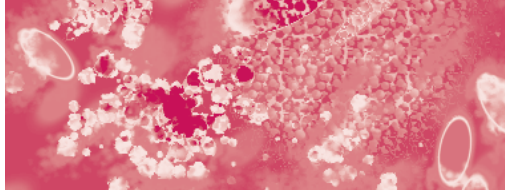

West Nile virus

Grant L Campbell, Anthony A Marfin, Robert S Lanciotti, and Duane J Gubler

West Nile (WN) virus is a mosquito-borne flavivirus and human, equine, and avian neuropathogen. The virus is indigenous to Africa, Asia, Europe, and Australia, and has recently caused large epidemics in Romania, Russia, and Israel. Birds are the natural reservoir (amplifying) hosts, and WN virus is maintained in nature in a mosquito-bird-mosquito transmission cycle primarily involving *Culex* sp mosquitoes. WN virus was recently introduced to North America, where it was first detected in 1999 during an epidemic of meningoencephalitis in New York City. During 1999–2002, the virus extended its range throughout much of the eastern parts of the USA, and its range within the western hemisphere is expected to continue to expand. During 1999–2001, 142 cases of neuroinvasive WN viral disease of the central nervous system (including 18 fatalities), and seven cases of uncomplicated WN fever were reported in the USA. Most human WN viral infections are subclinical but clinical infections can range in severity from uncomplicated WN fever to fatal meningoencephalitis; the incidence of severe neuroinvasive disease and death increase with age. Serology remains the mainstay of laboratory diagnosis. No WN virus-specific treatment or vaccine is available. Prevention depends on organised, sustained vector mosquito control, and public education.

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The dramatic appearance of epidemic West Nile (WN) meningoencephalitis (panel) in the New York City area in 1999[1]—with 59 hospitalised cases and seven deaths[2]—is an unsettling reminder of the ability of viruses, including arboviruses, to jump continents and hemispheres. Although preliminary serological tests of patients implicated the indigenous and closely related St Louis encephalitis (SLE) virus,[1] and preliminary gene amplification studies of human brain tissue implicated Kunjin virus (an Australian subtype of WN virus[3]), the accurate identity of the epidemic flavivirus strain was quickly resolved. The subsequent spread of WN virus throughout much of the eastern half of the USA and southern Ontario, Canada, during 1999–2001[4] emphasises the fact that, although arboviral transmission cycles and maintenance mechanisms are usually very complex, arboviruses that are introduced to new areas can become established if efficient vectors, suitable vertebrate amplifying hosts, and reliable overwintering mechanisms are available.[5] The fact that WN virus has caused widespread mortality in some North American bird species[4] is a reminder that a virus introduced into a new ecosystem, or new hemisphere, can produce unexpected results.

The 1999 New York epidemic also demonstrated that, without sustained vector mosquito control in urban areas, even the world's most affluent cities are at risk for epidemic arboviral disease. The intense publicity generated by this outbreak, which took place in a major economic and news media centre, largely overshadowed the fact that much larger, more deadly, and almost equally unexpected urban epidemics of WN meningoencephalitis occurred in Russia virtually simultaneously with the New York outbreak, in Romania only 3 years earlier, and in Israel only 1 year later.[6–8]

WN virus was first isolated in 1937 from the blood of a febrile patient in the West Nile district of northern Uganda.[9] During the 1940s, the close antigenic interrelationships of

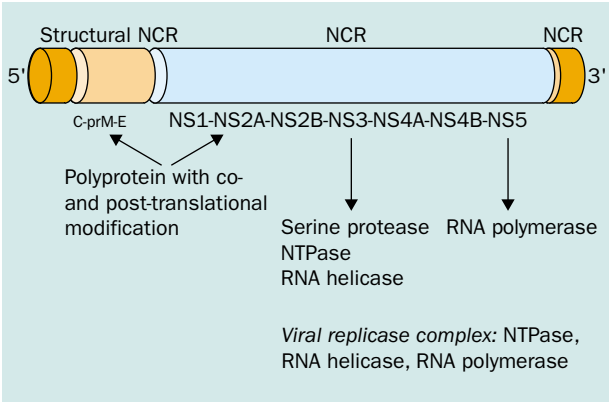

Figure 1. WN virus RNA genome consisting of 5′ non-coding region (NCR; 100 nucleotides), a single open reading frame coding for three viral structural proteins (capsid [C], membrane [M], envelope [E]) and seven non-structural proteins, and a 3′ NCR (600 nucleotides).

*In this review, the term "meningoencephalitis" is used to encompass encephalitis, meningitis, myelitis, and cases with overlapping features of these syndromes. Although some authors use "WN fever" to describe any illness caused by WN viral infection, including neuroinvasive illness, in this review "WN fever" refers only to the uncomplicated febrile illness.

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WN, Japanese encephalitis (JE), and SLE viruses were described,[10] the transmission of WN virus by mosquitoes under laboratory conditions was demonstrated,[11] and significant prevalences of neutralising antibody to WN virus and closely related flaviviruses were observed in residents of eastern central Africa.[12] During the next three decades, mosquito-borne transmission of WN virus was supported by field evidence,[13] birds were shown to be important amplifying hosts,[14] large epidemics of WN fever with few neuroinvasive disease cases were described in Israel and South Africa,[15,16] Europe experienced its first documented WN meningoencephalitis outbreak,[17] and WN virus emerged as an equine neuropathogen.[18] From 1975 through to 1993, no major epidemics of WN viral disease were documented. During 1994–2000, however, epidemics of WN meningoencephalitis occurred at an alarming rate in North Africa, Europe, North America, and the Middle East.[2,6,8,17,19] These outbreaks included several epizootics that primarily affected horses, as well as large urban epidemics such as the Israeli epidemic of 2000, where there were more than 400 cases and 35 deaths.[8] In retrospect, the 1996 Romanian epidemic seems to have been a singular event, signalling the emergence of epidemic WN viral disease in urban areas of the industrialised world.

Causative agent

WN virus is taxonomically placed within the family Flaviviridae, genus Flavivirus. Within the genus Flavivirus, WN virus has been serologically classified within the JE virus antigenic complex, which includes the human pathogens JE, Murray Valley encephalitis, SLE, and Kunjin viruses. The spherical WN virus particle is approximately 50 nm in diameter and consists of a host-derived lipid bilayer membrane surrounding a nucleocapsid core containing a single-stranded positive-sense RNA genome of approximately 11 000 nucleotides. Embedded in the virion membrane are the viral envelope (E) and membrane (M) proteins which are responsible for many of the important properties of the virus, including host range, tissue tropism, replication, assembly, and the stimulation of B and T cell immune responses. The RNA genome consists of a short 5′ non-coding region (about 100 nucleotides), followed by a single open reading frame coding for three viral structural proteins and seven non-structural (NS) proteins in the following order: capsid-membrane-envelope-NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5-3′ non-coding region (about 600 nucleotides) (figure 1). Virus replication occurs in the cytoplasm in close association with the rough endoplasmic reticulum (ER), followed by virus assembly in the ER lumen, and release from the cell via the cell secretory pathway apparatus.[20]

Phylogenetic analyses done on nucleic acid sequence data from a number of full-length genomes have demonstrated two distinct lineages of WN virus strains. Those in lineage 1 have a worldwide distribution, ranging from west Africa to the Middle East, eastern Europe, North America, and Australia (Kunjin virus); whereas lineage 2 consists of enzootic strains from Africa.[21]

Geographical distribution and epidemiology

WN virus *sensu lato* is indigenous to Africa, Asia, Europe, and Australia,[22,23] and was recently introduced to North America (figure 2), where it was first detected in New York City.[1] The likely origin of the introduced strain was the

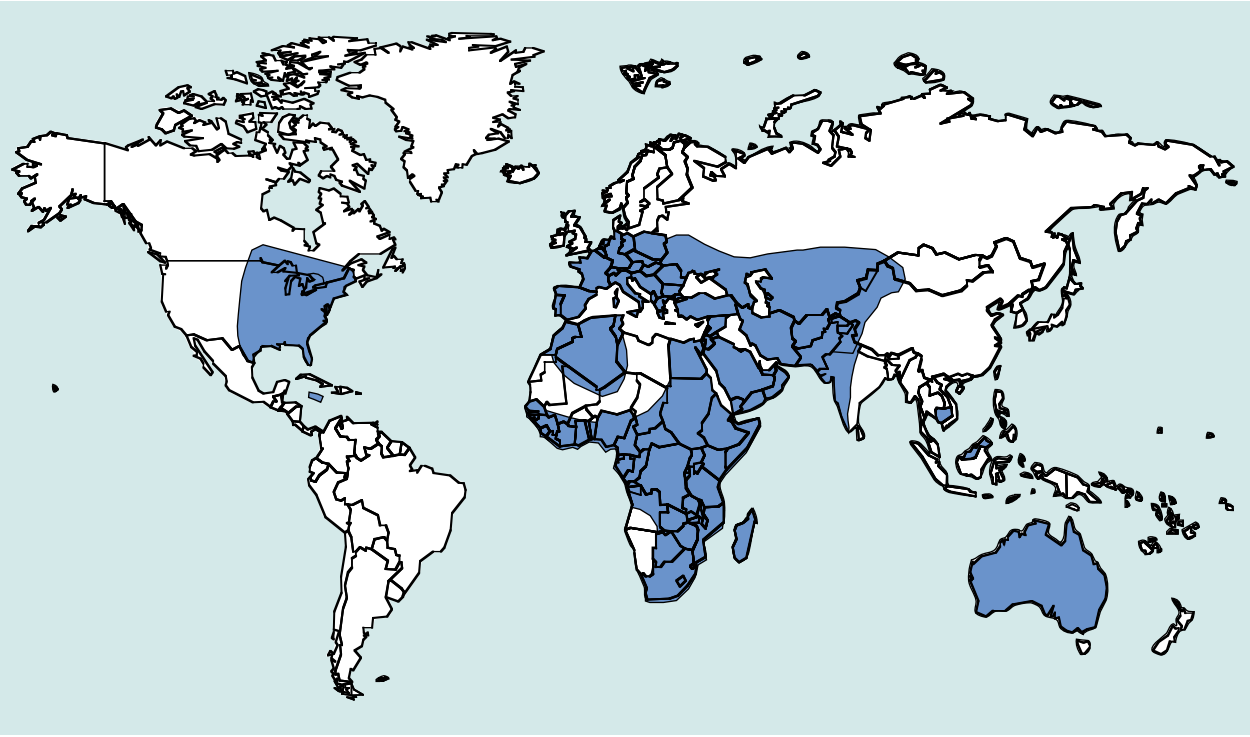

Figure 2. Approximate worldwide distribution (shown in blue) of West Nile (WN) virus and Kunjin virus, a subtype of WN virus.

Middle East,[24] but the mode of introduction is unknown. During 1999–2002, WN virus extended its range throughout much of the eastern parts of the USA, and has now been detected from Maine to the Florida Keys, and from the Atlantic coast to eastern North Dakota (unpublished data).[4] The virus has also been detected in south-central Canada, and, in 2001, a WN encephalitis case was serologically diagnosed in a resident of the Cayman Islands who had no recent travel history, which is circumstantial evidence that the virus has entered the Caribbean region.[4] Within a given region, the distribution of WN virus can be highly discontinuous and multifocal, depending on complex ecological factors.

Although mosquito-borne transmission of WN virus is by far the predominant mode, laboratory-acquired infections can occur via percutaneous inoculation or the airborne route.[25] Human-to-human or non-human-vertebrate-to-human transmission has not been documented.

In the temperate and subtropical zones, most human infections with WN virus occur in summer or early fall.[2,6–8,26] In the tropics, the incidence should be greatest during the rainy season when mosquitoes are most abundant, but little published information on the epidemiology or ecology of WN virus in the tropics is available. While all age groups and both sexes appear to be equally susceptible to WN viral infection, the incidence of encephalitis and death increase with age.[2,7,8] In recent urban epidemics, risk factors associated with WN viral infection included length of time spent outdoors, failure to regularly apply mosquito repellent, observing mosquitoes in the home, and living in an apartment building with a flooded basement.[27,28] These factors are obviously prerequisites for increased exposure to potentially infected mosquitoes. Other than age, host factors (eg, hypertension, smoking, and cerebrovascular disease) that increase the risk of developing meningoencephalitis in persons with WN or SLE viral infections have yet to be identified.

Epidemics or sporadic cases of WN viral disease in human beings or equines have been documented in Africa, the Middle East, Europe, west and south Asia, Australia, and North America.[2,6,8,16,17,19,23,29–31] In Australia, only sporadic human cases of Kunjin viral disease have been documented, including rare cases of encephalitis; therefore, Kunjin virus will not be further considered here.[23] Most human WN viral infections are subclinical and the remainder cause illnesses that can have a wide clinical spectrum.[7,27] In any population affected by WN virus, the proportions of different clinical syndromes observed will depend on the previous history of WN viral activity in the area and the consequent level of background immunity in the population (possibly including immunity to closely related flaviviruses), the age structure of the population, and the focus and completeness of surveillance efforts.[32] Based on extensive studies done in Egypt in the 1950s, at one epidemiological extreme are areas where WN virus circulates in most years; uncomplicated WN fever is a mild and common childhood disease that is easily overlooked among many other febrile conditions, high prevalences of background immunity are present and increase with age, and WN fever epidemics and WN meningoencephalitis cases are rare.[13] At the other extreme are the industrialised urban areas of the northern temperate zone, where little or no previous WN virus activity has occurred, ageing and largely immunologically naive populations are encountering this virus for the first time, and a preponderance of neuroinvasive disease cases has been observed. The proportion of about one meningoencephalitis case per 140 total WN viral infections estimated in the Borough of Queens (Queens County), New York,[27] is similar to proportions observed in WN meningoencephalitis epidemics in Bucharest, Romania,[7] and the Borough of Staten Island (Richmond County), New York,[33] as well as in some SLE epidemics in the USA.[34] Such proportions almost certainly depend on the age structure of the population studied, with higher proportions expected in older populations. In Queens, the estimated proportion among persons aged more than 65 years was 1/50, while among persons less than 65 years it was 1/300.[27]

Notably, the South African WN fever epidemic of 1973–1974 may be unique. Estimated to have involved thousands of cases, this was by far the largest epidemic of WN viral disease ever documented, yet for unknown reasons, only a single WN meningoencephalitis case was observed.[16] Whether strain-related variations in WN viral neurovirulence can contribute to such apparent differences between epidemics is unknown. Although recent WN meningoencephalitis epidemics have been associated exclusively with lineage 1 strains, the phylogeny of the strain(s) associated with the South African epidemic is undetermined.[21]

In the USA during 1999–2000, 78 WN meningoencephalitis cases were detected, all within the greater New York City metropolitan area with onsets in August and September.[2,35] The epicentres in 1999 and 2000 were the New York City boroughs of Queens (32 cases) and Staten Island (ten cases), respectively. Nationally in 2001, 64 such cases were detected but these exhibited greater geographical and temporal dispersion; cases occurred in 38 counties in ten states (figure 3), with no more than four cases in any one county, and with onset dates ranging from mid-July to early December (figure 4).[4] Based on reports to the ArboNET surveillance system of the Centers for Disease Control and Prevention (CDC),[36] among the 123 non-fatal cases detected in 1999–2001 for which information is available, median age of patients was 65 years (range 5–90), 73 (59%) were aged more than 60 years (figure 5), and 77 (63%) were males. Among the 18 (13%) fatal cases, median age of patients was 75 years (range 44–90 years), 16 (89%) were aged more than 60 years, and eight (44%) were males. One of the non-fatal cases was apparently laboratory-acquired. During 1999–2001, seven uncomplicated WN fever cases were detected in the USA, mostly serendipitously. Among these patients, the median age was 45 years (range 28–65 years) and only two (29%) were aged more than 60 years.

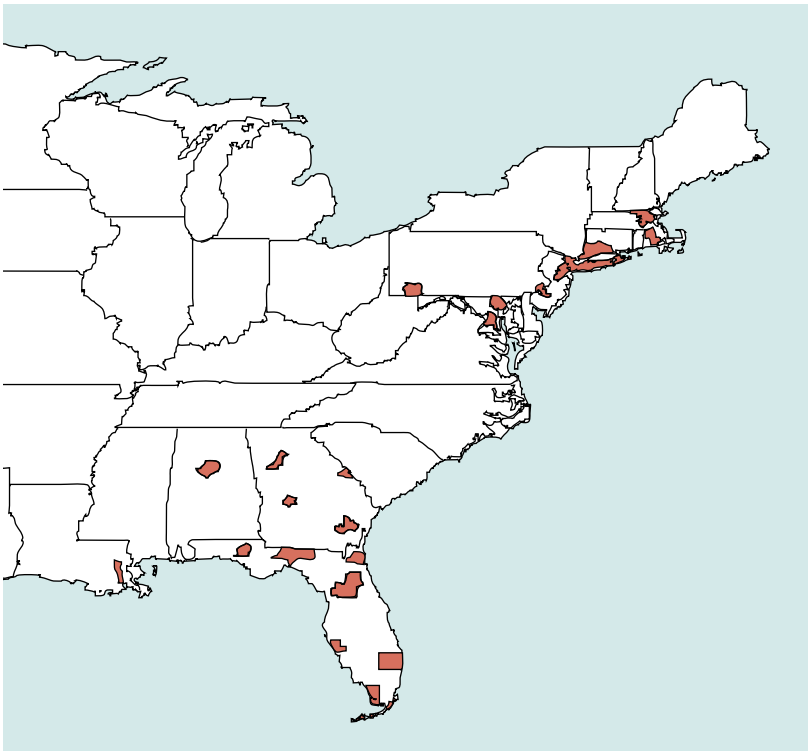

Figure 3. US counties reporting human cases of WN meningoencephalitis in 2001; 64 cases from 38 counties in ten states. Data are from the ArboNET surveillance system, Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC.

However, results of a 1999 post-epidemic serosurvey in New York City suggested that, in the outbreak's epicentre of Queens, roughly 110 asymptomatic WN viral infections and 30 WN fever cases had occurred for each WN meningoencephalitis case (ie, about 80% asymptomatic and 20% symptomatic infections).[27] Thus, it is likely that WN fever cases have been significantly underrecognised in the USA, probably because surveillance efforts have emphasised neuroinvasive disease, especially encephalitis cases. A similar phenomenon probably occurred during the Romanian epidemic.[7] By contrast, nearly one-fourth of patients hospitalised during the 2000 Israeli epidemic had WN fever.[37]

The prevalence of immunity to WN virus depends on geography and the human population studied. Comparing the results of different serosurveys is difficult because methods have varied. At one extreme are some endemic areas of Africa, where overall prevalences of background immunity to WN virus of roughly 50% in children and roughly 90% in adults have been observed.[13,22] By contrast, general background immunity to WN virus in Europe is probably very low,[38] and in much of North America it should be virtually absent. Moreover, the low post-epidemic seroprevalences estimated among residents of Queens, New York, in 1999 (3%),[27] Bucharest, Romania, in 1996 (2–4%),[7] and Staten Island and Suffolk County, New York, and Fairfield County, Connecticut in 2000 (0–1%),[33] suggest that no significant levels of background immunity resulted from recent epidemics in those areas.

Transmission cycle and host range

WN virus is maintained in nature in a mosquito-bird-mosquito transmission cycle primarily involving *Culex* sp mosquitoes (figure 6).[22,39] The virus, however, has been isolated from 29 mosquito species belonging to ten genera in the USA alone (unpublished data).[4,36,40] The vector status and epidemiological importance of many of these species are unknown. Although *Culex pipiens* (the northern house mosquito), a highly ornithophilic species that is often abundant in urban areas, was a major epizootic WN virus vector among birds in both Bucharest[41] and New York City,[40] its role in transmission to human beings is unclear.[19,42] *C quinquefasciatus* (the southern house mosquito) has yet to be implicated in epidemic urban WN virus transmission, but there is significant potential for this to occur.[43] Similarly, *C nigripalpus* and *C tarsalis* are likely to eventually serve as epidemic vectors of WN virus in Florida and the western parts of the USA, respectively, where they occur in rural agricultural and suburban areas, and where they are the major SLE virus vectors.[34,43] In Africa, *C univittatus* seems to be the most important vector of WN virus to human beings.[13,44] While WN virus has been isolated from both hard and soft ticks in the eastern hemisphere, ticks are probably not important epidemic/epizootic vectors of the virus.[22] Their role in virus maintenance is unknown.

Birds are the natural reservoir (amplifying) hosts for WN virus, which has been shown to infect at least 111 bird species in North America alone (unpublished data).[4,36] When infected with WN virus, many avian species develop transient high-titre viraemias that should allow transmission of the virus to feeding mosquitoes.[14] Infected birds commonly survive their infections and develop permanent immunity,[45] although some individuals of some species (especially in North America) become ill and die. In North America, WN virus seems to be particularly virulent in species belonging to the family Corvidae (eg, crows and jays), and these have a central role in dead-bird-based surveillance programmes for detecting and tracking the virus in the region.[46,47]

Transportation of WN virus strains between different areas by migratory viraemic birds along established flyways is probably a common occurrence.[48,49] In the eastern hemisphere, WN virus is thought to be regularly introduced in Mediterranean and European countries by birds. Whether such enzootic cycles and movement in migratory birds have become established in the Americas is unclear, but the following facts support this conclusion: (1) the virus has been active in the USA for four consecutive transmission seasons, which is evidence for an efficient overwintering

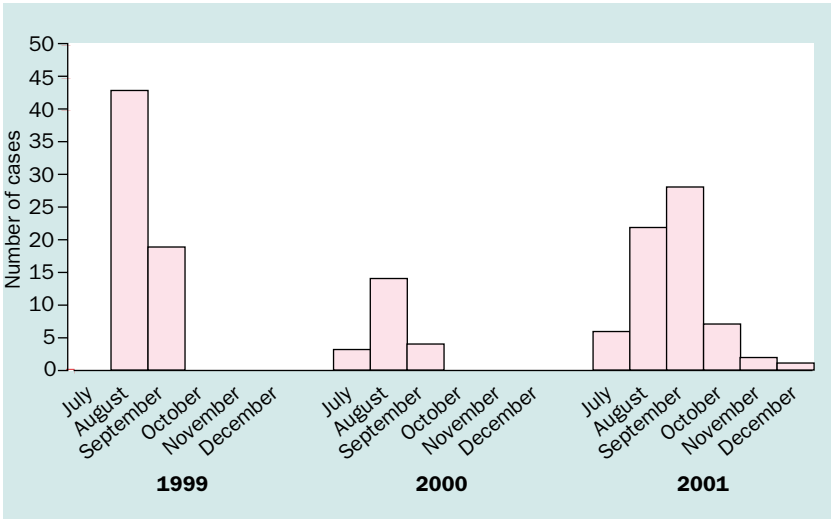

Figure 4. Number of WN meningoencephalitis or WN fever cases reported by month of illness onset, 1999–2001, USA. Data are from the ArboNET surveillance system, Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention.

mechanism;[50] (2) the virus was detected in Florida in June 2001, which suggests that it was introduced to that area in 2000 or earlier;[4] (3) the virus has exhibited explosive geographical expansion throughout most of the eastern parts of the USA (figure 7); (4) the virus has infected a broad range of avian hosts and potential vector mosquito species; and (5) year-round transmission of the virus has been documented in Florida during 2001–2002, suggesting the virus has become enzootic in subtropical areas of the USA.[4]

A broad range of mammalian species are susceptible to natural or experimental infection with WN virus, but naturally acquired disease in mammals has been conclusively demonstrated in human beings and equines only.[22] In the USA during 1999–2001, nine mammalian species (human beings, horses, cats, rabbits, skunks, squirrels, chipmunks, and two species of bats) were found to be naturally infected with WN virus.[36,51] The role, if any, that mammals play in the WN virus transmission cycle is unknown. In the USA in 2001, a large epizootic of WN encephalitis in horses occurred over a ten-state region—nearly two-thirds of the cases occurred in Florida.[4] Experimental studies suggest that horses are dead-end hosts for WN virus,[52] but this issue deserves further study.

Clinical features

Most WN viral infections are symptomless.[7,27] The incubation period is approximately 2–14 days for symptomatic infections overall, but 2–6 days is typical in WN fever cases.[53,54] The associated clinical syndromes are non-specific and a diagnosis cannot reliably be made on clinical grounds alone.

Uncomplicated WN fever typically begins with sudden onset of fever (usually >39°C), headache, and myalgia, often accompanied by gastrointestinal symptoms. The acute illness usually lasts less than a week, but prolonged fatigue is common. In earlier epidemics in which WN fever cases predominated—up to half of the patients had a generalised roseolar or maculopapular rash that lasted up to a week and resolved without desquamation—generalised lymphadenopathy was also common.[26,54–56] By contrast, in more recent epidemics in which WN meningoencephalitis cases predominated, no more than 22% of patients had skin rash and less than 5% had lymphadenopathy.[2,6,35,37,57] The reasons for these apparent differences are unknown.

In three recent WN virus epidemics, 58–69% of neurological disease cases were classified as encephalitis (or meningoencephalitis), while the remainder were classified as meningitis.[2,7,37] Comparing different epidemics in this respect is difficult because clinicoepidemiological criteria used by different researchers to classify cases may not be comparable or even described. Clinically, WN meningitis is a typical viral meningitis which will not be further described, except to note that in cases that do not progress to meningoencephalitis, the associated fatality rate is low.[57]

Clinically, WN encephalitis is generally typical of the arboviral encephalitides. A prodrome of fever, headache, and other non-specific symptoms (ie, typical WN fever) lasting from 1 to a few days occurs in some patients. In others, a more abrupt onset of fever accompanied by symptoms and signs of encephalitis, especially mental status changes and

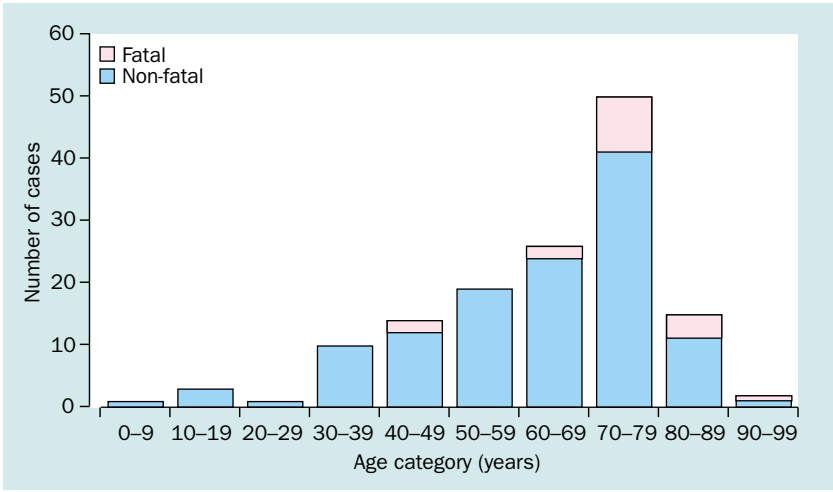

Figure 5. Age category and outcome for 141 reported cases of WN meningoencephalitis, 1999–2001, USA. Data are from the ArboNET surveillance system, Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC.

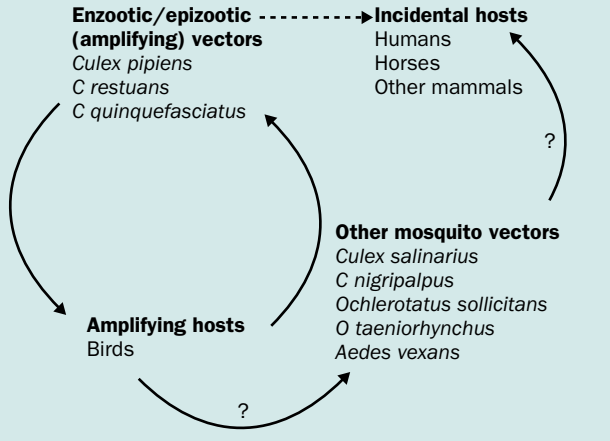

Figure 6. Postulated transmission cycle of WN virus. Primary cycle involves enzootic and epizootic amplification by avian hosts and mosquito vectors (primarily Culex *species). Human beings and other incidental hosts can become infected by bites from the amplifying vectors or other mosquito vectors with epidemic potential ("bridge" vectors). ?=postulated routes of transmission.*

vomiting, has been described. In about 15% of cases, cerebral dysfunction progresses to coma. Accompanying abnormalities can include depressed deep tendon reflexes, diffuse muscle weakness (often with profound proximal muscle weakness), flaccid paralysis, and respiratory failure. Seizures and focal neurological signs are uncommon.[2,37,57]

In patients with WN encephalitis, computer-assisted tomography often revealed pre-existing lesions and chronic changes in brain tissue but rarely showed signs of central nervous system (CNS) inflammation.[2,37,58] In about 30% of patients, enhancement of the leptomeninges or periventricular areas consistent with inflammation and encephalitis can be seen on magnetic resonance imaging scans.[2] Reminiscent of many other viral CNS infections, typical cerebrospinal fluid (CSF) findings in WN meningoencephalitis include: mild pleocytosis (30–100 cells/μL; range 0–1800 cells/μL) with lymphocytes usually in predominance; elevated protein concentration of 80–105 mg/dL (rarely, up to 1900 mg/dL); and normal glucose concentration. Peripheral blood cell counts are less remarkable. Although leucocyte counts of up to 30 000 cells/μL have been reported, leucocytosis usually occurs in less than 50% of patients. In about 10–15% of patients, a leucopenia and relative lymphocytopenia has been noted. Other typical laboratory findings include mild anaemia.[2,35,37,57]

Muscle weakness is a prominent part of the clinical presentation in many patients with WN encephalitis. In Romania and Russia, paresis or paralysis was seen in 15–20% of patients.[6,57] In New York City, paresis was documented in roughly 50% of hospitalised patients, and about 10% had flaccid paralysis.[2] The latter was associated with absent deep tendon reflexes and a high incidence of respiratory failure. For most patients who underwent electromyography, results were consistent with a motor axonal polyneuropathy, in which the sensory fibres were generally spared (ie, findings uncharacteristic of Guillain-Barré syndrome);[59] results in one case suggested demyelination polyneuritis consistent with Guillain-Barré syndrome.[60]

In recent WN meningoencephalitis epidemics, overall fatality/case ratios from 4–14% were reported, with higher ratios in older age groups and with virtually all deaths among encephalitis patients.[2,6,7,35,37,57] Considering encephalitis patients alone, however, ratios were significantly higher. In the recent Israeli epidemic, for example, the overall ratio in selected hospitals was 14% (33 of 233), but about 24% (33 of about 135) of encephalitis cases were fatal;[37] and in the USA during 1999–2000, the fatality/case ratio among all hospitalised patients was 12% (nine of 78), but 19% (nine of 48) of encephalitis cases were fatal.[2,35] Mortality in WN encephalitis significantly increases with age. In Israel, for example, the overall fatality/case ratio among patients aged more than 70 years was 29%, and 32 of the 33 deaths reported in this series were in patients aged more than 68 years.[37]

Rare neurological manifestations of WN viral infection include myelitis, optic neuritis, rhombencephalitis, and polyradiculitis.[61–65] Rare extraneurological manifestations include myocarditis, pancreatitis, and fulminant hepatitis[16,66,67]—the involved organs are sites of high viral replication.[67]

Only one long-term follow-up study of WN encephalitis survivors has been reported. At 12 months post-illness onset, 41–55% of patients self-reported that they had not recovered physically, functionally, or cognitively, and only 37% of patients reported full recovery in all three areas.[68] In another study, a medical records review found that only 37% of hospitalised patients had recovered fully at the time of discharge, 53% had improved but had not achieved their previous level of function, and 11% had died.[35]

Pathogenesis and pathology

The exact mechanisms and sites of WN virus replication following the bite of an infected mosquito are unknown but initial replication is thought to occur in the skin and regional lymph nodes and to produce a primary viraemia that seeds the reticuloendothelial system (RES).[20] Depending on the level of secondary viraemia that results from replication in the RES, virus may then seed the CNS. In healthy infected persons, virus can generally be isolated from blood during peak viraemia that occurs from about 2 days before until about 4 days after illness onset, but the success of virus isolation sharply decreases after the first day of illness;[67,69] this finding is most likely due to increased macrophage clearance and development of IgM antibody. WN virus was recovered from the blood of an immunocompromised patient up to 28 days post-inoculation, and some terminally ill persons intentionally infected with WN virus developed high-titre viraemia.[67,69] Studies of young, healthy people, however, suggest that viraemia resulting from naturally acquired infection is usually much lower and may be insufficient to infect mosquitoes.[56] More definitive studies of this issue are clearly needed.

Viraemia level is the result of virus-specific and host-specific factors and affects clinical manifestations and disease outcome. The WN virus envelope (E) protein mediates cell attachment and neuroinvasiveness and seems to be a primary virulence factor.[20,70] Host factors that allow CNS

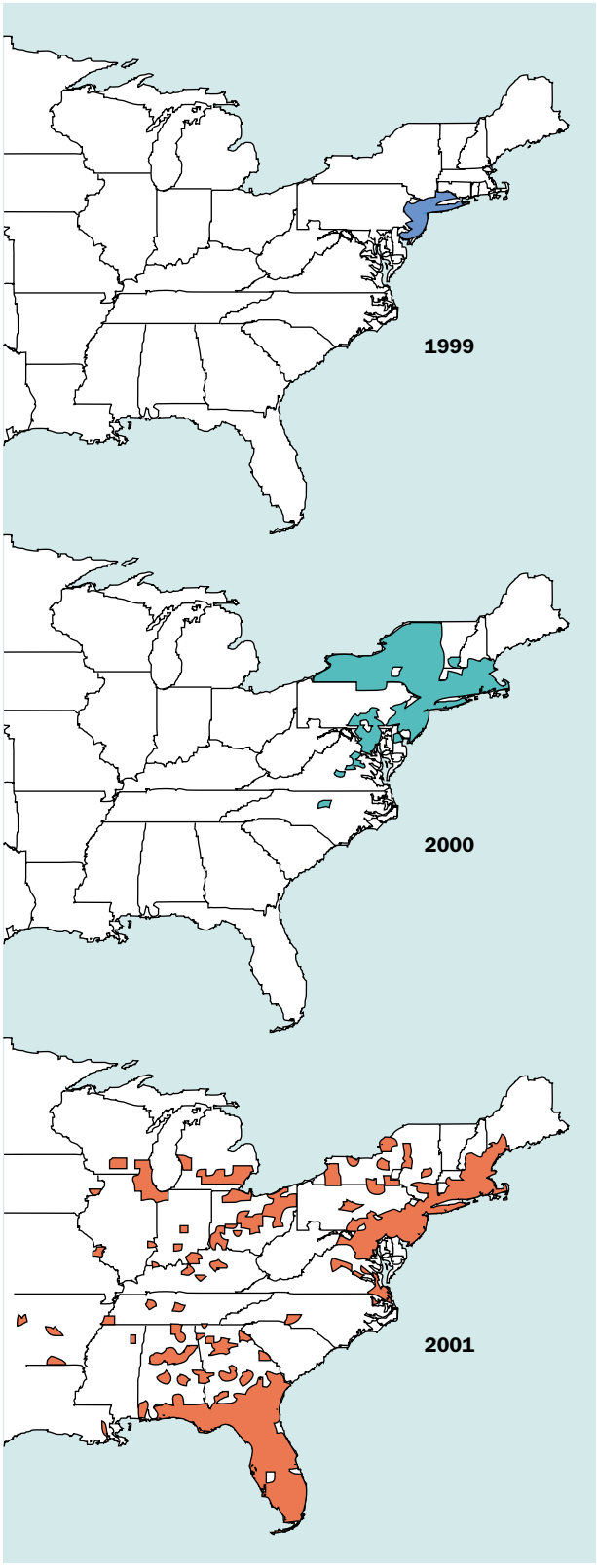

Figure 7. US counties reporting WN virus-infected birds in 1999 (blue), 2000 (green), and 2001 (red). Data are from the ArboNET surveillance system, Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC.

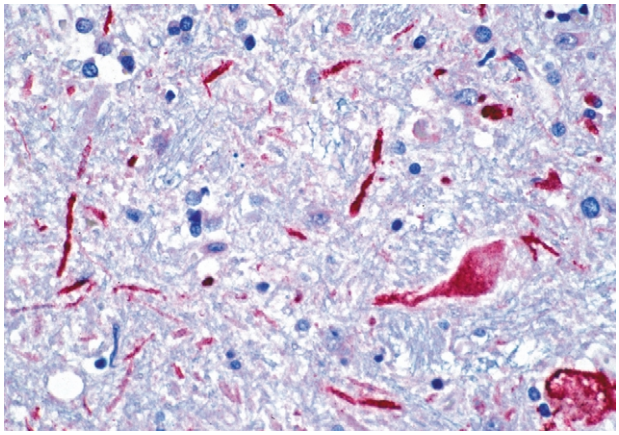

Figure 8. Positive staining of viral antigens in neurons and neuronal processes in fatal WN encephalitis case. Immunoalkaline phosphate staining, naphthol fast red substrate with light haematoxylin counterstain. Original magnification, x100. Photomicrograph provided by Wun-Ju Shieh and Sherif Zaki, Infectious Disease Pathology Activity, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, CDC. Reprinted with permission of Emerging Infectious Diseases.

entry of WN virus remain unknown but may include factors that promote virus entry into and replication in the endothelium at the blood-brain barrier.[20] Possible explanations for the higher incidence of WN meningoencephalitis in the elderly include factors that enhance viral entry into the CNS by disruption of the cerebral endothelium (eg, hypertension, cerebrovascular disease) or an increase in the magnitude and duration of viraemia (eg, immunosuppression, immune senescence). Other proposed mechanisms of viral entry into the CNS include axonal transport through olfactory neurons, cytokine-directed leukocyte diapedesis through endothelial tight-junctions, or viral shedding through the choroid plexus.[20] Based on SLE virus studies in laboratory animals,[71] the probability of neuroinvasion by WN virus is likely to be correlated with the level and duration of viraemia

The pathological changes in the CNS are the direct result of (1) viral proliferation within neuronal and glial cells (figure 8), (2) cytotoxic immune response to infected cells, (3) diffuse perivascular inflammation, and (4) microglial nodule formation (figure 9).[20,65,72] WN virus consistently causes diffuse inflammation of the thalamus, the medulla, other parts of the brain stem, and the proximal spinal cord where perivascular inflammation and microglial nodules predominate. Nodules are composed of lymphocytes and histiocytes and often occur in areas of extensive neuronal degeneration. CD8-bearing T-lymphocytes predominate within these nodules, perivascular infiltrates, and the lymphocytic infiltrates of the meninges and cranial nerve roots. CD4-bearing T-lymphocytes are present in lower density than CD8 cells. B-lymphocytes are predominantly found in areas of perivascular inflammation. At the time of clinical presentation, most patients with WN meningoencephalitis have already initiated an antibody response. CSF and serum IgM are detectable in 70–80% of patients by the 8th day of illness.[73,74] WN virus-specific antibody in CSF may decrease viral replication either by

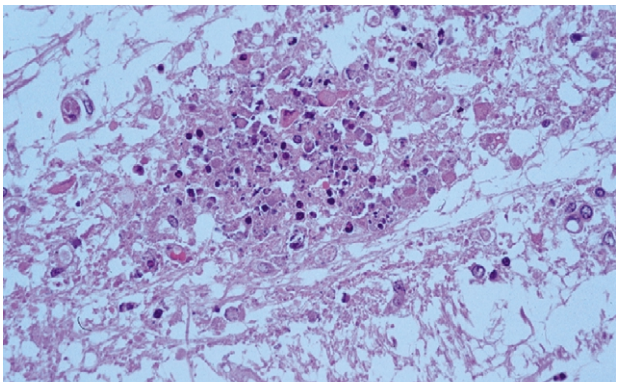

Figure 9. Neuronal necrosis with infiltrates of microglia and polymorphonuclear leucocytes in fatal WN encephalitis case. Haematoxylin-eosin staining. Original magnification, x100. Photomicrograph provided by Wun-Ju Shieh and Sherif Zaki, Infectious Disease Pathology Activity, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention. Reprinted with permission of Emerging Infectious Diseases.

interfering with viral attachment to the cell surface or by preventing intracellular structural rearrangement of the E glycoprotein that would normally allow endosomal fusion.[20] Little is known about the specific T-cell response.

Resolution of these pathological changes is believed to be complete in survivors of WN meningoencephalitis. Nevertheless, for poorly understood reasons, permanent neurological sequelae occur in some individuals. Although experimental inoculation with WN virus can produce persistent CNS infections in monkeys and hamsters,[75,76] there is no evidence, except for the persistence of WN virus-specific IgM,[73,74] for persistent WN viral infections in human beings.

Laboratory diagnosis

Serology continues to have a dominant role in the laboratory diagnosis of WN viral infections (and most other arboviral infections) in human beings.[77] The development of WN virus-specific neutralising antibody between the acute and convalescent phases of illness (as shown by a >four-fold rise in titre, typically by plaque-reduction neutralisation assay) remains the most convincing serological evidence of infection, and is associated with long-term immunity. A battery of other flaviviruses (selected as clinicoepidemiologically appropriate) should be included in the assay for comparison. Specificity is implied by the demonstration of neutralising antibody titres to WN virus that are more than four-fold higher than the corresponding titres to all flaviviruses with which it is compared. In second or subsequent flaviviral infections, a neutralising antibody response to a variety of flaviviruses is usually present, which often creates diagnostic uncertainty.[78,79] For neutralisation tests, the ideal acute-phase and convalescent-phase specimens are usually those collected on the first day of illness and more than 3 weeks later, respectively. Haemagglutination-inhibition tests are still used by some laboratories for serodiagnosis of arboviral infections, although they are becoming less common. Complement-fixations tests are now rarely used.[77]

A recent WN viral infection can be inferred by the detection of IgM in serum or CSF— the test battery should include other appropriately selected flaviral antigens for comparison. Antibody-capture enzyme immunoassays (EIA) are optimal for this purpose,[73,80] although immunofluorescent antibody (IFA) tests are also available. Positive results obtained with either method should be considered presumptive until confirmed by neutralisation tests of the same or a later specimen. Conservatively, negative IgM tests of specimens collected less than about14 days after illness onset should be corroborated by tests of a later specimen.[73]

Serum IgM can persist for extended periods in some WN encephalitis survivors.[73,74] The duration of IgM antibody in CSF, or in the serum of patients without meningoencephalitis, is unknown. In a recent study of patients recovering from WN meningoencephalitis, serum IgM was detectable in 77% (17/22) and 60% (7/12) of those tested approximately 12 months and 16 months post-onset, respectively (J Roehrig, CDC, personal communication). Therefore, in a patient with acute meningoencephalitis, WN virus-specific IgM detected in serum could theoretically be unrelated to the current illness. In most areas of North America and Europe, however, such occurrences should be very rare.

Barring laboratory error, an unambiguous diagnosis of WN viral infection can be made through virus isolation in cell culture or suckling mice from CSF, serum, or tissues, followed by virus identification by IFA using WN virus-specific monoclonal antibodies.[77] However, although viraemia is commonly detectable in WN fever patients, especially during the first 4 days of illness,[56] WN virus has rarely been isolated from the serum or CSF of meningoencephalitis patients. A number of molecular amplification assays with sensitivities surpassing that of virus isolation for detection of WN virus have been reported, but these are also of limited utility in human diagnostics due to the low magnitude and transient nature of viraemia. In a study of patients with serologically confirmed acute WN meningoencephalitis, the sensitivity of TaqMan RT-PCR for detecting WN viral nucleic acids in acute-phase CSF and serum specimens was 57% and 14%, respectively.[81]

In fatal WN encephalitis cases, WN virus can be readily detected in brain tissues by immunohistochemistry or molecular amplification methods,[72,81] and occasionally by culture.[6] Rarely, WN virus has been isolated from other solid organs that have a high concentration of reticuloendothelial cells (eg, liver, spleen, lung, and pancreas).[69]

Clinical management

Although the treatment of uncomplicated WN viral infections is symptomatic, all patients with suspected WN meningoencephalitis should be hospitalised for observation and supportive care, and to rule-out treatable CNS infections or conditions (eg, herpesvirus infection, Guillain-Barrè syndrome, and bacterial meningoencephalitis). The most frequent cause of death in WN encephalitis cases is neuronal dysfunction, respiratory failure, and cerebral oedema (following neuronal injury and death). No virus-

specific therapy is currently available, and no controlled studies of the prophylactic use of corticosteroids, anticonvulsants, or osmotic agents (eg, mannitol) have been reported. The potential benefits of short-course high-dose corticosteroids in cases with cerebral oedema must be weighed against the theoretical risk of potentiating the viral infection.

Several antiviral agents have been either studied in WN virus-infected cell lines in vitro, studied in laboratory animals, or administered empirically to some patients with WN encephalitis. These agents fall into three general categories: (1) purine and pyrimidine analogues (eg, ribavirin), (2) interferon α, and (3) human immunoglobulin.

Ribavirin is a guanosine analogue with in vitro activity against many RNA and DNA viruses, including the flaviviruses.[82] Preliminary evidence suggests that high ribavirin concentrations inhibit the replication and cytopathogenicity of WN virus in human neural cells in vitro.[83] Another nucleoside analog, pyridazine nucleoside, seems to have specific action against the flavivirus NTPase-helicase (a product of the NS3 gene) and to thereby greatly decrease WN viral replication in vitro.[84]

Interferon α has proven, but limited, clinical efficacy against hepatitis C viral infections. Species-specific interferon reportedly protects spinal cord cells from becoming infected with WN virus in vitro when given before inoculation. Interferon reportedly also increases the survival of Vero cells (ie, monkey kidney cells) when applied either before or after WN virus inoculation, and this effect was observed at levels that could be readily achieved in human beings.[85]

Although these in vitro studies have shown the potential clinical usefulness of these agents in WN viral infection, and except for limited evidence of a therapeutic effect of ribavirin in WN virus-infected mice, the effectiveness of these or other agents against this virus in vivo has yet to be demonstrated. No clinical trials in WN meningoencephalitis patients (or patients infected with closely related flaviviruses) have been reported, and relatively few such patients have ever received antiviral drugs empirically (with the probable exception of acyclovir for presumed herpes encephalitis). In a retrospective Israeli study of 233 WN meningoencephalitis patients, including 37 who empirically received ribavirin, multivariate analysis showed that this agent had no apparent effect on mortality.[37] An anecdotal report from Israel describes the apparently successful intravenous use of human immunoglobulin (from pooled Israeli donors) in a comatose WN encephalitis patient.[86]

Even if clinically effective agents are identified, the challenge will be to employ them early enough in the clinical course of WN meningoencephalitis to improve the outcome. This, in turn, will require a high index of clinicoepidemiological suspicion and the development of more rapid and widely available diagnostic tests. Meanwhile, supportive care (ie, respiratory support, management of cerebral oedema, and prevention of secondary bacterial infections) will remain the basis of clinical management.

Search strategy and selection criteria

Sources for this review were identified by searches of Medline, and citations from relevant articles and book chapters. Medline search terms were "arthropod-borne virus", "arbovirus", "flavivirus", and "West Nile virus". English and French language papers were reviewed. Unpublished data from the national arbovirus surveillance system (ArboNET) of the CDC were also used.

Prevention

No human vaccine for WN virus is available, although several laboratories are currently conducting vaccine research. Given the low incidence of WN viral disease in human beings in most areas, however, it is unlikely that such a vaccine would be cost-effective for public health use. Both inactivated and DNA-based vaccines have been developed for use in equines,[87] but their efficacy has yet to be demonstrated.

Effective prevention of human WN viral infections depends on the development of locally funded, comprehensive, integrated arboviral surveillance and vector mosquito control programmes in areas where the virus occurs.[47,88] It is essential to know which local mosquito species are important in transmission, including those that might serve as a "bridge" from birds to human beings. Breeding sites for all vector mosquito species should be mapped; surveillance and targeted control should be implemented early in the year in an attempt to disrupt springtime viral amplification in birds and mosquitoes. Emphasis should be on larval control using an integrated approach that includes source reduction, water management, chemicals, and biological control methods. Chemical spraying to control adult vector mosquitoes should be reserved for emergency application after WN virus activity has been documented in the community. The goal should be to implement mosquito control early enough to prevent or decrease the risk of human and domestic animal infection with WN virus.[47]

An important component of any prevention programme is public outreach to educate members of the community on how to avoid or decrease the risk of being bitten by potentially infected mosquitoes. The information conveyed to the public may vary, depending on the mosquito species involved in transmission. Generally, areas where mosquitoes are common should be avoided, individuals should limit outdoor activity during peak mosquito biting periods (usually from dusk to dawn, but this is species-dependent), and wear long-sleeved shirts and long trousers during periods when mosquito exposure is possible. Repellants containing DEET (N,N-diethyl-m-toluamide) as the active ingredient are recommended for application to clothing and exposed skin, while repellants containing permethrin can be applied to clothing.[89]

Predicting the future

WN virus will almost certainly continue to spread into the contiguous western parts of the USA over the next several years, primarily via the movement of viraemic birds. Similarly, it is likely that this virus will be introduced into

Central and South America and the Caribbean, if this has not already occurred. After many years or even decades, WN virus in the western hemisphere will likely achieve an ecological/epidemiological equilibrium resembling that of SLE virus. In the USA, this would mean regional or multifocal enzootic/epizootic WN viral activity and modest numbers of scattered clinical cases occurring most years, punctuated by occasional outbreaks that are difficult to predict.[90] During 1964–2000, a median of 26 SLE cases per year (range 2–1967) were reported in the USA (unpublished data). How WN and SLE viruses will interact epidemiologically and ecologically is difficult to predict.

In the summer and fall of 1975, roughly 2000 human SLE cases and nearly 170 deaths were documented, mainly in urban and suburban areas of the central and southern parts of the USA, and primarily in the elderly.[90] The ecological, climatological, and other factors that led to that epidemic are poorly understood, although urban *Culex* species (especially *C pipiens* and *C qinquefasciatus*) clearly had a prominent role. Whether a similarly large and geographically widespread WN meningoencephalitis epidemic will eventually occur is unknown, but this sobering prospect presents a significant challenge to communities and their leaders throughout much of the USA.

Within the expanding geographic range of WN virus, it is virtually certain that additional large, urban, *C pipiens* complex-driven WN meningoencephalitis epidemics will occur in the foreseeable future. Cities with relatively poor economic and infrastructural conditions, and those that lack effective arbovirus surveillance systems and vector mosquito control programmes, are particularly vulnerable.[19]

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Conflicts of interest

None declared.

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