

West Nile Virus Infection Facts And Threads

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ABSTRACT

West Nile virus (WNV) is a mosquito-borne zoonotic arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. The virus is found in temperate and tropical regions worldwide, but first identified in the West Nile sub-region in the East African nation of Uganda in 1937. Prior to the mid-1990s WNV infection was sporadically and considered a minor risk for humans, until an outbreak in Algeria in 1994, with cases of WNV-caused encephalitis, and the first large outbreak in Romania in 1996, with a high number of cases with neuroinvasive disease. Two main WNV genetic lineages are known: lineage 1, identified in the majority of the outbreaks in humans and horses in Europe and the United States and lineage 2, which until 2004 had not been detected outside Africa, but since then has repeatedly appeared – initially in Hungary in 2004 and 2005–09, in Russia in 2007 and in Austria in 2008–09.

WNV has now spread globally to Europe beyond the Mediterranean Basin and the United States, is now considered to be an endemic pathogen in worldwide especially in Africa. The WNV transmission is mainly by various mosquitos' species, also ticks were incriminated. The birds especially passerines are the most commonly infected animal and serving as the prime reservoir host.

WNV has now spread globally, with the first case in the Western Hemisphere being identified in New York City in 1999. Over the next 5 years, the virus spread across the continental United States, north into Canada, and southward into the Caribbean Islands and Latin America. WNV also spread to Europe, beyond the Mediterranean Basin a new strain of the virus was recently (2012) identified in Italy. WNV is endemic in Africa, the Middle East, Europe, Central Asia and most recently, North America. The Center for Diseases Control and prevention reported 4261 positive human WNV cases in a year in USA.

Keywords: Egypt, West Nile fever, Mosquitoes, Birds, Animals, Human.

INTRODUCTION

WN fever was considered a minor arbovirus, inducing in humans a nonsymptomatic disease or a mild flu-like illness. WN virions are small (~50 nm in diameter), spherical, enveloped, and have a buoyant density of -1.2 g/m^3 . The Spherical nucleocapsid is ~25 nm in diameter and is composed of multiple copies of the C protein. Cryo-electron microscopy data suggest that the virions envelope and capsid have icosahedral symmetry [1]. Recent data indicate that the symmetry is conferred on the virus particle by interactions between E proteins rather than by interactions between capsid proteins [2].

WN virus particles are spherical and 50 nm in diameter with an envelope and a single-stranded positive-sense RNA. The genome, 11,000–12,000 nucleotides long, has a single open-reading frame encoding 10 proteins: seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) and three structural proteins (core C, membrane M, and envelope E) [3]. The E protein is important in the detection of the viral receptor on the surface of the cell and in the installment of humoral immunity. Virus replication occurs in the cytoplasm in close association with the endoplasmic reticulum endothelium (ER) followed by viral assembly in the ER lumen and release from the cell by exocytosis [4]. The first epidemics of encephalitis were reported in Israel in the 1950s and then in France in 1962–1963, affecting both humans and horses [5,6]. For the past 10 years WNV has been recognized as the leading cause of arboviral encephalitis in the U.S. WNV has claimed 1,176 lives in the U.S. and has been responsible for 12,489 neuroinvasive disease cases between 1999 and 2010 [7].

During the last years, several human outbreaks have been reported in the Mediterranean basin, with fatal cases of encephalitis occurring principally among elderly people. Outbreaks have occurred in Algeria in 1994; Romania in 1996; Tunisia in 1997; Russia in 1999, and Israel in 2000 [6]. The unexpected **emergence** of WN virus during the summer of 1999 in New York City and its rapid spread throughout the USA highlight the ability of an arbovirus to become a major threat [8]. The connection with the WN viral strain identified previously from birds in Israel was established [9].

Since the emergence of WNF in Greece in 2010, the disease has spread in the country reaching both rural and urban areas. In 2011, cases of WNV infection occurred in the same districts as in

2010 [10]. In the subsequent summers from 2011 to 2013, the outbreaks did not subside in these areas [11]. WNV lineage 2 sequences (strain Nea Santa-Greece-2010) were obtained from blood donors, mosquitoes and birds in the transmission period (June to October) of both years [12,13].

Epizootics in horses have also been described in Morocco in 1996; Italy in 1998; France and Israel in 2000 [14,15]. In 1998, in Israel, an unusual mortality related to WN infection was observed in migrating white storks and domestic geese [16].

Surveillance programs of WN fever were initiated in Europe and several encephalitis cases related to WN infection are diagnosed every year in Romania, mostly around the Danube delta [7]. WN virus activity, as indicated by isolations of the virus, is reported annually in the Volga delta in France, some guards' birds in 2001 and 2002 indicating a low enzootic maintenance of the virus. Several dead were recorded in many local (resident) birds [17].

Regarding viral ecology of West Nile Virus and Mosquitoes, WN virus has been recovered from 11 genera of mosquitoes in Africa and America: *Culex*, *Ochlerotatus*, *Aedes*, *Anopheles*, *Coquilletidia*, *Aedemomyia*, *Mansonia*, *Mimomyia*, *Psorophora*, *Culiseta*, and *Uranotenia*. In the Mediterranean basin, the virus was isolated in Israel, Egypt, and Algeria, mostly from *Culex* mosquitoes: *Culex Antennatus*, *Culex univittatus*, and *Culex pipiens* [18,19]. In Europe, isolations from mosquitoes belonging to four genera have been reported in Portugal, France, Romania, the Czech Republic, southern Ukraine, Slovakia, and southern Russia. Only the mosquito species that replicate the virus and assure its transportation to the salivary glands via the haemolymph are potential competent vectors [20].

Members of the *Culex* genus are the most efficient for spreading the virus among birds and from birds to humans and mammals. Field evidence of natural vertical transmission of WN virus in *Culex* viremia in West Nile virus infection, 150 mosquitoes was reported in Kenya and persistence of the virus in over wintering mosquitoes in North America [21]. Some isolation from birds were reported in the Old World from native or migrant, marine, or birds (crow, pigeon, turtle, duck, teal, gull, starling, sandpiper, coot, ibis, and heron) in Egypt, Slovakia, Cyprus, Russia, and the Ukraine [22].

In Eilat (Israel) in September– October 1998, WN virus was isolated from several white storks (*Ciconia ciconia*) and domestic geese showing clinical symptoms of encephalitis and paralysis [23]. The emergence of WN virus in New York City was revealed by the death of thousands of native (crows, ravens, magpies, jays) as well as exotic birds [24-26]. Several species, including the blue jay, common grackle, house finch, house sparrow, and American crow, develop high viremia and are capable of infecting mosquitoes that feed on them [27].

The role of ticks in transmission of WN virus has been reported [28,29]. Adult Argas ticks artificially fed on bovine serum containing WN virus were able to transmit the virus to chickens 20 days later. Ticks may play a role in geographic distribution and maintenance of WN virus [30]. Antibodies related to WN virus was detected in a large variety of mammals [31]. In North

America, bats, cats, dogs, raccoons, rabbits, squirrels, chipmunks, mountain goats, reindeer, and alpacas were found positive for WN virus [32,33].

WNV is transmitted by the bite of infected mosquitoes that acquire the virus by feeding on infected birds. The intensity of transmission is dependent on large quantity and feeding patterns of infected mosquitoes, local ecology and behavior that influence human epidemics [34,35]. In Europe and Africa, principal vectors are *Cx. pipiens*, *Cx. univittatus*, and *Cx. antennatus*, and in India, species of the *Cx.vishnui* complex [21,36], while In Australia, Kunjin virus is transmitted by *Cx. Annulirostris* [37].

During the 2000 Staten Island epidemic, the MIRs in mixed *Cx.pipiens/restuans* pools ranged from 0.5% to 1.6% and the MIR in *Cx.salinarius* from 0.3% to 1.2%. . The estimated prevalence of infection, measured as the minimum infection rate (MIR), that is needed to produce epidemics is uncertain [38]. Relatively low MIRs in *Cx.restuans* (0.2%), *Cx.pipiens* (0.1%) and *Cx.salinarius* (0.1%) [39]. In 2001, moderate to high MIRs in *Cx.quinquefasciatus* (0.5%) and *Cx. nigripalpus* (1.1%) were associated with epizootic and epidemic transmission in Florida [40].

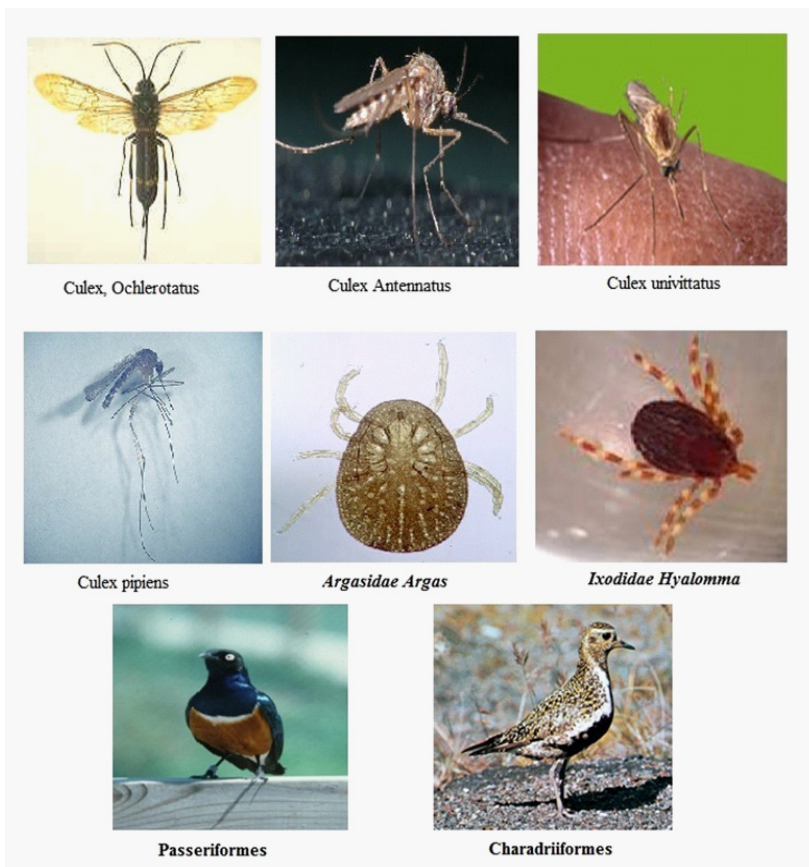


Figure 1

In North America, WNV has been found in 59 different mosquito species with diverse ecology and behavior; however, <10 of these are considered to be principal WNV vectors [41-43]. During 2003, as WNV activity progressed westward, *Cx.tarsalis* became the most commonly reported WNV positive mosquito species, followed by *Cx. pipiens*, *Cx.quinquefasciatus*, and *Cx.restuans* *Cx.salinarius* [44]. During 2004, when large epidemics occurred in the southwestern United States, the most commonly reported WNV-positive species was *Cx.quinquefasciatus*, *Cx.tarsalis* and *Cx.Pipiens*.

The intensity of transmission to humans is dependent on abundance and feeding patterns of infected mosquitoes and on local ecology and behavior that influence human exposure to mosquitoes [45]. Although both soft and hard ticks can become infected with WNV, they are unlikely to play a substantial role in WNV transmission. [38]. However, of the hard ticks *Amblyomma americanum*, *Ixodes scapularis*, *I. ricinus*, *Dermacentor variabilis*, and *D. andersoni*, the last 4 species became infected with WNV, but none transmitted the virus by subsequent bite [20,46].

Laboratory studies on California mosquitoes have demonstrated that 74%–100% of *Cx. tarsalis* mosquitoes become infected after consuming blood meals with WNV concentrations of 107.1 plaque forming Units (PFU)/mL, while only 0%–36% become Infected after consuming a female containing 104.9 PFU/mL The maximum estimated concentration of WNV in human blood tested during screening of blood donors in 2002 was approximately 103.2 PFU/mL Thus, it appears unbelievable that humans show WNV viremia levels of enough magnitude to infect mosquitoes [47].

Birds are accepted to be the most important amplifying hosts of WNV. Laboratory studies confirmed that type in the orders Passeriformes (songbirds) is an important host to WNV [38]. Charadriiformes (Shorebirds), Strigiformes (owls), and Falconiformes (Hawks) developed viremia levels sufficient to infect most feeding mosquitoes, whereas type of Columbiformes (pigeons), Piciformes (woodpeckers), and Anseriformes (Ducks) did not [44,48].

Field studies during and after WNV outbreaks. in several areas of the United States have confirmed that house sparrows were abundant and frequently infected with WNV, characteristics that would allow them to serve as important amplifying hosts [43,44,48].

Local movements of resident, non-migratory birds and long-range travel of migratory birds may both contribute to the spread of WNV [49]. Although WNV was isolated from rodents in Nigeria and a bat in India, most mammals do not appear to generate viremia levels of sufficient titer to be contributed to transmission [21,50,51].

Three reptilian (snake) and amphibian (frog) species were found to be useless as amplifying hosts of a North American WNV strain, and no signs of illness developed in these animals [52]. Viremia levels of sufficient titer to infect mosquitoes were found after experimental infection of young alligators (*Alligator mississippiensis*) [53]. In Russia, the lake frog (*Rana ridibunda*) appears

to be a capable reservoir [54]. This type of transmission has been observed or strongly suspected among farmed alligators, domestic turkeys in Wisconsin, and domestic geese in Canada [55].



Figure 2

The incubation period of WN virus infection is usually 3–15 days after the bite of an infected mosquito. Most cases are nonsymptomatic. In 15–20% of the cases, mild flu-like illness is reported, generally characterized by an abrupt onset of fever, headache, myalgia, malaise, anorexia, nausea, and vomiting [37]. A maculopapular or roseolar rash may be observed. The disease may last 2–5 days. In less than 1% of the cases, neurological symptoms such as meningitis, meningoencephalitis, or myelitis appear, generally associated with high fever [56]. Other neurological presentations are ataxia and extra pyramidal signs, polyradiculitis, seizures, and eye neuritis [57,58]. Muscle weakness is a prominent part of the clinical presentation of many patients with WN encephalitis [57,59].

In a very few cases, fulminant hepatitis, pancreatitis, and myocarditis have been reported with WN infection [60–62]. In the 1996 Romanian outbreak, 352 patients presented with acute central nervous system infection: meningoencephalitis (44%), meningitis (40%), or encephalitis (16%) [7].

Fatal cases were recorded in patients over 50 years of age. In the 2000, outbreak in Israel, the clinical features in hospitalized patients were encephalitis (58%), meningitis (16%), and febrile illness (29%) [63]. In the CSF, pleocytosis, predominantly with lymphocytes, elevated protein, and normal glucose, was commonly recorded. The median age of the 13 fatal cases was 80 years, ranging from 54 to 95 years [59]. Independent predictors of death were age over 70 years; change in the level of consciousness, and anemia following the mosquito bite has been reported [63]. The pathogenesis of WNV encephalitis has been studied in several animal models [51,64].

In animal models, as for human beings, neurons are the main target cell in the CNS, with preferential involvement of cerebellar Purkinje cells, anterior-horn cells in the spinal cord, and neurons of the thalamus and basal ganglia [64, 65]. In hamsters and mice, WNV causes meningoencephalitis with poliomyelitis-like features, mimicking the severe human disease. After subcutaneous inoculation, virus spreads sequentially to the draining lymph nodes, spleen, and serum, producing a high titre viraemia before disseminating to the CNS [64].

Mechanisms for this selective tropism among neuronal populations remain to be elucidated. Domain III of the envelope glycoprotein of WNV has been implicated in receptor binding [66]. Neuronal degenerative changes slightly precede the appearance of a lymphocytic inflammatory infiltrate predominantly consisting of CD8-positive T cells [64-67]. The available experimental evidence suggests that virus-induced apoptosis is an important cause of neuronal damage, although there may be an immunopathological component dependent on CD8- positive T cells [65,67]. Viral capsid protein causes caspase-9- dependent apoptosis in cultured neuronal cells [68]. A role for other innate immune responses in WNV pathogenesis is likely [70].

Diagnosis of WNV Infection can be done through virus isolation, serological assays, detection of WNV antigen and Nucleic acid amplification tests (NATs). Virus isolation of WNV from blood, other tissues or CSF, is rare due to the low concentration of virus in these samples and the transient nature of viremia. The success in isolating live virus from patients probably reflects the large number of cases observed in Canada 2002, as opposed to the modification of techniques of virus culture [69].

Hemagglutination inhibition (HI) assays and IgG ELISA can be used to show fourfold or greater rises in antibody levels in acute and convalescent serum samples. The HI test can detect both IgM and IgG antibodies but cannot differentiate between these two kinds of immuno globulins [71].

ELISA unlike the HI test as, there is several components (e.g., viral specific monoclonal antibodies) necessary for performing the assay. Although the cross-reactivity of IgM ELISA is significantly lower than that observed with HI or IgG ELISA, still we need confirmatory assays such as PRNT or NATs [65].

The decreased sensitivity of NATs is not due to the test itself (i.e., nucleic acid sequence-based amplification and real time Taqman (Taqman Laboratory, Pittsburgh) assays are able to detect as few as 0.1 to 0.05 plaque forming units/mL or 10 to 50 copies of genome) but the low titre

and transient nature of viremia decreases the likelihood that NATs will successfully detect virus in clinical samples [72]. A formalin-inactivated whole virus vaccine has been approved for use in horses. DNA vaccines Coding for the structural WNV proteins have also been assessed for veterinary use. Live attenuated Yellow fever WNV Chimeric vaccines have also been successful in animals and are currently undergoing human trials. Immunization with a relatively benign Australian Variant of WNV, the Kunjin virus, Provides protective immunity against the virulent North American strain [73].

Preventive measures in North America focus on the use of mosquito repellents. In addition, Killed vaccines have been used in the USA in horses with a classical immunization process: two initial intramuscular doses, 3–6 weeks apart, followed by a yearly booster. Recombinant DNA vaccine (pCBWN DNA) has been shown to be protective in horses, mice, and fish crows by intramuscular inoculation [74].

Other approaches include the use of live attenuated vaccines, which induce rapid immunity after a single dose and strong and durable immunologic memory. Chimeric vaccines are under construction using the 17D yellow fever vaccine strain as a vector and the prM and E genes of WN virus [69].

Avoiding human exposure to WNV-infected mosquitoes remains the cornerstone for preventing WNV disease. Source reduction, application of larvicides, and targeted spraying of pesticides to kill adult mosquitoes can reduce the abundance of mosquitoes. Persons in WNV-endemic areas use insect repellent on skin and clothes and avoid being outdoors during dusk to dawn when mosquito vectors of WNV are abundant. Of insect repellents recommended for use on skin, those containing *N,N*-diethyl- *m*-toluamide (DEET), picaridin (KBR-3023), or oil of lemon eucalyptus (*p*-menthane-3,8 diol) provide long-lasting protection [38,72]. Both DEET and permethrin provide effective protection against mosquitoes when applied to clothing [67]. To prevent transmission of WNV through blood transfusion, blood donations in WNV-endemic areas should be screened by using nucleic acid amplification tests. Screening of organ donors for WNV has not been universally implemented because of false-positive screening results [69].

CONCLUSION

The occurrence of outbreaks of WN fever remains unpredictable, as recently observed with the limited outbreak in humans and horses in La Riviera, Southern France, at the end of August 2003. West Nile virus remains a serious threat to the public health, especially to very young, elderly and immunocompromised individuals. The immediate adaptation of the WN virus in North America demonstrated the capacity of this arbovirus to distribute and persist in some vectors and hoststhat may allow the virus to survive during the cold season.

Increased awareness among clinicians and veterinarians about the possibility of WN virus causing cases of encephalitis and meningoencephalitis during periods of potential transmission. Suitable screening of blood Donors, including the exclusion of travelers returning from infected

areas, would avoid the risks of transmission of WN virus and other arboviruses. Low incidence of WNV neuroinvasive disease in healthy individuals and the focal WNV epidemics, vaccination target the groups at higher risk for WNV neuroinvasive infection. At the moment, the best way to prevent West Nile virus infection remains to avoid mosquito bites.

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