EXPERT REVIEWS

Risk factors for West Nile virus infection and disease in populations and individuals

Expert Rev. Anti Infect. Ther. 13(3), 317–325 (2015)

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West Nile virus (WNV) is a mosquito-borne enveloped positive-strand RNA virus that emerged in North America in 1999 in New York City. Over the past 15 years, WNV has become established throughout the USA and has spread into Canada, Mexico and the Caribbean. CDC reports indicate >41,000 clinical cases, including more than 1700 fatalities. An estimated 3 million people in the USA may have been infected to date. Infection with WNV is dependent on many factors including climate, mosquito habitats and immunologically naïve bird populations. In addition, variations within individuals contribute to the risk of severe disease, in particular, advanced age, hypertension, immunosuppression and critical elements of the immune response. Recent advances in technology now allow detailed analysis of complex immune interactions relevant to disease susceptibility.

KEYWORDS: aging • avian reservoir • epizootic spread • immune response • mosquito vector • mouse model • West Nile virus

West Nile virus (WNV) is a mosquito-borne enveloped positive-strand RNA virus that emerged in North America in 1999 in New York City. Over the past 15 years, WNV has become established throughout the USA and has spread into Canada, Mexico, the Caribbean and into South America. CDC reports indicate >41,000 clinical cases including more than 1700 fatalities. An estimated 3 million people in the USA may have been infected to date [1]. WNV has become an important public health concern in the USA because of its high prevalence, severe disease in humans and the absence of effective treatments or vaccines [2–5]. WNV patients exhibit considerable variation in clinical responses ranging from asymptomatic infection to severe neurological involvement and even fatal outcomes. There are currently no US FDA-approved treatments available. Infants, the immunocompromised and the elderly are more susceptible to neurological involvement that may result in death [6,7]. Many factors contribute to the risk of severe infection with WNV, including vector prevalence, exposure and individual host factors and immune responses. Here, we review recent studies that identify elements that contribute to infection with WNV and to divergent outcomes.

Emergence of WNV in North America

WNV was first recognized in the Western Hemisphere in the New York City area in 1999, with 62 human clinical cases reported (FIGURE. 1) [8]. In addition to human cases of disease, avian and equine morbidity and mortality from WNV infection were also reported [9,10]. By 2001, WNV had disseminated as far south as Florida, marking an adaptation from the *Culex* (*Cx.*) *pipiens pipiens* species mosquitoes present in the northeastern region of the US and Canada to the *Cx. pipiens quinquefasciatus* mosquito in the southern and mid-western regions of the US [11,12]. Between 1999 and 2001, a total of 149 human cases had been reported from 10 states [13]. In 2002, an unprecedented epizootic of WNV occurred, with the virus reaching 39 states plus the District of Columbia, resulting in 4156 reported clinical cases and 284 deaths. In 2002, the virus adapted to a new *Cx.* species and also underwent several nucleotide changes, with the new WN2002 strain of virus sweeping across North America, ultimately displacing the NY99 strain cases of disease [11]. Also during 2002, it became quickly evident that WNV could be transmitted through means other than from the

 10.1586/14787210.2015.1007043 ISSN 1478-7210

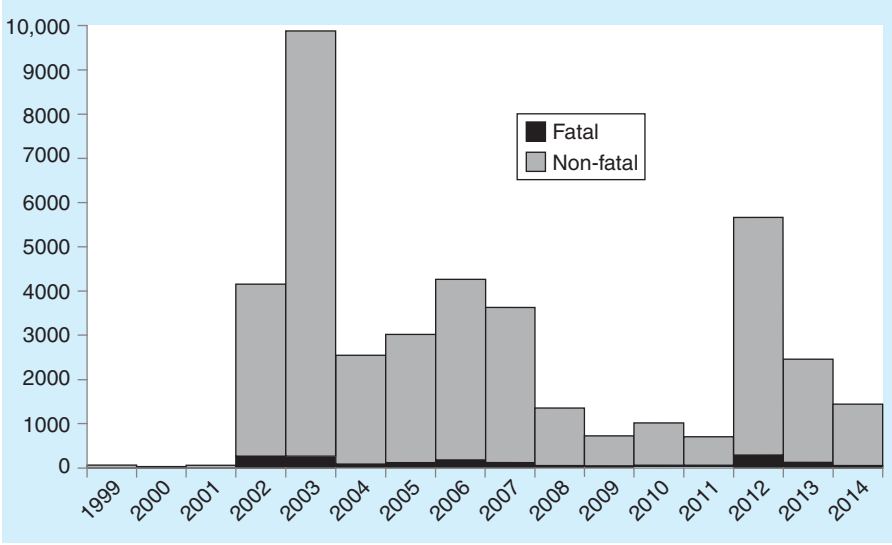

Figure 1. Total number of human cases of West Nile virus in the USA, 1999-2014.
†Number of WNV cases reported to CDC's Arbonet [13]; 2014 cases are as of 14 October, 2014.

bite of an infected mosquito, including blood transfusions, organ transplant, transplacental transfer from mother to fetus and through infected breast milk [14–16]. In 2003, blood banks across the US began to screen the blood supply for WNV using nucleic acid amplification testing, an amplification-based transcription technique, which identifies positive blood from WNV-infected individuals before they become symptomatic [17]. Since this testing, transfusion-transmitted infection is rare or absent, despite the persistence of WNV RNA for up to 3 months in red blood cells [18]; however, some cases of organ-transplant transmission have been reported [19].

Following the epizootic of 2002, WNV continued the wave of epizootic activity across the US, further adapting itself to the *Cx. tarsalis* mosquito and reaching the West Coast by 2003 [13,20]. By 2012, all lower 48 states plus the District of Columbia had reported locally acquired human WNV cases, and by 2014, more than 41,000 human cases of clinical WNV had been reported to CDC, including more than 1700 fatal cases, and avian and equine morbidity and mortality from WNV infection were also reported [13]. Although the virus appeared to reach endemic levels between 2008 and 2011, with an average annual report of 908 cases, another unprecedented and unexpected epizootic occurred in 2012, with 5674 cases (286 fatal) being reported to CDC, with Texas reporting 33% of cases (n = 1868) [21]. With continued high levels of virus activity reported in both 2013 and 2014, WNV will remain a substantial public health threat in North America.

Risk factors for populations

There are numerous factors, both known and unknown, that contribute to viral amplification in the environment and subsequent epizootic WNV activity [22]. Climate conditions, particularly ambient temperature and rainfall, are critical drivers of mosquito abundance and amplification of WNV [23–27]. Studies on *Cx. pipiens* have found a direct correlation between increases in ambient temperature and increases in vector populations [28]. Interestingly, elevated temperatures also affect the rate of virus replication, leading to more rapid infectivity of the mosquito [29]. Precipitation is another key component to promoting vector abundance by providing ample breeding environments for mosquitoes [31], although heavy rains and floodwaters can also reduce mosquito breeding grounds by flushing out their preferred stagnant water sources [32]. In contrast, drought conditions can promote WNV transmission by forcing birds and mosquitoes to share the same habitat through competition for scarce water sources; it was believed that the severe drought in Texas in 2011–2012 was the driver for the 2012 outbreak that resulted in 1868 human cases [21,33].

People living in close proximity to stagnant water sources where mosquitoes breed have been found to be at higher risk for infection. In Houston, Texas, where *Cx. quinquefasciatus* mosquitoes serve as the primary vector, a recent study found that WNV case patients were significantly more likely to reside near slow-moving/stagnant water sources with heavier vegetation [34]. Similarly, in El Paso, Texas, where *Cx. tarsalis* is the primary vector, WNV case-patients were close to yards that were flooded regularly by irrigation canals [35]. In northern Colorado, where *Cx. tarsalis* is also primary, irrigation and manual control of water on the landscape was also found to promote mosquito abundance [36]. In addition to being in areas with high vector abundance, risk for becoming infected can be influenced by time spent outdoors and decisions on whether to adopt personal precautions against mosquito bites. In Houston, a serosurvey of homeless individuals found that time spent outdoors greatly influenced infectivity, with 12.5% of those who reported spending >12 h outdoors being positive for WNV, compared with only 2% of those who reported spending <6 h outdoors [37]. Other studies have also found that increased time outdoors was associated with infection [38–40], in addition to inconsistent mosquito repellent use [38,39] and younger age, with children being five-times more likely to be infected with WNV when compared with adults [41]. Vector control measures may have value in reducing infection exposure [30].

Finally, viral amplification and infectivity of mosquitoes relies completely upon the presence of an immunologically naïve avian host reservoir population [21,42]. Birds are responsible for maintenance of virus in the environment and are the critical link to the transmission cycle. As seen with the rapid expansion of WNV across North America, birds spread the virus into new geographic areas [43] and *Cx. pipiens* species

mosquitoes are predominately ornithophilic, in that they prefer feeding on avian hosts, particularly passeriformes [44,45]. Passerine birds, including blue jay (*Cyanocitta cristata*), common grackle (*Quiscalus quiscula*), house finch (*Carpodacus mexicanus*), American crow (*Corvus brachyrhynchos*), house sparrow (*Passer domesticus*), cardinal (*Cardinalis cardinalis*), and American Robin (*Turdus migratorius*) have been found to contribute most to viral amplification and transmission of WNV to mosquitoes [46,47]. A study in Illinois found that 80% of blood meals from field collected *Cx. pipiens* mosquitoes were from avian sources, with 25 different avian species identified; American robin (*Turdus migratorius*) was the most overrepresented with 48% of avian blood meal sources [45]. Interestingly, they also found in this study that of the mammalian blood meal sources, 83% were from humans. In California, *Cx. tarsalis* species mosquitoes were found to have a much higher diversity in its host-seeking behaviors when compared with *Cx. pipiens* complex mosquitoes [48], though both species of mosquitoes were found again to be predominately ornithophilic. In contrast to the study in Illinois, the California mosquitoes rarely fed on humans, with human blood meal sources only found in 0.4% of *Cx. pipiens* and 0.2% of *Cx. tarsalis* mosquitoes, leading the authors to conclude that human cases only occur when there is substantial epizootic activity. In central and eastern Texas, the most common vector for WNV is *Cx. quinquefasiatus*, which has a remarkably different host seeking pattern. Blood meal analyses found that only 39% of blood meals came from an avian source [49]. Canines were the most common blood meal identified (41%), whereas humans were the least common (0.4%). Continued research focused on modeling amplification and predicting epizootic activity would be of extreme value to protect public health.

Risk factors for individuals

Clinical features & diagnosis

Human infection with WNV is often asymptomatic. WNV illness is characterized by fever and flu-like illness, which can include symptoms such as weakness, joint pain, chills. Rare cases (~1% of infected individuals) are diagnosed with severe WNV neuroinvasive disease characterized by meningitis, encephalitis, acute flaccid paralysis, and neurological sequelae may persist in some cases [50]. Diagnosis of WNV is based on laboratory findings of specific IgM and IgG antibodies by ELISA or pleocytosis/PCR-positive cerebrospinal fluid [50]. Identification of encephalitis can be challenging although specific radiologic patterns resemble other related virus infections (St Louis encephalitis, Kunjin and Japanese encephalitis) [51] and initially negative serology or CSF testing may bear repeating at a later interval to clarify the diagnosis.

Individual host risk factors including both innate and adaptive immune responses can contribute to severe neuroinvasive disease and death following infection with WNV. Risk for severe infection has been identified in particular with certain genomic determinants, advanced age, a history of cardiovascular disease, chronic renal disease, hepatitis C virus infection and immunosuppression [50,52,53]. Recent advances in high-throughput and bioinformatics technology now allow detailed analysis of complex interactions to generate a systems level understanding of disease susceptibility [54,55].

Genomic determinants of severe infection

Markers associated with susceptibility to severe WNV infection include single nucleotide polymorphisms in several genes. Certain HLA types appear to be associated with risk of more severe outcome [56], and interferon response pathway elements such as oligoadenylate synthetase 1b – involved in RNA degradation – interferon regulatory factor (IRF3), myxovirus (influenza virus) resistance 1, and a dominant negative splice variant of RNaseL, which functions in the anti-proliferative roles of interferon [5,57–59]. Another genomic study investigated >1500 symptomatic subjects (severe vs mild), and showed more severe neurological disease associated with single nucleotide polymorphisms in replication factor C (activator 1) 1, a replication factor; sodium channel, voltage gated, type I alpha subunit, a sodium channel; and alanyl aminopeptidase, an aminopeptidase, although even more differences might have been revealed when comparing asymptomatic and symptomatic cases [60]. And a deletion in chemokine (C-C motif) receptor 5 (CCR5), known to be protective in infection with HIV, was not associated with susceptibility to WNV, but did correspond to severity of infection, presumably due to reduced function of CCR5 pathways in infected hosts [61]. As more host factors are identified, there are sure to be a number of new determinants of WNV infection. One area of active investigation is identification of polymorphisms relevant to successful aging, and while mechanistic studies are only now in progress, the absence of these markers is likely to overlap with genes related to susceptibility to WNV [62].

Reduced response to WNV infection in elderly populations

Aging is associated with a progressive decline in immune function [63] and several anti-viral pathways in innate immunity show reduced efficiency in cells from older donors. Primary macrophages show age-dependent impairment in Toll-like receptor 3-mediated anti-WNV responses, leading to an early and sustained elevation of cytokines. In addition, WNV-induced type I IFN was significantly lower in dendritic cells from older donors compared with younger donors. These deficits in regulatory pathways in antiviral responses may contribute to the permeability of the blood–brain barrier (BBB) and enhanced susceptibility to WNV infections observed in aging [64,65].

Innate immune responses provide critical protection from severe disease

Protection from acute tissue injury is heavily reliant upon the innate immune system that functions early in the host response [66,67]. The first responding cells in infection, polymorphonuclear cells, show a paradoxical role in human WNV infection, where infiltrating polymorphonuclear cells are permissive for WNV replication and may serve as an early reservoir of WNV replication, but after exposure to IFN, contribute to viral

Table 1. Immune Components that protect from neurological infection with West Nile virus.

Component	Human	Mouse	Notes
Cytokines	IFN α/β IL-1 IL-4 [64,65,70,71,91,107]	IFN α/β IL-1 ifit2 [91–93,121]	IFN controls early virus replication in the brain IL-1 elevated during infection; critical in clearance in the brain; decreased in macrophages of severe human subjects
Chemokines	CCL2 CXCL10 [70,71]	CXCL10 CCR2 CXCR3 [87,88,90]	CXCL10 elevated in myeloid DCs Neuronal CXCL10 directs CD8 T cells
BBB permeability		Caspase 12 ICAM-1 IL-22 MIF MMP9 TLR3 TLR7 TNFα [78–86]	Decreased integrity of the BBB promotes viral entry to the brain

BBB: Blood–brain barrier; CCL: Chemokine (C-C motif) ligand; CXCL: Chemokine (C-X-C motif) ligand; DC: Dendritic cells; ICAM: Intercellular adhesion molecule; IFIT: Interferon-induced protein with tetratricopeptide repeats; MIF: Macrophage migration inhibitory factor; MMP9: Matrix metalloprotease 9; TLR: Toll-like receptors.

clearance [68]. Macrophages are also critical: mice depleted of macrophages are more susceptible to WNV infection and have higher and extended viremia and higher mortality [67]. The γδ T cell population rapidly expands after WNV infection and promotes a protective adaptive immune response by producing IFN and facilitating dendritic cell maturation [69]. Subjects with a history of severe disease had lower levels of plasmacytoid dendritic cells, which are critical for anti-viral responses [70].

Human studies show a role for chemokines chemokine (C-X-C motif) ligand (CXCL10) and chemokine (C-C motif) ligand 2 in control of early infection and an important role for IFN-mediated innate immunity in resolving acute WNV infection [71]. The production of Type I IFN and other cytokines that facilitate early control of viral replication follow recognition of the RNA of WNV through pathogen recognition receptors such as Toll-like receptors 3, 7, and 8 and cytoplasmic RNA helicases such as retinoic acid-inducible gene-I and melanoma differentiation associated gene 5 that activate transcription factors IRF3, IRF5 and IRF7. IFN-inducible interferon induced transmembrane protein also inhibits the early replication of WNV [5,72–75], and the retinoic acid-inducible gene-I-like receptors- and mitochondrial antiviral signaling protein-induced type I IFN response is inhibited by ubiquitin-regulatory-X (UBX)-domain-containing protein [76]. Relative deficiencies in the expression or function of these pathways would reduce the efficiency of anti-viral responses.

Factors contributing to neurological involvement

Permeability of the BBB, which is enhanced by cytokine responses, has been shown to be critical to susceptibility of neuroinvasive WNV infection [77]. Elements which decrease the integrity of the BBB contribute to susceptibility to infection with WNV (Table 1). Entry of WNV to the CNS may be afforded by cytokine responses, adhesion molecules or proteases [4,78–82], trafficking of infected $CD45^+$ leukocytes and $CD11b^+$ macrophages [83], T cells [84] or neutrophils [85]. TNFα may contribute to severe disease by promoting BBB permeability [78] or protect from severe WNV encephalitis by promoting monocyte entry into the brain [86]. CXCL10 from the brain directs CD8 T cell recruitment and viral control in specific regions of the brain [87,88]. During infection with WNV, $CD8^+$ T cells expand dramatically and migrate to the site of CNS infection [89].

In the murine model, the chemokine receptor CCR2 mediates monocyte accumulation that is critical to antiviral activity in the brain [90]. For control of virus in the brain, IL-1 and IL-1R1 signaling restricts WNV in the CNS [91,92], as does interferon-induced protein with tetratricopeptide repeats 2 in a cell-type specific manner [93]. Patients with severe WNV neurological involvement had elevated neutrophils in the cerebrospinal fluid that was only a modest predictor of disease outcome [94]. $CD8^+$ T cell clearance from the CNS is modulated by melanoma differentiation associated gene 5 [95]. Advances in our understanding of these pathways at the systems level will elucidate critical components of resistance to severe infection [70].

Adaptive immunity generates protective T cell responses & long-lasting antibody responses

The highest magnitude of specific T cell responses were $CD8^+$ cells [96] that were not apparently related to disease severity [97]. $CD8^+$ T cells, through c-Myc induced transcription factor AP4 [98], express perforin and/or granzyme B, and in response to infection with WNV, expand dramatically and migrate to the site of CNS infection [89,99]. WNV-specific murine CD4 T cells produced IFN-γ and IL-2 and also showed direct antiviral activity [100]. More cytolytic memory T cells were found in patients

with neurological disease [101]. Tregs play an important role in protecting against severe disease and it has been shown in human patients (and animal models) that WNV subjects with severe infection have reduced numbers of Th1/Th17 and Tregs [102]. Moreover, expression of T cell-inhibitory receptor TIM-3 during acute infection is associated with more severe disease [103].

Adaptive immunity provides protection against WNV via production of specific antibodies and both IgM and IgG antibodies are protective [104]. IgM and IgG antibodies develop rapidly after viremia and before RNA levels become undetectable, which occurred in a mean of 13.2 days [105]. Anti-WNV envelope antibody serum levels were relatively constant for >1 year [105,106], or even up to 8 years after infection, but no significant difference in antibody levels was observed between subjects with a history of asymptomatic or severe WNV infection [107]. This suggests that susceptibility to WNV was not the consequence of an inability to mount a humoral response, which has been noted for antibody responses to other viral antigens [108].

Biomarkers of susceptibility to severe WNV infection

Individual variations in serum cytokine levels were significantly different from WNV subjects with a history of severe infection (encephalitis) versus asymptomatic subjects. Subjects with a history of severe infection had significantly lower levels of IL-4 that were associated with altered gene expression patterns [107]. An integrated system-level study of transcriptional and functional datasets from WNV subjects identified a predictive signature of susceptibility (67% accuracy) that was detectable years after acute infection, with the most prominent alterations in severe susceptibility being decreased IL-1B production by macrophages and decreased CXCL10 expression from myeloid dendritic cells [70]. These results suggest that systems-level analysis of immune status can identify factors relevant for severe infections [70,107].

Prospects for biomarkers & treatments

A novel positive regulator for IFN production, E74-like transcription factor 4 (ELF4), may distinguish anti-WNV susceptibility. ELF4 is induced in primary human macrophages after infection with WNV and plays a role in signaling through the mitochondrial antiviral signaling protein–TANK-binding kinase 1 complex [109]. WNV subjects with severe disease showed higher levels of ELF4 in macrophages after infection *in vitro*, which may lead to increases in multiple cytokine responses and more severe infection [70]. Other antiviral signaling components have been identified *in vitro* but have not yet been shown to define susceptibility in human cohorts.

Many potential avenues for treatment show promise in animal models including RNAi, antibody therapy and interferon [5]. Both passive and active immunization strategies directed against the envelope (E) proteins of WNV can protect mice from lethal infection [110–113]. Single-chain antibody [112,114], targeted nanoparticles [115], attenuated virus vaccines [116] and plant-derived viral proteins have shown efficacy in animal models [117]. Indeed, in equine models, vaccination was both protective and cost-effective [118,119]. Although, Phase I clinical trials showed promise for antibody therapy [50,120], there are no FDA-approved vaccines or therapeutics. Limited case reports of use of IFNα or ribavirin, valuable treatments for other viral infections, have been inconclusive for use with WNV [50]. Future opportunities may benefit from advances in our understanding of individual immune susceptibility to target factors relevant for severe infections [70,107].

Expert commentary

WNV made a dramatic entrance to the US in 1999 and has rapidly spread throughout our region. Cases vary with climate conditions and are likely to increase. Prospects for a specific vaccine or therapeutic treatment are not immediately apparent but markers of immune susceptibility may provide directions for novel therapeutic interventions. Physical protective measures are valuable and our increased knowledge of immune determinants may provide clues to identify individuals at greater risk and modulate severity.

Five-year view: speculative viewpoint

The very dramatic introduction and rapid spread of WNV in the US and into neighboring countries has garnered considerable public attention and concern. As a result of investigations of the ecology and epidemiology of WNV reservoirs and cases, we have developed a detailed understanding of the method of infectivity, laboratory diagnostics and measures to reduce exposure. However, specific therapeutic measures are needed and not as yet available. With continued high levels of virus activity reported in both 2013 and 2014, WNV will remain a substantial public health threat in North America. This will be a valuable frontier for progress in the near future. In addition, WNV may serve as a model for other emerging pathogens such as Chikungunya or Ebola viruses and highlight critical steps in identification, testing, and remedies needed for public health and safety.

Acknowledgements

The authors regret omission of many important articles due to space limitations and thank many colleagues at their institutions for insightful discussions.

Financial & competing interests disclosure

This work was supported in part by the National Institutes of Health (HHS N272201100019C, U19AI089992, AI091816, AI057229), and the Gillson Longenbaugh Foundation. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Key issues

- People living in close proximity to stagnant water sources where mosquitoes breed have been found to be at higher risk for infection.
- Increased time outdoors and the absence of personal precautions against mosquitoes is associated with infection.
- Physical controls to limit exposure to mosquitoes such as use of screens and insect repellent will limit infection.
- Regional vector control programs, such as reducing standing water or spraying larvicides may reduce the reservoir of infected mosquitoes and limit human exposure.
- The elderly and immunocompromised individuals are particularly susceptible and should exercise heightened protection from mosquito exposure.
- Advances in our understanding of immune markers of susceptibility hold promise for screening the highest risk individuals.
- With continued high levels of virus activity reported in both 2013 and 2014, WNV will remain a substantial public health threat in North America.
- Developing therapeutic measures may include preventative vaccines as well as antibodies or other agents which act post-infection as therapeutics.

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population variables to predict future West Nile virus (WNV) outbreaks.

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