Flaviviruses

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Flaviviruses are a group of arthropod-borne viruses that have demonstrated a propensity to spread and establish in new geographic areas. This invasiveness of flaviriruses is due to the complex interplay of a number of different factors, some of which are properties of the viruses, and others that are the consequences of human activities. The viral factors include the ability of some members of the genus to use a wide spectrum of vector mosquito species and vertebrate host reservoirs, especially when establishing in new ecosystems, their ability to survive and replicate in vectors and hosts under a variety of environmental conditions, and the ability of some members to be transported between regions and continents through migratory bird hosts. Human activities are a major component of the interplay, and include the dispersal and spread of the viruses in infected mosquitoes and/or infected passengers on ships and planes, by commercial transport of animals and goods, and by providing new mosquito breeding sites through changes in land use such as in newly-developed rice paddies or areas of irrigated agriculture, or in urban shanty-towns. Also important has been the spread of major mosquito vector species and their subsequent colonization of new areas, as seen by the widespread global infestation of *Aedes albopictus* on all continents, frequently through the international trade in old automobile tires, and of *Ae. aegypti* mosquitoes which may move between regions and continents in containers on cargo ships or in aircraft holds. The latter is perhaps the most important vector mosquito species,

and its international spread has probably been the single most influential factor in the spread of yellow fever and the dengue viruses.

I. The biology of flaviviruses

Flaviviruses are small enveloped, positive single-strand RNA viruses which comprise one of the three genera within the family *Flaviviridae*. There are approximately 55 species in the genus *Flavivirus* and 70 recognised virus strains. Most members are transmitted by arthropod vectors, either mosquitoes or ticks, although a few have no known vector. The members of the genus and the genetic relationships are depicted in the Figure. The type-species of the genus is yellow fever virus, but the genus also contains a number of important human and animal pathogens, including dengue viruses, Japanese encephalitis, West Nile, St Louis encephalitis, and tick-borne encephalitis viruses. The other two genera in the family *Flaviviridae* are *Pestivirus* and *Hepacivirus*, the former containing species such as classical swine fever virus (or hog cholera virus) and bovine viral diarrhea virus, and the latter containing a single species, hepatitis C virus. The members of these two genera, however, do not require or utilise arthropod vectors for their transmission.

Most members of the *Flavivirus* genus exist in natural transmission cycles between a vertebrate host and an arthropod vector. The viruses can replicate in the cells of both host and vector and are transmitted to new hosts through the “bite” of the arthropod vector. Recent examples of the invasiveness of flaviviruses have been mosquito-borne viruses, three of which are described in more detail below. The female mosquito becomes infected with a flavivirus when it takes a blood meal from an infected vertebrate host, a requirement in its life cycle to enable it to lay eggs. The virus then replicates in the cells of the mosquito, eventually reaching

the cells of the salivary glands. Virus is then transmitted to the next vertebrate host in the saliva of the female mosquito when she bites the next host to acquire a further blood meal. There are two major groups of mosquito-borne flaviviruses, those with mammals as their primary vertebrate hosts and using *Aedes* spp. mosquitoes as their major vectors, and those with birds as their main vertebrate hosts and predominantly using *Culex* spp. mosquitoes as their vectors. The former include major pathogens such as yellow fever and the dengue viruses, both of which have jungle or forest mosquito-monkey transmission cycles, but which have also evolved human-mosquito urban transmission cycles, whereas the latter include Japanese encephalitis and West Nile viruses, two major encephalitic viruses, which use a variety of avian species. These viruses clearly demonstrate the invasive potential of flaviviruses, and their ability to establish subsequently in new areas using local mosquito species and vertebrate hosts, but they also demonstrate the different ways in which they invade new geographic niches. As examples, yellow fever, West Nile and Japanese encephalitis are described in more detail below.

II. Examples of the spread of mosquito-borne flaviviruses

A. Yellow fever virus – the type species of the genus *Flavivirus*

Historically, the most important member of the genus has been tellow fever virus, which is believed to have been spread on sailing vessels from its original home in the forest canopies of West Africa to the Americas by the slave trade during the period between the late 16th Century to the middle of the 19th century. It is hypothesised that the initial events may have been the transport of the anthropophilic mosquito vector, *Ae. aegypti* mosquito, to the New World on sailing ships where it probably rapidly became established. Subsequently, it is believed the virus was taken to the New World through mosquito-human transmission cycles on the sailing ships,

with mosquitoes breeding in the fresh water barrels and transmitting virus between the sailors and/or slaves on board. Many explosive outbreaks of yellow fever, a hemorrhagic illness with significant mortality, were recorded when these vessels reached ports in the Americas and Europe. Thus yellow fever has long demonstrated the propensity to spread and colonise new geographic areas, which has been a feature of other members of the genus. The natural sylvatic transmission cycle of the virus is in the forest canopy between monkeys as vertebrate hosts and *Aedes* spp. mosquitoes, usually referred to as jungle yellow fever, but the virus also occurs in urban mosquito-human transmission cycles with *Ae. aegypti* being the vector mosquito species These urban transmission cycles can result in explosive outbreaks with high mortality, especially in Africa, where annual cases can approach 200,000 with as many as 30,000 deaths. In South and Central America, the sylvatic cycle is maintained between *Haemagogus* spp. and *Sabethes* spp. mosquitoes and monkeys, but unlike the transmission cycles in Africa, American monkeys often die from the infection, probably because there has been insufficient time for adaptation to the virus since its relatively recent introduction. There has been little or no urban yellow fever for many decades in South and Central America, and almost all recent cases have been sporadic infections in jungle settings, but with the widespread infestation of *Ae. aegypti* mosquitoes in urban areas and around human habitation throughout much of Central and South America north of latitude 35^{o}S, there is a continuing threat of a major urban yellow fever outbreak in the region, which could have catastrophic consequences. Thus the invasiveness of yellow fever virus has been due largely to a dependence on human activities for the virus to move between continents and to the spread of *Ae. aegypti* mosquitoes, providing a very efficient vector for urban transmission. An excellent vaccine is readily available, the 17D live virus vaccine, which is

relatively inexpensive, and there is a continuing discussion about whether it should be included in the expanded program of immunisation of affected countries.

B. West Nile virus

West Nile virus, first isolated in Uganda in 1937, has long been a pathogen of humans and animals (some domestic bird species and horses) in Africa, parts of Europe, the Middle East, India, and Australia (where it was called Kunjin virus). It was responsible for outbreaks of relatively mild, febrile disease but with occasional cases of severe and sometimes fatal neurological disease. However, from 1994, it began to cause outbreaks of severe neuroinvasive disease in humans and horses in Europe, especially in Romania and Eastern Russia, and the Mediterranean basin including Israel, Italy, Morocco and Algeria. In 1997 and 1998, West Nile virus was reported for the first time as the cause of a neurological illness and death in geese in Israel. Phylogenetic studies of a range of isolates has suggested that there are frequent re-introductions of virus from Africa into Europe, probably by way of the Middle East, through the movement of migratory birds such as white storks (*Ciconia ciconia*). West Nile virus was detected in the New World for the first time in New York in 1999 where it caused 59 cases of encephalitis in humans, of whom 7 died, and the death of a number of different exotic bird species in the Bronx Zoo. Over the following years it dispersed throughout the U.S. and contiguous provinces of Canada, causing significant morbidity and mortality in humans and horses, an extensive and unprecedented avian mortality, and eventually becoming enzootic in all states except Alaska and Hawaii. The rapid spread was due in part to the large number of avian species able to act as vertebrate hosts and the wide range of mosquito species able to vector the virus, both probably exacerbated by the emergence of the virus in a novel ecosystem, thus providing a wealth of potential transmission cycles. West Nile virus has been responsible for

over 30,000 human cases with approximately 1300 deaths and for more than 27,500 equine infections since its arrival in North America. The equine infections would have been much greater if vaccines had not been developed and available from 2003. More recently the virus has spread southwards into Central and South America and the Caribbean, but its virulence seems to have significantly diminished in these areas. How West Nile virus reached North America still remains to be determined. The most likely scenario, however, is through an infected mosquito hitching a lift on board an aircraft.

C. Japanese encephalitis virus

Japanese encephalitis virus, the most important cause of viral encephalitis in southern and eastern Asia, is believed to be responsible for over 40,000 cases of encephalitis and 10,000 deaths annually, but these figures are generally regarded as a significant underestimate and the true figure may be closer to 175,000 cases of encephalitis annually. The natural transmission cycle of Japanese encephalitis virus alternates between marsh birds as the maintenance vertebrate hosts, particularly members of the Order *Ciconiiformes*, and Culicine mosquitoes, with domestic pigs being important amplifying hosts in both enzootic and epidemic situations. Recent phylogenetic analyses have indicated that Japanese encephalitis virus probably originated from an ancestral flavivirus in the Indonesia-Malaysia region, where it evolved into different genotypes that then spread out across Asia. Epidemics of encephalitis have been reported in Japan since the 1870s, and Japanese encephalitis virus was first isolated in 1935. Over recent decades, the spread of the virus may have been aided by deforestation and changes in land use, especially the development of rice paddy fields, which attract ardeid birds and provide conditions for culicine mosquito breeding, thus promoting the ideal ecological conditions for virus

transmission. In addition, as rice paddies are usually close to villages where pigs are raised, the environment has been ideal for epidemic development. However, phylogenetic studies have indicated that virtually identical strains of virus can be isolated many miles apart, indicating that the virus is dispersed frequently and rapidly by migratory birds. The most recent expansion of Japanese encephalitis virus into new areas has been into the eastern Indonesian archipelago in the early 1980s, Papua New Guinea in the late 1980s, Pakistan in the early 1990s, and the Torres Strait of northern Australia in 1995. Interestingly, there has been one human case in mainland Australia that has been associated with wind-blown mosquitoes from Papua New Guinea during a cyclonic weather pattern, a meteorological feature that is predicted to increase under climate change. An inactivated cell culture-derived vaccine is now available, and a live attenuated vaccine is used in China and most recently in India. In addition, a chimeric vaccine based on the yellow fever 17D genome but with the Japanese encephalitis envelope protein is undergoing clinical trials.

III. Future concerns: how and where will flaviviruses emerge next?

These examples of the way mosquito-borne flaviviruses can spread and colonise new areas provide a number of lessons that may be useful in understanding how these and other viruses might spread in the future. They demonstrate the invasive characteristics of flaviviruses and the interplay of biological and environmental factors with human activities that potentiate and assist their spread and establishment in new geographic regions. In addition, the effects of global warming might provide the warmth and humidity in southern and central Europe for increased epidemic activity of West Nile and possibly dengue, and the conditions potentiating further spread into new regions, such as northern Europe, and for dengue and even yellow fever into

North America. There is also the possibility of Japanese encephalitis virus spreading east across the Pacific into California.

See Also the Following Articles

Mosquitoes; Pathogens, Animal; Pathogens, Human

Glossary

arthropod-borne viruses viruses that are transmitted by ticks, mosquitoes, midges and sand-flies and that are able to replicate in the arthropod prior to transmission.

ardeids herons and egrets in the family Ardeidae (Order: Ciconiiformes).

Ciconiiformes an avian order of marsh birds including herons, egrets, bitterns, storks, ibises and spoonbills.

encephalitis inflammation of the brain.

febrile relating to or affected by fever.

hemorrhagic loss of blood or bleeding.

neurologic pertaining to the nervous system, including the brain, spinal cord and peripheral nerves.

sylvatic affecting only wild animals.

Further Reading

Blitvich, B.J. 2008. Transmission dynamics and changing epidemiology of West Nile virus. *Animal Health Research Reviews* 9: 71-86.

Bryant, J.E., E.C. Holmes, and A.D. Barrett. 2007. Out of Africa: a molecular perspective on the introduction of yellow fever virus into the Americas. PLoS Pathogens 3(5):e75.

Gould, E.A. and T. Solomon. 2008. Pathogenic flaviviruses. *Lancet* 371: 500-509

Gould, E.A., X. de Lamballerie, P.M. Zanotto, and E.C. Holmes. 2001. Evolution, epidemiology, and dispersal of flaviviruses revealed by molecular phylogenies. *Advances in Virus Research* 57: 71–103.

Gubler, D.J., G. Kuno, and L. Markoff. 2007."Flaviviruses". Pp.1153-1252 in *Fields Virology*, Volume 1, 5th Edition, eds.D. Knipe and P.M. HowleyPhiladelphia, Lippincott Williams & Wilkins.

Holmes, E.C. and S.S. Twiddy. 2003. The origin, emergence and evolutionary genetics of dengue virus. *Infection, Genetics and Evolution* 3:19-28

Kyle, J.L. and E. Harris. 2008. Global spread and persistence of dengue. *Annual Reviews of Microbiology* 62:71-92.

Mackenzie, J.S., D.J. Gubler, and L.R. Petersen. 2004. Emerging flaviviruses: the spread and resurgence of dengue, Japanese encephalitis and West Nile viruses. *Nature Medicine* 10(12): S98-S109.

Wilder-Smith, A. and D.J. Gubler. 2008.Geographic expansion of dengue: the impact of international travel. Medical Clinics of North America 92: 1377-1390.

Figure Caption

Figure 1. A phylogenetic tree of the *Flavivirus* genus based on genetic sequences from the NS5 gene, showing all major members of the genus except for Wesselsbron virus, a virus causing livestock disease and occasional human infections in Africa for which sequences in the NS5 gene were unavailable. Major vector clades are indicated. The three viruses described in the text, West Nile, Japanese encephalitis, and yellow fever viruses, are shown boxed. 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. TBE= tick-borne encephalitis. (This figure is based on the phylogenetic tree from J.S. Mackenzie and D.T. Williams (2009) Flaviviruses of Southern, South-Eastern and Eastern Asia, and Australasia: the potential for emergent viruses. *Zoonoses and Public Health* 56: 338–356.)