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Mosquito-Borne Arboviral Surveillance and the Prediction of Disease Outbreaks

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1. Introduction

Mosquito-borne viruses (arboviruses) have the capacity to cause widespread epidemics in humans, as well as epizootics in domestic animals and wildlife. These epidemics and epizootics can be extremely costly and disruptive. Because most mosquito-borne arboviruses can cause encephalitis and encephalomyelitis in host vertebrates, healthcare issues associated with human infections can result in significant economic impact on local economies and health care providers (Villari et al., 1995). Likewise, the loss of domestic animals to encephalitis infection can produce significant adverse financial impacts on local agricultural industries (Wilson et al., 1986; Anon, 2003). Recent mosquito-borne epidemics caused by West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) in North America and Chikungunya virus (family *Togaviridae*, genus *Alphavirus*, CHIKV) in the islands of the Indian Ocean are examples of how mosquito-borne viruses can severely impact local, regional, and national economies.

All mosquito-borne virus disease transmission cycles are driven by four factors: 1) the annual cycle of the pathogen (the specific arbovirus), 2) the annual cycle of the mosquito vector, 3) the annual cycle of the amplification, reservoir, and secondary vertebrate hosts that are infected with a specific viral pathogen, and 4) the environmental factors that drive each biological cycle independently and at times cause these three cycles to synchronize in ways that produce epidemics and epizootics (Day, 2001). Because three biological cycles, pathogen, vector and host, inform the transmission dynamics of a specific arboviral disease, surveillance protocols have been developed to monitor each of these cycles in order to estimate the local risk of arboviral transmission for a specific region. For example, it is possible to use sentinel animals to detect and measure the spatial and temporal distributions of specific arboviruses. Sentinel chickens, pheasants, quail, pigeons, and hamsters have been successfully used to monitor the transmission of St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, SLEV), WNV, eastern equine encephalitis virus (family *Togaviridae*, genus *Alphavirus*, EEEV), and Venezuelan equine encephalitis virus (family *Togaviridae*, genus *Alphavirus*, VEEV). Likewise, competent mosquito vectors can be identified for each mosquito-borne arbovirus transmission cycle. Once regional mosquito vectors have been identified, their populations can be tracked and important factors such as abundance, population age structure, and infection status can be monitored and used to

assess disease transmission risk. Similarly, the age structure and infectivity status of known amplification hosts can be monitored and used to measure arboviral transmission risk (Day and Stark, 1999). Finally, the environmental conditions that influence vector and amplification host population structures can be tracked and used to predict how and when vector, amplification host, and viral populations will synchronize and escalate to a point where epidemic disease transmission is inevitable (Shaman and Day, 2005; Day and Shaman, 2009).

A thorough knowledge of the three biological cycles (virus, vector, and amplification host) and the environmental factors that drive these cycles can allow the skillful prediction of when and where mosquito-borne arboviral disease outbreaks will occur (i.e. spatio-temporal disease transmission patterns). In this chapter we present a detailed analysis of the disease transmission cycles of SLEV, WNV, and EEEV in Florida, USA and WNV in Colorado, USA. We use these examples to illustrate how an intimate knowledge of the biotic cycles and the abiotic drivers of these biological cycles facilitate the accurate prediction of regional and local disease outbreaks. For example, SLEV was first isolated during an epidemic of human encephalitis in St. Louis, MO in the summer of 1933. Since 1933 at least 20 SLE epidemics have been reported in North America. In south Florida an SLE epidemic developed in 1990, lasted from August 1990 through January 1991, and resulted in 226 clinical cases and 11 deaths. A complete understanding of the SLEV transmission cycle in south Florida, including the mosquito and avian hosts responsible for SLEV amplification early in the summer of 1990, enabled prediction of epidemic transmission (an unusually elevated level of virus transmission to humans) eight weeks before the first human case was reported (Day and Lewis, 1992). In theory, all mosquito-borne encephalitis epidemics should have distinct biological and physical signatures that enable prediction of the spatio-temporal distribution of arboviral transmission risk prior to the appearance of human cases. To facilitate such real-time monitoring and risk prediction it is critical that these signatures are identified.

Epidemic prediction of vector-borne encephalitis diseases should be a priority for local vector control and public health programs. The development of systems for the skillful forecast of epidemic arboviral transmission is a desirable and attainable goal. Such forecasts help to minimize the impact of these dangerous disease agents on humans, domestic animals, and wildlife. Furthermore, the accurate early warning of impending arboviral epidemics allows increased vector control efforts and increased public awareness in the areas of highest risk for virus transmission. These predictive factors may allow vector control and public health officials the luxury of mitigating a potential arboviral epidemic before large numbers of humans and domestic animals are infected.

2. Biotic and abiotic factors associated with arboviral transmission cycles

Mosquito-borne viral cycles depend on the interaction of four distinct agents; three are biological (biotic) and one is a group of environmental factors (abiotic) that directly influence the biotic cycles. Under certain environmental conditions the three biotic cycles interact in ways that enhance viral abundance resulting in increased viral transmission. Depending on the exact local environmental conditions, viral transmission can be focal, sporadic, or epidemic. Focal transmission is constrained spatially and temporally and produces a localized outbreak of virus transmission. Sporadic transmission occurs intermittently over a broader geographic area than focal transmission but is often less locally intense. Epidemic transmission occurs when virus is transmitted intensely over a wide geographic area for an extended timeframe. These distinctions are somewhat qualitative but

provide a loose framework for assessing the distribution and duration of an outbreak of arbovirus transmission to humans and domestic animals.

There are a number of well-known mosquito-borne arboviruses. These include yellow fever virus (family *Flaviviridae*, genus *Flavivirus*, YFV), dengue viruses (four serotypes in the family *Flaviviridae*, genus *Flavivirus*, DENV), and the mosquito-borne encephalitis viruses including WNV, SLEV, and EEEV. Mosquito-borne arboviruses are usually restricted to specific habitats and are seasonally abundant with transmission corresponding to the abundance of competent vector species.

Mosquito vector species are generally matched to specific arboviruses in different regions of the world. For example, DENV (serotypes 1, 2, 3, and 4) are most commonly transmitted by *Aedes aegypti* (Linnaeus) in the tropics and subtropics around the world. However, a second DENV vector, *Aedes albopictus* (Skuse), has been responsible for recent epidemics in Hawaii (Kolivras 2006) and Singapore (Ooi et al. 2006). Likewise, WNV was introduced into North America in 1999 and rapidly spread across the continent (Marfin and Gubler 2001). In different parts of North America, WNV encountered and exploited different mosquito species that became the dominant vector for the virus in that particular part of the continent. *Culex pipiens pipiens* Linnaeus became the primary WNV vector in the north-eastern quarter of the continent, while *Cx. pipiens quinquefasciatus* Say became one of the primary vectors in the southern half of the continent. In Florida, the primary WNV vector is *Cx. nigripalpus* Theobald, and in the western half of the continent the primary WNV vectors are *Cx. pipiens quinquefasciatus* and *Cx. tarsalis* Coquillett.

The ability of a mosquito vector to acquire and then transmit a virus is referred to as vector competence (Hardy et al. 1983, Tabachnick 1994). To function as a competent mosquito vector, ingested virus must be able to escape the mosquito midgut, infect other organs, replicate, and eventually infect the mosquito's salivary glands from where it is then transmitted with each subsequent probe or blood meal. Intrinsic factors in all mosquitoes regulate viral escape from infected organs, the penetration of virus into new organs, and viral replication within those organs (Black et al. 2002). Extrinsic factors including the amount of virus ingested by the mosquito with its first infective blood meal and ambient temperature, which determines how quickly the virus replicates in the mosquito and infects the salivary glands (a process known as the extrinsic incubation period (EIP)), also influence mosquito vector competence (Anderson et al. 2010, Richards et al. 2009, 2010). Most mosquito species are poor arboviral vectors, primarily because of intrinsic and extrinsic factors that disfavor viral replication and mosquito infection processes. For example, there are currently 80 mosquito species known to occur in Florida. However, only two of these (*Cx. nigripalpus* and *Cx. quinquefasciatus*) serve as epidemic vectors of WNV in the state.

There are three types of vertebrate hosts involved in arboviral transmission cycles: amplification hosts, reservoir hosts, and dead-end hosts. Once infected, amplification hosts produce a viremia (virus in the circulating blood) at levels (titers) sufficient to infect competent vector mosquitoes. In the case of SLEV, wild birds serve as amplification hosts. Efficient amplification hosts need to be susceptible to viral infection, capable of producing a high level viremia, spatially and temporally abundant, and easily accessible to vector mosquitoes. Many species of wild birds are susceptible to infection with SLEV; however, few wild avian species serve as efficient amplification hosts for the virus, primarily because most avian species produce a viremia insufficient to infect vector mosquitoes. Viral titers above 4.0 logs (10,000 viral particles (virions) per millilitre of blood) are usually necessary to infect mosquitoes. In addition, efficient amplification hosts need to be abundant and have a

wide spatial distribution that insures frequent contact with infected and susceptible mosquito vectors. For instance, rare avian species may be infected, develop high viremias, and infect mosquitoes, but due to low prevalence in the environment, their mosquito contact rate is low and they are unlikely to infect mosquitoes at the levels necessary to sustain epidemic arboviral transmission. The major avian hosts involved in the amplification of SLEV in south Florida are Northern Cardinals, Common Grackles, Blue Jays, and Mourning Doves (Day and Stark 1999). These four avian species share three important characteristics that typify superior arboviral amplification hosts: 1) they are susceptible to SLEV infection, 2) they circulate virus at high enough titers to infect mosquito vectors, and 3) they are seasonally abundant and widely dispersed. This broad distribution and seasonal abundance ensures that these avian species have recurrent contact with mosquito vectors, which increases the probability that SLEV will be acquired by uninfected mosquitos from infected birds and that infected mosquitoes will contact susceptible birds and transmit SLEV. This cascade of viral transmission between infected mosquitoes and susceptible birds and, conversely, between infected birds and susceptible mosquitoes results in the amplification of virus in nature (Figure 1). As will be explained later in this chapter, the efficient amplification of an arbovirus, i.e. intense zoonotic transmission that increases arbovirus prevalence among vector mosquitoes and avian hosts, is a prerequisite for arboviral epidemics.
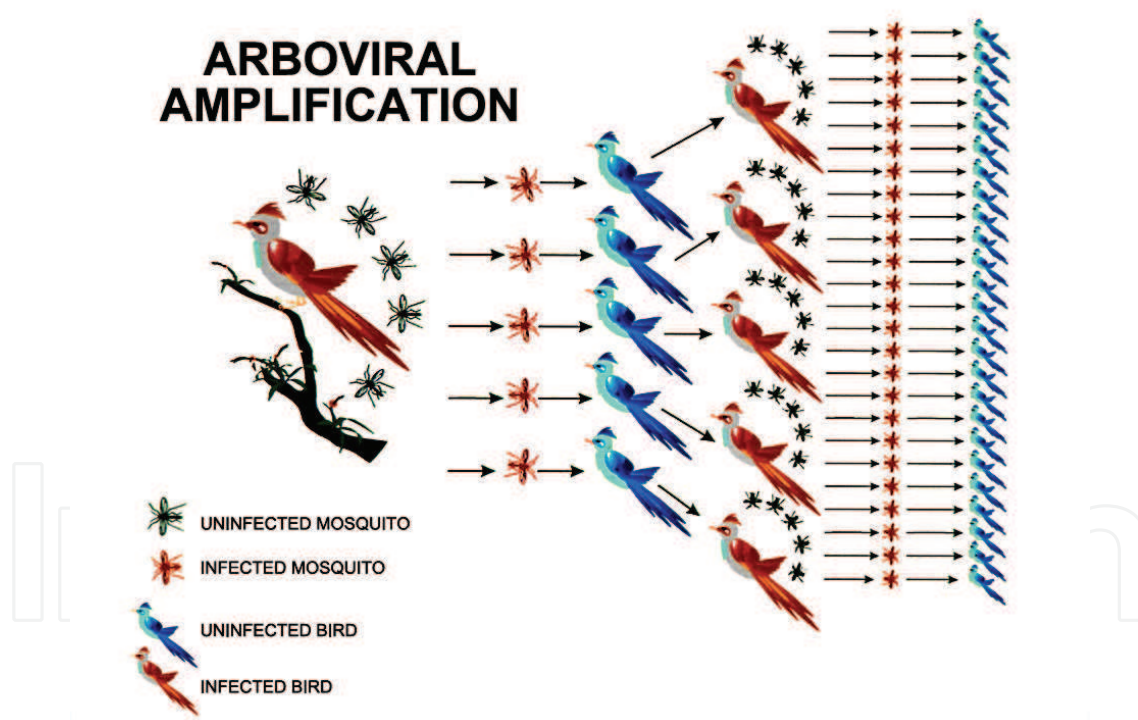

Fig. 1. An illustration depicting the sequence of events necessary for efficient arboviral amplification in nature. Epidemic amplification breaks down when there are too few competent mosquito vectors and when there are too few susceptible avian amplification hosts

A virus remains in an amplification host's circulating blood for a relatively short time, usually on the order of days. This is because the vertebrate host immune system usually clears the virus, rendering the infected host immune for life. In some cases there are vertebrate species that maintain long-term viremias at levels sufficient to infect vector

mosquitoes. Such hosts are sometimes referred to as reservoir hosts because they serve as a long-term local source for the virus. Garter snakes are known to maintain long-term viremias when infected with western equine encephalitis virus (family *Togaviridae*, genus *Alphavirus*, WEEV). It has been proposed that WEEV infected snakes may be the reservoir that allows WEEV to survive winters in temperate habitats (Burton et al., 1966).

Secondary or dead-end hosts are vertebrate hosts that do not have a high enough virus titer in the circulating blood to infect mosquitoes. Dead-end host infections are wasted in terms of viral survival and propagation. Humans are dead-end hosts for SLEV. Though a human infected with SLEV may show clinical signs of infection resulting from replication of virus in the nervous system, the amount of virus in the circulating blood is not sufficient to infect mosquito vectors. The normal SLEV amplification cycle in nature occurs between wild birds and mosquitoes. The fact that humans are involved at all in the SLEV transmission cycle is an accident resulting from the nonspecific blood feeding behavior of some vector species that feed readily on humans as well as wild birds. Blood meals by infective mosquitoes on susceptible wild birds may result in the transmission of virus to the bird and a new avian infection. Infected birds can infect additional susceptible mosquitoes. On the other hand, SLEV transmission to susceptible humans results in a dead-end infection for the virus; it is transmitted to a host where virus titers in the host's circulating blood remain too low to infect mosquito vectors.

The three biotic cycles discussed above are all influenced by environmental factors. The environmental factors that most directly impact arbovirus transmission cycles include rainfall, surface water accumulation, relative humidity, temperature, and land use practices. These environmental factors directly impact the reproductive biology and survivorship of mosquito vectors and vertebrate amplification hosts. By influencing the reproduction and longevity of vectors and amplification hosts, select environmental factors also influence arboviral abundance and transmission rates. For this reason, arboviral transmission cycles go through long periods when the viruses are rare and difficult to detect and quantify in nature. Likewise, due to environmental conditions that favor viral amplification and transmission, there are periods when arboviruses become extremely abundant and are transmitted at epidemic levels.

2.1 Biology of the virus

Most arboviruses are endemic to a particular environment in a specific region of the world. This means that arboviruses have integrated themselves into ecological habitats where they have coevolved with local mosquito vectors and vertebrate amplification hosts. For example, Ross River virus (family *Togaviridae*, genus *Alphavirus*, RRV) is found throughout Australia, Papua New Guinea, and other South Pacific Islands where it has coevolved with local mosquito vectors, especially *Aedes camptorhynchus* (Thomson), and large marsupial mammals that serve as amplification hosts (Russell 2002). The ecological conditions that occur throughout the distribution range of RRV, including the presence of suitable mosquito vectors and vertebrate amplification hosts, identify the environment most conducive for the seasonal amplification and transmission of this virus. Within this range, the RRV remains endemic and can survive throughout the year.

Survival from year to year is a prerequisite for viral endemicity. In the tropics and sub-tropics viruses survive from wet seasons, when mosquitos and amplification hosts are abundant, through dry seasons, when mosquitoes and amplification hosts are less abundant and arboviral transmission is less intense. For example, SLEV transmission to sentinel

chickens, used to monitor and document arboviral transmission in specific habitats throughout Florida, has been reported during every month of the year, indicating a year-round viral transmission pattern. In temperate habitats, arboviral survival through the winter is more problematic. In some cases, as for WNV in New York City, the virus survives in overwintering infected female mosquitoes (Nasci et al. 2001). For other viral cycles, like La Crosse virus (family *Bunyaviridae*, genus *Orthobunyavirus*, LACV) in the upper Midwest of the USA, the virus survives through a process known as transovarial transmission in which the LACV survives through winter in cold- and drought-resistant eggs deposited by infected female mosquitoes during the previous autumn (Watts et al. 1973). In other situations, viruses may be re-seeded into habitats by infected migrating birds. This may be the case for EEEV in Florida where infected birds that overwinter in tropical habitats where EEEV is endemic carry the virus north during spring migration and infect local mosquitoes in areas where the migrating birds stop to rest.

In some cases, viruses may be reintroduced into habitats where they have been absent for years or even decades. This happened in Key West, Florida sometime during 2009 when locally acquired human dengue (serotype 1) cases were reported for the first time in more than 50 years. The DENV may have been reintroduced through an infected human visiting the area. Whatever the source, the reintroduced DENV encountered an environment favorable for amplification (in susceptible humans) and transmission. The virus has since survived in a local transmission cycle in Key West for two years (MMWR 2010). It remains to be seen whether DENV will remain endemic in Key West for an extended period of time. In other situations a virus may be introduced into a completely new habitat where the ecology favors viral amplification and transmission. This happened with WNV in New York City in 1999 (Marfin and Gubler, 2001). The virus may have been introduced by an infected mosquito, an infected bird, or an infected human. Regardless of the mode of introduction, WNV found a habitat conducive for amplification and transmission in NYC. Not only did the WNV find the biotic and abiotic conditions in NYC favorable for long-term establishment, the virus spread and became established throughout the USA in less than four years.

2.2 Biology of the vector

To be an optimal epidemic arboviral vector, a mosquito species needs to be susceptible to infection, spatially and temporally abundant, long lived, and willing to blood feed on amplification as well as dead-end hosts. As discussed above, mosquito vectors need to be susceptible to viral infection such that, once ingested, virus must escape the blood meal in the midgut, penetrate the midgut wall, enter the mosquito hemolymph, infect and reproduce in other mosquito organs and tissues including the salivary glands, and exit the salivary glands with saliva during subsequent mosquito probing and blood feeding. This cycle of virus ingestion to salivary gland infection constitutes the mosquito extrinsic incubation period (EIP) discussed above (Figure 2). The EIP is temperature dependent. In general, warmer ambient temperatures result in shorter EIPs. Typical EIPs range from as low as 5 days for *Culiseta melanura* (Coquillett) infected with EEEV (Scott and Weaver 1989) to more than three weeks in SLEV-infected *Cx. nigripalpus*.

Competent mosquito vectors need to be spatially and temporally abundant in areas where they are sympatric with the arboviruses they transmit. For example, *Culex salinarius* Coquillett and *Cx. restuans* Theobald are both competent vectors of WNV in eastern North America. However, in south Florida these mosquito species are most abundant during the spring and adult numbers decline steadily throughout the summer months. It is possible that both species

are involved in the early season amplification of WNV in south Florida. But neither species is abundant during the mid-summer months when WNV is amplified and transmitted by the *Cx. nigripalpus* populations that are highly synchronized with avian amplification hosts.
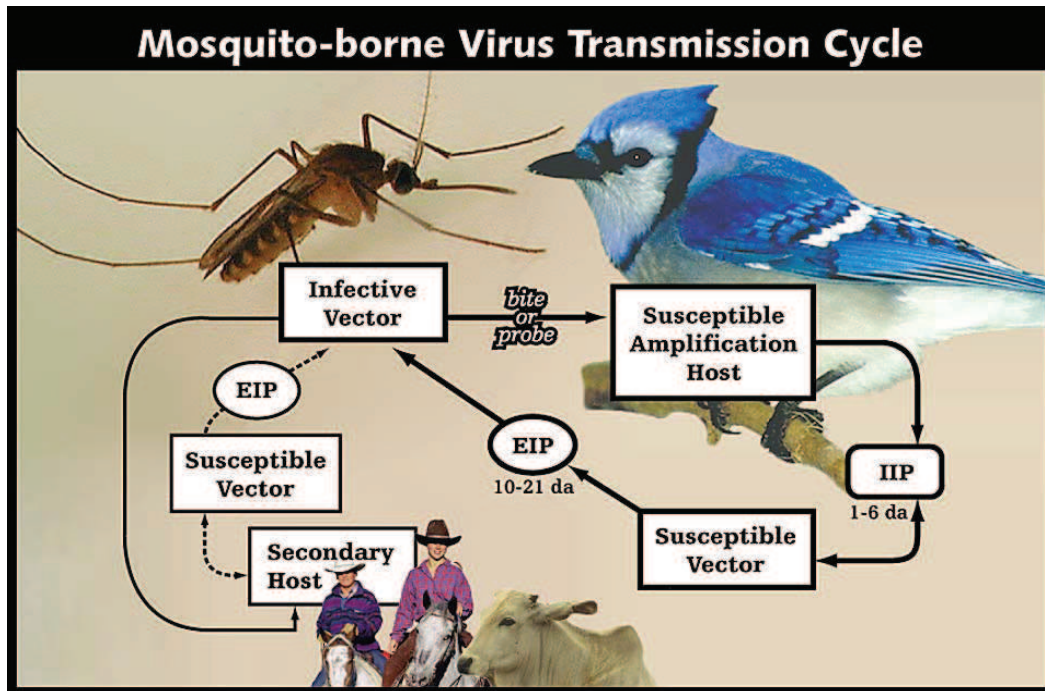

Fig. 2. An illustration depicting a typical mosquito-borne virus transmission cycle (e.g. SLEV). Abbreviations: IIP = intrinsic incubation period, EIP = extrinsic incubation period, secondary hosts are also often referred to as dead-end hosts

Short-lived mosquito species seldom serve as efficient arboviral vectors. This is because short-lived species do not have sufficient time to complete the temperature-dependent EIP. One of the most abundant mosquito species along the east coast of North America is *Aedes taeniorhynchus* (Wiedemann). Occasionally this species is found infected with SLEV or WNV; however, this mosquito species has never been implicated as a major arboviral vector along the east coast of North America. A major reason for this is that *Ae. taeniorhynchus* is a short-lived mosquito species, usually surviving as an adult for only two to three weeks. This short adult lifespan typically does not provide sufficient time for females to acquire an infective blood meal, complete the EIP, and blood feed a second time.

A final characteristic of an optimal arboviral vector is the willingness to blood feed on a variety of vertebrate hosts. Some mosquito species are efficient zoonotic vectors, that is, they feed selectively on certain vertebrate species, establishing an arboviral transmission cycle with those select species. For example, *Cs. melanura* feeds almost exclusively on birds. In areas of eastern North America where this mosquito species is abundant in hardwood swamps, efficient enzootic EEEV transmission cycles are readily established between resident wild birds and *Cs. melanura*. However, the transmission of EEEV to mammals involves secondary vectors, often referred to as "bridge vectors." Highly effective epidemic vectors blood feed willingly on amplification hosts as well as dead-end vertebrate hosts. *Culex nigripalpus* is an efficient epidemic vector of SLEV in south Florida where it readily blood feeds on avian amplification hosts as well as dead-end hosts including humans. In this instance a single mosquito species serves as both the amplification and the epidemic vector.

2.3 Biology of the amplification host

Many of the characteristics that make certain mosquito species optimal epidemic vectors are also characteristics that make some vertebrate hosts optimal arboviral amplification hosts. Efficient amplification hosts have to be susceptible to viral infection, maintain a high viremia in the peripheral blood, and maintain high viremias for a period of time that is long enough to ensure vector contact and successful blood feeding.

As with mosquito vectors, efficient amplification hosts need to be spatially and temporally abundant and readily available to host seeking mosquitoes. The primary mosquito vector of LACV in the upper Midwest of the USA is *Aedes triseriatus* (Say). This mosquito is day-active, which matches the day-active behavior pattern of the major LACV amplification host, the eastern chipmunk (*Tamias striatus*). In addition, the mosquito vector and vertebrate amplification host overlap in space through exploitation of similar woodland habitats. This spatio-temporal overlap of the mosquito and amplification host habitat preferences and behaviors maximizes the efficiency of LACV amplification and transmission.

The reproductive behavior of amplification hosts is also an important component of arboviral amplification and transmission. Young animals are highly susceptible to viral infection. Altricial birds are particularly susceptible to infection because they are virtually defenceless against blood feeding mosquitoes while on the nest and newly hatched birds have immune systems that are highly susceptible to viral infection. Many arboviral transmission cycles are synchronized with the reproductive patterns of primary amplification hosts. This is particularly true of arboviral cycles that rely on wild birds as their primary amplification hosts. As a consequence, years of high amplification host reproductive output may result in high levels of arboviral transmission. Avian and small mammal populations can cycle between years of extraordinary reproductive output and years of average or below average reproduction (Day and Stark 1999). When years of high amplification host reproductive output are synchronized with high vector output and high viral abundance, epidemic arboviral transmission can result. The vertebrate host and mosquito vector reproductive cycles are driven by environmental factors. Fortunately, it is uncommon for all three of the biological cycles associated with arboviral transmission patterns to be perfectly synchronized. This makes widespread arboviral epidemics rare events.

2.4 Environmental drivers of arboviral amplification and transmission

Arboviral cycles vary dramatically in their spatial and temporal distributions. There are times when it is difficult to find virus in habitats where specific viruses are known to be endemic. During tropical and subtropical dry seasons and during temperate winters it is almost impossible to make arboviral isolations from any source. During the hot summer months the appearance of virus may be focal, sporadic, or, at times, epidemic. The amount of virus present in an area during high volume transmission months is both directly and indirectly affected by the environmental factors that drive the biological cycles of the virus, the vector, and the amplification host. Perhaps the most important environmental factor affecting arboviral transmission cycles is rainfall. This is because rainfall directly impacts the reproduction, flight, and longevity of vector species. Mosquito larvae and pupae are fully aquatic, so eggs are laid in, on, or near water. Immature mosquitoes are aquatic, so eggs are laid on or near water, and larvae and pupae are fully aquatic. The lifecycle of all mosquitoes thus depends on the availability of standing water and it is precipitation that ultimately provides the land surface wetness that supports such standing waters. Similarly, rainfall cycles support the insect populations on which many birds rely for the nourishment of

nestlings. As will be discussed below, the cycling of rainfall and drought can directly impact the viral EIP in infected vectors.

Rainfall and drought also influence mosquito longevity and flight behavior, and have two direct effects on mosquito lifecycles and behavior. First, most small insects, and mosquitoes in particular, are subject to desiccation. The surface humidity associated with rainfall increases the potential flight range of host searching mosquitoes and their ability to blood feed once a host is located by reducing the desiccation rate in these small, fragile insects. Second, rainfall directly influences the abundance and quality of mosquito oviposition sites. Mosquito species that rely on temporary pools of water for oviposition are at the mercy of local rainfall events. During long periods of drought, these temporary oviposition sites are totally absent. This forces gravid female mosquitoes that deposit eggs directly in water or on the water surface to wait for a rainfall event sufficient to produce acceptable oviposition sites. Such drought-enforced oviposition delay may greatly enhance the transmission of certain arboviruses. For example, SLEV is transmitted by *Cx. nigripalpus* in Florida. This mosquito species depends on temporary pools of water for oviposition. In the absence of such pools, gravid female mosquitos will sit for weeks waiting for the proper oviposition cues. If a female mosquito is infected with SLEV during her first blood meal, she may complete the 15-21 day EIP before the occurrence of a rainfall event suitable to produce the temporary oviposition sites necessary for egg laying. Under these conditions, infected female mosquitoes can become infective in a single gonotrophic cycle. This makes it possible for an infected mosquito to transmit virus during her second blood meal.

This drought-delayed oviposition behavior serves two important epidemiological functions. First, extended periods of drought force infected gravid female mosquitoes to retain their eggs while they await the rains necessary to produce suitable oviposition sites. Once egg retention extends beyond the EIP, these infected female mosquitoes are ready to biologically transmit virus. Second, the drought-induced egg retention by infected mosquitoes serves to synchronize blood feeding once oviposition occurs. This means that virus transmission is focused into discrete time periods by patterns of rainfall and drought.

When oviposition sites are readily available to gravid *Cx. nigripalpus* females, eggs can be deposited five days after an infective blood meal and female mosquitoes are ready to immediately resume host searching behavior. In this situation, an infected female mosquito could potentially go through three or four gonotrophic cycles before she becomes infective. Host searching is one of the most dangerous activities undertaken by a female mosquito. Predation, vertebrate host defensive behavior (including the eating of attracted mosquitoes), and desiccation are all factors that terminate mosquito host searching flights. The ability to become infective after a single gonotrophic cycle greatly enhances the efficiency of arboviral amplification and transmission by vector mosquitoes.

Temperature is another environmental factor that can greatly influence arboviral transmission cycles. Because the EIP is temperature-dependent, even a small increase in daily ambient temperature can reduce the overall EIP in an infected female mosquito. Reduction of the EIP from 17 to 14 days may increase the probability that the mosquito will survive to become infective and transmit virus with subsequent host contacts (Watts et al. 1987). Environmental temperatures may also impact avian nesting behavior and reproductive success (Day and Stark 1999). Severe winter freezes in south Florida have been shown to enhance avian nesting, foraging, and reproductive success during the following spring (Day and Shaman, 2009). The exact ways in which winter freezes in south Florida impact avian nesting success the following spring will be discussed in detail in the SLEV in Florida section below.

A final environmental driver of arboviral transmission involves changes in landscape management practices. Human land management has long been known to influence mosquito species diversity, abundance, and age structure. Agricultural practices in particular are known to produce huge mosquito populations. Japanese encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, JEV) is endemic to Southeast Asia and the Far East where the primary vector is *Culex tritaeniorhynchus* Giles and domestic pigs and wild birds serve as amplification hosts (Erlanger et al. 2009). The JEV is the leading cause of mosquito-borne encephalitis in Asia where 30,000 to 50,000 cases are reported annually, primarily in rural settings. Rice paddies serve as the preferred oviposition site for *Cx. tritaeniorhynchus* and rice cultivation and associated agricultural practices, especially the production of domestic swine in areas adjacent to the rice paddies, greatly enhances the abundance, age structure, and JEV infection status of this important mosquito vector.

3. Specific examples of arboviral transmission cycles

To substantiate further the points discussed in Section 2, we will present examples of four mosquito-borne arboviral cycles from North America. These include: eastern equine encephalitis virus in the eastern USA; St. Louis encephalitis virus in Florida, USA; West Nile virus in eastern Colorado, USA; and West Nile virus in Florida, USA.

3.1 Eastern Equine Encephalitis virus in the Eastern USA

Eastern equine encephalitis virus is a complex of four viral lineages. Group I is endemic throughout the eastern half of the USA and the Caribbean Basin and is responsible for most human disease. Groups IIA, IIB, and III are endemic in Central and South America where they are primarily responsible for equine infections. In the USA, EEEV transmission occurs primarily east of the Mississippi River. As the name implies, EEE is primarily a disease of equines. Major epizootics that were consistent with the clinical definition of EEE infection in horses were reported in 1845 (Long Island and New York), 1902 (North Carolina), 1905 (New Jersey), 1908 (Florida), and 1912 (Maryland, New Jersey, and Virginia). In 1933 the EEEV was first isolated from the brain of an infected horse and recognized as the etiologic agent of EEE infection. Additional equine epizootics caused by EEEV were reported in 1933 (New Jersey), 1934 (Virginia), and 1935 (North Carolina). The largest equine epizootic in the USA occurred in 1947 in southern Louisiana and Texas where there were an estimated 14,334 equine cases with 11,727 deaths (Scott and Weaver 1989).

An average of six human EEE cases is reported annually in the USA. Most human cases are reported by health workers in coastal states from Texas east to Florida and north to New Hampshire. The first major human EEE epidemic was reported in eastern Massachusetts in 1938. During that year 34 human infections were reported with a case-fatality rate of 74%. A second human epidemic with 32 cases was reported in New Jersey during 1959 (Scott and Weaver 1989). Eastern equine encephalitis epidemics of this magnitude are exceedingly rare. In general, human EEE cases appear sporadically. For example, the highest number of human EEE cases reported during a single transmission season in Florida is five (this occurred on four separate occasions in 1978, 1980, 1991, and 2005). These annual groupings of five human cases were never clustered and during most years they were spread over three or four Florida counties.

The low number of human cases is a direct reflection of the complexity of the EEEV transmission cycle and the obstacles that this complexity places in the way of large-scale

EEEV amplification in vector mosquitoes. In the eastern USA, EEEV is endemic in hardwood freshwater swamps where the virus is cycled between resident and migratory wild birds by *Cs. melanura*. Because this mosquito species feeds almost exclusively on birds, mammals are rarely involved in the enzootic cycling of virus in these swamp habitats. If this were the extent of the EEEV amplification and transmission cycle, humans and horses would never be involved. However, the virus has two ways it can escape enzootic hardwood swamp habitats. First, it can move out of the swamp in infected birds as they disperse after fledging (Crans et al. 1994). Second, under the proper environmental conditions, where heavy rains saturate the open habitats surrounding the swamps, infected *Cs. melanura* and infected secondary mosquito vectors (bridge vectors) can disperse out of the swamps carrying the EEEV with them. Infected bridge vectors can blood feed on susceptible horses or humans in habitats adjacent to the swamps. In addition, infected bridge vectors and infected *Cs. melanura* females can blood feed on avian hosts at sites some distance from the original infection site and establish a secondary amplification focus. Once secondary mosquito vectors and amplification hosts become infected, new amplification foci are established. In Florida, secondary mosquito vectors include *Cx. nigripalpus*, *Coquillettidia perturbans* (Walker), *Mansonia* spp., and spring floodwater *Aedes* spp. All of these mosquito species are opportunistic blood feeders that will readily feed on a variety of wild bird species as well as mammals.

It is this two-tiered EEEV transmission cycle that makes it difficult to realize the large number of infected mosquito vectors required for epidemic transmission on the scale observed for WNV and DENV. Virus never escapes the hardwood swamps during many arboviral transmission seasons. During years when the virus does escape, secondary transmission foci need to become established or large numbers of infective bridge vectors need to escape the swamp habitats before human and horse cases appear. By their very nature, these transmission events involve fewer infected mosquitoes than what has been observed during epidemics caused by WNV. As a consequence, EEEV transmission foci remain small and isolated resulting in focal or sporadic transmission of virus to humans and horses.

3.2 St. Louis Encephalitis virus in Florida

St. Louis encephalitis virus is related to JEV, but its distribution is restricted to the New World. Transmission of SLEV has been reported throughout the USA and in Canada, Mexico, Central America, the Caribbean Islands, and South America. Transmission in North America is most commonly reported along the Mississippi and Ohio River basins. In Florida, SLEV is most commonly reported in the southern half of the state where epidemics were reported in 1959, 1961, 1962, 1977, and 1990 (Day 2001).

Prior to 1977 most SLEV transmission to humans in Florida occurred in urban transmission foci. The epidemics in 1959, 1961, and 1962 occurred in cities along the central west coast of Florida including St. Petersburg, Tampa, Clearwater, and Sarasota. Human infections during the 1977 and 1990 epidemics occurred mainly in suburban and rural settings. The shift of SLEV transmission from urban to rural foci may be a reflection of changes in urban public health practices, changes in the distribution and bionomics of the primary epidemic vector, *Cx. nigripalpus*, or changes in south Florida land use patterns.

The zoonotic transmission of SLEV occurs year round in south Florida where vector mosquitoes transmit virus to susceptible wild birds. The primary zoonotic vector is *Cx. nigripalpus*, a mosquito species that is present in a wide variety of Florida habitats throughout the year. Secondary zoonotic vectors may also be involved in the low level

transmission and maintenance of SLEV during the south Florida dry season (November-May) and during years where environmental factors do not favor the amplification and transmission of SLEV during the south Florida wet season (June-October). Secondary zoonotic vectors may include: *Cx. salinarius*, *Cx. restuans*, *Cx. erraticus* (Dyar and Knab), and *Anopheles crucians* Wiedemann. Low level viral transmission occurs between primary and secondary zoonotic vectors and wild birds. Transmission levels increase during the south Florida avian nesting season (April-June amplification) when nestling birds provide a steady source of blood for mosquitoes infected with SLEV.

Sentinel chickens serve as the best means of documenting viral transmission in the field (Day and Lewis, 1992). Flocks of 8 to 12 chickens are placed into different habitats throughout Florida where they are exposed continually to blood feeding mosquitoes. Weekly blood samples are drawn from each chicken and analysed for antibody to SLEV, WNV, EEEV, and Highlands J virus (family *Togaviridae*, genus *Alphavirus*, HJV). A positive antibody test is indicative of recent arboviral transmission to the chicken. Depending on environmental conditions, SLEV transmission, as measured by sentinel chickens, may be absent, focal, sporadic, or epidemic. The Florida sentinel chicken surveillance program provides a weekly spatial analysis of the distribution of virus transmission throughout Florida. More than 30 years of sentinel chicken arboviral surveillance in Florida has shown that the major SLEV transmission period occurs between August and October.

Occasionally, environmental conditions favor the enhanced early season amplification of SLEV. In particular, the Florida SLEV cycle appears to be sensitive to patterns of drought and rainfall. Spring droughts seem to be particularly important because drought and landscape fragmentation conspire to constrict vector activity to the more humid hammock environments exploited by nesting birds. Some surface waters remain within and at the fringes of these habitats, often in channels and canals that *Cx. nigripalpus* can exploit without having to travel far (and risking desiccation). The drought-forced cohabitation of vector mosquitoes and avian hosts, including nestlings, facilitates a rapid increase in the infection rate among both vector and host (Shaman et al., 2002). Late spring rainfall then facilitates the dispersal of these infectious mosquitoes and birds, enabling the establishment of secondary amplification foci.

When late spring and summer rainfalls occur at an optimal rate of once every 10-14 days, *Cx. nigripalpus* populations will increase efficiently. Furthermore, should temperatures be high, such that viral EIP is reduced, and major rainfall events are appropriately spaced, EIP will be completed in a single gonotrophic cycle and SLEV transmission will proceed optimally. *Culex nigripalpus* is a flood water species that prefers freshly flooded oviposition habitats. Rainfall cycles that temporarily flood oviposition sites, allowing mosquito egg-laying and the completion of immature mosquito development before the oviposition sites dry down, produce the maximum number of mosquitoes in age structure cohorts that favor viral transmission (Day and Curtis 1999). When rainfall, mosquito reproduction, and avian reproduction coincide early in the summer, the viral amplification and transmission begun during spring drought is further maximized. When such environmental conditions favorable for amplification persist through the summer, large numbers of infected vectors are produced resulting in epidemic SLEV transmission in the late summer and early fall.

There are additional environmental factors that may favor enhanced mosquito and avian reproduction. For example, flood irrigation in Florida citrus groves often occurs during the dry months of April and May. During this process, thousands of acres of citrus trees are flooded producing suitable *Cx. nigripalpus* oviposition sites that allow the mosquitoes to

begin reproducing early in the year, during the spring dry season, a period when there would normally be no available oviposition sites. In addition, it has also been shown (Day and Shaman 2009) that there is a significant relationship between severe south Florida winter freezes and avian reproduction during the three breeding seasons following the freeze. It is proposed that severe winter freezes clear cold-sensitive vegetation from the understories of woodland habitats. By clearing the understory vegetation, these habitats are opened to foraging by ground feeding birds, many of which are important SLEV amplification hosts that dramatically increase in abundance due to this increase in foraging and nesting habitat. It takes about three years for the understory vegetation to regrow after which avian reproductive rates return to normal (Day and Stark 1999).

The realization of epidemic SLEV transmission in south Florida depends on the precise synchronization of three biological cycles. The presence and abundance of virus depends on the abundance, distribution, and age structure of vector and amplification host populations. These population fluctuations are driven by winter freezes, spring drought, and the cycling of rainfall in a manner that produces periodic *Cx. nigripalpus* oviposition sites throughout summer.

3.3 West Nile virus in Eastern Colorado

West Nile virus is part of the Japanese encephalitis antigenic complex. Until 1999 the virus was enzootic throughout Africa and much of Asia, the Middle East, and the Mediterranean. A subtype of WNV is found in Australia where it is referred to as Kunjin virus (family *Flaviviridae*, genus *Flavivirus*, KUNV). In dramatic fashion, WNV was reported in New York City during the summer of 1999 (CDC, 1999). By 2001 the virus had spread west to the Mississippi River, north into Canada, and south to Florida. By 2002 it had spread to the front range of the Rocky Mountains, south into Mexico, and throughout the Caribbean Basin. By 2004 it was reported in all of the Continental United States (Hayes et al. 2005). Extremely large urban and rural epidemics were reported as WNV moved west across the USA. For example, in 2003 more than 6000 human clinical cases were reported in the Great Plains of the U.S. (i.e. Colorado, Nebraska, South Dakota, Wyoming, and Montana). West Nile virus and SLEV share similar viral transmission cycles, and states like Illinois, Louisiana, Texas, and California that had reported large SLE epidemics during the second half of the 20th century also reported large WN epidemics associated with establishment of the virus in those states.

Human cases of WN first manifested in Colorado during 2002. The following year, a major outbreak of WNV took place in Colorado with 2,947 human cases reported, and since then annual human cases in Colorado have numbered in the hundreds. The majority of cases have been in the eastern high plains of the state east of the Rocky Mountains (Shaman et al., 2010). This region is dominated by grasslands interspersed with river riparian zones that appear to be the nexus of WNV activity. These riparian zones provide habitat for avian host species and *Cx. tarsalis* and *Cx. pipiens,* the dominant vectors of WNV in the region (Bolling et al. 2007). In these riparian environments, WNV can be amplified and zoonotically transmitted between these vector mosquitoes and co-habitating avian hosts.

Both *Cx. tarsalis* and *Cx. pipiens* have been found to be more abundant in the riparian regions of the eastern Colorado plains than in the riparian regions of the foothills and higher elevations of the Rocky Mountains (Eisen et al., 2008; Barker et al., 2009). Thus, the geographic distribution of *Culex* vector abundance is consistent with higher WNV transmission risk in eastern Colorado (Winters et al., 2008). The distribution of *Culex* vectors

and the environments that support them provides an indication of where in space WNV transmission is more likely to occur; however, to understand *where and when* WNV activity will occur, temporal variations in environmental conditions must also be considered.

When environmental conditions are more favorable for mosquito breeding, increased vector abundance within riparian zones increases the ratio of vectors to hosts. This change facilitates the proliferation and amplification of WNV in both the vector and host populations (Shaman, 2007). In eastern Colorado, changes to local soil moisture conditions appear to be the catalyst for vector mosquito population increases. Specifically, wetter than usual spring conditions, including waters derived from the melting of late winter snow storms, and drier than usual summer conditions presage increased human WN cases (Shaman et al., 2010).

The wetter spring conditions provide a greater abundance of the cleaner, less eutrophic waters preferred by *Cx. tarsalis.* These vector mosquitoes then proliferate and in the presence of normal or above-normal numbers of avian hosts, as well as the virus, *Cx. tarsalis* can initiate early springtime epizootic amplification of WNV. Dry summer conditions then reduce stream flow and facilitate puddling and ponding of stagnant waters within the riparian zones. These eutrophic waters are favored by the other dominant vector, *Cx. pipiens* (Savage and Miller 1995), which can increase in abundance and continue the amplification and zoonotic transmission of WNV.

The eastern Colorado WNV transmission cycle appears to be further complicated by irrigation practices, which divert riparian flow into agricultural fields, in effect spreading water resources out and providing more eutrophic habitats during the summer. Areas with agricultural irrigation in eastern Colorado have been associated with increased incidence of human WN cases (Eisen et al., 2010). In addition, the watering of lawns and golf courses, as well as the presence of golf course water hazards, in the towns and cities along the Front Range in eastern Colorado (e.g. Fort Collins, Denver) provide additional habitats favorable for vector mosquitoes and avian amplification hosts. These land irrigation and management practices put humans in greater contact with infectious vector mosquitoes and support the heightened transmission of WNV to humans evident in this region. Thus, a combination of hydrological and land management practices appear to facilitate WNV transmission to humans in eastern Colorado.

3.4 West Nile virus in Florida

Florida is the only state with a history of SLE epidemics that has yet to see a major WN epidemic (Gubler 2007). West Nile virus entered Florida in late 2000 or early 2001 and was first detected in July, 2001 in Jefferson County, located in the Florida Panhandle. Horses and dead wild birds were the first animals from which WNV was isolated in Florida. There was no WN horse vaccine available in 2001 and the entire Florida horse population was naïve and susceptible to infection as WNV moved through the state. The vulnerable condition of the Florida horse population is evidenced by the WNV transmission data reported for 2001 through 2010. In 2001 there were 491 WNV-positive horses reported in the state. An experimental horse vaccine was introduced into Florida late in 2001, but the availability of the vaccine did not slow WNV transmission in 2002 when 499 positive horses were reported in the state. The number of WNV-positive horses started to decline in 2003 when 117 positive horses were reported (USDA—APHIS 2011). The number of WNV-positive horses remained low from 2004-2010 with an annual average of 6 positive horses per year. A combination of the availability of an efficacious vaccine along with environmental

conditions that did not favor the amplification of WNV in Florida appears to have been responsible for the reduced transmission observed between 2004 and 2010.

As was the case with WNV invasion in other states, human cases were associated with the first reports of virus in Florida. There were 12 clinical WN cases reported in Florida during 2001. These cases were reported from the western Florida Panhandle south to the Florida Keys, indicating that the virus was well-established in the state prior to the appearance of human cases. In 2002 there were 28 clinical human cases reported from throughout the state. The highpoint of WNV transmission to humans in Florida occurred in 2003 when a total of 94 clinical cases were reported from throughout the state. Since 2003 human cases have rarely been reported in Florida (CDC 2011).

The zoonotic transmission of WNV, as measured by sentinel chickens, is similar to that of SLEV and occurs year round in Florida. A major difference between the transmission cycles of the two viruses is the number of zoonotic mosquito vectors that transmit WNV. The WNV has been isolated from a wide variety of mosquito species in Florida and it is likely that many of these species can support the transmission of WNV to wild birds in nature. For example, *Deinocerites cancer* Theobald is a prolific mosquito species found in saltmarshes along the east coast of Florida where it blood feeds on marsh birds. Isolates of WNV have been made from pools of *De. cancer* collected in the Florida Keys (Hribar et al. 2004). It is very likely that this mosquito species supports WNV transmission in saltmarshes all along the east coast of Florida.

Even though a major WN epidemic has not yet been reported in Florida, the biological components of the WNV transmission cycle are already in place throughout the state. *Culex nigripalpus* is a proven vector of both SLEV and WNV in Florida (Shroyer 1991; Rutledge et al. 2003). The interaction of this mosquito species with WNV and avian amplification hosts, as well as the environmental drivers that affect WNV transmission, are very similar to those already reported for SLEV transmission in south Florida (Shaman et al. 2005).

As is the case with SLEV, the environmental conditions that favor a WN epidemic are rare occurrences. The exact sequence of events necessary to produce large numbers of nestlings along with abundant *Cx. nigripalpus* populations of just the right age structure are seldom realized. However, a knowledge of the biological and environmental conditions necessary to create a WN epidemic—winter freeze, ample bird abundance, spring drought and summer rainfall cycling—allow researchers to track seasonal biological events and predict when and where outbreaks may occur. Exactly how this is done is discussed in detail below.

4. Spatial-temporal arboviral surveillance and prediction

A thorough understanding of the biological and environmental components associated with any arboviral transmission cycle allows the tracking of those components and the formulation of a prediction about where and when arboviral amplification and transmission might occur. The biological components of an arboviral transmission cycle that can be tracked include: the abundance and spatio-temporal distribution of the virus; the abundance, spatio-temporal distribution, and age structure of all mosquito vectors involved with viral amplification and transmission; and the abundance, spatio-temporal distribution, and age structure of all amplification hosts associated with viral amplification and transmission. The physically-based components of an arboviral transmission cycle that can be easily tracked include: meteorological conditions (daily rainfall, temperature, and relative humidity; rainfall deviations from long term averages; and temperature deviations from long term

averages), hydrological conditions (soil moisture levels, land surface ponding and puddling, as well as the relationship between physical factors including topography, soil type, incident radiation, vegetation, rainfall, and temperature with surface groundwater levels), and land use (forest clearing and regrowth, housing and business development, agricultural practices including irrigation and ground surface manipulation, and other land management practices). Sampling techniques that assist the tracking of these biological and environmental components are discussed below.

4.1 Viral sampling

The abundance and spatio-temporal distribution of arboviruses can be measured in three ways: sentinel animal surveillance, isolation of virus from vectors, and virus and viral antibody isolation from wild and domestic animals.

Sentinel animal surveillance provides one of the easiest, cheapest, and most effective ways to detect and monitor arboviruses in the field. For most sentinel programs, naïve animals are placed in the field prior to the beginning of the arboviral transmission season and maintained at a fixed site for the duration of the transmission season. Weekly blood samples are collected from each sentinel animal and analysed for arboviral-specific antibody. Confirmation of antibody indicates recent arboviral transmission to that sentinel at that site. One of the most frequently used sentinel animals is the domestic chicken. Sentinel chickens have proven highly versatile for monitoring SLEV, WNV, EEEV, and HJV in Florida, where the state-wide program was initiated following the 1977 Florida SLE epidemic (Day and Lewis 1992). A great advantage of sentinel chicken surveillance for mosquito-borne encephalitis viruses is that the chickens serve as dead-end hosts without further amplifying the viruses in the environment.

The value of arboviral surveillance through well-run sentinel animal programs is that the sentinels are caged at predetermined sites in the field. When these sites remain constant from year to year, long term baseline seroconversion data sets can be established. Once baseline data sets are known for individual surveillance sites, monthly, or even weekly, deviations from normal can be calculated and monitored for each site. An arboviral surveillance program based on sentinel chickens was established in Indian River County (IRC), Florida in 1978. There are sentinel chicken flock sites in IRC that have been in the same location for nearly 35 years. These long term data sets prove invaluable during years when unusual levels of viral transmission are detected. They accurately measure weekly differences in viral transmission levels and also provide a spatial measurement that identifies viral transmission hotspots throughout the county.

Occasionally, completely naïve animal populations detect arboviral introductions into new areas. This happened in Florida in 2001 when WNV first entered the state. At that time, the sentinel chicken program was already in place for SLEV and provided a ready network for tracking the appearance and spread of WNV. In addition, in 2001 local horse populations were unvaccinated and previously unexposed to WNV. In many Florida counties, WNV-positive local horses (both antibody positive horses and WNV isolations from horses that died of WN infection) were the first to detect the presence of WNV. Once WNV became endemic in Florida and an efficacious WNV vaccine was introduced, the value of horses for monitoring the presence and movement of WNV was greatly reduced.

Horses are highly susceptible to infection with EEEV. Even though there is a highly efficacious EEEV vaccine, many horses remain unvaccinated and the monitoring of dead horses in EEEV endemic areas sometimes provides the first indication of seasonal EEEV transmission in regions along the east coast of North America. Humans also sometimes

serve as arboviral sentinels. The re-introduction of DENV into Key West, Florida in 2009 was first detected through an infected human because there are no avian or non-human reservoir or amplification hosts in the DENV transmission cycle in Key West, Florida.

In general, the isolation of virus from infected vectors provides a less sensitive measure of the spatiotemporal distribution of an arbovirus. Mosquitoes are able to fly and may move considerable distances from the point of infection. Still, viral isolation from an infected vector is a good way to detect the regional presence of a virus. Generally, mosquito species are assayed in groups or 'pools' of 50-100 mosquitoes. An increased number of infected mosquito pools collected at a site over a short period of time may be indicative of on-going arboviral amplification. However, positive mosquito pools do not provide evidence that the mosquito, or mosquitoes, responsible for the positive pool were able to transmit virus (Rutledge et al., 2003). During much of the year, mosquito infection rates are so low that viral detection in mosquito pools is difficult. Because mosquito pooling is labor intensive and expensive, it is more cost-effective to let sentinel animals screen large numbers of mosquitoes and to monitor viral presence and distribution through seropositive sentinels.

Because some viruses are highly pathogenic to certain vertebrate hosts, the mortality of these hosts sometimes provides a measure of viral transmission. For example, WNV can be highly pathogenic to many avian species, especially those in the Family Corvidae (crows, ravens, jays, and magpies). Thus, it is possible to analyse the tissues of dead birds for WNV to detect the presence of virus in an area (Nemeth et al. 2007) or to provide early warning of WNV amplification (Eidson et al. 2001). As with mosquito pooling, dead bird surveillance is labor intensive and expensive. Because birds can disperse from the original infection site, data interpretation is also sometimes difficult. However, dead bird surveillance was one of the major ways that the movement of WNV across the USA was monitored between 1999 and 2004 (Nemeth et al. 2007).

4.2 Vector sampling

Many mosquito control programs rely on adult and larval sampling to assess the spatial and temporal abundance of mosquito populations. Surveillance protocols are designed to monitor vector as well as nuisance species. A large number of adult and larval monitoring techniques, trapping devices, and sampling protocols have been developed (Service 1993). Each technique has its strengths and weaknesses, and individual surveillance programs rely on the technique that works best for the mosquito vectors and pests in their jurisdictions.

Light traps are among the most popular methods for sampling adult mosquitoes. The New Jersey light trap and the CDC light trap have both been used for decades by mosquito control programs to monitor adult mosquito populations. CDC light traps are commonly baited with CO_2 (in the form of dry ice or as bottled gas metered into the trap) as a secondary attractant. A disadvantage of light traps is that they capture a wide variety of non-target species including moths, beetles, and wasps. Non-target species in the collection greatly slows the sorting process. Carbon dioxide-baited suction traps usually make a pure collection of biting arthropods including mosquitoes and biting midges. Modified suction traps designed to capture gravid mosquitoes attracted to a tray containing oviposition media collect female mosquitoes that are more likely to be infected with an arbovirus because they have taken at least one blood meal and matured an egg batch. Mosquito control programs sometimes rely on landing rate count to quickly assess biting fly populations in selected areas of concern. Finally, mosquito control programs also rely on

sampling techniques designed to capture immature mosquitoes (larvae and pupae). Mosquito dippers are used to survey aquatic habitats for the presence of immature mosquitoes, usually in targeted larval habitats. Skilled technicians can easily identify the mosquito species, its abundance, the age (in days) of the immature mosquitoes, and the projected emergence date of the adult mosquitoes (Service 1993). When field identifications are in question, immature mosquitoes can be returned to the laboratory where they are allowed to mature and emerge as adults that are generally easier to identify.

One of the most versatile adult mosquito sampling techniques for monitoring vector populations is the ground aspiration of resting mosquitoes (Day and Curtis 1993, 1999). This technique relies on a battery powered aspirator that is used to make sweep collections of resting mosquitoes. These collections include newly emerged and older males as well as females in a number of different life stages including: newly emerged, unfed, freshly blood fed, half blood/half gravid, and gravid. By tracking the proportions of females in different life stages it is possible to calculate the age structure of a vector population and in so doing assess the risk of viral transmission (Day and Curtis 1994). Ground aspiration adult mosquito surveillance is most effective for monitoring nocturnally active mosquitoes that congregate in daytime resting sites. For example, many species of *Anopheles* mosquitoes rest in and around human habitations where they can be easily collected by aspiration during the daytime (Service 1993). Likewise, many *Culex* species congregate in humid, heavily-vegetated habitats where they are easily collected in large numbers by ground aspiration (Day and Curtis 1993).

4.3 Vertebrate host sampling

In cases where the species of an arboviral amplification host is known, it is possible to monitor individual populations to assess amplification risk. In Florida several wild bird species are known amplification hosts for WNV and SLEV. With the proper State and Federal permits, wild birds can be trapped, handled, banded, and bled. Blood samples can be analysed for virus and virus-specific antibody. The appearance of virus or antibody in recently fledged birds is an indication of recent local viral transmission and is sometimes helpful in assessing transmission risk. Avian sampling is also helpful in determining species abundance, population age structure, and the viral immune status of adult and immature birds (Day and Stark 1999). As is the case with infected mosquitoes, seropositive wild birds are usually rare and the collection and handling of wild birds is labor intensive, with a low yield of positive results, especially during inter-epidemic periods.

4.4 Meteorological, hydrological, and other environmental data

A wealth of meteorological and hydrological data is available from university, governmental, and international resources. These data are typically compiled by national meteorological services and, in some instances (e.g. the United States), are available free of charge (NCDC 2011). Typical records may include hourly or daily measurements of 2-meter above-ground temperature, precipitation rates, and 2-meter above-ground humidity, all of which may influence the abundance and distribution of arboviral pathogens, vectors, and hosts in a particular region. In addition, estimates of soil moisture content and land surface wetness and water pooling are routinely derived from both satellite observations and physically-based hydrology models that numerically simulate hydrological conditions. Maps of the distribution of soil and vegetation type, as well as land use classifications are also available from a variety of sources. These maps are increasingly available in digital format through resources such as Google Earth.

Temperature is typically measured at meteorological stations on a sub-hourly, hourly, or daily basis at a height of 1-2 meters above ground. These stations are distributed across the landscape at a density that usually reflects the level of funding provided to a nation's meteorological service. Dense networks replete with hourly temperature records are available in many developed countries. These temperature data can be used to derive an estimate of the local EIP for a given arbovirus vector and to document the magnitude and duration of local freezes and the level of vegetation die-off. As temperatures can vary considerably from the surface to several meters above ground and from the understory to open areas, such estimates of EIP would need to be qualified. Indeed, the arboviral pathogen, vector, and host are only subject to the conditions in their immediate surroundings, i.e. their microclimate, which can vary considerably over short distances. Both vectors and hosts can in part control this microclimate (e.g. temperature conditions) by simply moving.

Records of hourly or daily total precipitation are also often maintained by national meteorological station networks. Daily precipitation is the more commonly available measure, though this 24-hour measure does not always correspond with the end of the day; rather, not infrequently it represents the 24-hour rainfall accumulated prior to some unspecified hour (e.g. 7 am local time).

Humidity is much less commonly recorded than either temperature or precipitation, as not all meteorological stations are routinely equipped with hygrometers. Standard humidity measurements, where available, are taken 1-2 meters above the ground. There are numerous measures of humidity, which fall into two distinct categories: 1) relative humidity, which measures the amount of water vapor in the air relative to saturation, which is the point at which rates of evaporation and condensation are equivalent and a cloud or fog begins to form; 2) absolute humidity, which provides a mass-based measure of the amount of water vapor in the air. Relative humidity is typically given as a percent of saturation; absolute humidity comes in multiple forms such as vapor pressure and specific humidity.

There also exist many estimates of drought (NIDIS 2011). Some of these are very simple algorithms or indices that derive a measure of drought from recent meteorological conditions, including precipitation and temperature. Other measures are derived from networks of soil moisture monitoring stations. Satellite sensors can be used to estimate soil moisture content directly from bare soil or to infer water availability by measuring the greenness of land surface vegetation (Anyamba et al. 2009). In addition, hydrology models can provide more sophisticated and comprehensive physically-based estimates of the movement and pooling of water beneath the surface and at the land-atmosphere interface (e.g. Koster and Suarez 1996). All these estimates suffer shortcomings that must be recognized and accounted for when using these data.

Measurements are very often automated, which can lead to gaps in a record due to equipment failure; however, automated stations, when functional, provide more frequent measures of conditions and are not subject to certain human-related measurement errors. Biases within automated stations still may exist, for example due to instrumental bias or placement of the station in the shade or near building ventilation systems. It is best when using environmental data derived from national network sources, to investigate the specific collection protocols and placement for the station data to be used.

In addition, thermometers, hygrometers, rain gauge stations and soil moisture sensors are readily available for purchase from a variety of scientific supply companies. These systems

are easy to install and use, provide regular measures in the field at the site of interest, and typically come with software systems for automatically recording measured meteorological conditions. All systems should be calibrated prior to use.

4.5 The use of surveillance protocols to predict and mitigate arboviral transmission

A thorough knowledge of the biological and environmental components of an arbovirus transmission cycle allows the spatial and temporal tracking of the virus and a skillful prediction of where and when virus transmission will occur. The virus itself is tracked in a number of different ways. Sentinel surveillance uses virus-specific antibody in sentinel animals to narrow the spatio-temporal timeframe of viral amplification and transmission. An advantage of this technique is that it allows a better understanding of exactly where and when virus is being transmitted. A disadvantage is that there is often a significant time lag between the infective mosquito bite on the sentinel animal and the confirmation of positive virus-specific antibody in the sentinel's blood. Depending on the blood collection protocol, the delay may range from 10 days to a month (Day and Lewis 1992). Amplification host surveillance is a second way that specific viruses can be tracked in the field. Virus or antibody isolation from the blood of a known amplification host may help to determine recent viral transmission in the field. Disadvantages of this technique include the mobility of wild amplification hosts (they may be captured considerable distances from the original infection site), positive antibody tests in an adult animal that may represent an old infection, and the extensive permitting required for the handling and manipulation of most vertebrate amplification hosts.

The pooling of known mosquito vector species and subsequent viral isolation attempts is a third way that virus can be detected and monitored in the field. During inter-epidemic periods vector infection rates are usually very low. However, during epidemics or epizootics vector infection rates increase dramatically, especially during the early amplification phase of an epidemic, and it is possible to collect vectors in the field, pool them, and analyse the pools for the presence of virus (Day and Stark 1996). A positive mosquito pool indicates that virus was present in at least one of the mosquitoes contained in the pool. Large numbers of virus-specific positive mosquito pools from a localized area indicates that viral amplification may be on-going in that area (Shroyer 1991). Disadvantages of this technique are that vectors, like vertebrate amplification hosts, may disperse from the original infection site making it difficult to identify the exact geographical confines of the outbreak. In addition, a positive mosquito pool is not necessarily indicative of viral transmission by that mosquito species in the field (Rutledge et al. 2003) and positive mosquito pools may not accurately measure the viral transmission risk.

Dead animal surveillance is a fourth way that virus transmission can be monitored in the field. Focal die-offs of susceptible vertebrate species may indicate recent local viral transmission. Disadvantages of this technique include the fact that most amplification hosts do not die as a result of arboviral infection and there is a danger to the general public associated with the handling of animals that have died as the result of an arboviral infection. The environmental forces that influence the movement and infection of vectors and vertebrate hosts can be monitored to better understand the risks associated with viral amplification and transmission. Hydrological and surface wetness models have proven to be valuable in the monitoring and prediction of WNV transmission in Florida (Shaman and Day 2005; Day and Shaman 2008) and eastern Colorado (Shaman et al. 2010). It is important to understand and monitor the exact environmental drivers for individual arboviral

transmission cycles. For example, the environmental conditions responsible for high levels of WNV transmission in Texas are different than those responsible for WNV transmission in California. A careful study of the habitat-specific dynamics of viruses, vectors, and amplification hosts along with an understanding of the environmental conditions responsible for driving each of these cycles is necessary before transmission patterns are understood and outbreaks can be predicted.

Once the biological components of a transmission cycle and the environmental factors responsible for driving those biological components are understood, it is possible to attempt mitigation of an impending arboviral epidemic. Perhaps one of the best examples of how a thorough understanding of an arboviral transmission cycle can translate into epidemic mitigation comes from Pinellas County, Florida where a focal outbreak of WNV transmission resulted in 18 human cases in 2005 (Day and Shaman 2008). Pinellas County is located on the west coast of central Florida and consists of a peninsula that extends into the Gulf of Mexico to the west and Tampa Bay to the east. The main metropolitan districts in Pinellas County include St. Petersburg, Largo, and Clearwater. Pinellas County Mosquito Control (PCMC) is responsible for the arboviral surveillance program and mosquito control throughout the county. The Pinellas County Health Department (PCHD) and the PCMC are responsible for assessing arboviral transmission risk to humans and issuing mosquito-borne disease advisories and warnings. The first indication of a possible WNV transmission problem in Pinellas County during the summer of 2005 came on July 11, when seven sentinel chickens from three flocks located in the south western quarter of the county tested positive for WNV antibody. On July 25 an additional 12 sentinel chickens from the same flocks tested positive for WNV antibody, and on August 1 eight more sentinels from the same flocks tested positive. This intense, early season transmission of WNV to sentinel chickens indicated that WNV 1) was more abundant than normal in Pinellas County, 2) had undergone an efficient early season amplification cycle in the south western quadrant of the county, and 3) had infected mosquitoes that had completed the EIP and were transmitting the virus to sentinel chickens. In addition, a GIS modification of a hydrologic groundwater model developed for south Florida (Figure 3) (Shaman et al., 2005) indicated that the ground water conditions in the southern half of Pinellas County were favorable for WNV amplification during springtime, i.e. drought-induced restriction of *Cx. nigripalpus* activity to habitats exploited by nesting avian hosts (Day and Shaman, 2008).

The collection and analysis of arboviral surveillance data by PCMC initiated a series of events that most likely greatly reduced the impact of WNV transmission on the humans living in and visiting Pinellas County during the summer of 2005. As a result of the first group of WN-positive sentinel chickens reported on July 11, a West Nile Virus Advisory was issued by the PCHD in consultation with PCMC. On July 20, PCMC began extra mosquito control efforts focused around the area identified by the three positive sentinel chicken flocks. On August 1, 2005 the first human WN case was reported, and on that same day the PCHD upgraded the West Nile Virus Advisory to a Mosquito-borne Disease Alert. Elevated focused mosquito control efforts continued throughout August. The onset dates for the 18 human WN infections ranged from July 9 through August 12, 2005. It is suggested that the rapid responses to the WN surveillance data by PCMC and the PCHD mitigated a potential WN epidemic in Pinellas County during 2005. The peak WNV transmission months in Florida are July, August, and September. The fact that the final onset date for a human case in Pinellas County during the 2005 outbreak was August 12 indicates that the WNV transmission cycle was broken by a failure of sufficient rainfall cycling through the

summer and aggressive mosquito control efforts coupled with a public health campaign designed to alert and educate the residents of Pinellas County about the risk of an impending WN epidemic. The outbreak and control of WNV in Pinellas County, Florida during the summer of 2005 is one of the best examples of how a well-designed arboviral surveillance program can be used to monitor and mitigate a potentially severe mosquito-borne arbovirus transmission event.
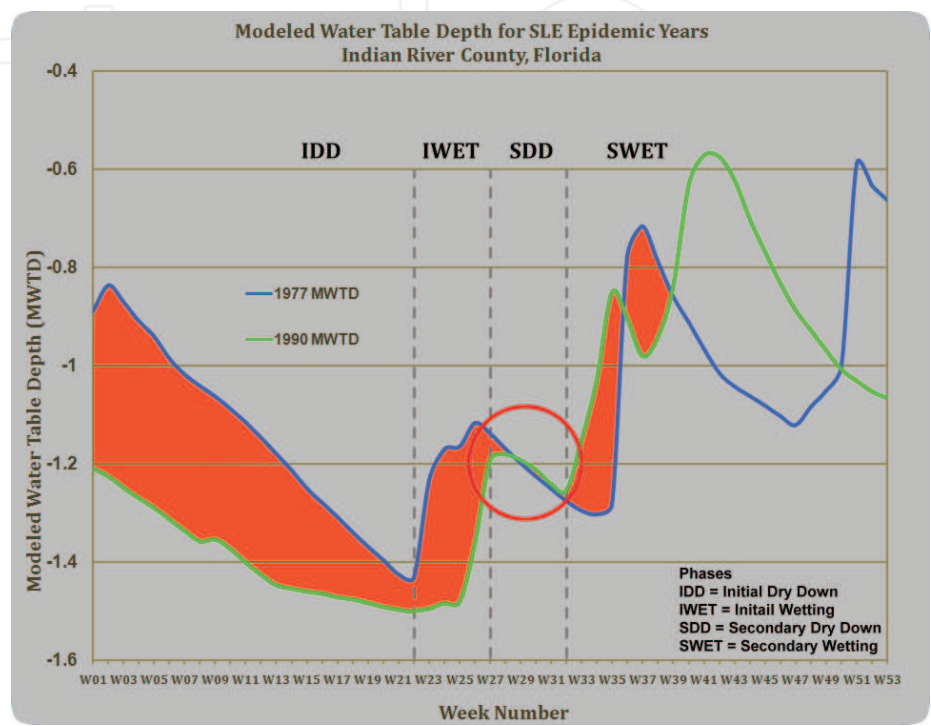

Fig. 3. Weekly Modeled Water Table Depth (MWTD) values in Indian River County, Florida for the SLE epidemic years 1977 (blue line) and 1990 (green line). The Florida Medical Entomology Laboratory Arboviral Epidemic Risk Model values (highlighted in orange) are compared to real-time WTD values collected throughout peninsular Florida. Modeled WTD values that fall continuously within the shaded area through the Initial Dry Down (IDD) and Initial Wetting (IWET) phases are favorable for the successful amplification of SLE and WN viruses. Areas where MWTD data closely follow the trends of the first two phases are considered at high risk for *focal* arboviral transmission. The Secondary Dry Down (SDD) phase, circled in red, along with a Secondary Wetting (SWET) phase, are considered critical for *epidemic* arboviral transmission. The last two phases provide conditions conducive for a second round of amplification followed by dispersal of virus out of the secondary amplification foci

5. Conclusions

Arboviral transmission cycles can be tracked in real time and the risk of an arboviral epidemic, based on surveillance data, can be predicted. The ability to accurately predict an arboviral outbreak is linked to the quality of the surveillance data, which depends greatly on an understanding of the biology and environmental conditions unique to each local disease transmission cycle. Our ability to mitigate arboviral transmission events is tied to the quality of the local long-term baseline data sets associated with annual measurements of

virus abundance and transmission; vector abundance, age structure, and infectivity status; and amplification host abundance and susceptibility to viral infection. Superimposed on these three biological cycles are the environmental factors that regulate the population biology of the virus, vector, and amplification hosts. There is a continued need to fine-tune our understanding of local arboviral transmission cycles. In a constantly changing environment, the importance of the primary vectors and amplification hosts to the dynamics of viral transmission are ever-changing. Vectors that were previously important to disease transmission become rare and disappear, while new vectors invade habitats where they were previously unknown. Amplification host populations that were previously important to transmission cycles may become less significant while new amplification hosts emerge and become important to the continuation of arboviral transmission cycles. Finally, viruses that were once abundant in particular habitats disappear and re-emerge to become major players in new habitats, sometimes on new continents. It is important that surveillance programs remain strong and vital in order to track these continually changing biological and environmental cycles that are responsible for disease outbreaks in humans, domestic animals, and wildlife around the World.

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7. References

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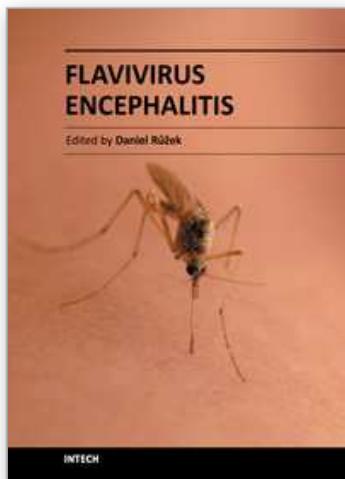
Flavivirus Encephalitis

Edited by Dr. Daniel Ruzek

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.

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