ORIGINAL ARTICLE

The Zoonotic Flaviviruses of Southern, South-Eastern and Eastern Asia, and Australasia: The Potential for Emergent Viruses

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Impacts

- Flaviviruses of southern, south-eastern and eastern Asia and Australasia comprise at least 30 viruses, ranging from major pathogens such as Japanese encephalitis (JE), West Nile (WN), Murray Valley encephalitis (MVE), and the four dengue viruses, to other viruses which either cause minor human febrile diseases or have not been associated with human disease.
- Flaviviruses have been shown to have a propensity to spread, emerge and establish in new geographic areas, and thus any member may have the potential to emerge unexpectedly anywhere if suitable vectors and vertebrate hosts occur.
- Most members of the genus have little or no known association with human or animal diseases, but they may all have an inherent potential to be more pathogenic in the future – indeed, with flaviviruses, one should expect the unexpected, they are unpredictable with respect to disease severity, unusual clinical manifestations, and unexpected methods of transmission.
- Phylogenetic studies suggest JE, MVE and Alfuy viruses may have derived from an ancestral African flavivirus and evolved in the Indo-Malay area to subsequently disperse throughout their geographic ranges, whereas WN virus probably evolved in Africa, and different genetic lineages spread to Australasia (lineage 1b), India (lineages 1a and 5), Malaysia (a newly identified lineage 6). In addition, a separate lineage evolved in Africa to become the closely related Koutango virus (lineage 7).
- Novel flaviviruses have emerged in the region, including New Mapoon virus in northern Australia, Sitiawan virus in Malaysia, and ThCAr virus from Thailand, which demonstrates that new flaviviruses might be found in different ecosystems.

Keywords:
Flavivirus; zoonotic; evolution; emergence; spread

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Summary

The genus Flaviviridae comprises about 70 members, of which about 30 are found in southern, south-eastern and eastern Asia and Australasia. These include major pathogens such as Japanese encephalitis (JE), West Nile (WN), Murray Valley encephalitis (MVE), tick-borne encephalitis, Kyasanur Forest disease virus, and the dengue viruses. Other members are known to be associated with mild febrile disease in humans, or with no known disease. In addition, novel flaviviruses continue to be discovered, as demonstrated recently by New Mapoon virus in Australia, Sitiawan virus in Malaysia, and ThCAr virus in Thailand. About 19 of these viruses are mosquito-borne, six are tick-borne, and four have no known vector and represent isolates from rodents or bats.

Evidence from phylogenetic studies suggest that JE, MVE and Alfuy viruses probably emerged in the Malaya-Indonesian region from an African progenitor virus, possibly a virus related to Usutu virus. WN virus, however, is believed to have emerged in Africa, and then dispersed through avian migration. Evidence suggests that there are at least seven genetic lineages of WN virus, of which lineage 1b spread to Australasia as Kunjin virus, lineages 1a and 5 spread to India, and lineage 6 spread to Malaysia. Indeed, flaviviruses have a propensity to spread and emerge in new geographic areas, and they represent a potential source for new disease emergence. Many of the factors associated with disease emergence are present in the region, such as changes in land use and deforestation, increasing population movement, urbanization, and increasing trade. Furthermore, because of their ecology and dependence on climate, there is a strong likelihood that global warming may significantly increase the potential for disease emergence and/or spread.

Introduction

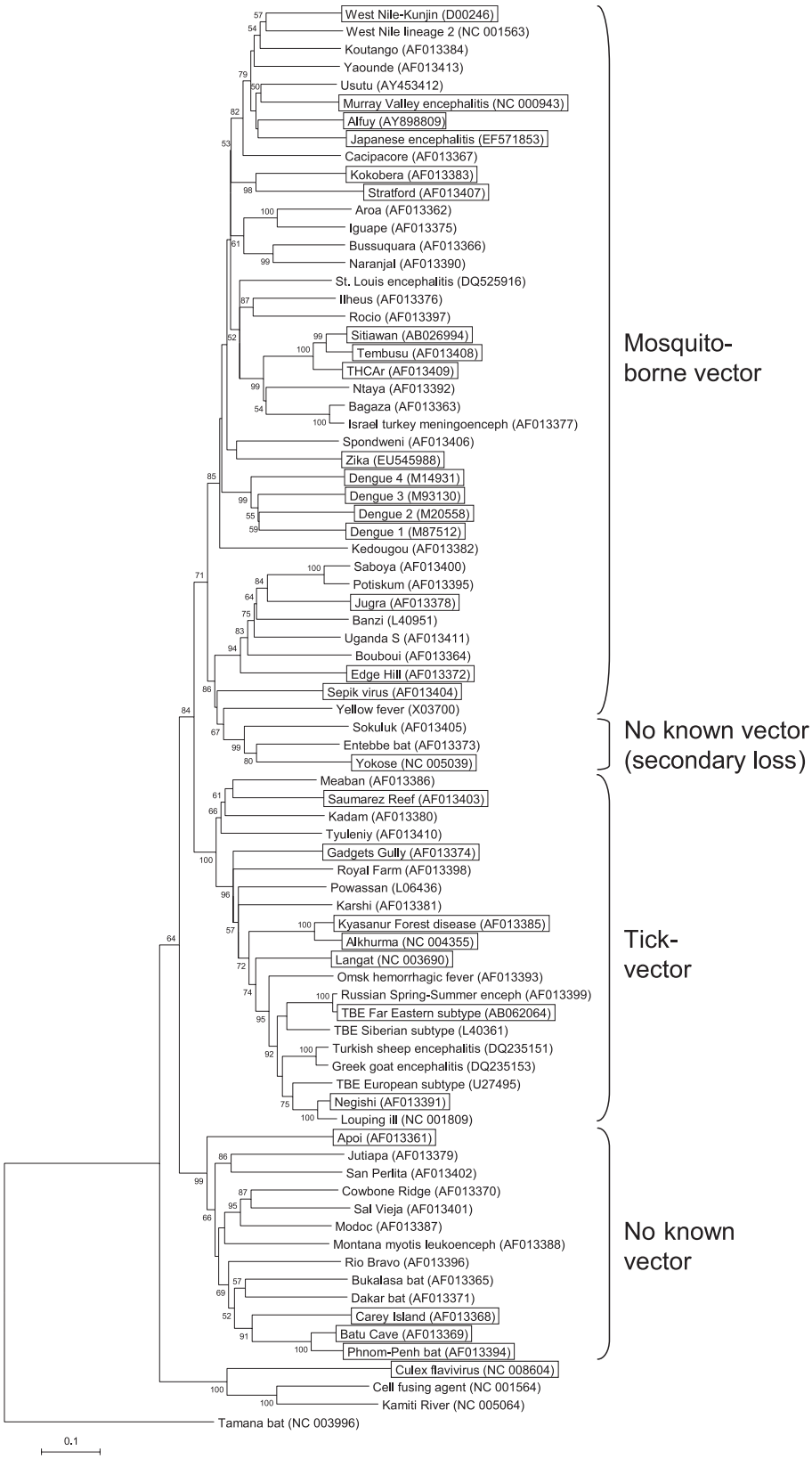
The family Flaviviridae comprises three genera; the Flavivirus genus, the Pestivirus genus, and the Hepacivirus genus. The viruses are 40–60 nm in diameter, spherical in shape, and contain a lipid envelope. All members of the family have a genome composed of a single molecule of linear positive-sense RNA, ranging in size from about 9.6 kb for Hepaciviruses, to 11 kb for Flaviviruses, and 12.3 kb for Pestiviruses. Members of each genus are serologically related to each other, but not to members of the other genera (Thiel et al., 2005). The Pestiviruses infect pigs and ruminants, but are not zoonotic. The major members of the genus include Bovine diarrhoeal disease virus and Classical swine fever virus. Hepaciviruses comprise the hepatitis C viruses and only infect humans. Thus, these two genera will not be discussed further.

The Flavivirus Genus

The Flavivirus genus comprises over 70 members (Thiel et al., 2005). The type species is yellow fever virus from which the genus (and family) name is derived (flavus, Latin for 'yellow'). Members of the genus Flaviviruses are found widely dispersed throughout the world on all continents except Antarctica. Most are transmitted by an arthropod vector, either mosquitoes or ticks, but a few viruses have no known vector species. The most important mosquito-borne viruses are yellow fever virus, which is found in west, central and east Africa and tropical South America; Japanese encephalitis (JE) which is found in east, south-east and southern Asia, and in northern Australasia (Mackenzie et al., 2007); West Nile (WN) virus which is found in Africa, the Middle East, Europe, southern Asia, Australasia, and most recently, in North America and now spreading into Central and South America (Kramer et al., 2007; Blitvich, 2008); and dengue viruses which are found throughout much of tropical and subtropical regions of the world. In addition, Murray Valley encephalitis (MVE) and St Louis encephalitis (SLE) are the major encephalitic viruses in their respective geographic regions, Australasia and the Americas respectively. The most important tick-borne virus is Tick-borne encephalitis (TBE) virus which is found in central Europe and across the central and northern Asian continent. Others are important regionally, such as Kyasanur Forest disease (KFD) virus in India. There are approximately 39 mosquito-borne members of the genus, 15 tick-borne members, and 17 with no known vector. The major criteria for determining the groupings of species and members within the genus are nucleotide and deduced amino acid sequence data, antigenic relationships, vector association and geographic incidence (Calisher and Gould, 2003; Thiel et al., 2005).

The mosquito-borne viruses have been grouped into seven groups: the Aroa virus group with four members, the JE virus group with 10 members, the Ntaya virus group with six members, the Kokobera virus group with two members, the Dengue virus group with five members, the Spondweni virus group with two members, and the Yellow fever virus group with 10 members (Thiel et al., 2005). Some members are defined as subspecies (e.g. Kunjin virus as a subspecies of WN virus, and Alfuy virus as a subspecies of MVE virus), however, the interpretation of the criteria for assigning a species level, and the definition of what constitutes a subspecies may be controversial. Thus, Kunjin virus is clearly a WN virus and a member of WN lineage 1, although forming a distinct clade, lineage 1b (Scherret et al., 2001, 2002), and Alfuy virus has recently been fully sequenced and is also clearly a separate species (May et al., 2006).

The tick-borne flaviviruses currently comprise 12 species divided into two groups, the mammalian and seabird groups, with two of the mammalian species comprising an additional seven subtypes (Thiel et al., 2005). In the mammalian tick-borne group, six species are capable of



causing disease in humans and animals, and include KFD virus, Langat (LGT) virus, Louping Ill virus, Omsk haemorrhagic fever virus, Powassan virus, and TBE virus. Of these, KFD and Omsk haemorrhagic fever viruses cause haemorrhagic fever in humans, while the remaining species are encephalitogenic. Gadget's Gully (GGY) virus and Royal Farm virus make up the remaining species of the mammalian tick-borne flavivirus group. The seabird tick-borne virus group contains the species Meaban, Saumerez Reef (SRE), Tyuleniy and Kadam viruses. However, a recent expanded phylogenetic analysis of these viruses identified Kadam virus as a distinct third group within the tick-borne flaviviruses (Grard et al., 2007). Transmission of tick-borne viruses to mammalian or avian hosts occurs through the bite of infected ticks; virus can be transmitted by infected larvae, nymphs or adult ticks. TBE virus infections of humans can also occur via untreated milk of goats, sheep and cows (Kohl et al., 1996). A major factor in virus transmission between ticks is transfer from infected to uninfected ticks during co-feeding (Jones et al., 1987). Virus is also maintained by transovarial and transstadial transmission (Danielová and Holubová, 1991; Nuttall and Labuda, 2003). Once a tick is infected, it carries the virus for the duration of its lifetime.

There are no major human or animal pathogens amongst the flaviviruses with no known vector. These viruses have been isolated from bats or rodents (Varelas-Wesley and Calisher, 1982).

Two additional viruses are listed as tentative species in the genus; Tamana bat virus and Cell fusing agent virus. The latter, a virus associated with Aedes mosquitoes, has often been used as an out-group in phylogenetic studies, but recent data on Tamana bat virus suggest that it may be more distant (de Lamballerie et al., 2002), and thus a more appropriate choice as an outgroup for phylogenetic analyses. In addition, a novel insect flavivirus, *Culex flavivirus* (CxFV), has recently been reported from Japan which was found to be widespread in *Culex pipiens* and other Culex mosquitoes (Hoshino et al., 2007). It was able to replicate in cultured mosquito cells but not mammalian cells. Genetic sequencing revealed that CxFV is closely related to Cell fusing agent virus and Kamiti River virus, the other two members of the genus associated with mosquitoes.

Perhaps the more useful indication of relationships between flaviviruses can be obtained from phylogenetic studies which tend to agree with antigenic and vector/host relationships (e.g. Kuno et al., 1998; Poidinger et al., 1996; de Zanotto et al., 1996; Mackenzie et al., 2002a). A phylogenetic tree of the major members of the genus is shown in Fig. 1.

Flaviviruses have shown a significant propensity to emerge and establish in new geographic areas. This was first observed some 400 years ago when yellow fever virus spread from West Africa to the New World through the slave trade, but more recently has been seen with a number of other members of the family. Thus WN virus jumped from the Middle East to North America in 1999 (Jia et al., 1999; Lanciotti et al., 1999); Usutu virus spread from Africa to emerge in Austria as a disease of blackbirds (Weissenböck et al., 2002); and JE virus has spread from south-east Asia into Australasia, first into Papua New Guinea (Johansen et al., 2000) and then into the Torres Strait of Northern Australia (Hanna et al., 1996).

Flaviviruses of Southern, South-Eastern, and Eastern Asia and Australasia

There are at least 30 flaviviruses found in Southern, south-eastern and eastern Asia and Australasia which have been recognized and classified into 11 of the 12 recognized groups; 19 of the viruses are transmitted principally by mosquitoes, seven by ticks, and four have no known vector (Mackenzie, 2000). These viruses and their serological group are shown in Table 1, and the phylogenetic relationships of the Asian and Australasian viruses compared with other members of the genus are shown in Fig. 1, with the Asian and Australasian viruses depicted within boxes. In addition, a map showing the location of their initial isolation or detection is shown in Fig. 2. They include some major pathogens, such as JE, WN, MVE and the dengue viruses, whereas some of the other members have not been associated with disease in humans or animals. With the exception of the dengue viruses, all of the flaviviruses have an animal reservoir, and thus either are, or have the potential to be, zoonotic. While the dengue viruses may also be epizootic in their sylvan habitats in south-east and southern Asia, or West Africa, with non-human primates as their vertebrate hosts leading to occasional human infections (Rudnick, 1965; De Silva et al., 1999; Wolfe et al., 2001), dengue is now primarily an urban and peri-urban disease with humans as the

Fig. 1. Phylogenetic tree of the *Flavivirus* genus based on the NS5 gene. Major vector clades are indicated. Available sequences from a 1077 nt region of the NS5 gene were aligned using ClustalW and manually edited with BioEdit. Phylogenetic tree construction was performed using the Kimura 2-parameter model and the Neighbor-Joining method implemented in MEGA4 (Tamura et al., 2007). Tamana bat virus was used as the outgroup. Percentage bootstrap values from 1000 replicates are indicated with a cut-off value of 50%. The scale bar represents 0.1 nucleotide substitutions per site. Genbank accession numbers for the genomic sequences used in this analysis are bracketed next to the strain name. There was insufficient sequence available in Genbank for one virus from Asia and Australasia, Wesselsbron virus. TBE, tick-borne encephalitis.

Table 1. Mosquito-borne Flaviviruses of Southern, South-Eastern and Eastern Asia, and Australasia

Serological group	Virus	Region/country[a]	Year of first isolation[b]	Major vector species[c]	Major reservoir host	Disease[d]
Japanese encephalitis virus group	Japanese encephalitis (JE)	Asia and Australasia	1935	*Culex tritaeniorhynchus*	Pigs, ardeid	Encephalitis (also horses)
	Murray Valley encephalitis (MVE)	Australasia, Indonesia	1951	*C. annulirostris*	Ardeid birds	Encephalitis
	Alfuy virus	Australasia	1966	*C. annulirostris*	Ardeid birds?	None known
	West Nile (WN) incl. Kunjin	Asia and Australasia	1955 (India)/ 1960 (Aust.)	*C. vishnui* (India)/ *C. annulirostris* (Australia)	Birds Ardeid birds	Encephalitis/Febrile
Yellow fever virus group	Edge Hill	Australasia	1961	*Aedes* sp.	Marsupials	Febrile?
	Sepik virus	New Guinea	1966	*Ficalbia* sp.	Not known	Febrile
	Wesselsbron virus	Asia	1966		Not known	None known (Asia)
	Jugra virus	Asia	1968	*Aedes* sp.	Bats?	None known
Dengue virus group	Dengue virus 1	Asia and Australasia	1944	*A. aegypti*	Humans	Febrile/HF[e]
	Dengue virus 2	Asia and Australasia	1944	*A. aegypti*	Humans	Febrile/HF
	Dengue virus 3	Asia and Australasia	1956	*A. aegypti*	Humans	Febrile/HF
	Dengue virus 4	Asia and Australasia	1956	*A. aegypti*	Humans	Febrile/HF
Spondweni virus group	Zika virus	Asia, Micronesia	1966	*A. aegypti*	Not known	Febrile
Ntaya virus group	Tembusu virus	Asia	1957	*C. tritaeniorhynchus*	Birds?	None known
	ThCAr virus[f]	Asia	1992	*C. tritaeniorhynchus*	Not known	None known
	Sitiawan virus[f]	Asia	2000	Unknown	Chickens	Encephalitis (in chickens)
Kokobera virus group	Kokobera virus	Australasia	1960	*C. annulirostris*	Marsupials	Febrile
	Stratford virus	Australasia	1961	*A. vigilax*	Marsupials	None known
	New Mapoon virus[f]	Australasia	1998	*C. annulirostris*	Not known	None known

[a]Region or country in Southern Asia, Eastern Asia, and Oceania. Where viruses have also been isolated in other Continents, these are not included in this Table.
[b]Year of first isolation in Asia or Oceania, or where isolation details have not included a year of isolation, the year of the first reported isolation.
[c]Major vector species have been listed but in many cases, such as Japanese encephalitis virus, the dominant species may vary between regions and localities.
[d]Human disease most often associated with each virus. Where a virus is shown to cause encephalitis, it may present with febrile disease in milder cases. In some cases of febrile disease, a rash and joint or muscle pains may also occur.
[e]Haemorrhagic fever.
[f]These viruses have not yet been named officially or classified by the International Committee for the Taxonomy of Viruses.

reservoir hosts and transmission effected most often by *Aedes* sp. mosquitoes, particularly *Aedes aegypti*. Because the incidence of zoonotic transmission of dengue is probably relatively infrequent in most of the region, the dengue viruses will not be further discussed in this presentation.

Mosquito-borne Flaviviruses

The most important mosquito-borne zoonotic flaviviruses in South-East Asia and the Western pacific are members of the JE serological group (Mackenzie et al., 2002a), and especially JE, MVE, and WN (including Kunjin viruses) viruses. The importance of the other mosquito-borne flaviviruses found in Asia and Australasia as causes of human or animal disease are less well defined, although at least one, Wesselsbron virus, is a major animal and human pathogen in Africa. Details of the viruses are summarized in Table 1, with the year and region of isolation, the major vector species and vertebrate hosts, and association with disease shown for each virus species.

JE virus

JE virus is the major mosquito-borne encephalitic flavivirus in Asia (Table 1). It is believed to be responsible for more than 40 000 cases of encephalitis annually, with at least 10 000 deaths, although these figures are generally regarded as a significant underestimate, and the true disease burden might be closer to 175 000 cases annually (Tsai, 2000). With the near eradication of poliomyelitis, JE virus is now the leading cause of childhood viral neurological infection and disability in Asia (Halstead and Jacobson, 2003). Its geographic range extends from Japan, Korea and Maritime Siberia in the north, through China to the Philippines in the east, and through south-eastern

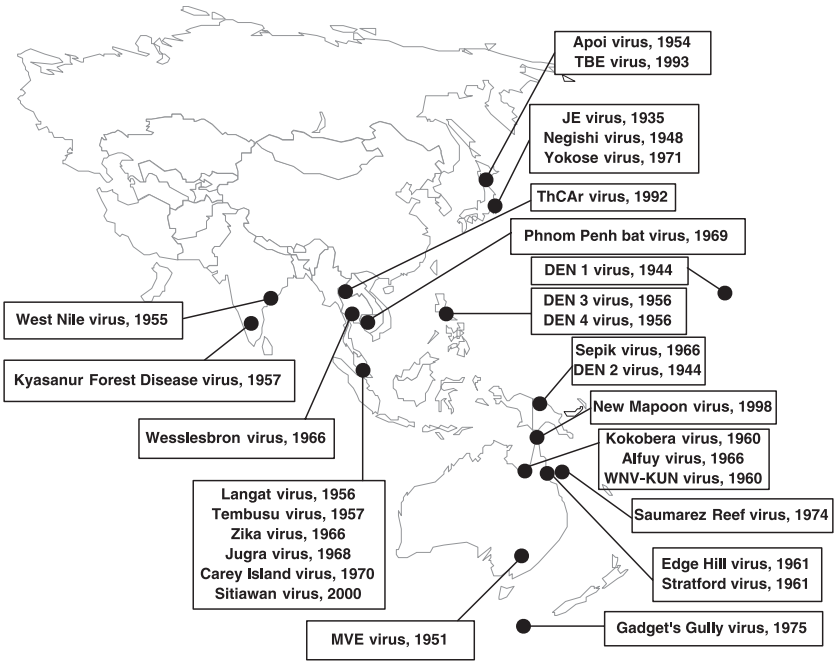


Fig. 2. First reported appearances of zoonotic flaviviruses of southern, south-eastern and eastern Asia, and Australasia.

and southern Asia to Indonesia, India and Sri Lanka (Umenai et al., 1985; Burke and Leake, 1988; Vaughn and Hoke, 1992; Endy and Nisalak, 2002; Mackenzie et al., 2007). In recent years, JE has spread westwards into Pakistan in 1994 (Igarashi et al., 1994), into Kerala State in southwestern India in 1996 (Dhanda et al., 1997), and eastwards into the Australasian region in 1995 and 1998 (Hanna et al., 1996, 1999; Ritchie et al., 1997; Johansen et al., 2000; Mackenzie et al., 2002b). Until the 1995 JE virus outbreak in the Torres Strait of northern Australia, and without good scientific reasons, the south-eastern limit of JE virus was believed to be restricted to the north and west of the Wallace Line, an imaginary line running between Bali and Lombok and separating the Oriental and Australasian zoogeographic regions, whereas MVE virus was believed to be restricted to the south and east of the line (Marshall, 1988; Mackenzie et al., 2002b). Much of the spread in Asia is believed to be due to deforestation for increased agriculture, and changes in land use with the large increase in rice fields and irrigated agriculture. JE is a zoonotic disease; the major vertebrate hosts for JE virus are pigs and ardeid birds, with pigs playing a central role in virus amplification. Indeed, pigs are often the drivers of epidemic activity, although epidemics can sometimes occur in their absence (Soman et al., 1977). The disease in pigs is relatively minor; transplacental transmission of JE virus is well established, leading to foetal encephalitis, abortion and stillbirth, and it also causes hypospermia and aspermia in boars (e.g. Takashima et al., 1988; Daniels et al., 2002). Ardeid birds are the maintenance hosts of JE virus, particularly Black-crowned night herons (*Nycticorax nycticorax*), Little egrets (*Egretta garzetta*), and Plumed egrets (*E. intermedia*), but the virus does not cause disease in birds. Pigs can also act as maintenance hosts in endemic areas. JE virus also causes encephalitis in horses, but as with humans, horses are 'dead-end' hosts and not involved in onward transmission (Ellis et al., 2000). Orangutans have also been implicated as possible vertebrate hosts in sylvatic cycles of JE transmission in Borneo (Wolfe et al., 2001). A number of mosquito species have been shown to be competent for transmitting JE, with *Culex tritaeniorhynchus*, *Cx. gelidus*, *Cx. vishnui*, and *Cx. annulirostris* being the most important species in various geographic locations.

Phylogenetic studies have suggested that at least four distinct genotypes of JE virus are circulating in Asia and northern Australasia (Fig. 3) (Chen et al., 1990, 1992; Ali and Igarashi, 1997), and there is limited evidence of a fifth genetic lineage isolated in Singapore (Uchil and Satchidanandam, 2001). Only one of these genotypes (G3) has been found in all regions encompassing the geographic range of JE virus, while G1 and G2 have been found in eastern and south-eastern Asia and northern Australasia. The circulation of the fourth genotype appears to have been restricted to Indonesia (Solomon et al., 2003).

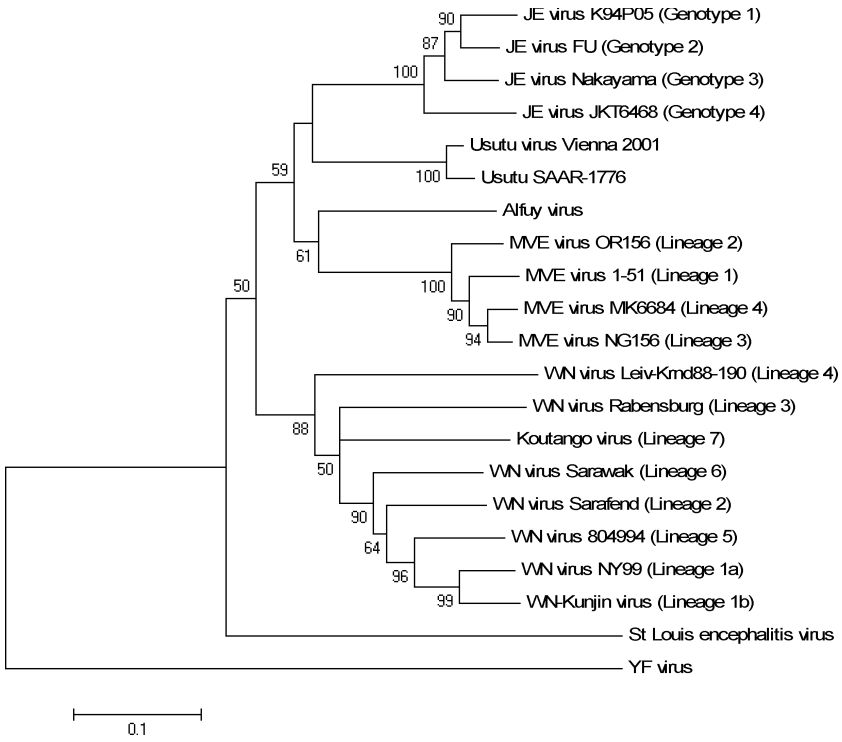


Fig. 3. Phylogeny of the Japanese encephalitis virus group based on available sequence information for the NS5–3′UTR (592 nt) genomic region. The Neighbor-Joining method was used for tree construction, as described in Figure 1. Yellow fever virus was used as an outgroup. The percentage bootstrap values from 1000 replicates are indicated with a cut-off value of 50%. The scale bar represents 0.1 nucleotide substitutions per site. Genotype or lineage is indicated in brackets. Distance-based maximum parsimony analysis also supported this analysis. Genbank accession numbers for the genomic sequences used in this analysis are: JE virus strains K94P05 (AF045551), FU (AF217620), Nakayama (EF571853), JKT6468 (AY184212); Usutu virus strains Vienna 2001 (AY453411), SAAR-1776 (AY453412); Alfuy virus (AY898809); MVE virus 1–51 (NC_000943); WN virus strains Leiv-Krnd88-190 (AY277251), Rabensburg (AY765264), Sarawak (L49311), Sarafend (AY688948), 804994 (DQ256376), NY99 (AF202541), Kunjin (D00246); Koutango virus (L48980); St Louis encephalitis virus (DQ525916); YF virus (X03700).

MVE and Alfuy viruses

MVE and Alfuy viruses are Australasian members of the JE serological complex (Mackenzie et al., 1994). MVE virus is the cause of Australian encephalitis and is the most important Australasian member of the complex. It is found in mainland Australia, New Guinea, and probably in the eastern Indonesian archipelago (Mackenzie et al., 1994; Spencer et al., 2001). It is endemic in northern Australia, especially in the Kimberley region of Western Australia and the north of the Northern Territory, and possibly endemic in parts of northern Queensland. It is epidemic in the Pilbara region of Western Australia, and very occasionally it spreads into south-eastern Australia where it causes major epidemics and from where the virus obtained its name. The two most recent epidemics in south-eastern Australia were in 1951 and 1974, but the virus also occurred in south-eastern Australia in 1956, and most recently, in 2008 although at the time of writing, no clinical cases have been reported. Since 1978, about 90% of human cases have occurred in the north-west of Australia, particularly the Kimberley and Pilbara regions, and in the Northern Territory, possibly due to the damming of the Ord River and the establishment of large areas of irrigated agriculture (Mackenzie and Broom, 1998). Indeed, the virus has been gradually moving further south in Western Australia, and is now within about 400 km of Perth in the south-west corner of the State (Broom et al., 2002). The major mosquito vector of MVE virus is *Cx. annulirostris*, although the virus has been isolated from a number of other mosquito species (Russell, 1995), some of which undoubtedly are involved in transmission cycles. The major vertebrate hosts are believed to be ardeid birds, of which the most important is the Nankeen night heron (*N. caledonicus*).

Limited sequence and antigenic analyses using monoclonal antibodies have suggested that at least four genetic lineages of MVE virus occur in Australasia (Fig. 3), two of which are found on the Australian mainland, lineages 1 and 2, and two in Papua New Guinea, lineages 3 and

4 (Coelen and Mackenzie, 1988; Poidinger et al., 1996; Johansen et al., 2007).

Alfuy virus has not been associated with human disease (Mackenzie et al., 1994), and is generally less neuroinvasive in murine experiments (May et al., 2006). Alfuy virus is also believed to utilize ardeid birds as its major reservoir host, and *Cx. annulirostris* as its major vector species. Recent antigenic, genetic and virulence studies have shown that it is closely related to MVE virus (Fig. 3), but is genetically, antigenically and phenotypically distinct (May et al., 2006). Indeed, these differences have led to the proposal that Alfuy virus be re-classified as a distinct virus within the JE virus group (May et al., 2006).

WN virus

WN virus is found in southern Asia, especially in India (Banerjee, 1996; Bondre et al., 2007; Mackenzie et al., 2007), and sporadically in South-East Asia, two examples being from Cambodia and Sarawak (Karabatsos, 1985; H.S. Hurlbut, unpublished data, cited by Simpson et al., 1970). Cases of WN encephalitis and febrile disease have been reported sporadically in India (Banerjee, 1996; Thakare et al., 2002; Mackenzie et al., 2007), which is a very different pattern to the epidemic activity displayed by JE virus. In Australasia, WN is found as Kunjin virus (Scherret et al., 2001; Hall et al., 2002), a genetically distinct sub-lineage of WN virus lineage 1. Kunjin virus can also cause encephalitis, Kunjin encephalitis, but the disease is generally milder than that caused by MVE virus. It can also cause a febrile illness, sometimes with polyarthralgia and polyarthritis (Hall et al., 2002). As described for MVE, the geographic range of Kunjin virus probably extends through New Guinea to parts of the eastern Indonesian archipelago, although this has only been indicated from serological data. Kunjin virus has also been reported from Sarawak, Borneo (Bowen et al., 1970), and Cambodia (J. Casals, personal communication to Bowen et al., 1970). Thus, the separate reports of WN virus and Kunjin virus in south-east Asia may be due to slight differences in the serological identification. However, sequence data have shown that a Sarawak strain of Kunjin virus is significantly different to other WN viruses (Scherret et al., 2001, 2002).

WN virus exists in a number of distinct genetic lineages and sub-lineages (Fig. 3). The strain of WN virus that recently emerged in New York, and the virus strains responsible for most of the major disease outbreaks, are members of WN lineage 1a, whereas the original isolate of WN virus from Uganda belongs to lineage 2 (Berthet et al., 1997; Lanciotti et al., 1999; Scherret et al., 2002). Within lineage 1, two additional sub-lineages has been recognized – lineage 1b which contained all the Australasian Kunjin viruses, and lineage 1c which comprised four Indian WN virus isolates (Lanciotti et al., 1999; Scherret et al., 2001). However, in a more recent detailed phylogenetic study, Bondre et al. (2007) have described five genetic lineages; lineages 1a, 1b and 2 remain as described but the Indian isolates previously comprising lineage 1c have been placed in a new lineage, lineage 5. In addition, two newly described flaviviruses from the Czech Republic and the Caucasus, Rabensburg (Bakonyi et al., 2005) and LEIV-Krns88-190 (Lvov et al., 2004) respectively, have been placed into lineages 3 and 4. The Sarawak Kunjin virus strain, however, is significantly different to the other Kunjin viruses, and it is suggested that this virus should be re-classified as lineage 6. Furthermore, the African virus, Koutango, is closely related to the WN virus lineages, and should perhaps be considered as a seventh lineage (Fig. 3).

The vertebrate hosts of WN virus are birds, and in the old world, especially ardeid birds. In Australasia, Kunjin virus shares vertebrate hosts and vectors with MVE virus; thus the vertebrate hosts and vectors are ardeid birds, especially the Nankeen night heron, and vectors, such as *Cx. annulirostris* mosquitoes, respectively. However, there appears to be ecological differences between Kunjin and MVE, with Kunjin spreading into southern Australia more frequently than MVE virus.

Tembusu, Sitiawan and ThCAr viruses

Tembusu virus was first described in 1957 from Kuala Lumpur, Malaysia (Table 1, Figs 1 and 2), where it had been isolated from *Cx. triteaniorhynchus*, *Cx. gelidus*, *Aedes linneatopennis*, and *Anopheles philippinensis* (cited in Karabatsos, 1985). It was subsequently isolated in Thailand from *Cx. gelidus*, *Cx. tritaeniorhynchus*, *Cx. vishnui*, and *Cx. sitiens* (P.K. Russell, personal communication in Karabatsos, 1985; Leake et al., 1986) and in Sarawak from *Cx. gelidus*, *Cx. pseudovishnui*, and *Cx. tritaeniorhynchus* (Simpson et al., 1970; Platt et al., 1975). The reservoir hosts of Tembusu remain unknown, but there has been serological evidence to suggest that birds and domestic chickens may act as reservoirs (Platt et al., 1975; Wallace et al., 1977). Tembusu virus has not been associated with human disease, but neutralizing antibody to the virus has been reported in human sera from Kuala Lumpur and Sabah indicating the occurrence of asymptomatic infections (Wallace et al., 1977; Karabatsos, 1985; Wolfe et al., 2001) and in Lombok, Indonesia (Olson et al., 1983), and Tembusu virus was shown to cause encephalitis in experimentally infected monkeys (C.E.G. Smith, unpublished results cited by Simpson et al., 1970).

Two other related viruses have been described, ThCAr virus (Pandey et al., 1999) and Sitiawan virus (Kono et al., 2000) (Table 1, Figs 1 and 2). Both viruses are

genetically related to Tembusu (Kono et al., 2000), but serological tests demonstrated that they were distinct subtypes of Tembusu. ThCAr virus was isolated from from *Cx. tritaeniorhynchus* mosquitoes trapped in Chiang Mai, Thailand, in 1992, but no disease associations have been observed (Pandey et al., 1999). Sitiawan virus was first described as a cause of encephalitis and retarded growth in broiler chickens in Perak State, Malaysia, in 2000 (Kono et al., 2000), but there has been no evidence of human infections.

Zika virus

Zika virus is a relatively common virus throughout most of Africa, and has been associated with occasional human disease consisting of fever with headache and rash (Karabatsos, 1985). In Asia, serological evidence of Zika virus infections had been reported from India, Thailand, Malaysia, Vietnam and Philippines, but the virus was not isolated until 1966 when it was obtained from *Ae. aegypti* mosquitoes in Malaysia (Marchette et al., 1969) (Table 1, Figs 1 and 2). It was subsequently implicated as a cause of fever in patients in Central Java (Olson et al., 1981), and neutralizing antibodies were found in human sera collected from Lombok (Olson et al., 1983). The complete genome sequence of Zika virus was reported recently (Kuno and Chang, 2007). Zika virus emerged unexpectedly in 2007 on the island of Yap and adjoining islands of Ulithi, Fais, Earpik, Woleai and Ifalik in the Federated States of Micronesia with over 150 cases (Press release from the Yap State EpiNet team, Yap State Department of Health Services, Colonia 96943, Yap State, FSM; Lanciotti et al., 2008). How and from where the virus moved to Yap State, remains unknown. Little is known about the reservoir hosts of Zika virus, although non-human primates have been implicated in Africa (McCrae and Kirya, 1982). Recent studies have suggested that non-human primates may also be reservoir hosts in Asia, especially orangutans (Wolfe et al., 2001; Kilbourn et al., 2003).

Wesselsbron and Jugra viruses

Wesselsbron virus, an important cause of disease in humans and livestock in Africa, causing abortion in sheep and cattle, was isolated in Thailand (T. Yuill and P.K. Russell, personal communication to Karabatsos, 1985) (Table 1, Fig. 2). A total of four isolates were obtained from *Aedes mediolineatus* and *Ae. lineatopeninis*, but these isolates appear to have been lost when stocks held at the Armed Forces Research Institute for Medical Science in Bangkok could not be revived. There have been no further reports of Wesselsbron virus in Asia, but it would have been interesting to determine whether these isolates were genetically closer to Sepik virus, a related virus found in Papua New Guinea. The Asian isolates of Wesselsbron were not sequenced, and there was insufficient sequence available in Genbank from the African isolates for inclusion in Fig. 1. Jugra virus (Table 1, Figs 1 and 2) was isolated in Malaysia from *Aedes* spp. and *Uranotaenaia* spp. of mosquitoes, and from the blood of the fruit bat, *Cynopterus brachyotis* (Karabatsos, 1985), but little is known about this virus.

Edge Hill virus

Edge Hill virus was first isolated in Cairns in 1961 (Table 1, Figs 1 and 2) (Doherty et al., 1963). It has subsequently been isolated from a range of mosquitoes trapped in north Queensland, New South Wales, the Northern Territory, and Western Australia, including *Cx. annulirostris*, *Ae. vigilax*, *Ae. normanensis*, *Ae. bancroftianus*, *An. amictus*, and *Coquillettidia linealis* (reviewed in Mackenzie et al., 1994; Russell, 1995; Macdonald et al., 2001). The major vertebrate hosts are believed to be marsupials (Doherty et al., 1964; Hawkes et al., 1985). A sero-epidemiological study in New South Wales has suggested that occasional human infections occur (Hawkes et al., 1985), and there has been one unconfirmed report implicating Edge Hill virus as the possible aetiological agent in a patient presenting with arthralgia, myalgia, and fatigue (Aaskov et al., 1993). Antigenic and genetic analyses have shown that distinct subtypes of Edge Hill virus exist in Western Australia and the Northern Territory (Macdonald et al., 2001).

Sepik virus

Sepik virus has only been isolated from Papua New Guinea (Table 1, Figs 1 and 2), although serological studies have suggested it might also occur elsewhere in the Indonesian archipelago. It was first isolated from *Mansonia septempunctata*, *Ficalbia flavens*, *Ficalbia* spp., and *Armigeres* spp. trapped in the Sepik district of Papua New Guinea (Woodroofe and Marshall, 1971; Karabatsos, 1985), and more recently, from *Cx. sitiens* subgroup mosquitoes trapped in Western Province of Papua New Guinea (Johansen et al., 2000; Nisbet et al., 2001). High neutralizing antibody titres in the convalescent serum of a New Guinea patient hospitalized with a febrile disease makes it a suspected human pathogen (Woodroofe and Marshall, 1971). In addition, from its close antigenic relationship to Wesselsbron virus and from seroepidemiological studies on Lombok (Olson et al., 1983), there is a suggestion that it might infect livestock. Sepik virus is the closest virus phylogenetically to yellow fever virus (Kuno and Chang, 2006), and is therefore particularly interesting on evolutionary grounds.

Kokobera, Stratford and New Mapoon viruses

Kokobera and Stratford viruses are the only two members of the Kokobera serological group of Flaviviruses (Thiel et al., 2005), and New Mapoon virus is a recently isolated member of the group (Table 1, Figs 1 and 2) (Nisbet et al., 2005). Both viruses had originally been classified as members of the JE serological group, but antigenic (Hall et al., 1991) and genomic sequence analysis (Poidinger et al., 1996) suggested that they should be reclassified into their own group. Further genomic studies demonstrated that Kokobera virus exists in several different genetic subtypes in different parts of Australia and Papua New Guinea (Poidinger et al., 2000). Kokobera virus is enzootic to Australia and Papua New Guinea. The virus was first isolated from *Cx. annulirostris* mosquitoes trapped at Kowanyama (Mitchell River Mission) in northern Queensland in 1960 (Doherty et al.,1963). Since that time, the virus has been isolated from various species of mosquitoes in Western Australia, Northern Territory, Queensland, New South Wales and Papua New Guinea (Mackenzie et al., 1994; Russell, 1995). Most recently, it has spread into the south-west of Western Australia (Poidinger et al., 2000). Serological evidence suggests that kangaroos and other macropods are the major wildlife reservoirs, although horses may also act as reservoir hosts (Doherty et al., 1964, 1971). Seroepidemiological studies in New South Wales, Queensland and Papua New Guinea have indicated that occasional human infections occur with Kokobera virus, and indeed, it was subsequently shown to be the cause of rare cases of acute polyarticular disease in Queensland, Victoria and New South Wales (Doherty et al., 1964; Hawkes et al., 1985, 1993; Boughton et al., 1986).

Stratford virus is enzootic in Australia and possibly in Papua New Guinea. It was first isolated from *Ae. vigilax* mosquitoes trapped in Cairns in north Queensland in 1961 (Doherty et al., 1963). Stratford virus has been isolated from several mosquito species, including *Ae. vigilax* in Queensland and New South Wales, and *Cx. annulirostris, Ae. procax* and *Ae. notoscriptus* in New South Wales. However, the role of each of these in transmission is unknown. Seroepidemiological studies carried out in New South Wales suggested that occasional human infections had occurred (Hawkes et al., 1985), but there has been no reported association with human disease.

Two other related viruses have been reported from Cape York, northern Australia. After monoclonal antibody analysis and sequencing studies, one of these viruses was deemed to be a distant subtype of Kokobera virus, but the other was shown to be a distinct yet related virus, and the name New Mapoon virus has been suggested (Nisbet et al., 2005).

Tick-borne Flaviviruses

Six tick-borne flaviviruses have been described from southern, south-eastern, and eastern Asia, and from Australasia (Table 2, Figs 1 and 2).

Two of the viruses were reported from Australasia, GGY virus and SRE virus. GGY virus was isolated from *Ixodes (Ceratixodes) uriae* ticks collected from 1975 to 1979 on Macquarie Island, in the southern Pacific Ocean, south of Tasmania (St George et al., 1985). Ticks were collected from areas inhabited by Royal penguins (*Eudyptes chrysolophus schlegeli*), however, disease association with seabirds has not been established and a limited serological survey of flavivirus antibodies in birds trapped on Macquarie Island was inconclusive (St George et al., 1985). Although GGY virus belongs to the mammalian tick-borne flavivirus group, it is associated with seabird transmission cycles. Further, GGY virus does not display the clinal geographic distribution reported for other mammalian tick-borne flaviviruses. It has therefore been suggested that it may constitute an evolutionary link between seabird-associated viruses and the more recently

Table 2. Tick-borne Flaviviruses of Southern, South-Eastern and Eastern Asia, and Australasia

Serological group	Virus	Region/country	Year of first isolation	Major vector species	Major reservoir host	Disease
Mammalian tick-borne virus group	Gadgets Gully	Macquarie Island	1975	*Ixodes uriae*	Birds (Penguins?)	None known
	Kyasanur Forest disease	India	1957	*Haemaphysalis spinigera*	Rodents, Monkeys	Haemorrhagic fever
	Langat virus	Southern Asia	1956	*I. granulatus*	Rodents	None known, but vaccine associated meningo-encephlaitis
	Negishi virus	Japan	1948	Not known	Not known	Encephalitis
	Tick-borne encephalitis (Far Eastern subtype)	Asia	1993 (Japan)	*I. granulatus*	Rodents	Encephalitis
Seabird tick-borne virus group	Saumarez Reef virus	Australia	1974	*Ornithodoros capensis*	Seabirds	None known

emerged mammalian-associated viruses (Grard et al., 2007). SRE virus was isolated in 1974 from soft ticks (*Ornithodoros capensis*) collected from the nests of seabirds, Sooty Terns (*Sterna fuscata*), on Saumarez Reef and Frederick Reef off the east coast of Queensland, and from hard ticks (*I. eudpyptidis*) collected from Silver Gulls (*Larus novaehollandiae*) in Tasmania (St George et al., 1977). This investigation was initiated following reports of febrile illness in meteorological workers operating on Saumerez Reef, who had been bitten by ticks. However, infection with SRE virus was not confirmed in these cases and association of SRE virus with human disease has not since been reported. A large sero-epidemiological survey of ~17 000 human sera collected from New South Wales also failed to find any evidence of human infection with either SRE virus, or GGY virus (Hawkes et al., 1985). Similarly, in a subsequent sero-survey of human and avian residents of the Coral Sea and the Great Barrier Reef, off the east coast of Queensland, no evidence for human infections with SRE virus was found, despite the presence of neutralizing (NT) antibodies in seabirds (Humphery-Smith et al., 1991). Interestingly, this study also revealed the presence of NT antibodies to GGY virus in equal proportions (4%) in the human and avian populations tested. The potential pathogenicity of SRE virus for seabirds was indicated by small scale experimental infections of little blue penguins (*Eudyptula minor*), which led to disease and mortality 9–13 days post-inoculation (Morgan et al., 1985). Further experimental and field investigations of SRE virus and GGY virus will be required to fully delineate the pathogenic potential of these viruses in avian and human populations.

Four tick-borne flaviviruses have been described from eastern, south-eastern and southern Asia. LGT virus was first isolated in Malaysia from *I. granulatus* hard ticks collected from two species of forest rats (*Rattus mulleri* and *R. sabanus*) (Smith, 1956). Subsequently, LGT virus was isolated in Thailand from *Haemaphysalis papuana* soft ticks associated with several species of forest rats (*R. rattus, R. fulvescens, R. niviventor, R. surifer*), implying that this virus may be widely distributed in forests throughout South-East Asia (Bancroft et al., 1976). No apparent human disease association has been found for endemic south-east Asian strains of LGT virus – a property that has led to the evaluation of the Malaysian TP21 strain as a vaccine candidate for TBE virus (Gritsun et al., 2003). However, in Russia during the 1970s, a small number of human cases of meningoencephalitis were observed in recipients of a live-attenuated LGT-based vaccine for TBE (reviewed in Gritsun et al., 2003). In Japan, Negishi virus – a mammalian tick-borne flavivirus – was first isolated in Tokyo from an encephalitis patient in 1948 (Ando et al., 1952). There was a second fatal case from Tokyo shortly after and human cases have been recognized in China. More recently, genetic characterization of Negishi virus indicated it is a strain of Louping ill virus (Venugopal et al., 1992). No further cases of Negishi virus infection have been reported and no endemic foci or tick vectors have been identified, and there has been speculation about its possible origin. The first human case of TBE caused by a subtype, Russian Spring Summer encephalitis virus, was reported in 1993 on the northern Japanese island of Hokkaido (Takashima et al., 1997), located in close proximity to Maritime Siberia where TBE virus is known to be prevalent. Further investigations on Hokkaido led to virus isolations from sentinel dogs, from *I. granulatus* ticks, and from small mammals (*Apodemus speciosus* and *Clethrionomys rufocanus*) (Takashima et al., 1997; Takeda et al., 1998, 1999), demonstrating endemic foci throughout the island. The Japanese strains of TBE virus are divided into three groups within the Far-eastern subtype and recent phylogenetic evidence indicated that transmission of the virus between Russia and Japan has occurred at least three times in the past 700 years – probably via the East Asian-Australian flyway of migratory birds (Suzuki, 2007). KFD virus is the major tick-borne virus from southern Asia. It emerged in 1957 in Karnataka State of southern India, and continues to be restricted to that State (Rodrigues, 1988; Pattnaik, 2006), although there is some suggestive evidence that silent transmission may also occur elsewhere in India and the Andaman and Nicobar islands (Pattnaik, 2006). It causes a prostrating human febrile disease with haemorrhagic and encephalitic manifestations and a case fatality rate of between 5% and 10% (Rodrigues, 1988; Pattnaik, 2006). Interestingly, a novel but closely related virus emerged in 1992 in Saudi Arabia, Alkhurma virus; over 60 human cases of Alkhurma haemorrhagic fever disease have been reported, mainly from Mecca and Jeddah, with several deaths (Zaki, 1997; Charrel et al., 2001; Pattnaik, 2006), demonstrating once again that novel pathogenic flaviviruses may emerge at any time.

Flaviviruses With no Known Vector

There are at least four flaviviruses reported from Eastern and South-Eastern Asia with no known vector (Thiel et al., 2005; Table 3, Figs 1 and 2). These include Apoi and Yokose viruses from Japan, Carey Island virus from Malaysia, Phnom Penh bat virus from Malaysia and Cambodia (Salaun et al., 1974; Karabatsos, 1985). An additional virus, Batu Cave virus, is believed to be a subtype of Phnom Penh virus.

Apoi virus was first isolated from rodents in 1954 in Japan. Isolates have been reported from *Apodemus speciosus* and *Ap. argentosus* hokkaidi, and from *Clethrionomys*

Table 3. Flaviviruses of Southern, South-Eastern and Eastern Asia, and Australasia, with No Known Vector

Serological group	Virus	Region/country	Host	Year of first isolation	Disease
Entebbe bat virus group	Yokose virus	Japan	Bats	1971	None known
Modoc virus group	Apoi virus	Japan	Rodents	1954	CNS symptoms
Rio Bravo virus group	Carey Island virus	Malaysia	Bats	1970	None known
	Phnom Penh bat virus (incl. Batu cave virus)	Cambodia, Malaysia	Bats	1969	None known

species. Apoi virus has been classified as a member of the Modoc virus group, and has only been found on the island of Hokkaido, Japan. Antibodies to Apoi virus have been found in humans and horses in Hokkaido, and the virus has been associated with human disease causing CNS symptoms and leg paralysis. The other three viruses have all been isolated from bats, and none have been associated with disease in either bats or humans (Karabatsos, 1985). Carey Island virus has been isolated from two species of fruit bat, *Macroglossus lagochilus* and *Cynopterus brachyotis*, in Peninsula Malaysia; Phnom Penh bat virus was first isolated from the salivary glands and brown fat of the *Cy. brachiotus angulatus* bats collected at the Chrui-Chang-War peninsula, Phnom Penh, Cambodia in June 1969, and from *Cy. brachyotis* and *Eonycteris spelaea* bats in Malaysia; and Yokose virus was isolated from an insectivorous bat, *Miniopterus fuliginosus* (Japanese long-fingered bat), caught on Kyushu Island, Japan, in 1971 (Karabatsos, 1985; Tajima et al., 2005). Carey Island and Phnom Penh bat viruses are species in the Rio Bravo virus group (Thiel et al., 2005), whereas Yokose virus is a member of the Entebbe bat virus group (Karabatsos, 1985; Kuno et al., 1998; Tajima et al., 2005). Interestingly, the Entebbe bat virus group members are not closely related phylogenetically to the other flaviviruses with no known vector, but are closely related to yellow fever virus and other related mosquito-borne viruses (Fig. 1). They can replicate in mosquito and mammalian cells (Kuno and Chang, 2006), so whether they can also be vectored by mosquitoes must remain a possibility.

Evolution and Phylogeny of Flaviviruses

The increasing availability of partial and full-length genomic sequence information for members of the Flavivirus genus have enabled the resolution of the phylogenetic relationships of these viruses. This work underlies current hypotheses on their origins, evolution and dispersal. Phylogenetic reconstructions have identified three distinct clades belonging to the mosquito-, tick-borne, and no known vector viruses (Zanotto et al., 1996; Kuno et al., 1998; Billoir et al., 2000; Gaunt et al., 2001; Fig. 1). Depending on the region of the genome analysed, the evolution of these groups can be explained by two conflicting theories: (i) the no known vector group diverged from an ancestor virus before the vector-borne viruses (NS5 gene), and (ii) the no known vector and tick-borne viruses evolved as a sister group from a common ancestor, with the mosquito-borne viruses arising independently (NS3 gene, complete open reading frame). The latter hypothesis has gained recent support from a comprehensive phylogenetic study by Cook and Holmes (2006), demonstrating that the NS3-based phylogeny provided statistically significant evidence of mosquito-borne viruses evolving as an outgroup to the remaining flaviviruses. In contrast, the NS5 data set did not contain sufficient phylogenetic signal to support either theory. Regardless, it is accepted that the acquisition of tick-borne transmission is a derived trait since all phylogenetic analyses show the tick-borne group to diverge from one of the other two flavivirus groups (Kuno et al., 1998; Billoir et al., 2000; Gaunt et al., 2001; Cook and Holmes, 2006).

Within the mosquito-borne group, viruses are sub-divided into *Aedes* and *Culex* clades, reflecting the predominant vector of the viruses in each group (Billoir et al., 2000; Gaunt et al., 2001). These groupings correlate with vertebrate host and disease syndrome. Thus, the *Culex* clade contains neurotropic viruses, such as JE and WN, associated with bird reservoir hosts, while viruses belonging to the *Aedes* clade, such yellow fever and dengue, associate with haemorrhagic disease and primate hosts (Gaunt et al., 2001). Two main clades have been identified for the tick-borne viruses, one associated with sea-birds and one associated with rodent hosts, the latter containing the TBE viruses. The no known vector viruses form three groups, of which one is associated with bats and two with rodent hosts (Kuno et al., 1998; Gaunt et al., 2001). Interestingly, the Entebbe Bat group of no known vector viruses clusters within the mosquito-borne group (Kuno et al., 1998) and is thought to indicate secondary loss of vector-borne transmission (Cook and Holmes, 2006), but may also reflect the failure to isolate mosquito vectors of these viruses.

There is compelling evidence to suggest that the flaviviruses have their origins in the Old World (Gaunt et al.,

2001; Gould et al., 2003). Thus, the earliest lineage of the mosquito-borne group is the *Aedes*-associated virus clade, from which the *Culex*-borne clade is descendent. Viruses from the *Aedes* group are thought to have African ancestry since the majority are found on this continent. Only yellow fever and the dengue viruses of the *Aedes* clade are found in the New World; their emergence there has been proposed to have occurred during the slave trade from the Old World to the Americas (Gaunt et al., 2001; Gould et al., 2003). Phylogenetic trees of the mosquito-borne viruses reveal a two-phase pattern in which a slow branching process is followed by rapid growth, with poor geographic structure and frequent lineage extinction (Zanotto et al., 1996). This was explained by the existence of mosquito-borne flaviviruses in low-prevalence endemic cycles up until recently (~200 years ago) when the availability of new and susceptible hosts, coupled with increasing worldwide movement and mixing of vectors, viruses and hosts led to a massive virus population expansion (Zanotto et al., 1996). Thus, in South-East Asia, some of the viruses of the JE group have been hypothesized to arise in the Indo-Malaysian region from an ancestral virus of African origin (Solomon et al., 2003; Gould et al., 2004). From this region, and aided by human activities promoting vector-bird transmission such as rice cultivation, dispersal and subsequent speciation has led to their current biogeography. Evidence of African ancestry of the JE group is observed by the close phylogenetic relationship of JE, MVE and Alfuy viruses of South East Asia and Australasia with the African virus Usutu (Fig. 3). Unlike other members of the JE group, WN virus is thought to have first emerged from Africa before spreading northward and eastward via migratory birds (Gould et al., 2003). How the South-East Asian types of West Nile became established is not fully understood, however, it has been hypothesised that Malaysian and Australian subtypes evolved from a common ancestor (Scherret et al., 2001). It is conceivable that this may have taken place in the Indo-Malaysian region, as proposed for JE virus (Solomon et al., 2003). It is notable that the African virus Koutango clusters within the WN virus group in phylogenetic reconstructions, indicating that this virus is a lineage of WN virus (Fig. 3). This may reflect the origin of the WN virus species; however, further sequence information for Koutango will be required to better establish the phylogenetic relationships of this virus.

Of the tick-borne flaviviruses, virtually all are found in the Old World. Phylogenetic analyses have provided evidence that the mammalian tick-borne viruses have evolved gradually along a geographical cline over the past 2000 years after an initial dispersal event from Africa (Zanotto et al., 1995, 1996; Gould et al., 2004). These viruses dispersed north and westwards across the forests of Asia and Europe from an ancestral lineage in the eastern part of its geographic range, with the more recent species occurring in the north-western end of its range. This contrasts with the pattern of evolution and dispersal of the mosquito-borne flaviviruses and is thought to reflect the lifecycle of ticks (Zanotto et al., 1996). For example, the tick generation time can be measured in months to years and direct virus transmission can occur between vectors during co-feeding on non-viraemic hosts, enabling viral lineages to survive for relatively long periods. While phylogenetic evidence indicates that the Far-eastern subtype of TBE virus found in Japan originated from Russia (Suzuki, 2007), the origins of other mammalian tick-borne flaviviruses listed in Table 2 are not clear. The seabird tick-borne viruses are associated with seabird ticks and have mainly been isolated in the Old World. In contrast with the mammalian tick-borne flaviviruses, there is no evidence of a close relationship between genetic evolution and geographic distribution (Grard et al., 2007). It is therefore assumed that seabird tick-borne flaviviruses have been disseminated by independent migratory flights and that their observed genetic evolution reflects adaptation to the different ecological niches they have reached (Grard et al., 2007). The emergence of the Australian seabird-associated viruses GGY and SRE may have thus occurred via migratory seabirds from the northern hemisphere. Indeed, St George et al. (1985) suggested that GGY virus represents a genetic link between subarctic and subantarctic tick-borne viruses based on the identification of the seabird species *Oceanites oceanicus* and *Sterna paradisaea*, which breed in the Arctic and migrate to the Antarctic and vice versa (respectively).

The no known vector viruses are found in either the Old World or the New World, implying that they were dispersed widely following their emergence (Gaunt et al., 2001; Gould et al., 2003). While bat-associated no known vector viruses are found in the Old World and New World, the rodent-associated no known vector viruses are found mainly in the New World, with the notable exception of Apoi virus, which is found in Japan. The latter distribution is consistent with a single dispersal event (such as bat migration) from the Old World followed by local infection of rodents and subsequently restricted distribution (Gaunt et al., 2001). Thus, the no vector viruses of South-East Asia (Table 3) are likely to have emerged from migratory bats and established localized transmission cycles in bat colonies or rodent populations.

Emergence and Spread of Flaviviruses

Flaviviruses have a strong predeliction to spread and establish in new areas, as recently described for WN virus

in the Americas (reviewed by Gubler, 2007), for JE virus in Papua New Guinea and the Torres Strait of northern Australia (reviwed by Mackenzie et al., 2007), and for Usutu virus in Austria (Weissenböck et al., 2002), and subsequently elsewhere in Europe, including Spain (Busquets et al., 2008), Hungary (Bakonyi et al., 2007), and Italy (Rizzoli et al., 2007). This latter spread is particularly interesting as Usutu virus is one the closest viruses phylogenetically to MVE virus. Thus known flaviviruses may emerge in a new locality unexpectedly and sometimes over long distances, probably due largely to the migratory habits of avian vertebrate hosts.

However, over the past decade, a number of new, previously unrecognized flaviviruses have also been reported, including Rabensburg virus in the Czech Republic (Bakonyi et al., 2005), Alkhurma virus in Saudi Arabia (Zaki, 1997; Charrel et al., 2001; Pattnaik, 2006), Sitiawan virus in Malaysia (Kono et al., 2000), ThCAr virus in Thailand (Pandey et al., 1999), LEIV-Krns88-190 in the Caucasus region (Lvov et al., 2004; and New Mapoon virus in Australia (Nisbet et al., 2005). It is difficult to assign a specific reason for the emergence of these novel viruses, and in most cases their detection has been due either to a newly recognized disease entity, as described for Akhurma and Sitiawan viruses, or serendipity through the field collection of arthropods for studies of other and often related viruses. As an example, Rabensburg virus was isolated from mosquitoes trapped during a flood in 1997 near the Czech-Austrian border, and although cases of febrile illness due to WN virus had been reported previously from the Czech Republic, there had been no disease outbreak at the time of collection (Bakonyi et al., 2005). New hosts may also lead to new genetic variants, as seen with LEIV-Krns88-190 which was isolated from ticks rather than the more usual mosquito vectors. With the ease with which these viruses have been detected, it is probable that many other flaviviruses exist and remain to be discovered. A number of factors may contribute to the risk or potential of emergence, including changes in land use, especially if involving new ecological niches, increasing travel and trade, increasing urbanization and population density, and changes to climate and weather through global warming. Many of these factors have been important in national developments in the Asia-Pacific regions, especially the changes in land use, including deforestation, to provide increased agricultural land for rice growing, and increased urbanization and population density giving rise to mega-cities. The strong inter-dependence of mosquito-borne virus ecology with climatic factors would suggest that the global warming and climatic changes may have a significant affect on the potential for new virus emergence and for the increased spread and incidence of many known viruses. Rabensburg virus may be viewed as an example of this as it was first described following severe flooding. Thus, new previously unrecognized flaviviruses may arise anywhere and at anytime, and there is little doubt that with these viruses we should expect the unexpected.

Conclusions

As shown in this brief review, the genus Flaviviridae consists of a number of viruses, some of which are associated with severe human disease including encephalitis and haemorrhagic fever, some with mild, febrile disease, but most have no known association with disease in either humans or animals. The members of the genus have a well recognized propensity to spread and establish in new areas, and although their characteristics are well defined, they are still unpredictable and may exhibit increases in disease severity, unusual clinical manifestations, unexpected methods of transmission, and long-term persistence (Gould and Solomon, 2006). Furthermore, and as exemplified by New Mapoon in Australasia, by Sitiawan virus in southern Asia, by Alkhurma virus in Saudi Arabia, and by Rabensburg in the Czech Republic, new flaviviruses continue to be found. Thus as a genus, they are well suited to be the source of new, zoonotic emergent diseases.

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