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Aaron C. Brault

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Aaron C. Brault. Changing patterns of West Nile virus transmission: altered vector competence and host susceptibility. *Veterinary Research*, 2009, 40 (2), 10.1051/vetres/2009026 . hal-00903121

HAL Id: hal-00903121

<https://hal.science/hal-00903121>

Submitted on 11 May 2020

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Changing patterns of West Nile virus transmission: altered vector competence and host susceptibility

Aaron C. BRAULT^{*,**}

Center for Vector-Borne Diseases and Department of Pathology, Microbiology and Immunology,
School of Veterinary Medicine, University of California, 5327 VM3A,
One Shields Ave, Davis, CA 95616, USA

(Received 4 February 2009; accepted 29 April 2009)

Abstract – West Nile virus (WNV) is a flavivirus (*Flaviviridae*) transmitted between *Culex* spp. mosquitoes and avian hosts. The virus has dramatically expanded its geographic range in the past ten years. Increases in global commerce, climate change, ecological factors and the emergence of novel viral genotypes likely play significant roles in the emergence of this virus; however, the exact mechanism and relative importance of each is uncertain. Previously WNV was primarily associated with febrile illness of children in endemic areas, but it was identified as a cause of neurological disease in humans in 1994. This modulation in disease presentation could be the result of the emergence of a more virulent genotype as well as the progression of the virus into areas in which the age structure of immunologically naïve individuals makes them more susceptible to severe neurological disease. Since its introduction to North America in 1999, a novel WNV genotype has been identified that has been demonstrated to disseminate more rapidly and with greater efficiency at elevated temperatures than the originally introduced strain, indicating the potential importance of temperature as a selective criteria for the emergence of WNV genotypes with increased vectorial capacity. Even prior to the North American introduction, a mutation associated with increased replication in avian hosts, identified to be under adaptive evolutionary pressure, has been identified, indicating that adaptation for increased replication within vertebrate hosts could play a role in increased transmission efficiency. Although stable in its evolutionary structure, WNV has demonstrated the capacity for rapidly adapting to both vertebrate hosts and invertebrate vectors and will likely continue to exploit novel ecological niches as it adapts to novel transmission foci.

West Nile virus / vector competence / temperature / host competence / virus-host interaction

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* Corresponding author: acbrault@ucdavis.edu, abrault@cdc.gov

** Current address: Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, 3150 Rampart Rd/Foothills Campus, Fort Collins, CO 80521, USA

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1. INTRODUCTION

The purpose of this review is to present some of the potential factors that have been associated with the emergence of West Nile virus (WNV) from an endemic virus, generally associated with childhood disease, to an agent that has rapidly expanded its geographic range, becoming established in the New World with novel, more serious disease manifestations. Ecological, virological, epidemiological and entomological avenues are explored to address this phenomenon.

1.1. Viral classification

West Nile virus is a member of the genus *Flavivirus* within the family *Flaviviridae* [59] and was first isolated from a human experiencing a febrile syndrome in the West Nile district of Uganda in 1937 [142]. Serological cross reactivity has grouped WNV within the Japanese encephalitis (JE) virus serocomplex [27]. In addition to WNV, the JE serocomplex includes three other viral species responsible for human disease including Japanese encephalitis (JEV), St. Louis encephalitis (SLEV) and Murray Valley encephalitis (MVEV) viruses [94].

Despite the name “serocomplex”, classification of these viruses is based on a combination of serological cross reactivity, nucleotide sequence data, and vector association [59]. The WNV species contains one viral subtype (Kunjin), comprised of isolates from Australia

and Malaysia [157]. West Nile viruses have a pandemic distribution having been isolated on every continent with the exception of Antarctica [57]. Despite the fact that serological assays distinguish WNV isolates from India as well as Kunjin from other WNV, only Kunjin is classified as a distinct viral subtype [156]. Nucleotide sequence data has subsequently demonstrated Kunjin viruses to have a greater genetic identity with newly emergent WNV genotypes than Old World strains including those from India [115, 135]. Altered serological reactivity is most likely due to the lack of an additional glycosylation motif within the envelope glycoprotein of Kunjin viruses [1].

1.2. Structure and replication

West Nile virus is comprised of an enveloped spherical virion with a diameter of approximately 50 nm. The virus has a linear, single-stranded, message-sense genome of approximately 11 Kb with a 5'(7-methylguanosine) cap and no polyadenylation at the 3' end of the genome [129]. The 5' and 3' non-coding regions (NCR) have conserved nucleotide elements that form stem-loop structures necessary for viral RNA transcription, translation and packaging [25, 72, 139]. Viral proteins are translated as a single polyprotein that is post-translationally cleaved by host and viral proteases to produce 3 structural (C, prM and E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The 5' third of the genome encodes the structural



Figure 1. Genomic organization of the WNV genome. Three structural proteins (C, prM, E) and seven nonstructural proteins (NS1, 2A, 2B, 3, 4A, 4B and 5) are translated as a single polyprotein directly from the 11 Kb positive-sense RNA genome.

proteins necessary for encapsidation of the viral RNA and the surface proteins required for viral receptor interaction and fusion with host cells (Fig. 1) [76]. The remainder of the genome encodes the nonstructural proteins that form the viral replication complex for the generation of negative-sense template RNA as well as positive-sense genomic RNA [161]. The largest of the NS proteins, NS3 and NS5, have been characterized for interactions and replicase activity; however, the function of the remaining NS proteins is largely unknown. The NS5 protein has both RNA polymerase and methyltransferase motifs necessary for viral replication. Helicase and serine protease domains have been described for the NS3 protein [129]. Interestingly, the NS1 protein is a secretory nonstructural protein that does not have a designated function in RNA replication or as a control element [162].

1.3. Molecular epidemiology of WNV

Phylogenetic trees based on nucleotide sequence data indicate the existence of five potentially distinct genetic lineages of WNV that have diverged by up to 29% at the nucleotide level [18, 89]. Sequence analysis of viral strains from North America, Africa, the Middle East, Asia, and Australia (Kunjin) constitute lineage 1 based on structural [18, 63, 89] or nonstructural gene regions [14, 29]. Phylogenetic analyses clearly indicate that Kunjin is a member of lineage 1 and that the second lineage WNV, restricted to a sub-Saharan African distribution, are more distantly related to other lineage 1 WNV than to Kunjin virus [135]. Lineage 3 WNV is represented by a Czech Republic mosquito isolate (Rabensburg; RabV)

[11], lineage 4 is comprised of *Dermacentor* spp. tick isolates from the Russian Caucasus [117], and lineage 5 is represented by a separate genotype from India [21]. Phylogenetic grouping of isolates does not correlate with geographic distribution, indicating the potential importance of birds in viral dispersal [18].

All of the major outbreaks of human encephalitis have been associated with viruses genetically categorized as lineage 1, making an important correlation between viral genetics and disease phenotype. The preponderance of genetic evidence suggests that the introduction of WNV into North America occurred from the Middle East. Complete genomic sequencing of an isolate from an outbreak in geese in Israel in 1998 and NY99 strains of WNV group them in the same genetic clade with a 99.7% shared nucleotide identity [29, 90]. The identification of bird carcasses found to be positive for WNV from a number of species in Israel provides evidence that naturally occurring bird virulent strains could be circulating within the Middle East and were introduced into North America [95].

1.4. Disease and distribution

Clinical disease in humans ranges from mild fever to the development of severe neurological disease [143]; however, retrospective serological studies have indicated that the majority of WNV infections are actually asymptomatic [6, 154]. West Nile fever is the most common clinical manifestation and is characterized by the development of high fever, chills, rash, headache, myalgia and nausea. Symptoms typically abate within 3–5 days of onset and result in lifetime immunity. WNV infection also may result in

the development of severe neurological symptoms that include encephalitis, meningitis, myelitis or combinations of these symptoms [58, 98]. Acute flaccid paralysis of the limbs and respiratory muscles has been described in North America [8] and Romania [28].

Elderly individuals are at a greater risk for developing severe neurological disease, with younger patients more commonly developing WN fever [106]. It is not readily clear whether the more severe neurological symptoms are the result of increased virulence of the introduced strain or a reflection on the immunologically naïve status of the population whose age structure might be more predisposed to severe disease manifestations. Postulated mechanisms of viral entry to the CNS and subsequent development of more severe disease include impaired structural elements of the blood-brain barrier (BBB) or impaired immunity with increased age [143]; however, data generated with microvascular endothelial cells indicates the maintenance of BBB integrity following infection with WNV, indicating the potential of free virus to pass this barrier. Furthermore, increased cell adhesion molecule expression observed in this study indicated the potential for infected immune cells to also traverse the BBB [158].

West Nile virus has been found to be endemic to Europe [80], Africa [45, 101], Asia and Australasia [69]. Within its geographic range, outbreaks of febrile disease in humans have occurred sporadically with well documented outbreaks being reported in Egypt [149], France [64] and South Africa [68] with outbreaks occurring in 2008 in Romania [116], Hungary [84] and Italy [93, 133]. Like in the USA [4, 5, 7], recent disease outbreaks occurring in Romania [154], Italy [10, 104], Russia [92] and Israel [30] have been associated with the severe disease symptomology. Since its introduction to the USA in 1999, a total of 28 975 human cases including 1 124 deaths (through 2008) have been documented (www.cdc.gov).

West Nile virus has demonstrated a wide host range, infecting, for example, equines, alligators [62], dogs [9], sheep [71], llamas [86], alpacas [35, 87]; however, few animals consistently demonstrate overt disease. The attack rate

for equines is approximately 5% with an estimated 1 in 3 horses that demonstrate encephalitis succumbing to the disease [10, 26]. Other than birds, some squirrel [51, 60, 78, 111, 113], chipmunk [112] and rabbit species [153], and potentially alligators [79] most serve as dead-end hosts in which circulating viremias are not sufficient for infection of mosquito vectors.

1.5. Transmission of WNV

Since its identification in 1937 from the West Nile district in Uganda [142], WNV transmission cycles have been extensively described in Egypt [149], South Africa [68], Israel [109] and Pakistan [3, 55, 121]. These maintenance cycles have all included ornithophilic mosquito species and mostly passerine avian hosts (Fig. 2) that maintain sufficient viremias for the infection of subsequent mosquitoes. Isolations have been made most extensively from mosquitoes of the genus *Culex*, not only in Egypt [148] but also throughout its pandemic distribution [121]. In South Africa, although isolations have been made from a wide range of mosquitoes, *Culex univittatus* is the only mosquito vector that is efficiently infected at titers that are present in birds infected with WNV. The role of alternative mosquito populations in viral transmission is presumably quite low [66, 67]. In endemic transmission foci of Australia, *Culex annulirostris* is the major mosquito vector [53], while the *Culex vishnui* complex and *Culex tritaeniorhynchus* have been implicated with transmission in India and Pakistan [3, 102]. Evidence of seroconversions of birds in endemic foci [81, 100, 165] and viremia [164] and antibody [22] production following experimental inoculation of bird species has incriminated avian hosts as the vertebrate reservoir for WNV throughout its distribution. Additionally, certain mammalian species have been found to, in some cases, develop sufficient viremia for infection of mosquito vectors [112, 113, 132]; however, the role that these hosts play in the transmission dynamics of WNV are currently uncertain but of significant interest due to the potential infection of mammalophilic mosquito vectors.

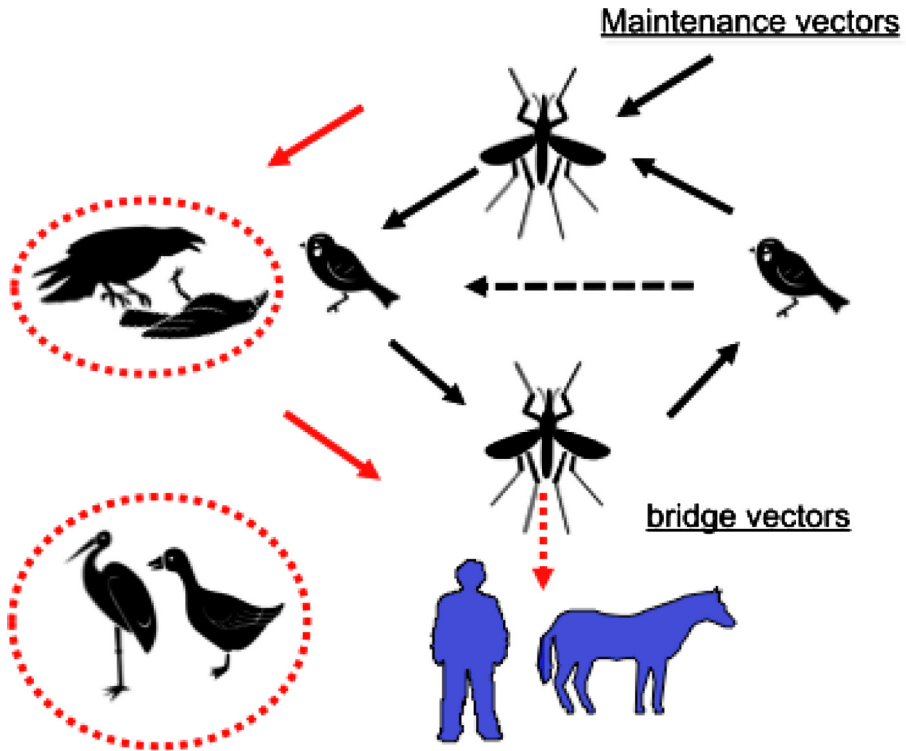


Figure 2. Transmission cycles of WNV. Transmission occurs between ornithophilic mosquito species (*Culex* spp.) and passeriform avian hosts. Birds typically manifest high viremias capable of exceeding oral infection thresholds for many mosquito vectors. Humans and equines fail to generate sufficient viremia for the infection of mosquito hosts and are deemed “dead end” or tangential hosts. Red: Avian mortality was first identified in a migratory stork and subsequently in commercially farmed geese in the late 1990s and has subsequently been associated with widespread mortality among numerous North American avian species (in particular, corvids). (A color version of this figure is available at www.vetres.org.)

Bird mortality has been a unique epidemiological finding for WNV transmission in Israel [19] and North America [131], having never before been reported during outbreaks of WN fever in Europe [134] the Middle East [105] or with endemic transmission in Australia [53] or South Africa [68] (Fig. 2). In contrast to Old World transmission, isolations of WNV have been made from numerous species of North American mosquitoes representing 11 genera. Mosquitoes most commonly infected in the eastern USA have been members of the *Culex pipiens* species complex, and this species complex was also associated with transmission during a human urban epidemic

in Romania in 1996 [154]. However, isolations have been made from marginally competent vectors such as the catholic vectors *Aedes albopictus* and *Aedes vexans* [155].

In North America, West Nile virus rapidly expanded its geographic range from four states in 1999 to all contiguous 48 and Canada by 2004 [120]. With this westward transition, WNV has adopted *Culex tarsalis* as its predominant rural vector in western North America. The mechanism for viral dispersal has not been experimentally addressed; however, the extremely high viremia titers present within both resident and migratory bird species (> 150) indicate the probable role of some of these bird

taxa in viral dissemination [131] with the potential for limited local dispersal by mosquitoes [118].

1.6. Use of crow mortality to track WNV activity

Deaths in American crow (AMCR) were especially prevalent in New York City in 1999 [4] and monitoring of crow mortality has been adopted as an epidemiological indicator for the tracking of WNV transmission in the USA and has proven useful in predicting increased risk for human infections [39, 40, 65]. Dead crows with confirmed WNV infections preceded the first human cases by three months in New York in 2000. Despite this fact, there is no knowledge regarding the genetic and/or pathological mechanism(s) of susceptibility for WNV in crows or any other bird species.

1.7. Virulence and immunity studies with WNV

West Nile virus neurovirulence has been studied in primate [114] as well as mouse [13, 52, 160] and hamster models [166]. Age-related resistance to neuroinvasion is typical of mosquito-borne flaviviruses [42, 48]; however, intraperitoneal infection of 15–16 week-old mice and adult hamsters with the newly introduced North American genotype induces fatal encephalitis [13]. This finding is in contrast to a study in which WNV encephalitis was rare in mice older than 7 weeks of age following inoculation with a WNV strain from Egypt [160]. Genetic mapping studies have indicated the importance of the IFN-inducible 2'-5'-oligoadenylate synthetase genes with resistance to WNV infection [99].

Development of encephalitis is identified at or near the time of clearance of virus from the peripheral circulation concurrent with the production of neutralizing antibody in a hamster model of WNV encephalitis. This model has also demonstrated that hematogenous spread of the virus across the blood-brain barrier is the most likely route of entry to the brain as the olfactory ganglia were not reported to have become infected [166]. Intranasal inoculation is

a highly inefficient mode of inducing neurovirulence in mice, further indicating that infection of the olfactory bulb is likely not the most efficient mechanism of viral entry to the brain [13, 52, 110]. Inoculation of mice with different WNV strains has demonstrated a correlation between viral genotype and neurotropism [13]; however, genotypes with increased neurotropism do not correlate with increased binding to either human or mouse brain receptor preparations [12, 107]. Unlike AMCR that have generated titers of up to $10 \log_{10}$ PFU/mL serum and uniformly succumb to infection within 5 days of infection [83], hamsters have peak viremias of approximately $5 \log_{10}$ TCID₅₀/mL of serum, have approximately 50% survival rates and typically die 7–14 days after infection with the NY99 WNV genotype [166]. Antibody development may play an important role in protecting hamsters from potentially fatal encephalitis, since peripheral viral titers dissipate as circulating neutralizing antibody is produced [151, 166]. Immunization of hamsters with heterologous flaviviruses such as Japanese encephalitis and St. Louis encephalitis viruses has demonstrated a protective effect against lethal encephalitis [152]. Administration of anti-flaviviral antibodies within 4–6 days post-infection has also been demonstrated to be efficacious for preventing flaviviral encephalitis in a mouse model [130]. This time frame correlates with viral invasion of neural tissues [52, 130, 166]. Intraperitoneal inoculation of WNV in a hamster model also demonstrates that serum neutralizing antibody development within five days of inoculation correlates with decreased peripheral viral circulation [166].

2. FACTORS ASSOCIATED WITH WEST NILE VIRAL EMERGENCE

2.1. Virological factors associated with emergence

2.1.1. Emergence of avian virulence

An Egyptian strain, Ar-248, was isolated from a moribund pigeon (*Columba livia*) squab in a field study conducted from 1952-1954.

This strain produced morbidity and mortality in experimentally infected hooded crows (*Corvus corone*) and house sparrows (*Passer domesticus*) [164]. High seroprevalence rates within hooded crow populations in Egypt coupled with the rapid deaths of the experimentally inoculated bird (some within 1 to 2 days of infection), indicate the possibility that mortality could have been the result of experimental handling of the birds rather than natural virulence from infection [149].

No reports of natural WNV-associated mortality in adult birds occurred during the first six decades since the discovery of the virus in 1937 [57]. However, significant avian morbidity and mortality have been recent hallmarks of WNV outbreaks in Israel [19, 147] and North America [17]. In 1998, WNV was identified in tissues taken from dead migratory storks in Israel [97] and later that year, an outbreak within goose farms across Israel was identified. Affected flocks had severe mortality rates in goslings between 3 and 8 weeks of age [96]. Experimental infection of goslings with viruses later isolated from North America demonstrated similar mortality and resulted in viremias that were sufficient to infect mosquito vectors [147]. Evaluation of the virulence of WNV isolated from New York in 1999 demonstrated mortality in 8 of 25 species experimentally infected. The most susceptible species were the blue jay (*Cyanocitta cristata*), common grackle (*Quiscalus quiscula*), house finch (*Carpodacus mexicanus*), AMCR (*Corvus brachyrhynchos*), and house sparrow (*Passer domesticus*). Corvids (including AMCR, blue jays and magpies) all developed circulating viremias in excess of $8 \log_{10}$ PFU/mL blood and in some cases exceeded $10 \log_{10}$ PFU/mL blood [83]. Necropsy tissues taken from naturally [145] and experimentally infected [83] AMCR have demonstrated that the North American WNV is not limited to infection of particular organ systems, with virus being identified in the brain, heart, kidney, lungs, gonads, spleen, liver, intestines, esophagus and skin.

High mortality rates in AMCR and the quantity of virus identified in various organs have been factors for the adoption of crow mortality as a surveillance tool to identify areas with

recent WNV activity [39, 40]. WNV infection with the NY99 genotype has been identified in more than 150 species of birds representing over 20 bird families in North America, causing morbidity in some cases but not necessarily mortality [82, 83, 136]. Chickens, for example, demonstrate no mortality following infection with WNV, yet titers as high as $5 \log_{10}$ PFU/mL of serum have been identified and virus can be isolated from myocardium, spleen, kidney, lung, and intestine. Additionally, histopathological examination has identified myocardial necrosis, nephritis, and pneumonitis most notably in young birds [136].

The genetic changes observed in different WNV strains can modulate their virulence potential in avian species [23, 91]. Kunjin viruses have demonstrated low peak viremia thresholds compared to alternative lineage 1 WNV strains in both AMCR [23] and HOSP [91]. Other lineage 1 WNV, closely related to but different from the highly avian virulent NY99 WNV strain, have also demonstrated reduced viremia and subsequently lowered mortality rates in AMCR following experimental infection [77]. These data in accordance with the finding that WNV strains circulating in Israel that are genetically most similar to the NY99 strain have been associated with avian virulence [19] indicate that viral genetics play a crucial role in host susceptibility to lethal infection.

2.1.2. Genotype associated with avian virulence

Positive selection modeling of WNV representing members of all of the lineages has identified a single genetic loci (NS3-249) to be under adaptive evolution [24]. This residue lies within the helicase domain of the NS3 protein in an area not associated directly with RNA binding or ATP hydrolysis; however, incorporation of a NY99 substitution (NS3-T249P) into a closely related lineage 1 WNV that elicited a low level of virulence in AMCR was sufficient to elicit a viremia and mortality response in crows indistinguishable from the NY99 strain. Interestingly, this avian virulence mutation, NS3-249P, has emerged on at least three independent occasions (Fig. 3). In each case, the viruses with this

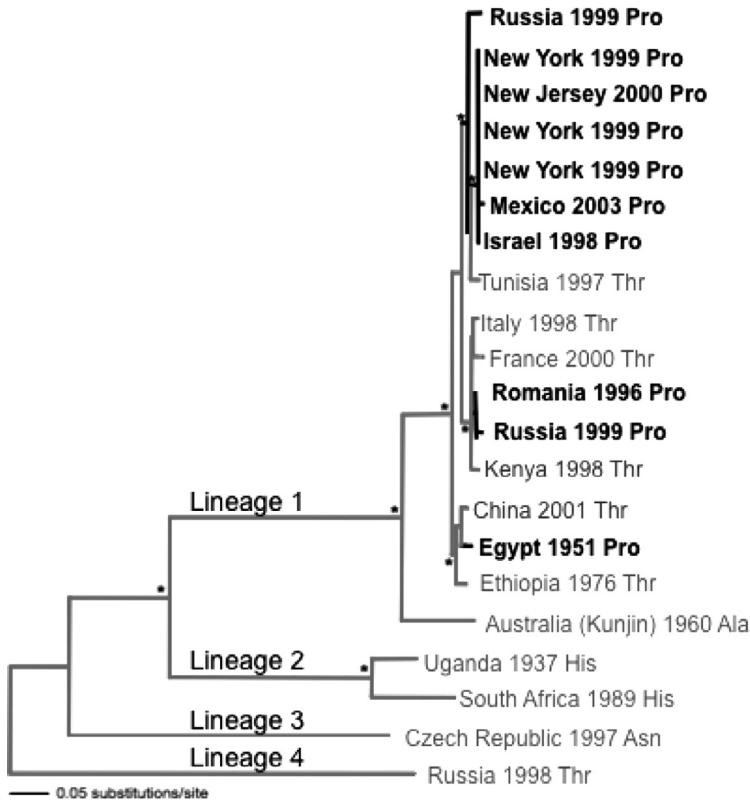


Figure 3. Maximum likelihood phylogenetic tree of 21 complete genomes of WNV. Viruses are grouped according to four described lineages of WNV (excluding the lineage 5 Indian group) and the genetic residue at each NS3-249 site is indicated. The three independent emergences of the NS3-249P genotype has been denoted on the tree. In each case an NS3-249Pro genotype emerged (designated in bold black) from a predecessor genotype (Thr; designated in grey). The asterisks represent nodes with bootstrap support values > 95%.

substitution have been associated with human disease outbreaks; however, only the Egyptian 1951 isolate [164] as well as viruses from the Israeli/North American clades [89] have been associated with avian virulence. The preponderance of these data indicated the potential importance of avian viremia for the emergence of lineage I WNV and suggests the potential for the emergence of alternative WNV genotypes following small genetic changes. Interestingly, several WNV lineages contain the NS3-249P substitution but have not been associated with avian virulence or

the emergence of disease outbreaks indicating the possible limitations that particular WNV genetic backbones could impose on emergence potential of alternative genotypes as well as the potential role of the NS3-249P for increased replication in a wider array of avian species.

2.1.3. Increased virulence as a factor for increased transmission

A number of avian species are highly susceptible to WNV infection, exhibiting high

viremias and associated mortality rates, and decreases in abundance [88]. Although it could be concluded that this resultant reduction of the breeding population would diminish transmission, high avian mortality is postulated to potentially increase transmission in three ways: (i) survival of previously infected and now immune birds could dilute the effect of subsequent transmission events [50], (ii) mortality in birds is associated with high peripheral viremia leading to the efficient infection of moderately susceptible mosquito vectors [23, 77, 83], and (iii) increased pathological manifestations could favor increased infection of mosquitoes due to reduced anti-mosquito defensive behavior of moribund avian hosts [33] as well as increased attractiveness to mosquitoes as a result of hyperthermia [77]. The time period in which avian hosts such as AMCR are the most viremic coincides with maximal viremia and moribund birds, presenting with elevated viremias on the ground could result in increased efficiency for infectivity of contact with vector mosquitoes, thereby increasing transmission potential of WNV to humans or equines [108]. Furthermore, it has been hypothesized that vector-borne agents have the potential for tolerance of higher levels of mortality. This has been theorized to result from the need for disseminated replication for the infection of arthropod vectors that often results in more serious clinical manifestations within the vertebrate host. The transmission of vector-borne viruses by mobile vectors has also been postulated to compensate for any dampening of transmission due to the lack of mobility of moribund vertebrate hosts [34, 43].

In contrast to WNV transmission in North America in which low oral infection rates of *Culex* spp. vectors are likely offset by high viremias in avian hosts, limited avian mortality and low oral infection thresholds of *Cx. univittatus* and *Cx. tritaeniorhynchus* vectors are transmission hallmarks within endemic foci. Although a few studies have demonstrated geographic differences in susceptibility of Old World endemic mosquito populations [2, 56], vector competence experiments with North American mosquitoes have indicated considerable temporal and spatial variability for oral susceptibility with no discernable pattern that could be linked

to amplification [125]. These data taken together indicate the importance of avian amplification by invasive WNV for infection of marginally susceptible North American *Culex* spp. while low oral infection thresholds of specific vectors can allow for transmission in the absence of high avian viremias within endemic transmission. These differing evolutionary strategies are also evident with a closely related flavivirus, SLEV. Comparatively, despite the fact that they utilize the same avian hosts and mosquito vectors, SLEV has an approximately 10-fold greater oral susceptibility than WNV but produces low magnitude viremias and is less virulent in birds [122].

2.1.4. Viral adaptation for replication at higher temperature

One factor that has classically defined the limit of the host range utilized by an RNA virus has been its ability to replicate at elevated temperature [36, 61]. Arboviruses such as WNV have a wide range of temperatures in which they must replicate in order to be propagated within both vertebrate and invertebrate hosts. An arbovirus such as dengue that replicates in primates is incapable of replicating at temperatures above 39 °C. This could be an important factor in the inability of these viruses to utilize birds (that have higher body temperatures) as vertebrate hosts [85]. Much of our knowledge in reference to replication at alternative temperatures is the result of the generation of temperature sensitive (*ts*) viral mutants for the identification of the functional role of viral proteins in the replication process [140] or the development of mutants that have impaired replication capacity [20, 167] for the development of vaccines. Replication of arboviruses in a two host system can moderate the amount of selection that can take place in either the vertebrate or invertebrate host [159]. Evidence also suggests that in vitro selection for increased replication in vertebrates can result in a decreased ability to replicate in mosquito tissues [54]. The temperature spectrum for which different arboviruses can replicate (and subsequently the hosts that they are capable of utilizing) is poorly understood.

Previous studies have defined the potential importance of both temperature constraints within avian hosts [77] as well as mosquito vectors for WNV transmission. Degree-day models have identified the replication limit of WNV in mosquito vector to be constrained by temperatures below 14 °C and further demonstrated that the strain of WNV introduced to North America requires warmer temperatures for dissemination and subsequent transmission [124] than a WNV strains from sub-Saharan Africa [31]. Further data has demonstrated that the genotype of WNV that has predominated in North America since 2002, the WN02 genotype, seems to disseminate more rapidly and at a more efficient level at elevated temperatures than the prototype NY99 North American strain [75, 103]. This shortening of the extrinsic incubation period (EIP), the time between a mosquito imbibing an infectious bloodmeal and being capable of transmitting virus by bite, could have been the factor by which this genotype of virus has been selected. Analyses of temperature patterns in the USA have demonstrated an association between above-normal temperatures and epidemics of WNV in northern latitudes [124]. These data coupled with the finding of increased efficiency of vector dissemination at elevated temperatures of the predominant WNV genotype indicate temperature as a potential important selection criterion for WNV evolution.

2.2. Environmental factors associated with emergence

2.2.1. Climate perturbation

Climate change can have a direct effect on WNV amplification rates in poikilothermic mosquito vectors as external temperature has been documented to have an inverse relationship to the duration of the EIP [124]. Additionally, increases in transmission season can result as well as expansion of transmission to more extreme latitudes and elevations. Although increasing temperature is negatively correlated with longevity of mosquitoes, in most instances the increased dissemination and transmission rates actually result in increased transmission

efficiency. Patterns of WNV transmission are significantly linked to the abundance of vector populations. It has been determined that heavy rainfall in the spring and warm, dry temperatures during the summer are optimal for *Culex* spp. population increases and are positively correlated with WNV transmission [120, 126, 137, 138].

2.2.2. Species diversity, species behavior and transmission dynamics

One explanation for the lack of WNV-associated disease in Latin America is the increased avian species diversity in the tropics. It is hypothesized that increased species diversity could negatively impact transmission of WNV if there was a high proportion of incompetent hosts. One study has made such a connection by identifying a negative correlation between non-passerine species diversity and the force of WNV transmission, presumably owing to a dilution effect of vertebrate hosts with incompetent non-passerine avian species [44]. Other studies have suggested the importance of “super-spreaders” or certain avian species such as the American robin (*Turdus migratorius*) in temperate habitats that appear to not only be highly susceptible to infection with WNV but also are preferential sources for bloodmeals [73]. These concepts will require further investigation as a number of competing theories for the reduced impact of WNV in Latin America have been proposed including the circulation of WNV strains with reduced virulence phenotypes [15] and the potential inhibitory role of acquired immunity to heterologous flavivirus circulation in Mexico, Central and South America [47].

Shifts in mosquito host selection have been documented and associated with the potential for increased infection. One study indicated that *Culex pipiens* mosquitoes in the northeastern USA shift their feeding behavior from highly competent American robins to mammals and humans in the late summer to early fall, coinciding with the emigration of this avian species [74]. This host switching has also been observed with *Culex tarsalis* in the western USA [150] and *Cx. nigripalpus* in Florida

[38], indicating the importance of feeding behavior in the tangential transmission of WNV. Utilizing microsatellite markers to genetically distinguish Nearctic and Palearctic *Culex* spp. mosquitoes, one study inferred that Palearctic European species limit their feeding to avian hosts while North American Nearctic species feed indiscriminately upon avian and mammalian hosts [49] and concluded that this differential feeding behavior was the explanation for the lack of persistent WNV activity in Europe. However, a number of other studies [144] have provided evidence that Palearctic European *Culex* spp. feed on both mammalian and avian hosts, which contradicts the conclusion that feeding behavior could modulate epidemiological patterns.

Previously, WNV infection in AMCR has been uniformly lethal. However, the detection of seropositive AMCR has been reported, indicating that survival of individual birds in these highly susceptible avian species can occur [163]. Resistance to lethal infection could be associated with lower peak viremia, thus leading to selection for WNV genotypes with reduced oral infective thresholds for mosquitoes. As such, the effect that these modulations in vertebrate host population susceptibility could have on a concomitant selection for increased oral susceptibility of mosquito vectors should be monitored closely.

2.2.3. Role of heterologous flaviviruses on WNV transmission

The effect that the presence of heterologous flaviviruses has had on WNV transmission is a subject of some debate; however, it is apparent that increased avian herd immunity and mortality has had a detrimental effect on the transmission of, for instance, SLEV in southwestern transmission foci [127]. Cross-protection with WNV has been demonstrated to preclude House finches (HOFI) from mounting detectable viremia following SLEV challenge. Conversely, inoculation of HOFI with SLEV prior to WNV infection substantially reduces WNV viremia to levels below that associated with oral infection in most mosquito vectors [46, 123]. The potential effect of selective pressures from

heterologous flaviviral immunity is currently not known. Heterologous infection of vertebrate hosts and mosquito vectors with alternative flaviviruses could also result in potential recombination events as well as block mosquito infectivity through barriers to super-infection. Barriers to super-infection have been described previously for arthropod-borne viruses [16, 32, 37, 41, 70, 141, 146]. For example, *Aedes triseriatus* mosquitoes experimentally infected with LaCrosse virus (LACV) have been shown to be resistant to infection with another closely related bunyavirus, Snowshoe Hare virus (SSHV), approximately two days post-infection [16]. A resistance period for dual infection was identified for ticks co-infected with Thogoto virus (THOV) for a period ranging between 24 h and 10 days of the initial infection with an alternative THOV strain [32]. An *Aedes albopictus* cell (C6/36) model further demonstrated that the inhibition of the secondarily infecting alphavirus is sequence specific. Alternative alphaviruses were blocked from super-infection of C6/36 cells, while unrelated bunyavirus or flaviviruses could establish infection in alphavirus-infected C6/36 cells within the time period that these cells were resistant to infection with heterologous alphaviruses [37, 70]. The mechanism for this “super-infection exclusion” has not been identified; however, it has been hypothesized that competitive exclusion through template scavenging during RNA replication as well incompatible interactions between viral proteins exclude replication of the secondarily infecting virus. Inhibitory effects on the super-infecting virus have been identified in the processes of binding to the cellular surface receptors, low pH fusion with the endocytic vesicle, viral uncoating, viral replication as well as viral maturation and budding [141].

2.2.4. Alternation of transmission patterns

Since WNV was introduced into the USA in 1999, an identified pattern has been recognized in which the season of introduction is characterized by mild activity with few human cases. This is followed by an epidemic year characterized by large numbers of cases, high infection

rates in mosquitoes with subsequent avian sero-conversions and mortality. The third season of activity has been referred to as the “subsidence year” in which human cases and enzootic indicators drop precipitously, presumably owing to increased herd immunity and avian depopulation of highly competent amplification hosts [119]. Decreased avian herd immunity resulting from the presence of naïve hatchlings as well as waning immunity in after-hatch year birds is then conducive for continued transmission during subsequent seasons. In contrast, areas lacking “super spreaders” or highly susceptible corvids have failed to amplify enzootic transmission to outbreak levels [127], whereas other areas have experienced recurring epidemic level transmission during sequential seasons [128]. The identification of more severe neurological syndromes in North America could be the result of an increased virulence of the WNV strain introduced as well as age-related exposure rates in a completely naïve population. Within endemic areas of WNV or SLEV transmission in which youth exposure rates are high, neuroinvasive disease is uncommon. While the virulence of the imported strains deserves further attention, the role of a “virgin soil” effect should not be overlooked as an explanation for the altered disease phenotypes observed in North America.

3. CONCLUSION

Since its introduction to North America, WNV has undergone a dramatic increase in geographic range and has demonstrated a greater association with a more severe neurological clinical presentation. Undoubtedly a number of ecological, virological and social factors have contributed to this emergence. Virological factors highlighted in this review have included genetic adaptation for increased replication in avian hosts as well as increased dissemination in mosquito vectors at elevated temperatures. As with other RNA viruses, the presence of an error-prone polymerase that lacks proofreading function coupled with the large population sizes occurring in both infected avian hosts and mosquito vectors allow for the

rapid selection of novel genotypes. Novel ecological niches that have been created by climatic perturbations and social or demographic changes assuredly will result in the emergence of novel genotypes to occupy these habitats and thus perpetuate the continually changing landscape of WNV epidemiology.

Acknowledgements. I would like to thank Dr Eddie Holmes for the construction of the Phylogenetic tree presented in [Figure 3](#) as well as Dr Bill Reisen for his many helpful suggestions.

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