Unraveling a Complex Transmission Cycle: Implications for Control

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Abstract The ability of an arbovirus such as the West Nile virus to be transmitted depends on interactions among a large number of factors including host population structure and susceptibility, mosquito population structure, feeding patterns, and vectorial capacity and the genetic makeup of the virus. The interaction of these genetic components with environmental factors at any given time plays a significant role in viral transmission, as well as viral evolution and adaptation.

Keywords West Nile virus • Culex • Mosquito • Arbovirus transmission

An understanding of vector – virus – vertebrate interactions will allow us to begin to make predictions of public health risk and consequently allow us to make informed decisions regarding mosquito control. The plethora of factors influencing epidemic activity of a pathogen include host factors such as population composition and density, competence and immunity; vector factors including species composition and competence, longevity; and virus factors including strain and genotype (Fig. 1). The genetics of the vector, virus, and vertebrate have a critical impact on the intensity of virus transmission. Additional layers of complexity are the influence of environmental factors such as rainfall and temperature, and the need for synchronicity leading to contact between the vector and vertebrate at a time when one of them is infectious. The focus of this paper is on the consequences of these layers of interactions on viral evolution and adaptation, and determination of the most significant drivers of virus transmission with an emphasis on the impact of heterogeneity. Recent field studies and experimental research on West Nile virus (WNV) conducted by our laboratories will be used to address these points, and in the process honor the ecological approach to control fostered by Mir Mulla.

West Nile virus (Flavivirus: Flaviviridae) is spherical, enveloped, and approximately 50 nm in diameter Rice 1996. It contains a host-derived lipid bilayer surrounding a nucleocapsid consisting of the viral RNA complexed with multiple
copies of the capsid protein (Fig. 2) (Mukhopadhyay et al. 2003) as depicted in Kramer et al. (2007a). The viral genome is linear, positive sense, single-stranded RNA, 11,029 kb in length. Sequence data indicate that the first WNV isolates from New York City in 1999 were 99.8% identical to an Israeli isolate from 1998 (Lanciotti et al. 1999). This introduced genotype of WNV has been termed the “Eastern” or NY99 genotype.

The primary vectors of WNV are Culex spp. mosquitoes, although the virus has been isolated from at least 60 additional species of ten genera (http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm). Some of the non-Culex species are undoubtedly incidental vectors which are not competent to transmit virus. The predominant Culex species in WNV transmission varies regionally: Culex pipiens Linnaeus is the most important vector in the northeastern, central, and mid-Atlantic US (Kilpatrick et al. 2005), while the sibling species Culex quinquefasciatus Say in the southern US, and Culex tarsalis Coquillett in agricultural regions in the west (Bernard and Kramer 2001). The importance of Cx. restuans Theobald is unknown as its distribution overlaps with Cx. pipiens and since their adults are difficult to distinguish, they are commonly grouped together in surveillance testing. Studies of them are uncommon, but indicate they are equally competent as Cx. pipiens (Ebel et al. 2005). In the southeast, Cx. nigripalpus

Theobald is also a significant vector and Cx. erraticus Dyar and Knab may play a role in some areas (Cupp et al. 2007). Culex species, including Cx. pipiens, are also the most important vectors outside the US. It has been postulated that the lack of intense transmission of WNV in northern Europe is a consequence of the lack of hybridization between two biotypes of Cx. pipiens, form pipiens and form molestus (Fonseca et al. 2004).

Viruses have been detected in over 300 native and captive avian species. Although most species appear susceptible to infection, mortality varies greatly. Mammals have been implicated as susceptible hosts, although they are thought to play a less important role, if any, in viral amplification because of their relatively lower viremia titers. Evolutionary pressures on the virus, therefore, are applied predominantly by the mosquito and avian environments. WNV is one of the most widely distributed arboviruses, WNV has been isolated in Africa, the Middle East, Europe, Russia, Asia, Australia, and most recently the United States, Canada, Mexico, the Caribbean, Central and South America (Kramer et al. 2007b). The viruses are separated into two major lineages: Lineage-1 viruses include all Africa, Middle East, Europe, Russia, India, Australia, and the US. This lineage is further subdivided into Kunjin and India subtypes. Lineage-2 viruses include Sub-Saharan Africa and Madagascar (Fig. 3) (Lanciotti et al. 2002; 1999). Recently 3 additional lineages have been proposed, III [Czechoslovakia; Rabensburg virus (Bakonyi et al. 2005)], IV [Russia (Lvol et al. 2004)], V [India (Bonvic et al. 2007)].

The first topic my laboratory addressed was the evolution of WNV over time in New York State where the virus has been active since 1999. To study this, we sequenced isolates from American crows (Corvus brachyrhynchos) and Culex pipiens mosquitoes submitted by counties in diverse locations in NYS to the Arbovirus Lab since 1999 (Ebel et al. 2004). The entire envelope gene (1,503 nt) of more than 88 isolates of virus was sequenced. Neighbor joining analysis was conducted
using 1,000 replicates. During the spread of WNV across North America, a new genotype ("North American dominant" or WNO2) emerged and rapidly became dominant among circulating WNV strains (Davis et al. 2005; Ebel et al. 2004). The WNO2 genotype consensus sequence contains three consensus nucleotide sequence changes from the NY99 genotype: a U–C change at position 1442 in the E gene, a C–U change at position 2466 in the E gene, and a C–U change at position 9352 in the NS5 gene (Davis et al. 2005; Ebel et al. 2004). The U1442C is the only nonsynonymous change, resulting in a valine to alanine change at amino acid position 159 in the E protein. This conserved amino acid change is not located within the predicted receptor binding domain or any region predicted to be critical for efficient fusion of the viral envelope with the host cell membrane. The North American dominant genotype was first identified in Texas in 2001, and in New York in 2002.

Fig. 4. Displacement of the genotype of West Nile virus introduced into the US in 1999 ("NY99") by a new genotype, "North American dominant" or "WNO2". Reproduced with permission from Snapp et al. (2007). □ NY99 genotype, ■ WNO2 genotype

It rapidly displaced the introduced genotype over the next 2 years so that by 2004, there was no evidence of the NY99 genotype virus in circulation (Fig. 4) (Snapp et al. 2007). In order to understand the mechanism of this rapid displacement of one genotype with another, we began with an examination of the simplest explanation, that the WNO2 genotype replicates more efficiently than the NY99 genotype. We examined this first in vitro, using multi-step growth analysis, and found no differences in growth in mosquito or avian cell culture of strains of the two genotypes (Fig. 5) (Moudy et al. 2007). We then evaluated fitness of the viral strains of the two genotypes following modified protocols of Holland as described (Ciota et al. 2007) and also saw no consistent differences (Fig. 6) (Moudy et al. 2007). Studies then were conducted in vivo using *Culex pipiens* and *Culex tarsalis*. A greater proportion of both species of mosquitoes became infected with WNO2 than NY99 at early time points, and this continued out to 14 days post-feeding in *C. tarsalis*. In early experiments, proportions of infected *C. pipiens* equalized by 9 days post-feeding. However later studies demonstrated the WNO2 advantage continued over time and accelerated with temperature (Kilpatrick et al. 2008). Viral transmission of both genotypes begins very early at temperatures of 32°C, with a consistently greater fraction of mosquitoes transmitting WNO2 than NY99. In summary, we observed a change in the basic reproductive ratio of WNV through impact on extrinsic incubation period (EIP) as well as overall vector competence when experiments were carried out beyond 14 days. These differences are most likely due to genotype-specific differences in replicative efficiency and/or cell to cell spread in the mosquito.

There are several points during the mosquito infection process that differences could occur in virus-vector interactions. When a mosquito imbibles an infectious bloodmeal, the virus enters the midgut lumen and infects and replicates in the midgut epithelial cells. It then escapes from the midgut and infects and replicates in
Fig. 5 Replication efficiencies of NY99 and WN02 genotypes in vitro. Confluent (a) DF-1 or (b) C6/36 cells were infected with a single strain of WNV at an MOI of 0.01, and samples of supernatant were taken at the indicated times for virus titration in Vero cells. Viral titers at each time point are shown as $\log_{10}$ pfu/mL. Reproduced with permission from Moudy et al. (2007)

9 days post-inoculation. This indicates that WN02 viruses exhibit increased replication efficiency in vivo as compared to NY99 viruses. Together with previous data, it suggests that WN02 viruses can better overcome the midgut barriers due to their increased replication efficiency. However, these studies measure overall viral replication, which can be affected by several aspects of the viral life cycle including entry, RNA replication, and exit from the host cell. Therefore, future work will focus on viral interactions with the midgut.

As with the mosquito vectors, several aspects of virus interaction with the avian amplifying hosts could play a role in strain displacement, such as a difference in levels of avian viremia or differences in virus shedding or persistence within the birds. Braught and colleagues (Braught et al. 2007) have demonstrated the importance of a single point mutation in viremia in American crows. We are in the process of comparing the two WNV genotypes in house sparrows, which were the second most important host in our study sites in the Baltimore/Washington DC area, with American robins being first (Kilpatrick et al. 2006b).

These differences between the two WNV genotypes in mosquitoes could lend a significant advantage to amplification of WN02 over NY99 strains. Since Culex species can take a bloodmeal every 5 days, on average, a virus that can be transmitted 5 days after infection would be able to infect susceptible avian hosts during the first bloodmeal after infection, whereas a virus that isn’t transmitted until seven to nine days post-infection would not be able to infect birds until the second bloodmeal after infection. Therefore, the WN02-infected mosquito would have the potential to infect more birds than the NY99-infected mosquito, leading to greater numbers of WN02 infected birds, and subsequently increased minimal infection rates.
in mosquitoes. Similarly, higher viremias in birds would lead to a larger fraction of mosquitoes biting a host becoming infected.

A second focus of our research is to determine the spatio-temporal drivers of WNV transmission in North America, focusing initially on the northeast and mid-Atlantic region of the US. It was initially demonstrated that Cx. pipiens is not only the predominant enzootic vector of WNV in the northeast where some of the studies took place. Minimal infection rate (MIR) and host feeding patterns of mosquitoes in New York analyzed in conjunction with vector competence data indicated Cx. pipiens is also an important bridge vector to humans (Kilpatrick et al. 2005). Taking into account abundance, vector competence, feeding patterns, and minimal infection rate of different species of mosquitoes, Cx. pipiens represents the greatest threat to humans. The species-pair Cx. pipiens and Cx. restuans accounts for >80% of the total Risk, a surrogate for human WNV infections in the New York region, over the transmission season. The combined Risk of four other important species evaluated, Aedes japonicus, Aedes vexans, Aedes trivittatus, and Culex salinarius, represented one quarter the threat posed by Cx. pipiens and Cx. restuans.

In the mid-Atlantic, the relative abundance of birds at two residential and three urban sites were compared to the avian hosts identified in blood meals from Culex pipiens and Culex restuans mosquitoes, the predominant enzootic vectors in the mid-Atlantic region of the country. It was observed that a mosquito’s feeding pattern did not match the relative abundance of bird species at each of the study sites. American robins (Turdus migratorius), which made up only 3.7% (range among sites 1.0–7.5%) of the birds, were highly preferred at all sites, and accounted for 43.4% (range 24–71%) of Cx. pipiens blood meals (Kilpatrick et al. 2006b). Fish crows were also preferred at two sites, but were relatively uncommon and made up only 4% of blood meals at these sites. In contrast, house sparrows (Passer domesticus) were extremely abundant at all sites (56% of birds; range 42–67%) but appeared to be avoided by mosquitoes and made up 11% (range 0–21%) of the blood meals. The feeding preferences were then integrated with information from the literature on the host competence for each species, which estimates the probability of infecting a mosquito while viremic. Competence takes into account avian susceptibility, mean infectiousness, and days infectious (Komar et al. 2003). Using a conservative set of assumptions, the results indicated that American robins were likely responsible for 59.3% (range 35–88%) of the WNV-infectious mosquitoes at the five sites, whereas the much more abundant house sparrows were only responsible for 24% (range 4–40%) (Kilpatrick et al. 2006b). The impact of the focused feeding on American robins, and the heterogeneity in host competence, was to intensify transmission to this species and speed up the viral amplification. The pathogen reproductive ratio, R0, was increased by 10.4 fold (range 4.3–15.3) compared to the situation where mosquitoes fed on each host according to their abundance, and all hosts were equally competent. A possible consequence of the increased R0 was seen in earlier detection of WNV in mosquitoes at the sites where R0 was increased the most by heterogeneity in mosquito feeding and host competence.

One pattern that arose in the feeding data was a seven-fold increase over the season in the fraction of Cx. pipiens mosquitoes that had fed on humans (Kilpatrick et al. 2006c). Feeding shifts previously had been observed over the season in other mosquitoes, including Cx. tarsalis in California (Tempelis et al. 1965) and Colorado (Tempelis et al. 1967), and Cx. nigripalpus in Florida (Edman and Taylor 1968), but the cause for these previous feeding shifts was unknown. In the mid-Atlantic, the feeding shift coincided with the dispersal of American robins from urban and residential areas, and a decrease in feeding on robins. It was thus plausible that the feeding shift to humans occurred at least partly because of the decrease in the abundance of robins, Cx. pipiens’ most preferred host.

The consequence of the feeding shift for WNV transmission to humans was determined using data collected at the same sites on mosquito abundance, and WNV infection prevalence. The abundance of WNV-infected mosquitoes that fed on humans was strongly correlated with the number of human WNV cases in the region two weeks later (the approximate length of the time from infection to the onset of illness), suggesting that this product was a valuable measure of the risk of human infection (Fig. 7). In fact, this was the first time a risk index had been used to successfully predict temporal variation in the number of human cases. An analysis using this risk index suggested that the number of human cases was increased 4.5 fold as a result of the feeding shift compared to the situation in which the mosquitoes fed on humans at the frequency observed in June (Fig. 7). The feeding shifts previously noted in other areas (as well as that recently observed in Cx. erraticus in Alabama (Hassan et al. 2003)) all likely contributed to the relatively intense epidemics of WNV observed across North America.

An alternate explanation for the shift in feeding patterns is that mosquito feeding behavior is determined by genetic ancestry. Previous work had shown that Cx.
pipiens in North America were hybrids between two old world forms, Cx. p. p. form pippipes, which was thought to feed primarily on birds, and Cx. p. p. form molestus which was known to feed readily on humans (Fonseca et al. 2004). Thus, it was possible that the feeding shift in Cx. p. p. could have been a result of a shift in mosquito genetics over the season with form pippipes predominating earlier in the summer and form molestus later. In collaboration with Dina Fonseca (Rutgers University) we tested this hypothesis. Dr. Fonseca used microsatellites to determine the genetic ancestry of the same mosquitoes that were used in the feeding shift study (Kilpatrick et al. 2006a). This analysis showed that the fraction of alleles from form molestus was a significant predictor of whether the mosquito blood meal came from humans or birds (Kilpatrick et al. 2007). Thus, genetic ancestry was important in determining mosquito feeding patterns. However, the analysis also showed that there was no change in the genetic composition of the mosquito populations over time, so the feeding shift could not be explained by a shift in mosquito genetics. Taken together, these data from two studies showed that both genetic ancestry and host abundance influence mosquito feeding patterns and determine the transmission of pathogens between hosts.

A third focus of our research is to determine if and how the virus might spread to distant island groups including Hawaii, Galapagos, and Barbados (Douglas et al. 2007; Kilpatrick et al. 2006a; Kilpatrick et al. 2004). The pathways of spread that were considered included infected humans, wind transported mosquitoes, human transported mosquitoes, human transported vertebrate hosts, and migratory birds. These analyses showed that for Hawaii and Galapagos mosquitoes transported on airplanes were by far the greatest risk of introduction, whereas for Barbados migratory birds and mosquitoes on airplanes were of similar risk (Fig. 8). Other pathways were of lower risk because the concentration of virus in the blood of infected humans is too low to infect mosquitoes, very few mosquitoes have reached these islands by wind, and few vertebrate animals that could be infectious are brought to

these islands. Mosquitoes on boats are usually in the larval stage and the probability of vertical transmission is low. Finally, the numbers of migrating birds moving between the mainland and Hawaii and Galapagos is relatively low compared to Barbados, and both of the former island groups receive substantial flights from mainland areas where WNV is circulating. The primary management tools available to prevent the introduction of WNV and other vector borne pathogens to these island groups are (1) disinfection: killing insects on airplanes through residual chemical coatings, especially on the walls of the cargo holds where most mosquitoes are transported, or aerosols which appear to be less effective (Naumann and Michalich 1999) and (2) control of mosquitoes around airports and areas where migratory birds arrive to decrease the chance of establishment if a mosquito or bird were to reach the island.

In summary, combined field – laboratory research on each of the three components of arbovirus transmission cycles, i.e., virus, vector, vertebrate, is critical to expanding our understanding of virus amplification leading to high risk to humans. This knowledge is essential to the development of effective control strategies.

References


![Fig. 8](image-url) Estimated risk, on a logarithmic scale, of WNV introduction to Galapagos, Hawaii, and Barbados by five pathways. Risk is quantified as the number of infectious mosquitoes or bird days (the number of infected animals transported, multiplied by their infectiousness, and the length of their infectious period). Data from [Kilpatrick et al. 2004], [Kilpatrick 2006], [Douglas et al. 2007].
Sustainable Mosquito Control in California: A Template for the World

David Brown

Abstract Mosquito control in California is achieved through interactions with local mosquito abatement districts, the California State Department of Health and the University of California. This interaction has resulted in sustainable mosquito control across a large state that contains a diversity of mosquito environments. Effective mosquito control has also prevented the establishment of invasive mosquito species into California though its ports of entry and despite that large volume of freight shipped to California. The unique structure of the relationship that has effectively controlled mosquitoes in California can serve as an example to other regions of the world.

Keywords Mosquito control · California · Arbovirus · University of California

Introduction

Sustainable mosquito control has been a standard in California for over 75 years. Mosquito control has remained sustainable in California in large part through an innovative partnership involving local mosquito control districts, the State of California’s Department of Public Health, and the University of California. This partnership has provided a foundation that allows the implementation of research-based control efforts at the local level that are endorsed by the State of California.

This chapter will examine the three components of this partnership and how it could be a template for other parts of the world.