

Review

West Nile virus—an old virus learning new tricks?

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West Nile virus (WNV) has spread across the United States causing annual outbreaks since its emergence in 1999. Although severe disease develops only in about 1% of infections, WNV has claimed a total of 564 lives in the 5 years from 1999 to 2003. Observation of flaccid paralysis due to WNV infection at a higher incidence than previously documented and the devastating mortality recorded in infected American bird species triggered concerns about a potentially enhanced virulence of this virus. Here we summarize recent observations made during the American outbreaks regarding host range and transmission modes of WNV, and discuss epidemiological aspects of the emergence of this pathogen in the new habitat. *Journal of NeuroVirology* (2005) **11**, 469–475.

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Identification of West Nile virus (WNV) in the continental USA during the summer of 1999 has attracted remarkable attention, both domestically and internationally, mainly due to two facts: the indeterminate mode of introduction and the significant disease observed in humans, horses, and birds. The emergence of this old world virus into a new habitat has resulted in numerous cases of West Nile fever (in approximately 20% of infections) and West Nile encephalitis (in approximately 1% of infections). The case fatality rate increases in the elderly. Furthermore, compared to outbreaks in Africa, the Middle East, and Europe, a previously undocumented high incidence of muscle weakness and flaccid paralysis has been recorded. This striking disease manifestation, in conjunction with the unprecedented mortality observed in American corvid species, has sparked concerns that the virus may represent an emerging variant with enhanced virulence. Hence, WNV qualifies as an emerging pathogen not only based on its geographic transfer into a virgin environment, but also through a possibly evolving pathogenic potential. In this short review we intend to highlight recent findings from the ongoing WNV epidemic in the United States.

WNV is a positive-strand RNA virus that together with Alfuy, Japanese encephalitis, Kokobera, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Stratford, and Usutu viruses is grouped in the Japanese encephalitis (JE) virus group, a serologically closely related group of arthropod-borne viruses (arboviruses) in the genus *Flavivirus* of the family *Flaviviridae* (van Regenmortel *et al*, 2000). WNV is endemic in large parts of Africa, where early childhood infection is presumed to result in high immunity throughout the population (Darwish and Ibrahim, 1975; Smithburn *et al*, 1940; Taylor *et al*, 1956). Transmission is mediated by mosquitoes primarily between birds, which serve as the main amplifying host for WNV. Migrating bird species are thought to disperse the virus widely throughout northern Africa, the Mediterranean, and southern Europe (Malkinson *et al*, 2001, 2002; Taylor *et al*, 1956; Work *et al*, 1955), where outbreaks of severe disease resulting in hospitalization and occasional fatalities have been recorded over the past 50 years with increasing frequency (Ceccaldi *et al*, 2004; Hubálek and Halouzka, 1999). No outbreaks are documented in Asia, and the Australian variant, Kunjin virus, is reported to cause fever with flulike symptoms that are usually less severe than those caused by Murray Valley encephalitis virus, the other Australian flavivirus (Mackenzie and Broom, 1995). Humans and other mammals are incidentally infected by mosquito species that feed on birds

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and on mammals (bridge vectors) and are considered dead-end hosts because they usually develop a viremia too low for successful infection of feeding mosquitoes.

The first WNV outbreak in the Americas in the late summer of 1999 presented initially as a focused event, localized to the States of New York, New Jersey, and Connecticut, with 61 documented human cases (7 fatalities) primarily found in the New York City area (CDC, 2000a). Identification of a mosquito-borne virus as the causative agent (CDC, 1999) triggered intense vector control measures including spraying of malathion from trucks and helicopters during the height of the outbreak and widespread application of larvicide during the Spring of 2000, in an attempt to eradicate the virus before it could establish itself in the new environment. Despite the fact that the outbreak occurred in an urban area of an industrialized nation, such hopes were unrealistic, as became clear in early 2000 with the detection of WNV in overwintering mosquitoes in New York City, and the isolation of live virus from a hawk that died in New Jersey in February (CDC, 2000b; Garmendia *et al*, 2000). Over the next 2 years, WNV slowly spread along the eastern seaboard and then to the southeast and Midwest, resulting in 21 human cases (2 fatalities) in 2000 and 66 cases (9 fatalities) in 2001 (Bernard and Kramer, 2001; Campbell *et al*, 2002; CDC, 2002e; Marfin and Gubler, 2001; ProMED-mail, 2001). In 2002, 3 years after the first detection of WNV in the United States, the situation changed dramatically and one of the largest WNV outbreaks ever was recorded with 4156 human cases (2946 neuroinvasive, 284 fatal; <http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount02.htm>), was overshadowed only by the 2003 outbreak that resulted in 9858 reported human infections causing 262 fatalities. By the end of 2003, WNV had spread to all states of the continental United States, sparing only Oregon (<http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm>).

During the 2002 outbreak several modes of WNV transmission in addition to mosquito bites were identified in the United States. In August, cases of encephalitis and febrile illness were reported in two recipients of organ transplants derived from a common donor (CDC, 2002f). Four organs were transplanted from that donor, and subsequent investigation identified WNV infection also in the two recipients. Retrospective analyses found the donor to be viremic by reverse transcriptase-polymerase chain reaction (RT-PCR) assay and virus isolation, but not positive for immunoglobulin M (IgM) antibodies, at the time of organ recovery (Iwamoto *et al*, 2003). As a victim of a fatal injury, the organ donor received multiple transfusions during his 2-day hospitalization, and one stored plasma unit from 1 of 63 donors of the blood products was identified as positive for WNV RNA by RT-PCR. This likely represents the first

documented case of WNV infection through blood transfusion. Subsequently, a total of 23 cases of blood product-transmitted WNV infection were confirmed during the 2002 season; 43% occurred in individuals who were immunocompromised as a result of various conditions including organ, stem cell, and bone marrow transplantation or advanced cancer, and another 35% occurred in individuals who were 70 years of age or above. All infections were linked to 16 donors shown retrospectively to be viremic at the time of blood donation. The donations were used in a total of 26 transfusions of red blood cells, platelets, and fresh-frozen plasma performed on 25 recipients, including all 23 confirmed cases and 2 individuals that were not available for testing (Pealer *et al*, 2003).

Identification of WNV transmission through blood products demonstrated a considerable threat to the blood supply. Although experimental studies verified high safety margins in the manufacturing process of plasma derivatives with regard to WNV (Kreil *et al*, 2003), survival of WNV in chilled, nontreated blood products for more than 42 days, and productive infection of white blood cells was demonstrated (Mather *et al*, 2003). The 2002 experience led to changes in the donor deferral questionnaire by explicitly asking about febrile illness in the week before donation (<http://www.fda.gov/cber/gdlns/wnvguid.htm>), and to the implementation in June 2003 of investigational donor-screening by nucleic acid amplification tests (NATs). Two assays were introduced, a RT-PCR-based quantitative TaqMan assay (Roche) and a quantitative isothermal transcription-mediated amplification assay (GenProbe-Chiron) that use minipools of 6 or 16 samples, respectively (CDC, 2003a). During the 2003 season approximately 6 million units were screened leading to the removal of over 800 viremic donations (CDC, 2004b).

In connection with the above investigations, additional sources for WNV infection were identified. A potential infection through breast milk has been suggested as the most likely scenario in one case of a mother who subsequent to delivery received red blood cells from a donation that tested positive by real time RT-PCR (CDC, 2002a). Nine days later the mother, who was breastfeeding since delivery, developed symptoms of WNV encephalitis, tested positive for anti-WNV IgM by day 14, and showed evidence of WNV-specific RNA and IgM in her breast milk. The infant remained healthy, but tested positive for anti-WNV serum IgM at 25 days of age. Intrauterine transmission was identified as the source of WNV infection in another case. In this single case of an infection during the second trimester, the full-term born infant was diagnosed with chorioretinitis and bilateral white matter loss in conjunction with congenital WNV infection as indicated by the presence of WNV RNA in placenta and umbilical cord, and specific antibody in the infants blood and cerebrospinal fluid (CSF) (CDC, 2002b). Although

abortion and severe disease have previously been reported in some cases of intrauterine infection with JE and dengue virus (Chaturvedi *et al*, 1980; Chye *et al*, 1997; Thaithumyanon *et al*, 1994), this single event does not prove a causative role of WNV infection in the observed congenital abnormalities. Furthermore, in three other investigated cases of WNV infection during pregnancy healthy infants were born at full-term with negative WNV laboratory results (CDC, 2004a).

An immunocompromised status constitutes a serious risk factor for WNV infection. Not only does the rate of infection appear higher, but also the disease seems more severe in the immunocompromised individual, including more frequent neuroinvasion, and prolonged duration of viremia at potentially increased levels combined with less reliable serologic diagnosis (Baden and Rubin, 2004; DeSalvo *et al*, 2004; Kumar *et al*, 2004). A prolonged viremia in immunocompromised individuals was demonstrated in experiments performed in the 1950s on human volunteers (Southam and Moore, 1951, 1954). Transplantation recipients and cancer patients must therefore be considered high-risk populations, and they may potentially transmit virus, if their viremia reaches levels high enough to support infection of mosquitoes.

Occupational WNV infections occurred after percutaneous injury in laboratory workers (CDC, 2002c). In addition, a potential occupational risk has been identified for turkey farm workers and people working on alligator farms. High seroprevalence of WNV neutralizing antibodies in farmed turkeys, accompanied by a high incidence of febrile illness and WNV-specific IgM serum antibodies among farm workers, suggested an occupational infection source for the workers (CDC, 2003b). The mode of transmission, however, remains to be identified. Experimentally infected turkeys developed mostly a low level viremia, considered too low to efficiently sustain a mosquito-borne transmission cycle (Swayne *et al*, 2000). Infectious virus was demonstrated in feces, albeit at low levels and no contact transmission was observed. However, bird-to-bird transmission, presumably via virus shed in fecal-uric or oral secretions, has been demonstrated experimentally in geese, chickens, and crows (Banet-Noach *et al*, 2003; Komar *et al*, 2003a; Langevin *et al*, 2001; McLean *et al*, 2001; Swayne *et al*, 2001). Thus, non-vector-borne transmission modes including percutaneous, fecal-oral, or aerosol infection must be considered. This potential of WNV infection by non-mosquito-borne transmission may also apply to other settings where large numbers of potentially infected birds are housed and handled. Non-vector-borne transmission through fecal shedding of virus has also been suggested to occur in the spread of WNV infection in farmed alligators. A single confirmed human infection associated with infected alligators in Idaho, however, occurred most likely by direct transmission during a necropsy per-

formed without personal protection before WNV infection was considered. All other workers concerned with daily handling of the animals on that farm did not show evidence of WNV infection.

WNV infection of alligators causing movement disorder, neurological symptoms, and death has been reported mainly from Florida, Georgia, and Louisiana. Although virus isolation or RT-PCR analysis was performed only on a small number of animals, it is assumed that WNV was the etiologic agent during significant epizootics that occurred in 2001 and 2002. Out of approximately 10,000 animals housed on a single farm in Georgia, about 250 died in 2001 and more than 1000 in 2002 (Miller *et al*, 2003). There are no published studies indicating the peak viremia levels achieved in alligators, and it is unclear to what extent these animals may serve as an amplifying reservoir. Experimental infections of American snake, frog, and iguana species suggest a rather low viremia in ectotherms in a range of 10^2 to 10^3 pfu/ml, lower than the 10^4 to 10^5 pfu/ml level considered necessary for infection of *Culex pipiens* mosquitoes (Klenk and Komar, 2003). However, mosquitoes, including species in the genera *Culex* and *Aedes*, do feed on reptiles and/or amphibians (ProMED-mail, 2003b). It should be emphasized that all confirmed cases of WNV infection in alligators were among farmed animals housed under environmentally controlled, artificial conditions, and that no infection has been reported among free-ranging alligators thus far. Investigation of the Georgia case indicated an introduction of the virus through infected feed (Miller *et al*, 2003). Alligators are fed a horse meat diet, and horses have been heavily affected with approximately 730 WNV reported cases in 2001 and more than 15,000 cases in 2002 (<http://www.aphis.usda.gov/lpa/issues/wnv/wnv.html>; CDC, 2002e). The epizootics in alligators began in both years during the fall, at a time when infected horse meat may appear in the supply. Meat fed during the outbreak tested positive for WNV RNA by RT-PCR analysis, whereas samples from shipments subsequent to the outbreak were negative (Miller *et al*, 2003). These findings are compatible with an oral route of infection, which has been suggested previously as a transmission mode for cases of ingestion of infected prey by raptors (Komar *et al*, 2003a), for mice that ate infected offspring or were orally treated with WNV suspension (Odelola and Oduye, 1977), and for cats that consumed infected mice in experimental settings (Austgen *et al*, 2004).

Particular features of the U.S. epidemic may also result from differences in vector competence and feeding behavior. As a result of active surveillance, WNV RNA has been detected in over 30 mosquito species in the United States since 1999, but the primary enzootic vectors are *Cx. pipiens*, *Cx. restuans*, *Cx. quinquefasciatus*, and *Cx. tarsalis* (Bernard and Kramer, 2001; CDC, 2002d). Mosquito transmission of WNV to humans and horses requires a mosquito that feeds

on both birds and mammals, and is competent for WNV. Both *Cx. quinquefasciatus* and *Cx. tarsalis* readily feed on birds and mammals. *Cx. pipiens* and *Cx. restuans*, although primarily ornithophilic, feed on mammals occasionally, with variable frequency observed in different mosquito populations (Apperson *et al*, 2004). A recent genetic analysis of the *Cx. pipiens* complex indicated a separation of two physiological and behavioral populations in northern Europe that correspond to preferential bird-biting and human-biting behavior (Fonseca *et al*, 2004). In contrast, hybrids of these populations are common in the United States and may represent an expansion of feeding behavior in *Cx. pipiens*. If this proves true, the spread of WNV from its avian reservoir into the human population may be facilitated by this enzootic vector. Other mosquitoes, such as *Cx. salinarius* and *Ae. albopictus*, may also act as important epidemic vectors (Kulasekera *et al*, 2001).

WNV has also repeatedly been isolated from ticks, though a vector competence of ticks is not well characterized (L'vov *et al*, 1975, 2002; Schmidt and Said, 1964). The susceptibility of ixodid and argasid ticks to WNV infection was confirmed in experimental studies (Abbassy *et al*, 1993; Anderson *et al*, 2003). A recent report compared the vector competence of a hard tick species (*Ixodes ricinus*) to that of argasid *Ornithodoros moutaba* ticks. Although both species became infected, the hard tick species did not support a productive infection, whereas the soft tick species maintained the virus for more than three months and was able to infect mice (Lawrie *et al*, 2004). This suggests that soft ticks are a potential reservoir for WNV.

The virus circulating in humans, horses, birds, and mosquitoes in 1999 was shown to be genetically homogeneous and closely related to a WNV isolate circulating at the same time in the Mediterranean (WNV-Isr98) (Ebel *et al*, 2001; Jia *et al*, 1999; Lanciotti *et al*, 1999). This finding is compatible with a novel, single source introduction of WNV. Only recently have minor genetic variants been characterized in the United States (Beasley *et al*, 2003; Davis *et al*, 2003), and a recent study has shown an increase in the replication kinetics in the mosquito for one of the New York variants (Ebel *et al*, 2004). Future analyses will indicate whether these changes are random mutations reflecting the normal genetic variability of WNV, or specific adaptive changes in response to distinct selective pressures in the new habitat. It will be interesting to learn what genetic variation future isolates will reveal (Estrada-Franco *et al*, 2003). Over the past years WNV has invaded Canadian Provinces bordering the United States (Nosal and Pellizzari, 2003), the Caribbean, and Central America most likely through spread by migrating birds. By 2002 WNV reached Jamaica and the Dominican Republic as indicated by serologic data from resident birds (Dupuis *et al*, 2003; Komar *et al*, 2003b). In 2002 WNV-specific antibodies were shown in horses and birds in Guadeloupe,

with increasing prevalence in 2003 (Quirin *et al*, 2004). At the end of 2002 infection of horses was confirmed in Mexico (Blitvich *et al*, 2003; Loroño-Pino *et al*, 2003) and El Salvador (ProMED-mail, 2003a). Strangely however, no overt bird mortality has been reported, symptomatic infection in horses appears to be infrequent, and no human cases have been observed thus far. It is possible that immunity to other endemic flaviviruses may alter the transmission and pathogenicity of WNV in Central and South America (Tesh *et al*, 2002).

Phylogenetic analyses of the species *West Nile virus* indicate considerable genetic variability, and two lineages are distinguished. Lineage II comprises viruses isolated exclusively in Africa and Madagascar, and lineage I comprises WNV-NY1999 and viruses isolated in northern Africa, the Mediterranean, and southern Europe primarily during outbreaks, as well as Kunjin virus and Indian isolates (Berthet *et al*, 1997; Briese *et al*, 2002; Jia *et al*, 1999; Lanciotti *et al*, 1999, 2002; Scherret *et al*, 2001). The isolation of lineage I viruses in the context of outbreaks has been viewed as the reflection of a higher intrinsic pathogenicity of the Euro-African lineage I viruses in comparison to endemic lineage II viruses. A recent analysis of numerous South African isolates, however, demonstrated the isolation of lineage II viruses in the context of severe disease and suggested, in contrast to previous findings, that the major South African outbreaks of 1974 and 1983/1984 were likely caused by lineage II viruses (Burt *et al*, 2002). Despite the fact that no fatalities were recorded during these two outbreaks, these findings accentuate our fragmentary understanding of the determinants involved in WNV pathogenicity.

Two of the most prominent features of the U.S. outbreak are the extraordinarily high mortality observed among American bird species and an unusual high frequency of muscle weakness and paralysis observed in human infection (Asnis *et al*, 2000; Bernard *et al*, 2001; Jeha *et al*, 2003; McLean *et al*, 2001; Sejvar *et al*, 2003; Steele *et al*, 2000; Yaremych *et al*, 2003). These features as well as the characterization of previously unreported transmission modes and host species have been discussed as possible indicators for the emergence of a more virulent WNV variant (Ceccaldi *et al*, 2004). However, on the background of the introduction of WNV into a new ecological niche, it is difficult to discern to what extent features of the American epidemic(s) correlate to intrinsic changes affecting virulence or to the unique situation of WNV interacting with a naive environment with heightened surveillance.

Transmission of WNV through blood products or organ transplantation, though previously not reported, should not be surprising given the natural mode of WNV transmission. The detection of cases may relate to the significantly larger size of the outbreak in comparison to many previous epidemics, linked possibly to the complete absence of immunity

in the population, and a more frequent use of blood products in modern medicine. Infection also correlates strongly to conditions of increased host susceptibility, such as immunosuppressive treatment or elevated age. Although the devastating outbreak among farmed alligators may indicate biologically relevant genetic differences between WNV and Kunjin virus, which is not known to affect farmed crocodiles in Australia, there are also no reports on WNV killing Nile crocodiles. In fact, recent analyses suggest a high rate of infection among farmed crocodiles in Israel, presumably with a virus closely related to WNV-Isr98, without any reported mortality (Steinman *et al*, 2003). Differences in the U.S. alligator farms may relate to feeding or other husbandry practices. In addition to alligators, infection of skunks, chipmunks, alpacas, and more than 10 other nonavian species has been viewed to indicate an extended host range

and high virulence of WNV-NY1999. However, this could also mark the first opportunity for WNV to infect such species in the absence of previous coevolution between virus and host, or a 'spill-over' into incidental species in areas of high vector density and viral activity.

It remains to be seen whether certain changes in clinical course and pathogenicity recognized during recent outbreaks associated with the NY1999/Isr98 genotype of WNV relate to evolutionary selection of a more virulent virus, or alternatively reflect increased medical alertness, the effectiveness of active surveillance programs, and immunologically naïve host populations. Valuable new insights into host-virus coevolution can be anticipated from the thorough real time analysis of the ongoing Darwinian experiment created by the introduction of WNV into the Americas.

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