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West Nile virus

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Summary
This review covers the basic biology of the West Nile virus and the host–vector–pathogen interactions that result in significant disease in wild birds, horses and humans. The review describes the basic properties of the virus, cellular infection and the pathogenesis of the disease, and the ecology of virus maintenance, amplification and transmission. Disease epidemiology and risk estimation strategies that are currently in use are also examined, and host immune responses and vaccination practices described. The principles of vector control, exposure control and long-term risks caused by climatic and habitat factors are also included.

Keywords
Arbovirus – Avian – Emerging disease – Encephalitis – Equine – Mosquito – Vaccine – West Nile virus – Zoonosis.

Introduction

West Nile virus (WNV) continues to circulate among mosquitoes and has been reported in a wide range of wild and domestic animals, including birds, reptiles and mammals. This virus causes disease in birds, horses and people (1, 2, 3, 4), and is transmitted by mosquitoes. The disease emerges and re-emerges in horses and humans in the Mediterranean and Eastern Europe, Africa, Asia and North America. Many species of birds have been identified as potential amplification hosts (5). In regions where the disease has more recently emerged, multiple species of mosquitoes have been identified as potential vectors. This virus continues to be a significant cause for concern because of its epizootic and zoonotic potential. The spread and emergence of WNV infection and disease has provided strong natural evidence of the global risk of future epidemics caused by other arboviruses (4, 6, 7). The following websites provide additional information:

– www.nwhc.usgs.gov/disease_information/west_nile_virus/affected_species.jsp

– www.cdc.gov/westnile/index.html

– www.ecdc.europa.eu/en/healthtopics/west_nile_fever/pages/index.aspx.

Virology and pathogenesis

The genus *Flavivirus* contains the viruses of the Japanese encephalitis virus (JEV) antigenic complex (serogroup), which includes JEV, WNV, St Louis encephalitis virus, and Murray Valley encephalitis virus (MVEV) (6). All are human pathogens but WNV, MVEV and JEV are very important veterinary pathogens. All of these viruses are mosquito-transmitted viruses. The spherical virions are 50 nm in diameter. The nucleocapsid has typical icosahedral symmetry (8). There is a tightly adherent lipid envelope surrounding the nucleocapsid that displays the transmembrane and spike glycoproteins. The genome is a single molecule of linear, positive-sense, single-stranded RNA of approximately 11 kilobases. The genome contains one long open reading frame. The reading frame encodes for ten proteins that are generated from both co- and post-translational processing (Fig. 1). The translated structural proteins are: C, the nucleocapsid protein; M, the transmembrane glycoprotein; and E, the major spike glycoprotein. The E glycoprotein is the principal target site for antibody-mediated neutralisation (9).

West Nile virus, like most flaviviruses, replicates well in primary and adapted cell cultures. In animals and birds, the virus is neurotropic (8). In susceptible species

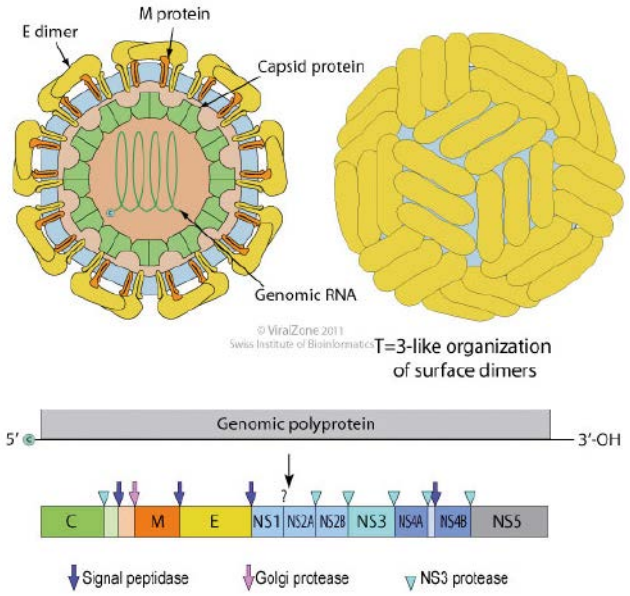

Fig. 1
Flavivirus
Flaviviruses are enveloped, spherical and about 50 nm in diameter. The surface proteins are arranged in an icosahedral-like symmetry
Mature virions contain two virus-encoded membrane proteins (M and E), while immature virions contain a membrane protein precursor

Source: Courtesy of the ViralZone project of SwissProt group, Swiss Institute of Bioinformatics

of birds, especially some corvids, infection results in an overwhelming, high-titre viraemia, severe inflammation and subsequent tissue necrosis with haemorrhaging in multiple internal organs and the central nervous system (CNS) (10, 11). Horses typically have a relatively low-titre viraemia with lesions only in the CNS (8). Tangential infections and subsequent epizootics have occurred in horses, but the attack rate is probably below 10% (12). However, the case fatality rate is close to 50%. Clinical signs are associated with the encephalitis and include weakness, posterior ataxia, recumbency and muscle fasciculation. Human infections are mostly asymptomatic, with the majority of clinical cases presenting flu-like symptoms; however, severe cases with meningo-encephalitis or meningitis can occur in people with certain medical conditions and the elderly, resulting in 10% mortality (13). There is no human vaccine currently available nor any specific antiviral treatment for WNV infections. As the WNV epidemic spread westwards across North America from 1999 to 2003, its emergence was closely associated with very high crow mortality and large numbers of equine encephalitis cases (neuroinvasive/high-mortality WNV infections) (14, 15, 16, 17, 18). In the years from 1999 to 2001, most infections occurred in the north-eastern United States and along the Atlantic coast. In 2002 and 2003, the infection had spread west across the United States (19). After 2003, the number of cases decreased significantly, probably due to immunity from natural exposure or vaccination (18). However, the disease has periodically re-emerged (2005–2007 and 2012) (19). In the summer of 2014, there have been many reports of disease in North America (www.cdc.gov/westnile/statsMaps/preliminaryMapsData/).

There are two pathogenic lineage strains of WNV: 1 and 2 (20). Lineage 2 strains commonly infect horses and are in circulation in mosquitoes in sub-Saharan Africa and Europe, and infection with WNVs of this lineage may cause significant disease in equines (21, 22, 23, 24). The lineage 1 virus strains that emerged in Europe and North America are also very often virulent in horses (21, 22). These, more recently emerged, lineage 1 WNV strains are the leading cause of viral encephalitis in horses and people in the United States (25, 26). The CNS lesions observed include scattered foci of necrotic neurons and non-suppurative inflammation (encephalomyelitis).

Apoptosis is an important mechanism of cell death associated with WNV infection in the CNS (27). The extent of neuronal apoptosis and encephalitis in infected tissue is influenced by the intensity of the initial innate inflammatory response. Hence, an over-aggressive inflammatory response may potentiate cellular death (27, 28). However, a delayed or suppressed immune response may allow more extensive development and spread of infection, leading to more severe pathology in the CNS, as observed in older horses (27, 29). Recent neuropathogenesis studies in mice have shown that phylogenetically distant strains induce partially distinct lesions in the brain, suggesting that the virulence of a WNV strain is also associated with its tissue tropism (22).

Vector and virus ecology

The ecology of WNV has been reviewed from both a North American and a European/Western Asian perspective (1, 5, 19, 30). Before 1999, WNV was limited to Africa, with occasional outbreaks in the Mediterranean region, Romania and Russia (31, 32, 33, 34). The migration of people, birds and mosquitoes from Africa and Asia led to the establishment of vectors, hosts and the virus in Europe and North America. Initially, multiple species of the *Culex pipiens* complex adapted to both temperate and tropical climates (35, 36, 37, 38, 39, 40). Many of these mosquito species, such as *C. tarsalis,* subsequently successfully adapted to multiple agricultural and urban landscapes (35, 36, 37, 38, 39, 40).

In Africa and Asia, multiple species of mosquitoes and birds have been associated with the maintenance of WNV infection and transmission to horses, with very little disease in birds or horses (41, 42, 43). Many of the lineage 1 isolates that have spread to Europe contain the NS3-249P mutation,

associated with increased virulence in Corvidae (44). Even so, WNV introductions into Europe have not generally been associated with a high incidence of avian mortality (34). A similar WNV lineage 1 strain was isolated from storks and geese in Israel in 1998, which was associated with high mortality in geese (45). This strain was very closely related to the lineage 1 strain that emerged in 1999 in New York City (46). The virus may have been transported there from the Middle East. In 2002, the emerging NY99 virus acquired an additional non-synonymous mutation in the envelope protein (V159A) that allowed the virus to multiply within *Culex* mosquitoes and reach the salivary glands more efficiently (47, 48, 49).

During the summer of 1999, large numbers of crows were dying in and around New York City (50). *Culex pipiens*-complex mosquitoes were identified as the principal vectors and house sparrows as an important maintenance host (51, 52, 53). Crows (and some other corvids) suffered fulminating systemic disease and have been identified as critical amplification hosts (11, 44, 54). The virus spread rapidly through the United States and Canada (Fig. 2). The rapid movement of the virus has been attributed to migratory birds and the post-nesting movement of multiple species of resident birds (15, 16). Previous experience in temperate regions of Europe had suggested that introduced strains of WNV from Africa or the Mediterranean did not persist and re-introduction was necessary for repeated outbreaks of disease (55). However, in North America, WNV persistence was achieved and sustained by long-term infection of both mosquitoes and birds (19). Importantly, *C. pipiens* is capable of facultative diapause and surviving cold conditions (56). The females can be vertically infected (57). In addition, viral RNA persists in the tissues and blood of birds for months, especially in passerines (58). Some birds may have incomplete immune responses (poor virus neutralisation activity) that could allow transmission to mosquitoes over a long period of time (across seasons) (59, 60). The onset of disease in humans and horses is largely limited to the summer months (58). This is primarily because of thermodynamic limitations on vector behaviour and reproduction, as well as on virus multiplication in female mosquitoes. Persistent infections of avian hosts may recrudesce at nesting season as temperatures increase (61, 62).

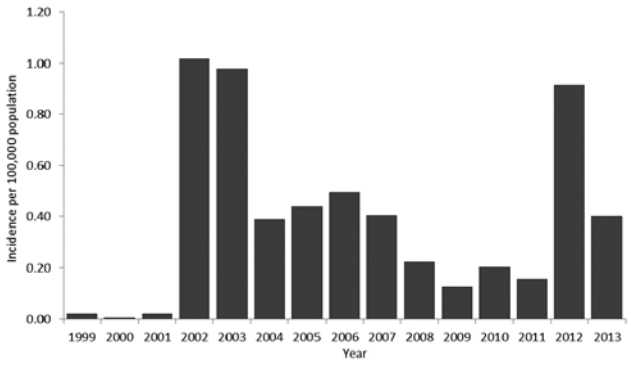

Fig. 2
Incidence of West Nile virus neuroinvasive disease, reported to the Centers for Disease Control and Prevention, by year, for the United States, 1999–2013
Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention

Detection, surveillance and epidemiology

Laboratory diagnosis of tissue specimens or body fluids from suspect animals is straightforward and methods are well established. The detection of IgM antibodies by capture enzyme-linked immunosorbent assay (ELISA) or serum virus neutralisation is indicative of infection. A positive test in the IgM capture ELISA is strong presumptive evidence of very recent infection. Viral RNA from tissues can be detected by reverse transcription polymerase chain reaction (PCR), in-situ hybridisation or by immunohistochemistry (8). Polymerase chain reaction techniques and virus isolation have been used to detect viral infection of mosquitoes (63).

Mosquito surveillance for WNV infection is a useful tool to estimate the risk of infection in humans and horses (64, 65). These surveillance data are also useful as justification for emergency control measures. Furthermore, the use of predictive models that include data associated with climate variations (temperature, rainfall); the history of the incidence of previous human or equine disease; vector density; and viral virulence (avian mortality) can predict the relative risk of transmission, disease and regional persistence of the virus (66, 67, 68, 69). In general, increasing temperature, increasing rainfall, the previous occurrence of disease and field surveillance data (on avian mortality/infection of mosquitoes) are all associated with an increased risk of transmission to, and disease in, horses and people. Therefore, a decision can be made to manage vector control through the use of insecticide and recommendations on avoiding insect bites, based on the degree of relative risk (64). These techniques have had limited success but improvements in tools and methods may be beneficial (70).

Decreasing the availability of potential mosquito habitats can be achieved through improved agricultural and urban surface control methods, such as reducing sewer overflow, improving surface drainage, improving water quality and avoiding the occurrence of water pooling. These and similar measures are documented methods of reducing the risk of virus transmission (71). Such approaches primarily reduce the number of available sites for oviposition and aquatic larval maturation.

Immunology and vaccines

Both haemagglutination-inhibiting and virus-neutralising antibodies are detectable soon after infection (6). However, some birds may not develop neutralising activity after infection (59, 60). The neutralisation activity is directed towards the E glycoprotein (9). Antibody responses to natural WNV infection are associated with recovery and long-term protection. Cellular immune responses are largely directed at non-structural proteins of the virus. The cytotoxic and regulatory T-lymphocytes are important for viral clearance. After animals recover from infection, immunity is lifelong (72). This effective, lifelong immunity is related to large pools of resident memory T-cells in the CNS and secondary lymphoid organs (72).

The flaviviruses have developed multiple mechanisms to evade innate immune responses in the host, such as pattern recognition signalling and Type I interferon responses (29). These innate immune responses are vital components of the host's immediate ability to limit the establishment or spread of infection. In general, these responses include intracellular mechanisms to limit virus multiplication and to signal activation to an array of host innate defence cells, such as resident macrophages (73, 74). Multiple pattern recognition system receptors are crucial activation signals in inflammatory leukocytes and also in the cells of the CNS (75). These impaired innate responses play a role in increasing host susceptibility to infection and are also associated with subsequent impaired adaptive immune responses. While these mechanisms are essential components of the innate response to viral infection of the CNS, they may also contribute to immunopathology (76). Nevertheless, most animals and humans are able to clear infections of WNV with no apparent clinical disease. Older horses and people are more susceptible to infection, although the specific mechanisms causing this state of increased susceptibility are not clear (29, 77). Presumably, age-related reductions in innate and adaptive responses may account for this increase in susceptibility.

There are four vaccines for veterinary use that have been registered for use as prophylaxis against WNV (78, 79). They include a killed (inactivated) WNV in adjuvant (two separate manufacturers); a live, canarypox-vectored vaccine; a live *Flavivirus* chimera (yellow fever) vaccine, and a DNA vaccine construct (12). The DNA vaccine has been discontinued by the manufacturers (Table I) (79). All of these vaccines have demonstrated efficacy (80, 81, 82). The canarypox-vectored vaccine provides immunity to disease caused by both lineage 1 and 2 WNV (83). West Nile virus candidate vaccines that elicit humoral and cellular immune responses would be expected to provide cross-lineage protection. Unlike natural infection, the available vaccines do not provide lifelong immunity and annual revaccination is recommended (79). A subunit glycoprotein E vaccine in a saponin/alum adjuvant is under development and this experimental vaccine protects against challenges from both lineage 1 and 2 viruses in laboratory animals (83). Other strategies are also in development, but for most recombinant antigens, annual boosters would probably be required (79).

Table I
Vaccines for immunising horses against West Nile virus disease

Type	Name	Protective component
Inactivated whole virus in adjuvant	West Nile-Innovator® (USA) Vetera WNV® (USA) EQUIP WNV® (Europe)	Whole virus antigen
Chimeric recombinant canarypox virus	Recombitek® Equine WNV (USA) Proteq West Nile® (Europe)	prM-E antigen
Chimeric vaccine virus containing yellow fever virus NS proteins and WNV prM-E	PreveNile® (USA) (discontinued)	prM-E antigen
DNA vaccine with adjuvant	West Nile-Innovator® DNA (USA) (discontinued)	prM-E antigen

NS: non-structural proteins
prM-E: surface glycoproteins M and E of West Nile virus
USA: United States of America
WNV: West Nile virus

Conclusions

Clearly, WNV has become established in the western hemisphere and remains a threat to people, birds and horses in North America. There is also a continuing risk of new epidemics as climate change, agricultural land use practices, urban landscape management practices, vector-control measures, rainfall patterns and host population dynamics are never constant. Subtle mutations in the WNV that affect host virulence or replication within vectors could also tip the balance towards re-emergence and new epidemics. Similar vector mutations could also significantly increase disease transmission. Therefore, continued research is needed to develop tools for interventions in infection (vaccines, therapeutics, etc.) and vector control. Furthermore, continued environmental surveillance and epidemiological research are needed to predict, limit and avoid future epidemics caused by WNV.

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Résumé
Cette synthèse décrit l'essentiel de la biologie du virus West Nile et des interactions hôte–vecteur–pathogène qui déclenchent une maladie grave chez les oiseaux sauvages, les chevaux et l'homme. Les auteurs décrivent les principales propriétés du virus, les modalités de l'infection cellulaire et la pathogénie de la maladie ainsi que les facteurs écologiques à l'œuvre dans la persistance du virus, son amplification et sa transmission. Ils examinent également l'épidémiologie de la maladie et les diverses stratégies d'appréciation du risque mises en œuvre, ainsi que la réponse immune des hôtes et les pratiques de vaccination. Sont également abordés les principes de la lutte antivectorielle, le contrôle de l'exposition et les risques à long terme liés aux facteurs climatiques et à l'habitat.

Mots-clés
Arbovirus – Aviaire – Encéphalite – Équidé – Maladie émergente – Moustique – Vaccin – Virus West Nile – Zoonose.

El virus West Nile

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Resumen
Los autores examinan la biología básica del virus West Nile y las relaciones hospedador–vector–patógeno que dan lugar a una importante enfermedad en aves salvajes, caballos y humanos. También describen las propiedades básicas del virus, así como la infección celular, la patogénesis de la enfermedad y los aspectos ecológicos del mantenimiento, la amplificación y la transmisión del virus. Asimismo, tras exponer la epidemiología de la enfermedad y las estrategias de estimación del riesgo empleadas actualmente, describen la respuesta inmunitaria de los hospedadores y las prácticas de vacunación. Por último examinan los principios de la lucha contra el vector, el control de exposiciones y los riesgos a largo plazo derivados de factores climáticos o relacionados con el hábitat.

Palabras clave
Arbovirus – Aviar – Encefalitis – Enfermedad emergente – Equino – Mosquito – Vacuna – Virus West Nile – Zoonosis.

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