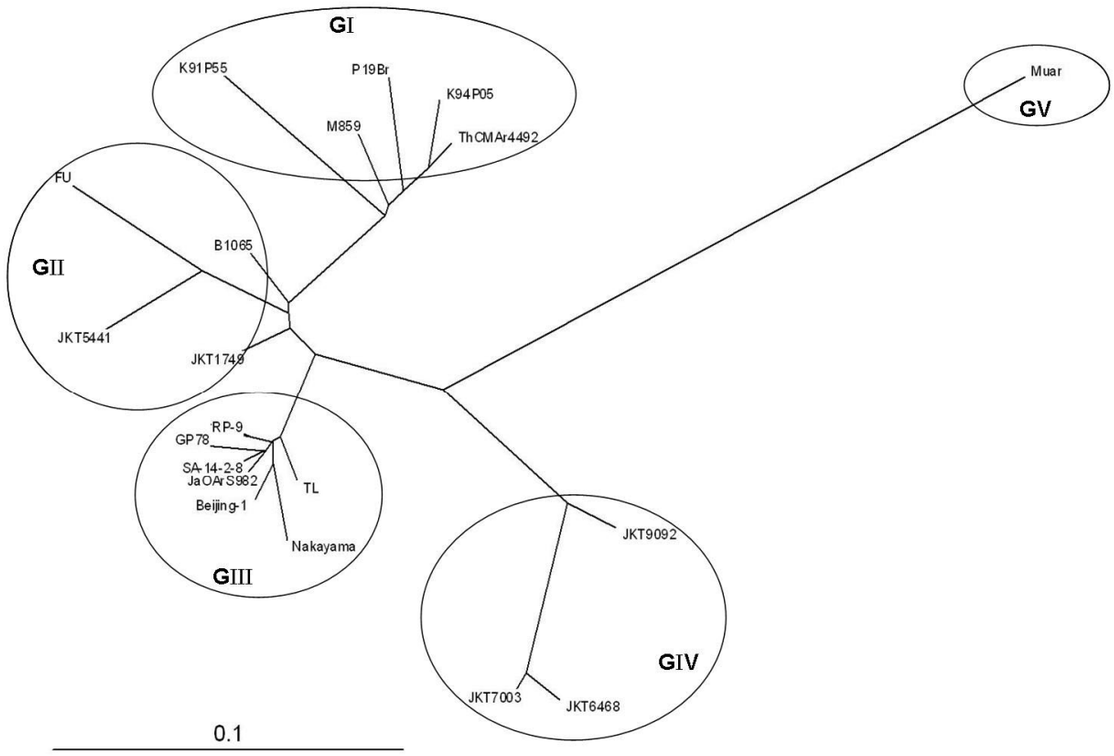


Fig. 2. Unrooted phylogenetic analysis of 17 homologs of protein E of the Japanese encephalitis virus showing that this protein can be divided into 5 clades or genotypes. Genotype I: K91P55 (U34928), M859 (U70410), P19Br (U70416), K94P05 (AF045551), and ThCMAr4492 (D45362); genotype II: B1065 (U70388), FU (AF217620), JKT5441 (U70406), and JKT1749 (U70405); genotype III: RP-9 (AF014161), GP78 (AF075723), SA-14-2-8 (U15763), JaOArS982 (M18370), Beijing-1 (L48961), Nakayama (EF571853), and TL (AF098737); genotype IV: JKT7003 (U70408), JKT6468 (AY184212), and JKT9092 (U70409); and genotype V: Muar (HM596272). The analysis used the Neighbor-joining method within the PHYLIP 3.6 software package with 500 bootstraps (Felsenstein, 2005) after sequence alignment with the Clustal W package (Thompson et al., 1994)

In fact, intra-genotypic groupings can occur (Ali & Igarashi, 1997; Wang et al., 2007), among which genotypes IV and V are the most divergent genotypes and are thought to represent the oldest lineages (Misra & Kalita, 2010). Genotype III can further be divided into 3 clusters among strains isolated in Taiwan (Huang et al., 2010). It is interesting that genotype III was the major one in Japan before 1991, while most isolates after 1995 were identified as belonging to genotype I (Ma et al., 2003; Morita, 2009). A similar phenomenon or even genotype replacement was also seen in many other countries in Asia (Huang et al., 2010; Morita, 2009; Nitatpathana et al., 2008). This suggests that the flow or a shift of genotypes

between endemic regions is possible (fig. 3). Eventually, genotype I, which is genetically close to those from Malaysia, was found to have been introduced and co-circulated with and/or ultimately replaced the existing genotype III in many Asian countries (Fulmali et al., 2011; Huang et al., 2010; Ma et al., 2003; Nga et al., 2004; Pyke et al., 2001; Wang et al., 2007; Yoshida et al., 2005; Yun et al., 2010). Putatively, the introduced genotype I originated from Southeast Asia (Nabeshima et al., 2009; Nga et al., 2004); which has been an important region for emerging pathogens (Solomon et al., 2003). Wind-blown infected mosquitoes flying with air currents during the typhoon season supposedly played a role in the geographic expansion (Ritchie & Rochester, 2001).

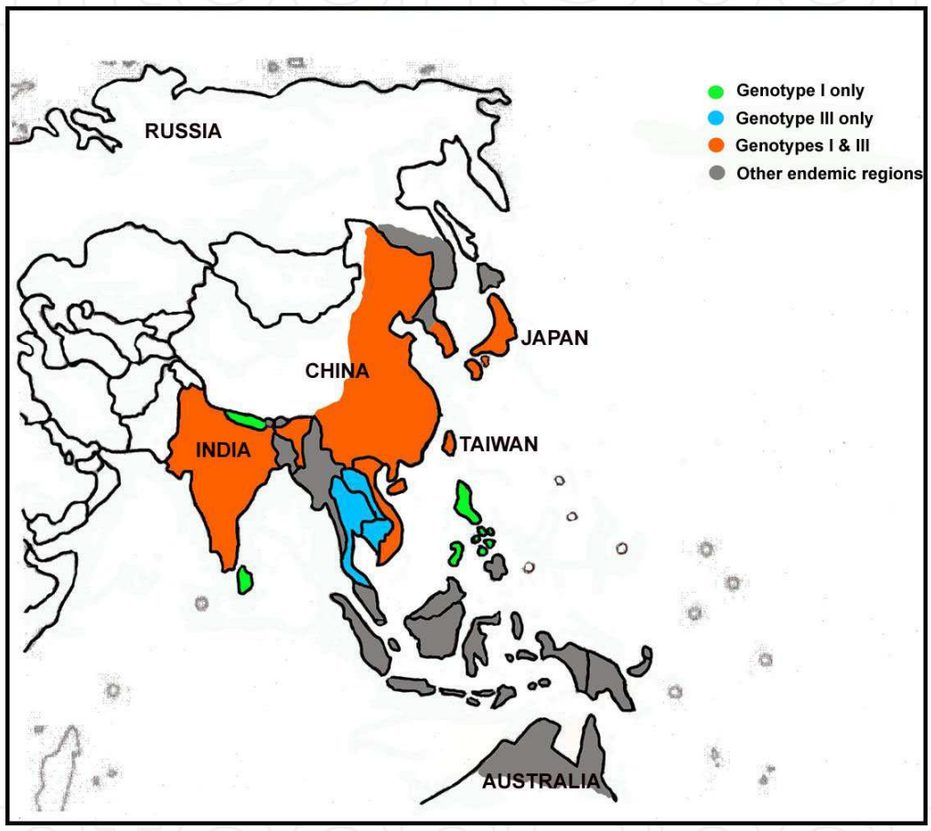


Fig. 3. Japanese encephalitis virus (JEV) is widely distributed in most Asian countries (gray). Among the 5 genotypes of JEV, genotype III (blue) has been predominant in most northeast Asian countries such as Taiwan, China, Japan, Korea, Viet Nam, Thailand, and even India. Genotype I (green) was putatively introduced by wind-blown infected mosquitoes, for example, during the typhoon season. Nowadays, the mixed occurrence of both genotypes (orange) has become common in such regions

5. Mutations and evolution of the JEV in nature

RNA genomes of flaviviruses evolve rather rapidly compared to DNA-based organisms. Generally, RNA viruses evolve rapidly, with approximately 6 orders of magnitude higher rates of nucleotide substitutions compared to DNA viruses (Jenkins et al., 2002). It seems that horizontal gene transfer of the virus between host cells increases its genetic diversity.

Therefore, it is important to see how genetic diversity occurs, either by investigating the molecular epidemiology or evolution of flaviviruses. Unlike re-assortments of RNA segments that frequently occur in the influenza virus (Khiabanian et al., 2009), flaviviruses readily change nucleotides in their genomes due to a lack of proof-reading and mismatch repair abilities (Holland, 1996). This error-prone nature of RNA synthesis triggers mutations which are an important source of RNA virus diversity. They, in turn, form a variety of genetically related variants known as "quasispecies" and are responsible for the pathogenesis and tissue distribution of viruses (Holmes, 2004).

Natural mutations with lower virulence were isolated from field-caught mosquitoes (Chiou & Chen, 2001), leading to circulation in a human population with a low incidence of the disease (Chen et al., 2000). Among most viruses, mutations serve as the major source of genetic change; which in turn creates strains that may be either lethal or advantageous. The E protein is known to be the major component on the surface of flaviviral virions (Adams et al., 1995; Rey et al., 1995), and generally plays a role in interactions with molecule(s) or receptor(s) on cell membranes when the virus infects host cells (Martinez-Barragan & del Angel, 2001; Ramos-Castaneda et al., 1997; Rothwell et al., 1996). Passage of flaviviruses in cultured cells was demonstrated to induce genetic changes in the E protein (Marchette et al., 1990), causing phenotypic alterations, *e.g.*, viral virulence (Cao et al., 1995; Hasegawa et al., 1992).

As mentioned, phenotypic changes may be caused by mutations in the E protein (fig. 4), which include antigenicity, pathogenicity, virulence, persistence, or interactions with host cells (Cecilia & Gould, 1991; Chiou et al., 2007; Chung et al., 1996; Hasegawa et al., 1994; Lee & Lobigs, 2002; Mangada & Takegami, 1999; Ni & Barrett, 1996; Nitayaphan et al., 1990; Wu et al., 1998). It was reported that a single mutation at E-123 (Ser→Arg) in the JEV significantly increases the virulence in mice, indicating that this nucleotide may be responsible for the pathogenicity of the JEV (Tajima et al., 2010). In Neuro-2a cells, a higher-virulent variant (CJN-S1) of the JEV with a mutation at E-138 (Glu→Lys) was isolated from human brain tissues in a relatively short time of passage. The same mutation was also reported to evidently affect multiple steps of the life cycle of the JEV (Zhao et al., 2005), including reduced neurotropism and attenuated virulence (Chen et al., 1996; Ni & Barrett, 1998). The specific step of such an effect is its ability to attach and penetrate (Liu et al., 2004), leading to lower virulence in host cells (Chiou & Chen, 2007). In the meantime, mutations at E-306 (Glu→Lys) (T1P1-S1) and E-389 (Asp→Asn) (CC27-S6) were also observed to possess a higher affinity for heparin sulfate and to form small plaques (Chiou et al., 2005). Apparently, the E protein is not the only site of the viral genome related to phenotype determination of the JEV. A single N-linked glycosylation site in the prM protein of the JEV was documented to be critical for the pathogenicity of the virus in mice (Kim et al., 2008).

Factors affecting the genetic diversity of the JEV may be complex and remain to be worked out. In a study of E/NS1 protein of another flavivirus (dengue), the genetic composition changed with passages of cultured cells (Chen et al., 2003). In fact, 1 (E-53) and 2 amino acids (NS1-178 and NS1-181) had mutated after 30 passages in Vero cells, while no changes were observed in viruses serially passaged in C6/36 cells (Chen et al., 2003). This reflects a feature by which mutations occur more slowly in arboviruses, either alphaviruses or flaviviruses, than in other RNA viruses (Weaver et al., 1992). Perhaps, antioxidant defenses that protect mosquito cells from apoptosis in response to the virus may play an important role in reducing the occurrence of viral mutations (Chen et al., 2011).

Heparan sulfate, a glycosaminoglycan (GAG), on the surface of all adherent cells is known to modulate the actions of a number of extracellular ligands (Bernfield et al., 1999). Clusters

consisting of E protein residues 49, 138, 306, or 389/390 on the surface of JE virions may define molecular determinants for GAG binding and concomitant virulence attenuation in human adenocarcinoma (SW13) cells (Lee et al., 2004). Additionally, the JEV strain with the mutation of Glu-306-Lys in the E protein was found to increase its binding ability with highly sulfated forms of GAGs in Neuro-2a cells (Chiou et al., 2005). This implies that GAGs, particularly highly sulfated forms (Su et al., 2001), in host cells can be important as a tool to screen viral strains (Chiou et al., 2005).

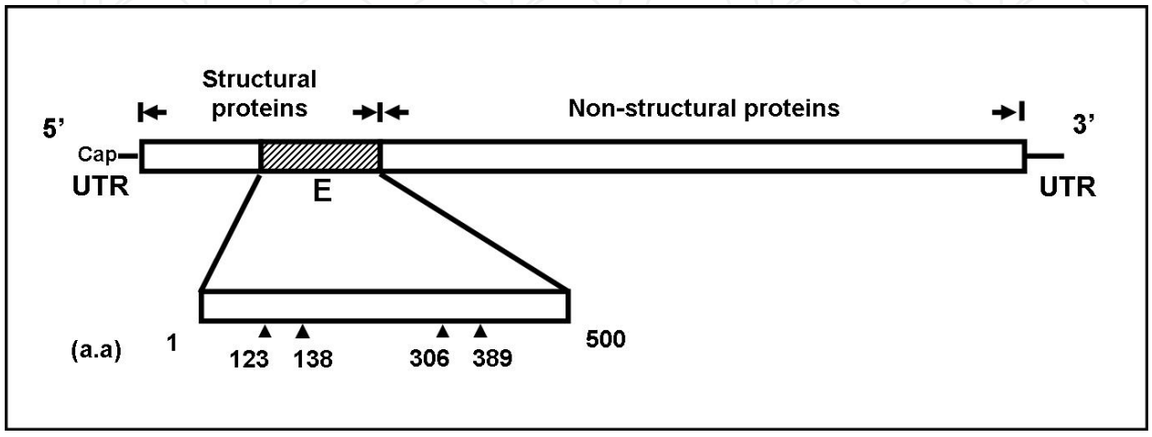


Fig. 4. Phenotypic changes through mutations in the E protein may determine viral characteristics, such as the antigenicity, pathogenicity, virulence, persistence, and interactions with host cells. Several amino acids, including E123, E138, E306, and E389, were demonstrated to be important in the ability of the Japanese encephalitis virus to interact with a host

Viral evolution generally occurs as a result of genetic variations and selection of variants from large viral populations, producing a new genetic makeup which can pass from generation to the next. Due to the differential fates of flaviviruses in vertebrate and mosquito cells (Chen et al., 2003), patterns of genetic evolution may differ between viruses replicating in these 2 types of host cells. Experimental evidence showed that slower mutations of arboviruses, either alphaviruses or flaviviruses, may occur in mosquito cells by applying the brake on the evolution of these viruses (Vasilakis et al., 2009). The evolution of viruses depends on the mutation-selection balance. It was hypothesized that the JEV changes its genetic composition under certain circumstances in vertebrate cells. In contrast, either positive or negative selection can occur in mosquito cells, selecting fitter variants.

6. RNA recombination in the JEV

As mentioned, RNA viruses evolve rapidly compared to DNA viruses, with approximately 6 orders of magnitude higher rates of nucleotide substitutions (Jenkins et al., 2002). In addition, genetic exchanges via re-assortment of RNA segments are known to occur in segmented RNA-containing viruses such as the influenza virus (Khiabanian et al., 2009), rotavirus (Gentsch et al., 2005), bluetongue virus (Carpi et al., 2010), and so forth. RNA recombinations are now believed to be one mechanism exploited by many viruses, which serve as a strategy for evolution (Worobey & Holmes, 1999). This was found in the poliovirus (Ledinko, 1963), flaviviruses (Cristina & Colina, 2006; Twiddy & Holmes, 2003),

and others (Rohayem et al., 2005). Most notably, western equine encephalitis virus, a member of the genus *Alphavirus*, is thought to have originated from a recombination between an eastern equine encephalitis-like virus and a Sindbis-like virus, giving rise to a completely new virus (Hahn et al., 1988). Similarly, the Singapore S275/90 strain of dengue 1 virus very likely arose from a recombination between a Djibouti/Cambodia lineage ancestor and an Abidjan lineage ancestor (Tolou et al., 2001).

Genetic diversity derived from spontaneous mutations and the introduction of new strains through migratory birds increase the possibility of RNA recombination in the JEV (Rosen, 1986). Accumulating data from phylogenetic analyses indicate that the recombinant forms of the JEV may have appeared in Korea and Thailand (Twiddy & Holmes, 2003). It is now believed that 2 putative recombinants isolated from Korea (K82P01 and K91P55) appear to have parental strains originating in Japan and Korea, while one from Thailand (Thailand 82) has parental strains originating in Thailand and China (Twiddy & Holmes, 2003).

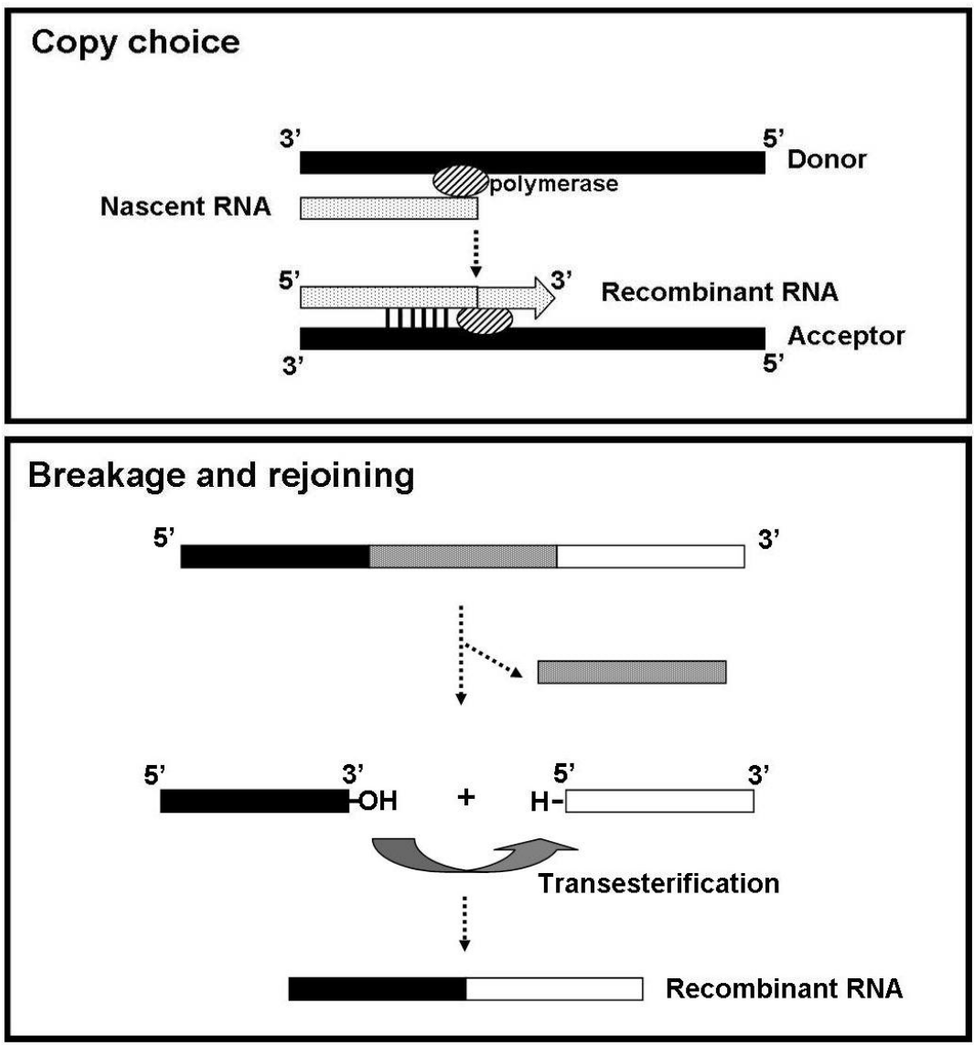


Fig. 5. Two possible mechanisms were proposed for the occurrence of RNA recombination: (i) the copy choice mechanism and (ii) breakage and rejoining (Lai, 1992)

In general, recombination follows 2 possible mechanisms (fig. 5): (1) a copy choice mechanism and (2) breakage and rejoining (Lai, 1992). The breaking and rejoining pathway is commonly seen with DNA but usually not with RNA recombinations (Jarvis & Kirkegaard, 1991, 1992). In contrast, a copy choice mechanism for RNA recombination was demonstrated to occur in the poliovirus; in which templates are switched by viral RNA-dependent RNA polymerase (RdRp) switches during negative-strand synthesis (Kirkegaard & Baltimore, 1986). This may also be the mechanism of RNA recombination in other RNA viruses such as coronaviruses and plant viruses (Makino et al., 1986; Sztuba-Solunsk et al., 2011).

There are 3 types of RNA recombinations (fig. 6), including precisely homologous, imprecisely (aberrantly) homologous, and non-homologous, which are known to occur in RNA viruses (Alejska et al., 2001). Of these, the precisely homologous recombination through a template-switching (copy-choice) mechanism is probably the most common type (Wierzchoslawski & Bujarski, 2006). We recently provided experimental evidence showing that genetically different JEV strains can simultaneously infect a single BHK-21 or C6/36 cell, resulting in the occurrence of genetic exchange or RNA recombination. It probably follows the copy choice mechanism with the precisely homologous recombination type (Chuang & Chen, 2009).

Secondary structures are probably essential for the occurrence of RNA (Hsue et al., 2000; Wierzchoslawski & Bujarski, 2006). It is particularly important that the structure of the acceptor RNA affects the occurrence of RNA recombinations in the case of HIV (Abdeladim et al., 2003). The stability of the secondary structure eventually promotes the frequency of template-switching RNA recombinations (Nagy & Jozef, 1997; White & Morris, 1995). In the JEV, changes in specific secondary structures eventually affect the frequency of RNA recombinations (Chuang & Chen, 2009).

Recently, AU-rich sequences were mentioned as being more efficient at promoting recombination, compared to GC-rich sequences, in single-stranded, positive-sense genomic RNA (Shapka & Nagy, 2004). However, AU-rich sequences do not really exist, and the AU content was < 50% in the region favoring crossovers in Japanese encephalitis according our recent study (Chuang & Chen, 2009). Perhaps the nucleotide composition of the sequence is not crucial in determining RNA recombinations of the JEV. Because neither deletions nor insertions of nucleotides were shown to occur in the crossover region, the precisely homologous recombination is believed to be the predominant, if not the only, type of RNA recombination between JEVs. This is consistent with the inference from previous observations that AU-rich sequences usually decrease the accuracy of crossovers, leading to imprecisely or aberrantly homologous recombinations of viral RNA (Nagy & Bujarski, 1996).

Rates of recombination in many single-stranded RNA viruses appear to be low (Chare et al., 2006; Worobey & Holmes, 1999), and RNA recombination is thought to have played some roles in the co-evolution of viruses with genetic conflicts (Turner & Chao, 1998) and the development of strains with increased virulence and transmission potential (Holmes et al., 1999). Moreover, RNA recombination was found more frequently in BHK-21 than C6/36 cells, indicating that mammalian cells are more crucial in determining RNA recombinations (Schneider & Roossinch, 2001). This causes higher rates of genetic variants in mammalian cells, and only those with fitness are selected, which possibly also operates in mosquito cells.

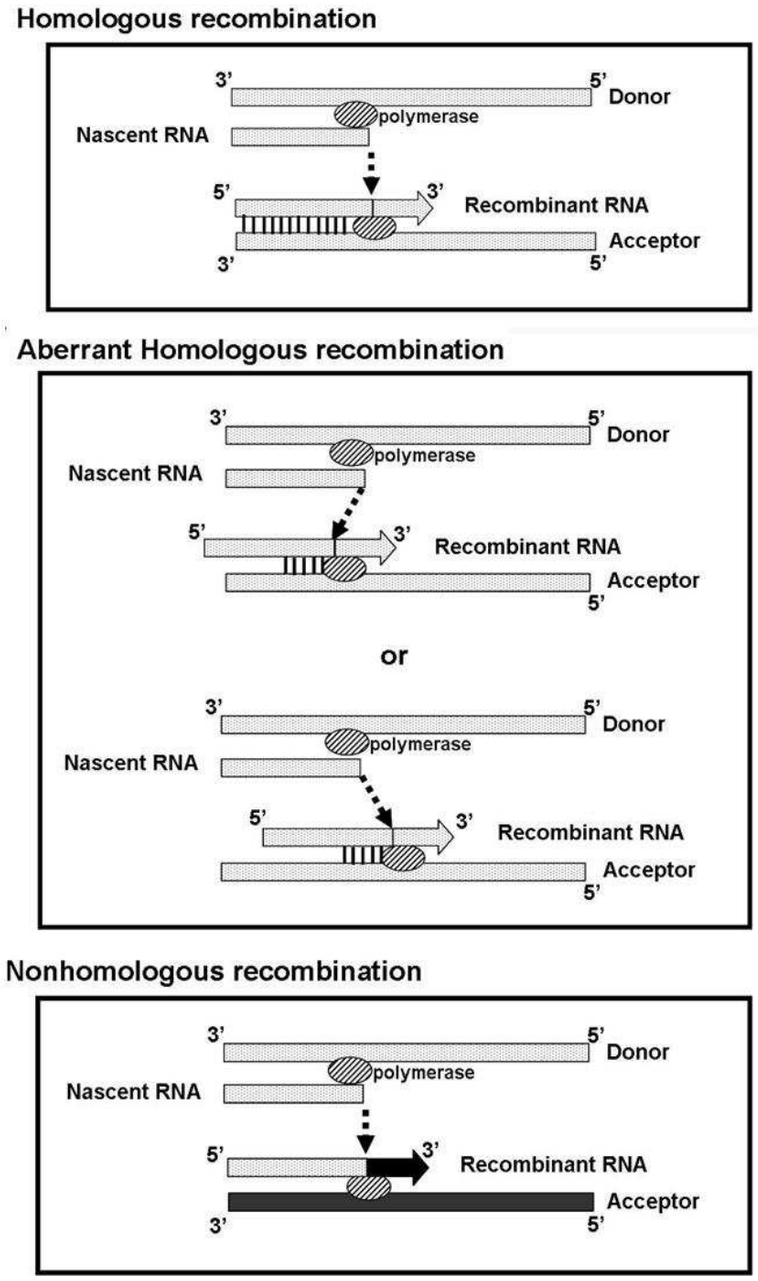


Fig. 6. Putatively, there are 3 types of RNA recombinations: (i) homologous (precisely), (ii) aberrantly (imprecise) homologous, and (iii) non-homologous (Alejska et al., 2001)

7. Conclusions and perspectives

Because of underlying mutation and genetic recombinations, the JEV probably evolves in a very similar way to that which governs other organisms. Nowadays, new mass-sequencing techniques are more popular and have revealed a greater diversity of flaviviruses than ever thought (Kuno et al., 1998). Due to the small size and probably the fragility, virus evolution

cannot possibly be investigated from so-called fossil viruses, if there are any. However, genetic diversity in nature provides information, facilitating an understanding of the existence of viral evolution. Maintenance of genetic diversity can theoretically reduce the rapid loss of fitness due to Muller's ratchet during passage of the virus from 1 host to another. Evolutionary, this is beneficial for a virus that has shifted to a new environmental niche or selective regimen, serving as a factor helping a virus escape from accumulated deleterious effects in a virus population.

Most flaviviruses comprise multiple genotypes or strains which potentially determine the virulence and epidemiology of the diseases. Mutation(s) of the viral genome followed by selection in host cells are the commonest way to form a new viral strain, the phenotypes of which may be more virulent or attenuated. Understanding changes in genomic RNA can help better elucidate evolving viruses as well as their clinical significance. Accumulating evidence suggests that RNA recombination is also one mechanism creating diversity of viruses, producing more unpredictable results during their evolution. A special concern is the possibility of genetic exchange between different populations of the virus (Tolou et al., 2001). As a result, it is particularly worthwhile noting whether or not live-attenuated vaccine strains experience crossing-over with wild viruses during the synthesis of new RNA (Becher et al., 2001; Holmes et al., 1999).

8. Acknowledgment

This work was financial supported by National Science Council of Taiwan (NSC99-2320-B-182-012-MY3) and partially by Chang Gung Memorial Hospital (CMRPD190161). The authors would like to thank Mr. Wei-Ting Chen for figure sketching.

9. References

Abdeladim, M.; Lucette, P.; Torsten, U.; Michel, V.; Henri, B. & Matteo, N. (2003). Evidence for a mechanism of recombination during reverse transcription dependent on the structure of the acceptor RNA. *Journal of Biological Chemistry,* 278, pp. 15973-15982.

Adams, S. C.; Broom, A. K.; Sammels, L. M.; Hartnett, A. C.; Howard, M. J.; Coelen, R. J.; Mackenzie, J. S. & Hall, R. A. (1995). Glycosylation and antigenic variation among Kunjin virus isolates. *Virology,* 206, pp. 49-56.

Alejska, M.; Kurzyñska-Kokorniak, A.; Broda, M.; Kierzek, R. & Figlerowicz, M. (2001) How RNA viruses exchange their genetic material. *Acta Biochimic. Polonica,* 48, pp. 391-407.

Ali, A. & Igarashi, A. (1997). Antigenic and genetic variations among Japanese encephalitis virus strains belonging to genotype 1. *Microbiology and Immunology,* 41, pp. 241-52.

Assenberg, R.; Mastrangelo, E.; Walter, T. S.; Verma, A.; Milani, M.; Owens, R. J.; Stuart, D. I.; Grimes, J. M. & Mancini, E. J. (2009). Crystal structure of a novel conformational state of the flavivirus NS3 protein: implications for polyprotein processing and viral replication. *Journal of Virology,* 83, pp. 12895-12906.

Ballabh, P.; Braun, A. & Nedergaard, M. (2004). The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiology of Disease,* 16, pp. 1-13.

Becher, P.; Orlich, M. & Thiel, H. J. (2001). RNA recombination between persisting pestivirus and a vaccine strain: generation of cytopathogenic virus and induction of lethal disease. *Journal of Virology,* 75, pp. 6256-6264.

Bernfield, M.; Götte, M.; Park, P. W.; Reizes, O.; Fitzgerald, M. L.; Lincecum, J. & Zako, M. (1999). Functions of cell surface heparan sulfate proteoglycans. *Annual Review of Biochemistry,* 68, pp. 729-777.

Burke, D.S. & Leake, C. J. (1988). Japanese encephalitis, In: *The Arboviruses: Epidemiology and Ecology,* T. P. Monath, (Ed.), 63-92, CRC, Boca Raton, Florida, USA.

Cao, J. X.; Ni, H.; Wills, M. R.; Campbell, G. A.; Sil, B. K.; Ryman, K. D.; Kitchen, I. & Barrett, A. D. (1995). Passage of Japanese encephalitis virus in HeLa cells results in attentuation of virulence in mice. *Journal of General Virology,* 76, pp. 2757-2764.

Carpi, G.; Holmes, E. C. & Kitchen, A. (2010). The evolutionary dynamics of bluetongue Virus. *Journal of Molecular Evolution,* 70, pp. 583-592.

Cecilia, D. & Gould, E. A. (1991). Nucleotide changes responsible for loss of neuroinvasiveness in Japanese encephalitis virus neutralization-resistant mutants. *Virology,* 181, pp. 70-77.

Chambers, T. J.; Hahn, C. S.; Galler, R. & Rice, C. M. (1990). Flavivirus genome organization, expression, and replication. *Annual Review of Microbiology,* 44, pp. 649-688.

Chare, E. R. & Holmes, E. C. (2006). A phylogenetic survey of recombination frequency in plant RNA viruses. *Archive of Virology,* 151, pp. 933-946.

Chen, L. K.; Lin, Y. L.; Liao, C. L.; Lin, C. G.; Huang, Y. L.; Yeh, C. T.; Lai, S. C.; Jan, J. T. & Chin, C. (1996). Generation and characterization of orang-tropism mutants of Japanese encephalitis *in vivo* and *in vitro*. *Virology,* 223, pp. 79-88.

Chen, T. H.; Tang, P.; Yang, C. F.; Kao, L. H.; Lo, Y. P.; Chuang, C. K.; Shih, Y. T. & Chen, W. J. (2011). Antioxidant defense is one of the mechanisms by which mosquito cells survive dengue 2 viral infection. *Virology,* 410, pp. 410-417.

Chen, W. J.; Wu, H. R. & Chiou, S. S. (2003). E/NS1 modifications of dengue 2 virus after serial passages in mammalian and/or mosquito cells. *Intervirology,* 46, pp. 289-295.

Chen, W. J.; Dong, C. F.; Chiou, L. Y. & Chuang, W. L. (2000). Potential role of *Armigeres subalbatus* (Diptera: Culicidae) in the transmission of Japanese encephalitis virus in the absence of rice culture on Liu-Chiu Islet, Taiwan. *Journal of Medical Entomology,* 37, pp. 108-113.

Chen, W. R.; Rico-Hesse, R. & Tesh, R. B. (1992). A new Genotype of Japanese Encephalitis Virus from Indonesia. *American Journal of Tropical Medicine and Hygiene,* 47, pp. 61-69.

Chen, W. R.; Tesh, R. B. & Rico-Hesse, R. (1990). Genetic variation of Japanese encephalitis virus in nature. *Journal of General Virology,* 71, pp. 2915-2922.

Chiou, S. S. & Chen, W. J. (2001). Mutations in the NS3 gene and 3'-NCR of Japanese encephalitis virus isolated from an unconventional ecosystem and implications for natural attenuation of the virus. *Virology,* 289, pp. 129-136.

Chiou, S. S. & Chen, W. J. (2007). Phenotypic changes in the Japanese encephalitis virus after one passage in Neuro-2a cells: Generation of attenuated strains of the virus. *Vaccine,* 26, pp. 15-23.

Chiou, S. S.; Liu, H.; Chuang, C. K.; Lin, C. C. & Chen, W. J. (2005). Fitness of Japanese encephalitis virus to Neuro-2a cells is determined by interactions of viral envelope protein with highly sulfated glycosaminoglycans on the cell surface. *Journal of Medical Virology,* 76, 2005, 583-592.

Chiou, S. S.; Tsai, K. H.; Huang, C. G.; Liao, Y. K. & Chen, W. J. (2007). High antibody prevalence in an unconventional ecosystem is related to circulation of a low-virulent strain of Japanese encephalitis virus. *Vaccine*, 25, pp. 1437-1443.

Chuang, C. K. & Chen, W. J. (2009). Experimental evidence that RNA recombination occurs in the Japanese encephalitis virus. *Virology,* 394, pp. 186-197.

Chuang, C. K.; Chiou, S. S.; Liang, L. C. & Chen, W. J. (2003). Detection of Japanese encephalitis virus inside peripheral blood mononuclear cells of mouse using *in situ* RT-PCR. *American Journal of Tropical Medicine and Hygiene,* 69, pp. 648-651.

Chuang, Y. M.; Kwan, S. Y.; Lirng, J. F.; Tiu, C. M. & Pan, P. J. (2002). Radiological and manometric diagnosis of cricopharyngeal dysphagia in a Japanese encephalitis survivor. *European Journal of Neurology,* 9, pp. 407−411.

Chung, Y. J.; Nam, J. H.; Ban, S. L. & Cho, H. W. (1996). Antigenic and genetic analysis of Japanese encephalitis viruses isolated from Korea. *American Journal of Tropical Medicine and Hygiene,* 55, pp. 91-97.

Cristina, J. & Colina, R. (2006). Evidence of structural genomic region recombination in hepatitis C virus. *Virology Journal,* 3, pp. 53.

Deng, S.; Zhang, H. & Li, J. (2009). Distribution characteristics of mosquitoes and their natural infection with Japanese encephalitis virus in Yunnan province. *Chinese Journal of Vector Biology and Control,* 20, pp. 344-348.

Desai, A.; Shankar, S. K.; Chandramuki, A. & Gourie-Devi, M. (1995). Japanese encephalitis virus antigen in the human brain and its topographic distribution. *Acta* Neuropathologica, (Berl.) 89, pp. 368-73.

Deubel, V.; Nogueira, M.; Drouet, M. T.; Zeller, H.; Reynes, J. M. & Do, Q. H. (1993). Direct sequencing of genomic cDNA fragments amplified by the polymerase chain reaction for molecular epidemiology of dengue 2 viruses. *Archive of Virology,* 129, pp. 197-210.

Diamond, S. M., & Klein, R. S. (2004). West Nile virus: crossing the blood-brain barrier. *Nature Medicine,* 10, pp. 1294-1295.

Erlanger, T. E.; Weiss, S.; Keiser, J.; Utzinger, J. & Wiedenmayer, K. (2009). Past, present, and future of Japanese encephalitis. *Emerging Infectious Diseases,* 15, pp. 1-7.

Fan, J. M.; Luo, J.; Chen, L.; Teng, M.; Bu, D.; Wang, F. Y.; Wang, L.; Wang, C. Q. & Zhang, G. P. (2010). Genetic analysis of strains of Japanese encephalitis virus isolated from swine in central China. *Virus Gene,* 40, pp. 357–361.

Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package) version 3.6: Department of Genome Science, University of Washington, Seattle, USA.

Fulmali, P. V.; Sapkal, G. N.; Athawale, S.; Gore, M. M.; Mishra, A. C. & Bondre, V. P. (2011). Introduction of Japanese encephalitis virus genotype I, India. *Emerging Infectious Diseases,* 17, pp. 319-321.

Gentsch, J. R.; Laird, A. R.; Bielfelt, B.; Griffin, D. D.; Banyaim, K.; Ramachandran, M.; Jain, V.; Cunliffe, N. A.; Nakagomi, O.; Kirkwood, C. D.; Fischer, T. K.; Parashar, U. D.; Bresee, J. S.; Jiang, B. & Glass, R. I. (2005). Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. *Journal of Infectious Diseases,* 192 (Suppl 1), pp. S146-S159.

Gould, E. A. (2002). Evolution of Japanese encephalitis serocomplex viruses. *Curr. Top. Microbiology and Immunology,* 267, pp. 391-404.

Gould, E. A.; Moss, S. R. & Turner, S. L. (2004). Evolution and dispersal of encephalitic flavivruses. *Archive of Virology, (Suppl.)* 18, pp. 65-84.

Griffin, D. E.; Levine, B.; Tyor, W. R. & Irani, D. N. (1992). The immune response in viral encephalitis. *Seminars in Immunology,* 4, pp. 111-119.

Grigoriadis, N. & Hadjigeorgiou, G. M. (2006). Virus-mediated autoimmunity in multiple sclerosis. *Journal of Autoimmune Diseases,* 3, pp. 1.

Hahn, C.S.; Lustig, S.; Strauss, E. G. & Strauss, J. H. (1988). Western equine encephalitis virus is a recombinant virus. *Proceedings of the National Academy of Sciences,* 85, pp. 5997-6001.

Hasegawa, H.; Yoshida, M.; Fujita, S. & Kobayashi, Y. (1994). Comparison of structural proteins among antigenically different Japanese encephalitis virus strains. *Vaccine,* 12, pp. 841-844.

Hasegawa, H.; Yoshida, M.; Shiosaka, T.; Fujita, S. & Kobayashi, Y. (1992). Mutations in the envelope protein of Japanese encephalitis virus affect entry into cultured cells and virulence in mice. *Virology,* 191, pp. 158-165.

Hickey, W. F.; Hsu, B. L. & Kimura, H. (1991). T-lymphocyte entry into the central nervous system. *Journal of Neuroscience Research,* 28, pp. 254-260.

Holland, J. J. (1996). Evolving virus plaques. *Proceedings of the National Academy of Sciences,* 93, pp. 545-546.

Holland, J.; Spindler, K.; Horodyski, F.; Grabau, E.; Nichol, S. & VandePol, S. (1982). Rapid evolution of RNA genomes. *Science,* 215, pp. 1577-1585.

Holmes, E. C. (2004). The phylogeography of human viruses. *Molecular Ecology,* 13, pp. 745-756.

Holmes, E. C.; Worobey, M., & Rambaut, A. (1999). Phylogenetic evidence for recombination in dengue virus. *Molecular Biology and Evolution,* 16, pp. 405-409.

Hsue, B.; Hartshorne, T. & Masters, P. S. (2000). Characterization of an essential RNA secondary structure in the 3′ untranslated region of the murine coronavirus genome. *Journal of Virology,* 74, pp. 6911-6921.

Huang, J. H.; Lin, T. H.; Teng, H. J.; Su, C. L.; Tsai, K. H.; Lu, L. C.; Lin, C.; Yang, C. F.; Chang, S. F.; Liao, T. L.; Yu, S. K.; Cheng, C. H.; Chang, M. C.; Hu, H, C. & Shu, P. Y. (2010). Molecular epidemiology of Japanese encephalitis virus, Taiwan. *Emerging Infectious Diseases,* 16, pp. 876-878.

Jarvis, T. C. & Kirkegaard, K. (1991). The polymerase in its labyrinth: mechanisms and implications of RNA recombination. *Trends in Genetics,* 7, pp. 186-191.

Jarvis, T. C. & Kirkegaard, K. (1992). Poliovirus RNA recombination: mechanistic studies in the absence of selection. *EMBO Journal,* 11, pp. 3135-3145.

Jenkins, G. M.; Rambaut, A.; Pybus, O. G. & Holmes, E. C. (2002). Rates of molecular evolution in RNA viruses: a quantitative phylogenetic analysis. *Journal of Moleculaar Evolution,* 54, pp. 152-161.

Johnson, L. J.; Halliday, G. M. & King, N. J. (2000). Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. *Journal of Investigative Dermatology* 114, pp. 560-568.

Johnson, R. T. (1987).The pathogenesis of acute viral encephalitis and postinfectious encephalomyelitis. *Journal of Infectious Diseases,* 155, pp. 359-364.

Johnson, R. T.; Burke, D. S.; Elwell, M.; Leake, C. J.; Nisalak, A.; Hoke, C. H. & Lorsomrudee, W. (1985). Japanese encephalitis: Immunocytochemical studies of viral antigen and inflammatory cells in fatal cases. *Annals of Neurology,* 18, pp. 567-573.

Kalita, J. & Misra, U. K. (2000). Comparison of CT and MRI findings in the diagnosis of Japanese encephalitis. *Journal of Neurological Science,* 174, pp. 3-8.

Kalita, J. & Misra, U. K. (2000). Neurophysiological changes in Japanese encephalitis. *Neurology India,* 50, pp. 262-266.

Khiabanian, H.; Trifonov, V. & Rabadan, R. (2009). Reassortment patterns in swine influenza viruses. *PLoS ONE,* 4, pp. e7366.

Kim, J. M.;Yun, S. I.; Song, B. H.; Hahn, Y. S.; Lee, C. H.; Oh, H. W. & Lee, Y. M. (2008). A single N-linked glycosylation site in the Japanese encephalitis virus prM protein is critical for cell type-specific prM protein biogenesis, virus particle release, and pathogenicity in mice. *Journal of Virology,* 82, pp. 7846-7862.

Kimura-Kuroda, J.; Ichikawa, M.; Ogata, A.; Nagashima, K.; Yasui, K. (1992). Specific tropism of Japanese encephalitis virus for developing neurons in primary rat brain culture. *Archive of Virology,* 130, pp. 477-484.

Kirkegaard, K. & Baltimore, D. (1986). The mechanism of RNA recombination in poliovirus. *Cell,* 47, pp. 433-443.

Konishi, E. & Suzuki, T. (2002). Ratios of subclinical to clinical Japanese encephalitis (JE) virus infections in vaccinated populations: evaluation of an inactivated JE vaccine by comparing the ratios with those in unvaccinated populations. *Vaccine,* 21, pp. 98-107.

Kuno, G.; Chang, G. J.; Tsuchiya, K. R.; Karabatsos, N. & Cropp, C. B. (1998). Phylogeny of the genus *Flavivirus*. *Journal of Virology*, 72, pp. 73-83.

Lai, M. M. (1992). RNA recombination in animal and plant viruses. *Micribiology and Molecular Biology Review*, 56, pp. 61-79.

Ledinko, N. (1963).Genetic recombination with poliovirus type 1: studies of crosses between a normal horse serum-resistant mutant and several guanidine-resistant mutants of the same strain. *Virology,* 20, pp. 107-119.

Lee, E. & Lobigs, M. (2002). Mechanism of virulence attenuation of glycosaminoglycans-binding variants of Japanese encephalitis virus and Murray Valley encephalitis Virus. *Journal of Virology,* 76, pp. 4901-4911.

Lee, E.; Hall, R. A. & Lobigs, M. (2004). Common E protein determinants for attenuation of glycosaminoglycan-binding variants of Japanese encephalitis and West Nile viruses. *Journal of Virology,* 78, pp. 8271-8280.

Lescar, J. & Canard, B. (2009). RNA-dependent RNA polymerases from flaviviruses and Picornaviridae. *Current Opinion in Structural Biology,* 19, pp. 759-767.

Lindenbach, B. D. & Rice, C. M. (2001). Flaviviridae: the viruses and their replication, In: *Fields Virology, vol. 1. 4th ed,* D. M. Knipe & P. M. Howley, (Eds.), 991-1041. Lippincott Williams & Wilkins Publishers, Philadelphia, PA, USA.

Liou, M. L. & Hsu, C. Y. (1998). Japanese encephalitis virus is transported across the cerebral blood vessels by endocytosis in mouse brain. *Cell and Tissue Research,* 293, pp. 389-394.

Liu, H.; Chiou, S. S. & Chen, W. J. (2004). Differential binding efficiency between the envelope protein of Japanese encephalitis virus variants with heparan sulfate on the cell surface. *Journal of Medical Virology,* 72, pp. 618-624.

Liu, T. H.; Liang, L. C.; Wang, C. C.; Liu, H. C. & Chen, W. J. (2008). The blood-brain barrier in the cerebrum is the initial site for the Japanese encephalitis virus entering the central nervous system. *Journal of Neurovirology,* 14, pp. 514-521.

Liu, Y.; Chuang, C. K. & Chen, W. J. (2009). *In situ* reverse-transcription loop-mediated isothermal amplification (in situ RT-LAMP) for detection of Japanese encephalitis viral RNA in host cells. *Journal of Clinical Virology,* 46, pp. 49-54.

Ma, S. P.; Yoshida, Y.; Makino, Y.; Tadano, M.; Ono, T. & Ogawa, M. (2003). A major genotype of Japanese encephalitis virus currently circulating in Japan. *American Journal of Tropical Medicine and Hygiene,* 69, pp. 151-154.

Mackenzie, J. S. (1999). Emerging viral diseases: an Australian perspective. *Emerging Infectious Diseases,* 5, pp.1-8.

Mackenzie, J. S.; Gubler, D. J. & Petersen, L. R. (2004). Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature Medicine,* 10, pp. S98-S109.

Mackenzie, J. S.; Williams, D. T. & Smith, D. W. (2007). Japanese encephalitis virus: the geographic distribution, incidence, and spread of a virus with a propensity to emerge in new areas. In: *Emerging viruses in human populations*, E. Tabor, (Ed.), 201-268, Elsevier, Amsterdam, Netherlands.

Mangada, M. N. M. & Takegami, T. (1999). Molecular characterization of the Japanese encephalitis virus representative immunotype strain JaGAr 01. *Virus Research,* 59, pp. 101-112.

Marchette, N. J.; Dubois, D. R.; Larsen, L. K.; Summers, P. L.; Kraiselburd, E. G.; Gubler, D. J. & Eckels, K. H. (1990). Preparation of an attenuated dengue 4 (341750 Carib) virus vaccine. I. Pre-clinical studies. *American Journal of Tropical Medicine and Hygiene,* 43, pp. 212-218.

Martinez-Barragan, J. J. & del Angel, R. M. (2001). Identification of putative coreceptor on Vero cells that participates in dengue 4 virus infection. *Journal of Virology,* 75, pp. 7818-7827.

Mathur, A.; Kulshreshtha, R. & Chaturvedi, U. C. (1987). Induction of secondary immune response by reactivated Japanese encephalitis virus in latently infected mice. *Immunology,* 60, pp. 481-484.

McMinn, P. C.; Dalgarno, L. & Weir, R. C. (1996). A comparison of the spread of Murray Valley viruses of high or low neuroinvasiveness in the tissues of Swiss mice after peripheral inoculation. *Virology,* 220, pp. 414-423.

Meyer, R.; Weissert, R.; Diem, R.; Storch, M. K.; Graaf, K. L.; Kramer, B. & Bähr, M. (2001). Acute neuronal apoptosis in a rat model of multiple sclerosis. *The Journal of Neuroscience,* 21, pp. 6214-6220.

Misra. U. K. & Kalita, J. (1997). Movement disorders in Japanese encephalitis. *Journal of Neurology*, 244, pp. 299−303.

Misra. U. K. & Kalita, J. (2010). Overview: Japanese encephalitis. *Progress in Neurobiology,* 91, pp. 108-120.

Monath, T. P. (2002). Japanese encephalitis vaccines: current vaccines and future prospects. *Current Topics in Microbiology and Immunology,* 267, pp. 105-138.

Monath, T. P., and F. X. Heinz. (1996). Flaviviruses, In: *Fields Virology*, 3rd ed., B. N. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, J. P. Melnick, T. P. Monath, B.

Roizman, & S. E. Strauss, (Eds.), 961-1034, Lippincott-Raven Publishers, Philadelphia, USA.

Morita, K. (2009). Molecular epidemiology of Japanese encephalitis in East Asia. *Vaccine,* 27, pp. 7131-7132.

Muller, D. M.; Pender, M. P. & Greer, J. M. (2005). Blood-brain barrier disruption and lesion localization in experimental autoimmune encephalomyelitis with predominant cerebella and brainstem involvement. *Journal of Neuroimmunology,* 160, pp. 162-169.

Myint, K. S. A.; Gibbons, R. V.; Perng, G. C. & Solomon, T. (2007). Unraveling the neuropathogenesis of Japanese encephalitis. T*ransactions of the Royal Society of Tropical Medicine and Hygiene,* 101, pp. 955-956.

Nabeshima, T.; Loan, H. T. K.; Sumiyoshi, M.; Haruda, Y.; Nga, P. H.; Huong, V. T.; Parquet, M. C.; Hasebe, F. & Morita, K. (2009). Evidence of frequent introduction of Japanese encephalitis virus from south-east Asia and continental east Asia to Japan. *Journal of General Virology,* 90, pp. 827-832.

Nagy, P. D. & Bujarski, J. J. (1996). Homologous RNA recombination in brome mosaic virus: AU-rich sequences decrease the accuracy of crossovers. *Journal of Virology,* 70, pp. 415-426.

Nagy, P. D. & Jozef, J. B. (1997). Engineering of homologous recombination hotspots with AU-rich sequences in Brome mosaic virus. *Journal of Virology,* 71, pp. 3799-3810.

Nargi-Aizenman, J. L. & Griffin, D. E. (2001). Sindbis virus-induced neuronal death is both necrotic and apoptotic and is ameliorated by N-methyl-D-aspartate receptor antagonists. *Journal of Virology,* 75, pp. 7114-7121.

Nett, R. J.; Campbell, G. L. & Reisen, W. K. (2009). Potential for the emergence of Japanese encephalitis virus in California. *Vector Borne and Zoonotic Diseases,* 9, pp. 511-517.

Nga, P. T.; del Carmen Parquet, M.; Cuong, V. D.; Ma, S. P.; Hasebe, F.; Inoue, S.; Mikino, Y.; Takagi, M.; Nam, V. S. & Morita, K. (2004). Shift in Japanese encephalitis virus (JEV) genotype circulating in northern Vietnam: implications for frequent introductions of JEV from Southeast Asia to East Asia. *Journal of General Virology,* 85, pp. 1625-1631.

Ni, H. & Barrett, A. D. T. (1998). Attenuation of Japanese encephalitis virus by selection of its mouse brain membrane receptor preparation escape variants. *Virology,* 241, pp. 30-36.

Ni, H. & Barrett, A. D. T. (1996).Molecular differences between wild-type Japanese encephalitis virus strains of high and low mouse neuroinvasiveness. *Journal of General Virology,* 77, pp. 1449-1455.

Nidaira, M.; Taira, K.; Okano. S.; Shinzato, T.; Morikawa, T.; Tokumine. M.; Asato, Y.; Tada, Y.; Miyagi, K.; Matsuda, S.; Itokazu, K.; Kydaka, J.; Nakamura, M. & Tamanaha, K. (2009). Survey of Japanese encephalitis in pigs on Miyako, Ishigaki, Kume, and Yonaguri islands in Okinawa, Japan. *Japanese Journal of Infectious Diseases,* 62, pp. 220-224.

Nitatpattana, N.; Dubot-Pérès, A.; Gouilh, M. A.; Souris, M.; Barbazan, P.; Yoksan, S.; de Lamballerie, X. & Gonzalez, J. P. (2008). Change in Japanese encephalitis virus distribution, Thailand. *Emerging Infectious Diseases,* 14, pp. 1762-1765.

Nitayaphan, S.; Grant, J. A. & Chang, G. J. (1990). Nucleotide sequence of the virulent SA-14 strain of Japanese encephalitis virus and its attenuated vaccine derivative, SA14-14-2. *Virology,* 177, pp. 541-552.

Pyke, A. T.; Williams, D. T.; Nisbet, D. J.; van den Hurk, A. F.; Taylor, C. T.; Johansen, C. A.; Macdonald, J.; Hall, R. A.; Simmons, R. J.; Mason, R. J.; Lee, J. M.; Ritchie, S. A.; Smith, G. A. & Mackenzie, J. S. (2001). The appearance of a second genotype of Japanese encephalitis virus in the Australasian region. *American Journal of Tropical Medicine and Hygiene,* 65, pp. 747-753.

Ramos-Castaneda, J.; Imbert, J. L.; Barron, B. L. & Ramos, C. (1997). A 65-kDa trypsin-sensible membrane cell protein as a possible receptor for dengue virus in cultured neuroblastoma cells. *Journal of Neurovirology,* 3, pp. 435-440.

Ravi, V.; Taly, A. B.; Shankar, S. K.; Shenoy, P. K.; Desai, A.; Nagaraja, D.; Gourie-Devi, M. & Chandramuki, A. (1994). Association of Japanese encephalitis virus infection with Guillain–Barré syndrome in endemic area of South India. *Acta Neurologica Scandinavica,* 90, pp. 67-72.

Ren, J.; Ding, T.; Zhang, W.; Song, J. & Ma, W. (2007). Does Japanese encephalitis virus share the same cellular receptor with other mosquito-borne flaviviruses on the C6/36 mosquito cells? *Virology Journal,* 4, pp. 83.

Rey, F. A.; Heinz, F. X.; Mandl, C.; Kunz, C. & Harrison, S. C. (1995). The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. *Nature,* 375, pp. 291-298.

Ritchie, S. A. & Rochester, W. (2001).Wind-blown mosquitoes and introduction of Japanese encephalitis into Australia. *Emerging Infectious Diseases,* 7, pp. 900-903.

Rohayem, J.; Münch, J. & Rethwilm, A. (2005). Evidence of recombination in the norovirus capsid gene. *Journal of Virology,* 79, pp. 4977-4990.

Rosen, L. (1986). The natural history of Japanese encephalitis virus. *Annual Review of Microbiology,* 40, pp. 395-414.

Rothwell, S. W.; Putnak, R. & LaRussa, V. E. (1996). Dengue-2 virus infection of human bone marrow characterization of Dengue-2 antigen-positive stromal cells. *American Journal of Tropical Medicine and Hygiene,* 54, pp. 503-510.

Samuel, M. A.; Morrey, J. D. & Diamond, M. S. (2007). Caspase 3-dependent cell death of neurons contributes to the pathogenesis of West Nile virus encephalitis. *Journal of Virology,* 81, pp. 2614-2623.

Sarma, J. D. (2010). A mechanism of virus-induced demyelination. *Interdisciplinary perspectives on infectious diseases,* Article ID 109239.

Schneider, W. L. & Roossinck, M. J. (2001). Genetic diversity in RNA virus quasispecies is controlled by host-virus interactions. *Journal of Virology* 75, pp. 6566-6571.

Schuh, A. J.; Tesh, R. B. & Barrett, A. D. T. (2011). Genetic characterization of Japanese encephalitis virus genotype II strains isolated from 1951 to 1978. *Journal of General Virology,* 92, pp. 516-527.

Sejvar, J. J.; Bode, A. V.; Marfin, A. A.; Campbell, G. L.; Ewing, D.; Mazowiecki, M.; Pavot, P. V.; Schmitt, J.; Pape, J.; Biggerstaff, B. J. & Petersen, L. R. (2005). West Nile virus-associated flaccid paralysis. *Emerging Infectious Diseases,* 11, pp. 1021-1027.

Shapka, N. & Nagy, P. D. (2004). The AU-Rich RNA recombination hot spot sequence of Brome mosaic virus is functional in tombusviruses: implications for the mechanism of RNA recombination. *Journal of Virology*, 78, pp. 2288-2300.

Shope, R. E. & Sather, G. E. (1979). Arboviruses, In: *Diagnostic procedures for viral, rickettsial, and chlamydial infections, 5th ed.*, E. H. Lennette & N. J. Schmidt , (Ed.), 767-814. American Public Health Association, Inc., Washington, D.C., USA.

Solomon, T. & Vaughn, D. W. (2002). Pathogenesis and clinical features of Japanese encephalitis and West Nile virus infections. *Current Topics in Microbiology and Immunology,* 267, pp. 171−194.

Solomon, T. (2004). Flavivirus encephalitis. *New England Journal of Medicine,* 351, pp. 370-378.

Solomon, T.; Dung, N. M.; Kneen, R.; Gainsborough, M.; Vaughn, D. W.; Khanh, V. T. (2000). Japanese encephalitis. *Journal of Neurology, Neurosurgery & Psychiatry*, 68, pp. 405-415.

Solomon, T.; Ni, H.; Beasley, D. W. C.; Ekkelenkamp, M.; Cardosa, M. J. & Barrett A. D. T. (2003). Origin and evolution of Japanese encephalitis virus in Southeast Asia. *Journal of Virology,* 77, pp. 3091-3098.

Stamatovic, S. M.; Shakui, P.; Keep, R. F.; Moore, B. B.; Kunkel, S. L.; Van Rooijen, N. & Andjelkovic, A. V. (2005). Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. *Journal of Cerebral Blood Flow & Metabolism,* 25, pp. 593–606.

Stephens, E. B.; Singh, D. K.; Kohler, M. E.; Jackson, M.; Pacyniak, E. & Berman, N. E. J. (2003). The primary phase of infection by pathogenic simian-human immunodeficiency virus results in disruption of the blood-brain barriers. *AIDS Research and Human Retroviruses,* 19, pp. 837−846.

Stiasny, K. & Heinz, F. X. (2006). Flavivirus membrane fusion. *Journal of General Virology,* 87, pp. 2755–2766.

Stohlman, S. A. & Hinton, D. R. (2001). Viral induced demyelination. *Brain Pathology,* 11, pp. 92-106.

Su, C. M.; Liao, C. L.; Lee, Y. L.; Lin, Y.-L. (2001). Highly sulfated forms of heparin sulfate are involved in Japanese encephalitis virus infection. *Virology,* 286, pp. 206-215.

Sumiyoshi, H.; Mori, C.; Fuke, I.; Morita, K.; Kuhara, S.; Kondou, J.; Kikuchi, Y.; Nagamatu, H. & Igarashi, A. (1987). Complete nucleotide of the Japanese encephalitis virus genome RNA. *Virology,* 161, pp. 479-510.

Suri, N. K. & Banerjee, K. (1995). Growth and cytopathic effect of Japanese encephalitis in astrocyte-enriched cell cultures from neonatal mouse brains. *Acta Virologica,* 39, pp. 143-148.

Sztuba-Solinska, J.; Urbanowicz, A.; Figlerowicz, M. & Bujarski, J. J. (2011). RNA-RNA recombination in plant virus replication and evolution. *Annual Review of Phytopathology,* 49, (in press)

Tajima, S.; Nerome, R.; Nukui, Y.; Kato, F.; Takasaki, T. & Kurane, I. (2010). A single mutation in the Japanese encephalitis virus E protein (S123R) increases its growth rate in mouse neuroblastoma cells and its pathogenicity in mice. *Virology,* 396, pp. 298-304.

Thompson, J. D.; Hinggins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acid Research*, 22, pp. 4673-4680.

Tolou, H. J. G.; Couissinier-Paris, P.; Durand, J. P.; Mercier, V.; de Pina, J. J.; de Micco, P.; Billoir, F.; Charrel, R. N. & de Lamballerie, X. (2001). Evidence for recombination in natural populations of dengue virus type 1 based on the analysis of complete genome sequences. *Journal of General Virology,* 82, pp. 1283-1290.

Trent, D. W.; Grant, J. A.; Rosen, L. & Monath, T. P. (1983). Genetic variation among dengue 2 viruses of different geographic origin. *Virology*, 128, pp. 271-284.

Trent, D. W.; Kinney, R. M.; Johnson, B. J.; Vorndam, A.V.; Grant, J. A.; Deubel, V.; Rice, C.M. & Hahn, C. (1987). Partial nucleotide sequence of St. Louis encephalitis virus RNA: structural proteins, NS1, NS2a, and NS2b. *Virology*, 156, pp. 293-304.

Tseng, Y. F.; Wang, C. C.; Liao, S. K.; Chuang, C. K. & Chen, W. J. (2011). Autoimmunity-related demyelination in infection by Japanese encephalitis virus. Journal: *Journal of Biomedical Science,* 18, pp. 20.

Tsunoda, I.; Kuang, L. Q.; Libbey, J. E. & Fujinami, R. S. (2003). Axonal injury heralds virus-induced demyelination. *The American Journal of Pathology,* 162, pp. 1259-1269.

Tung, W. H.; Tsai, H. W.; Lee, I. T.; Hsieh, H. L.; Chen, W. J.; Chen, Y. L. & Yang, C. M. (2010). Japanese encephalitis virus induces matrix metalloproteinase-9 expression via the ROS/MAPKs-dependent NF-κB signaling pathway in rat brain astrocytes. *British Journal of Pharmacology,* 161, pp. 1566-1583.

Turner, P. E. & Chao, L. (1998). Sex and evolution of intrahost competition in RNA virus φ6. *Genetics,* 150, pp. 523-532.

Twiddy, S. S. & Holmes, E. C. (2003). The extent of homologous recombination in members of the genus Flavivirus. *Journal of General Virology,* 84, pp. 429-440.

Uchil, P. D. & Satchidanandam, V. (2001). Phylogenetic analysis of Japanese encephalitis virus: envelope gene based analysis reveals a fifth genotype, geographic clustering, and multiple introductions of the virus into the Indian subcontinent. *American Journal of Tropical Medicine and Hygiene,* 65, pp. 242-251.

Umenai, T.; Krzysko, R.; Bektimirov, T. A. & Assaad, F. A. (1985). Japanese encephalitis: current worldwide status. *Bulletin of World Health Organization,* 63, 625-631.

Unni, S. K.; Růžek, D.; Chhatbar, C.; Mishra, R.; Johri, M. K.; Singh, S. K. (2011). Japanese encephalitis virus: From genome to infectome. *Microbes and Infection,* 13, pp. 312-321.

van den Hurk, A. F.; Ritchie, S. A. & Mackenzie, J. S. (2009). Ecology and geographical expansion of Japanese encephalitis virus. *Annual Review of Entomology,* 54, pp. 17-35.

van den Hurk, A. F.; Ritchie, S. A.; Johansen, C. A.; Mackenzie, J. S. & Smith, G. A. (2008). Domestic pigs and Japanese encephalitis virus infection, Australia. *Emerging Infectious Diseases,* 14, pp. 1173-1178.

Vasilakis, N; Deardorff, E. R.; Kenney, J. L.; Rossi, S. L. & Hanley, K.A. (2009) Mosquitoes put the brake on arbovirus evolution: experimental evolution reveals slower mutation accumulation in mosquito than vertebrate cells. *PLoS Pathogens,* 5, pp. e1000467.

Wang, H.; Takasaki, T.; Fu, S, Sun, X.; Zhang, H.; Wang, Z.; Hao, Z.; Zhang, J.; Tang, Q.; Kotaki, A.; Tajima, S.; Liang, X.; Yang, W.; Kurane, I. & Liang, G. (2007). Molecular epidemiological analysis of Japanese encephalitis virus in China. *Journal of General Virology,* 88, pp. 885-894.

Wang, L.; Fu, S.; Zhang, H.; Ye, X.; Yu, D.; Deng, Z.; Yuan, J.; Zhai, Y.; Li, M.; Lv, Z.; Chen, W.; Jiang, H.; Gao, X.; Cao, Y.; Wang, H.; Tang, Q. & Liang, G. (2010). Identification and isolation of Genotype-I Japanese encephalitis virus from encephalitis patients. *Virology Journal,* 7, pp. 345.

Wang, T.; Town, T.; Alexopoulou, L.; Anderson, J. F.; Fikrig, E. & Flavell, R. A. (2004). Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nature Medicine,* 10, pp. 1366−1373.

Weaver, S. C.; Rico-Hesse, R. & Scott, T. W. (1992). Genetic diversity and slow rates of evolution in New World alphaviruses. *Current Topics in Micriobiology and Immunology,* 176, pp. 99-117.

Weng, M. H.; Lien, J. C.; Wang, Y. M,.; Lin, C. C.; Lin, H. C. & Chin, C. (1999). Isolation of Japanese encephalitis virus from mosquitoes collected in Northern Taiwan between 1995 and 1996. *Journal of Microbiology, Immunology, and Infection,* 32, pp. 9-13.

Westaway, E. G.; Brinton, M. A.; Gaidamovich, S, Y.; Horzinek, M. C.; Igarashi, A.; Kääriäinen, L.; Lvov, D. K.; Porterfield, J. S.; Russell, P. K. & Trent, D. W. (1985). Flaviviridae. *Intervirology,* 24, pp. 183-192.

White, K. A. & Morris, T. J. (1995). RNA determinants of junction site selection in RNA virus recombinants and defective interfering RNAs. *RNA,* 1, pp. 1029-1040.

Whitley, R. J. & Gnann, J. W. (2002). Viral encephalitis: familiar infections and emerging pathogens. *Lancet,* 359, pp. 507−513.

Wierzchoslawski, R. & Bujarski, J. (2006). Efficient *in vitro* system of homologous recombination in Brome mosaic bromovirus. *Journal of Virology,* 80, pp. 6182-6187.

Worobey, M. & Holmes, E. C. (1999). Evolutionary aspects of recombination in RNA viruses. *Journal of General Virology,* 80, pp. 2535-2543.

Wu, S. C.; Lian, W. C.; Hsu, L. C.; Wu, Y. C. & Liau. M. Y. (1998). Antigenic characterization of nine wild-type Taiwanese isolates of Japanese encephalitis virus as compared with two vaccine strains. *Virus Research,* 55, pp. 83-91.

Wu, Y. C.; Huang, Y. S.; Chien, L. J.; Lin, T. L.; Yueh, Y. Y.; Tseng, W. L.; Chang,K. J. & Wang, G. R. (1999). The epidemiology of Japanese encephalitis on Taiwan during 1966-1997. *American Journal of Tropical Medicine and Hygiene,* 61, pp. 78-84.

Xia, S. Y.; Guzman, Ha.; Zhang, H.; Travassos, da Rosa. A. P. A. & Tesh, R. B. (2001). West Nile virus infection in the golden hamster (*Mesocricetus auratus)*: a model for West Nile encephalitis. *Emerging Infectious Diseases,* 7, pp. 714−722.

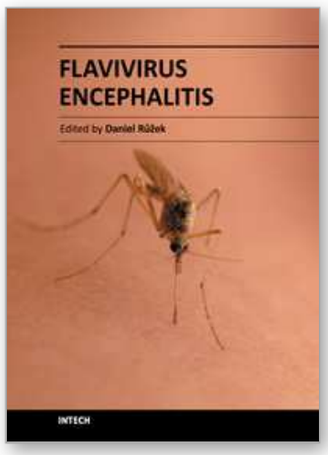
Yamada, M.; Nakamura, K.; Yoshii, M. & Kaku, Y. (2004). Nonsuppurative encephalitis in piglets after experimental inoculation of Japanese encephalitis flavivirus isolated from pigs. *Veterinary Pathology,* 41, pp. 62−67.

Yoshida, Y.; Tabei, Y.; Hasegawa, M.; Nagashima, M. & Morozumi, S. (2005). Genotypic analysis of Japanese encephalitis virus strains isolated from swine in Tokyo, Japan. *Japanese Journal of Infectious Diseases,* 58, pp. 259-261.

Yun, S. M.; Cho, J. E.; Ju, Y. R.; Kim. S. Y.; Ryou, J.; Han, M. G.; Choi, W. Y. & Jeong, Y. E. (2010). Molecular epidemiology of Japanese encephalitis virus circulating in South Korea, 1983-2005. *Virology Journal,* 7, pp. 127.

Zhao, Z.; Date, T.; Li, Y.; Kato, T.; Miyamoto, M.; Yasui, K. & Wakita, T. (2005). Characterization of the E-138 (Glu/Lys) mutation in Japanese encephalitis virus by using a stable, full-length, infectious cDNA clone. *Journal of General Virology,* 86, pp. 2209-2220.

Zhou, Y.; Ray, D.; Zhao, Y.; Dong, H.; Ren, S.; Li, Z.; Guo, Y.; Bernard, K. A.; Shi, P. Y. & Li, H. (2007). Structure and function of flavivirus NS5 methyltransferase. *Journal of Virology,* 81, pp. 3891-3903.



Flavivirus Encephalitis

Edited by Dr. Daniel Ruzek

ISBN 978-953-307-669-0
Hard cover, 478 pages
Publisher InTech
Published online 30, September, 2011
Published in print edition September, 2011

Encephalitis is an inflammation of the brain tissue associated with clinical evidence of brain dysfunction. The disease is of high public health importance worldwide due to its high morbidity and mortality. Flaviviruses, such as tick-borne encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, or St. Louis encephalitis virus, represent important causative agents of encephalitis in humans in various parts of the world. The book Flavivirus Encephalitis provides the most recent information about selected aspects associated with encephalitic flaviviruses. The book contains chapters that cover a wide spectrum of subjects including flavivirus biology, virus-host interactions, role of vectors in disease epidemiology, neurological dengue, and West Nile encephalitis. Special attention is paid to tick-borne encephalitis and Japanese encephalitis viruses. The book uniquely combines up-to-date reviews with cutting-edge original research data, and provides a condensed source of information for clinicians, virologists, pathologists, immunologists, as well as for students of medicine or life sciences.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen