Emerging Infections: West Nile Virus in the New World

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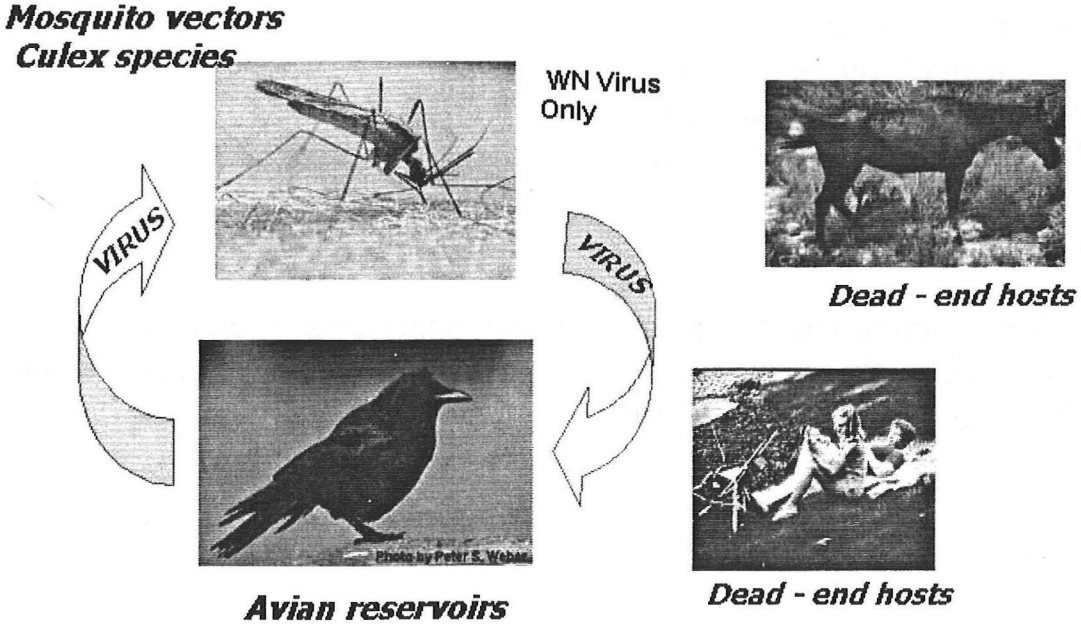
West Nile and St. Louis Encephalitis Virus Transmission Cycles



Disclosure: This is to acknowledge that Elizabeth Race, M.D., M.P.H. has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Race will be discussing off-label uses in her presentation.

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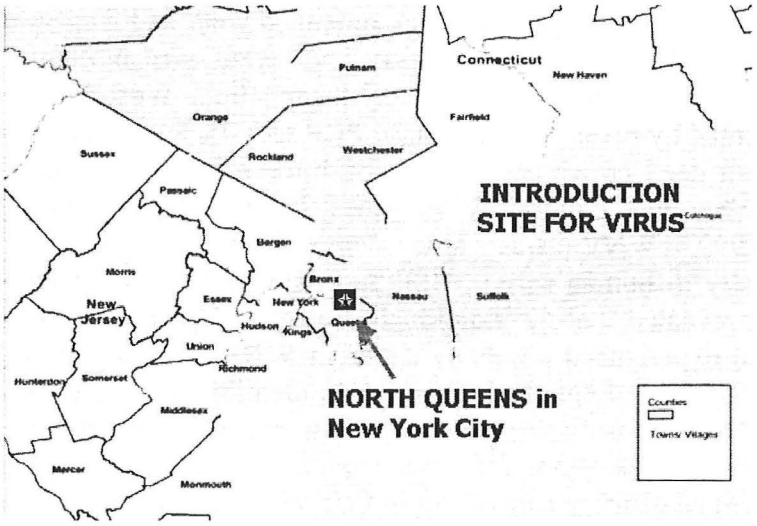
EXOTIC WEST NILE VIRUS INTRODUCED IN NEW YORK CITY IN 1999



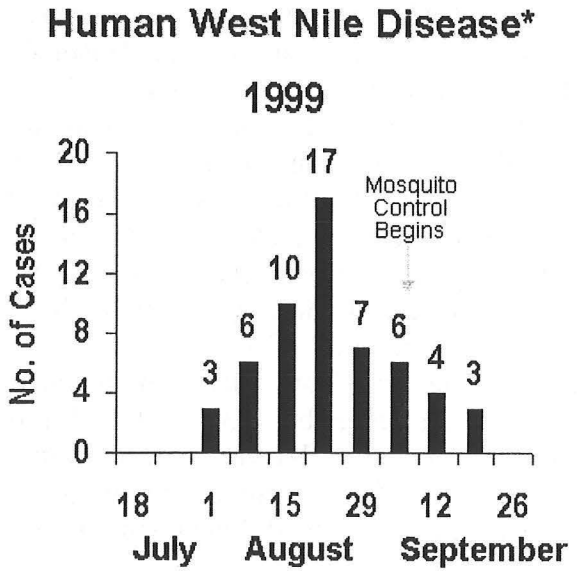
INTRODUCTION: THE 1999 WEST NILE VIRUS OUTBREAK IN NEW YORK CITY

On August 23, 1999, the New York City Department of Health received a call from Dr. Deborah Asnis, an infectious disease specialist at Flushing Hospital in Queens, New York, to report two cases of viral encephalitis.[1-3] Both individuals had presented with fever, altered mental status and lymphocyte-predominant cerebrospinal fluid (CSF). One of the two individuals suffered from profound muscular weakness as well. The Department of Health initiated an epidemiological investigation, which included active case finding at local hospitals. Their efforts resulted in the detection of six additional cases, all within a 16 square mile area of Northern Queens. All eight patients carried a clinical diagnosis of viral encephalitis, and three had initially been diagnosed with Guillain-Barre syndrome due to the presence of flaccid paralysis. Serum and CSF specimens were submitted to both the New York City Department of Health and the Centers for Disease Control (CDC). On September 2-3, 1999, both groups reported that the initial serological tests were consistent with flavivirus infection; most likely St. Louis encephalitis (SLE). Mosquito control efforts were begun in Queens and expanded citywide when active surveillance mechanisms detected laboratory-positive cases in multiple boroughs.

Several weeks prior to the human outbreak, a large avian die-off occurring in the same vicinity was observed by veterinary and wildlife officials. Termed an epizootic, the excessive avian mortality, which primarily affected crows, was recognized in early to mid August by Dr. Tracy McNamara, a veterinary pathologist at the Bronx Zoo.[4] However, the connection between the human and avian outbreaks was not appreciated until media

coverage of the human cases triggered an intensified local investigation into the etiology of the epizootic. Dr. McNamara doubted the diagnosis of SLE, as this virus does not normally carry a high mortality in birds. In addition, she doubted the presence of Eastern Equine encephalitis (EEE), since the emu flock remained well, and their species is highly susceptible to EEE.[5] Ultimately, both human and avian post-mortem examinations revealed a common pathological diagnosis of encephalitis. West Nile virus (WNV) infection was confirmed by reverse transcriptase-PCR (RT-PCR) and genome sequencing of neural tissue from dead crows and exotic zoo birds submitted to the CDC and the National Veterinary Services Laboratory in Ames, Iowa. Soon after this discovery, on September 24, 1999, WNV was also detected by serology, RT-PCR and immunohistochemistry in human serum, CSF and neural tissue specimens. Subsequent case-finding efforts revealed that 62 individuals from New York City, Westchester and Nassau counties had experienced clinically apparent WNV infection, and seven deaths, all in patients over 65 years of age, had occurred. Epidemiological investigation revealed that no person-to-person transmission was occurring, and thus household contacts were unaffected. The common risk factor for virus acquisition was determined to be outdoor activity. A subsequent serosurvey carried out in Queens revealed that 2.6% of individuals tested in that area had evidence of WNV infection.[2]

The NYC Department of Health implemented a series of prevention and control measures, including intense mosquito spraying, elimination of peridomestic mosquito breeding sites, free distribution of DEET-containing mosquito repellants, and public health awareness and education efforts focusing on personal protective measures.



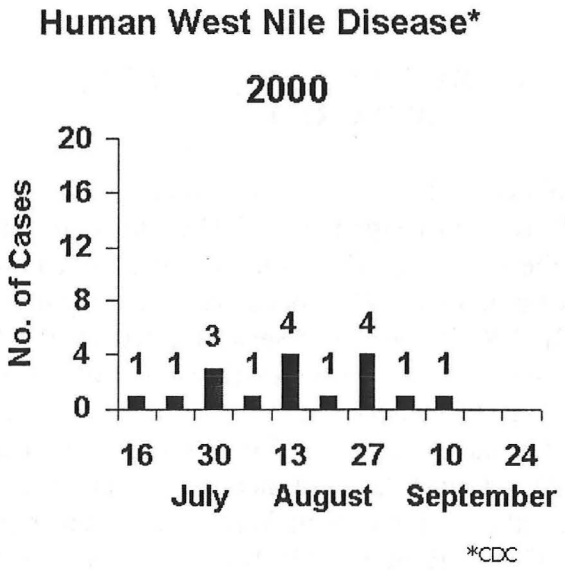
*CDC

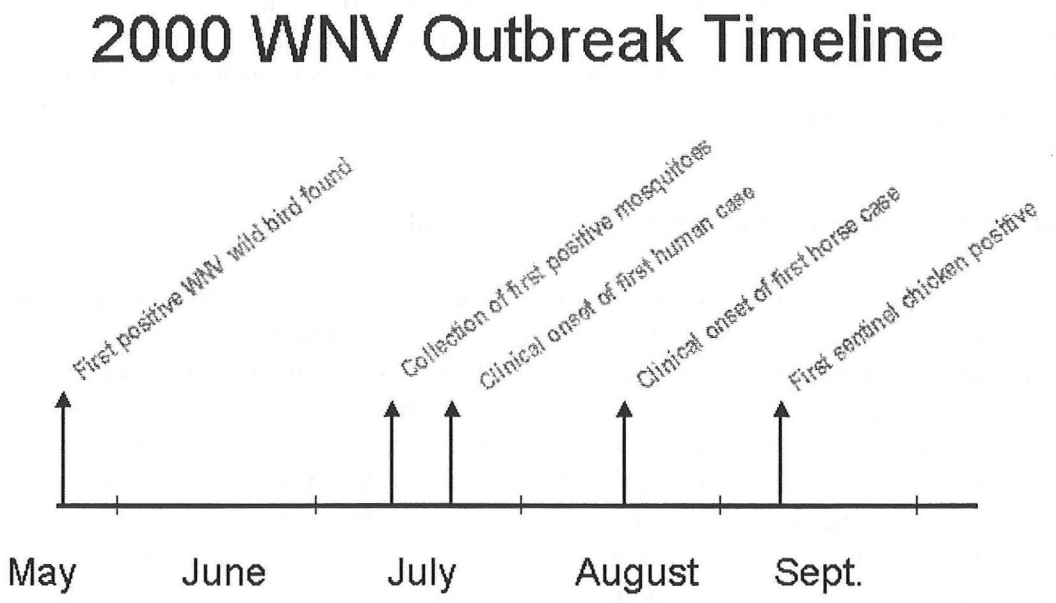
The occurrence of the 1999 WNV epidemic in New York City has raised multiple issues regarding the preparedness of public health officials, hospitals and physicians for dealing with outbreaks of new and unexpected diseases. Several avenues to improving the recognition of epidemics and the response time to implementing control measures

have been discussed. Among areas targeted for improvement, better communication between veterinary and public health officials has been foremost, as the initial failure to connect the human epidemic and the avian epizootic may have delayed the implementation of surveillance and control measures. *It should be noted, however, that flaviviruses such as SLE and WNV do not normally kill their primary reservoir avian hosts, and that simultaneous human and avian arboviral encephalitis outbreaks had not been previously described.*[1]

Proof of Overwintering: the West Nile Outbreak of 2000

The intensive prevention and control efforts implemented by the NYC Department of Health, the CDC and multiple federal agencies paid off. Despite the spread of WNV-positive birds from the initial four states in 1999 to twelve states in 2000, only 19 human cases were recognized in New York (Staten Island) and New Jersey, and only one fatality occurred. Serological testing after the 2000 outbreak revealed that a relatively small proportion of Staten Island residents (0.46%) had evidence of recent exposure to WNV. Another contributing factor may have been the relatively mild Staten Island avian epizootic in 2000: the presence of WNV-neutralizing antibody had been six times greater in Queens in 1999 than in Staten Island in 2000.[44]





HISTORY AND GEOGRAPHIC DISTRIBUTION: WEST NILE VIRUS IN THE OLD WORLD

West Nile virus was first isolated in 1937, in a febrile adult woman from Omogo, the West Nile district, Northern Province of Uganda. [1-3,6] The mosquito transmission cycle was characterized in Egypt in the 1950's. In 1957, the first recognized outbreak of severe human meningoencephalitis attributable to WNV occurred in elderly individuals in Israel. Shortly thereafter, in the early 1960's, equine disease related to WNV infection was reported in both Egypt and France. WNV continues to be one of the most widespread flaviviruses, extending throughout Africa, the Middle East and Europe, South Asia and Oceania (subtype Kunjin). The most extensive human epidemic occurred in Cape Province, South Africa, in 1974. At least 3,000 clinically apparent cases were described in that outbreak.[6, 7] More recently, epidemics of WNV have been reported in Algeria (1994), Romania (1996), the Czech Republic (1997), the Congo (1998), and Russia (Volgograd) and the U.S. in 1999.

The epidemic in southeastern Romania between July 15 and October 12, 1996 provides a good example of a large outbreak of human neurological disease due to WNV infection. Of an estimated 527 cases, 393 were laboratory-confirmed, and a staggering 60% had meningoencephalitis or encephalitis. The fatality rate was reported as 4.3 to 7.6%, and was greatest in elderly patients with encephalitis. Subsequent serosurveys revealed that as many as 43,000 to 96,000 individuals were infected, yielding a ratio of clinical to

subclinical infection of 1:140 to 1:320. Infected persons were more likely than seronegative persons to report noticing mosquitoes in their homes and to have experienced flooded basement apartments. In addition, they recalled a higher number of mosquito bites during the epidemic period. Of note, patients with meningoencephalitis were more likely to have spent more time outdoors than those with subclinical infections.[8]

In addition to recognized human cases, large epizootics have continued to occur throughout the world, sometimes with devastating economic impact. Equine outbreaks were recorded in Egypt and France in the 1960's (as above); and more recently in Morocco (1996), in Italy (1998) and in the U.S. (in 1999).[6]

MOLECULAR BIOLOGY AND TAXONOMY

West Nile virus is a positive-stranded ribonucleic acid (RNA) virus, consisting of a 40 nm-diameter particle containing 11.3kb of genomic RNA. The RNA is associated with a nucleocapsid core protein and enclosed by host-derived membrane expressing viral envelope proteins.[6] WNV is a member of the family *Flaviviridae*, genus *Flavivirus*. This group of viruses is comprised of more than 70 antigenically related viruses of global public health importance, such as the Yellow Fever and Dengue viruses. WNV belongs to the Japanese encephalitis virus (JEV) serocomplex, which contains JEV, SLE virus, Murray Valley encephalitis virus, Stratford virus, Kunjin virus; and Alfuy, Koutango, Cacipacore, Usutu and Yaounde viruses. Even within the JEV serocomplex, WNV and Kunjin virus (found in Australia and southern Asia) are very closely related antigenically, and Kunjin virus is considered to be a subtype of WNV. [6,9-10]

West Nile virus taxonomy is also based on the flaviviral envelope (E) protein, which is a primary target of the humoral immune response. Subtypes of WNV are determined by antigenic variation in the E protein, encoded by the E gene. Two lineages of WNV have been described based upon phylogenetic analysis of E glycoprotein nucleic acid sequence data. Critical areas of differentiation include specific signature amino acid motifs in the E glycoprotein, along with the presence or absence of a 12 nucleotide deletion site which codes for an N-glycosylation site (Asn-Tyr-Ser) at amino acid positions 154-156. [11,12,30]

Lineage 1 encompasses West Nile and Kunjin viruses from Europe, the Middle East, Africa, Australia and southern Asia, and are strongly associated with acute human disease. Lineage 2 consists of WNV isolates from overlapping areas in Africa as well as Madagascar. In general, lineage 2 isolates are not associated with human disease outbreaks, but are instead associated with endemic, enzootic cycles. [6,11,30]

INVESTIGATION INTO THE PHYLOGENY AND ORIGIN OF THE WEST NILE VIRUS NY1999 STRAIN

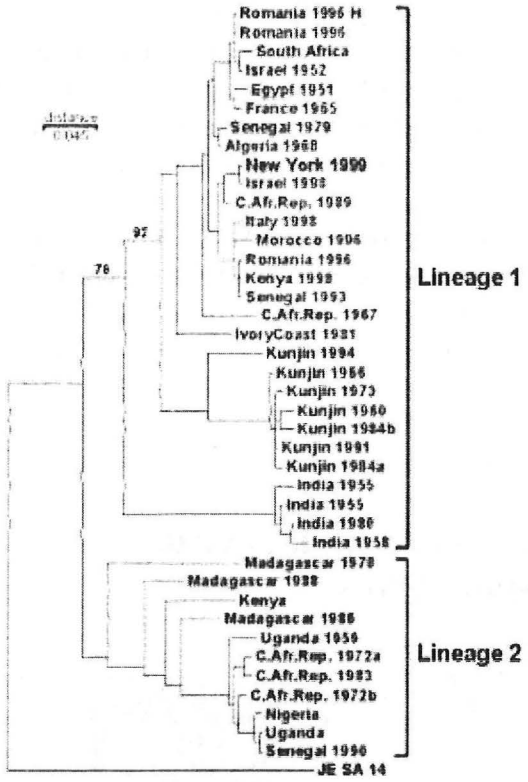
In September, 1999, Jordan, Briese, Lipkin and colleagues at UC Irvine were provided with brain specimens from 5 individuals who died in the New York outbreak. Their group was investigating a new technique for amplification of viral nucleic acids, known as domain-specific differential display, or DSDD. DSDD utilizes consensus PCR with differential display, employing highly degenerate family-specific primers. The PCR products are then analyzed by chromatography or by polyacrylamide gel electrophoresis. On September 22, 1999, flavivirus sequences were detected in 4 of 5 brain specimens. PCR products from two of these individuals were amplified and sequenced, revealing the presence of a Kunjin-like isolate of WNV.[11-13]

The investigators cloned the genome of WNV NY1999 strain from a brain specimen by RT-PCR. Subsequent analysis of the deduced E protein sequence indicated the presence of the N-glycosylation site, along with the characteristic amino acid motifs, signifying a lineage 1 virus (Ala172, Asn199, Thr205, Thr208 and Thr210). Additional phylogenetic analysis of the NY1999 E protein gene sequence also confirmed the lineage 1 origin of the virus. [11] Comparison with E gene sequence data from multiple strains of WNV revealed a near-perfect match with a 1998 isolate implicated in a domestic geese outbreak in Israel in that year (Israel-98). In fact, 227 E gene nucleotides were identical, and only two mismatches were detected in the analysis of a longer 1,278 nucleotide sequence. [11-13] As was subsequently learned, WNV had been isolated in Israel from the neural tissue of four dead white storks, a vulture, and the domestic geese, all in 1998. [6]

As the information regarding the high degree of similarity between the Israel-98 and the NY1999 strains became available, hypotheses were put forth as to the origin of the New World strain. Chief among them was the suggestion that an infected bird (or mosquito) had been legally or illegally imported into the U.S. from Israel. The notion that an infected human could have introduced the virus after a trans-Atlantic flight seemed very unlikely, given that the level of viremia normally achieved in humans precludes their ability to serve as amplifying hosts or to initiate an epidemic cycle. Another possibility included trans-Atlantic storm activity, also felt to be unlikely. The most ominous possibility was the theory of intentional introduction as an act of bioterrorism.

Of note, these results also prompted Israeli agriculture officials to undertake an enhanced surveillance program. A concurrent WNV epizootic was subsequently detected in domestic goslings in November 1999 in the Ramala and Yizre'el districts of Israel. A 40% avian mortality rate was reported, and approximately 8,000 geese were exterminated. [11,13]

Phylogenetic Tree Based on Envelope Glycoprotein Sequence Data



MEGA, distance tree, Kimura 2-parameter, neighbor-joining

Lanciotti et al. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern U.S. [Science 286:2333-337.]

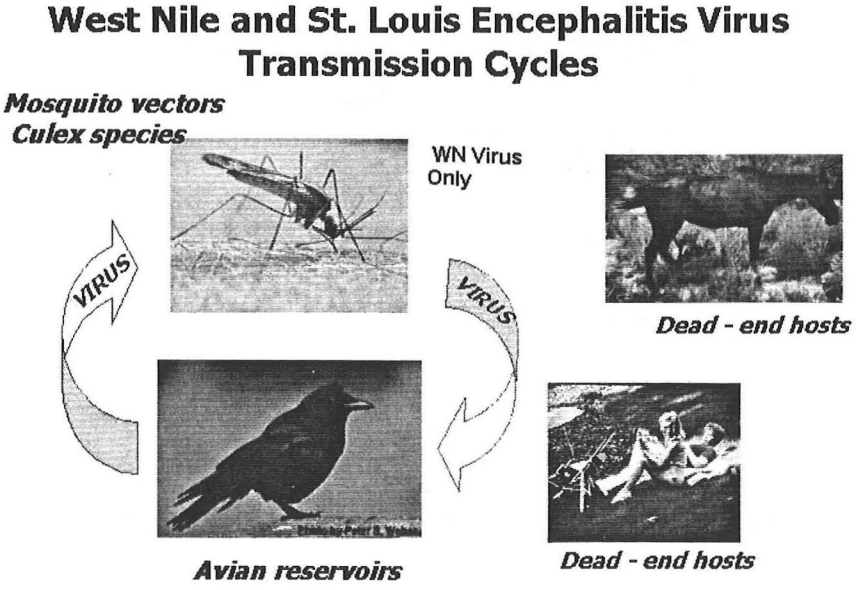
TRANSMISSION CYCLE OF WEST NILE VIRUS

WNV transmission is characterized by amplification during episodes of adult mosquito feeding upon avian hosts in a continuous cycle. Viral particles are transported from the salivary glands of infectious mosquitoes to susceptible hosts during blood-meal acquisition. Competent avian vectors will maintain an infectious level of viremia for 1 to 4 days, after which life-long immunity is generated. In order to perpetuate the transmission cycle, newly infected mosquitoes must survive the extrinsic incubation period of approximately two weeks. This length of time is required for viral development to the point at which the mosquito is fully infectious to the next susceptible host. Most mammals, including humans and equines, rarely manifest an infectious level of viremia, rendering them "dead-end" hosts. Under certain ecological and climatological

circumstances, the transmission cycle may become amplified, resulting in an epizootic or even a human epidemic. However, the specific set of circumstances do not normally persist for longer than a few months, after which the epizootic or epidemic extinguishes itself. [6]

West Nile virus transmission cycles vary considerably by geographic location, utilizing different primary vector species and vertebrate reservoir host species. In long-standing enzootic cycles, the reservoir host species usually involves passerine birds, and the mosquito vector is often of the *Culex* species. West Nile virus persistence between periods of ongoing transmission has been attributed to multiple mechanisms: overwintering in hibernating adult *Culex* species mosquitoes, transovarial transmission in certain species of mosquitoes, and via migration of viremic birds to temperate zones. [6]

Non-culicine arthropods have also been found to harbor West Nile virus, but are thought to be of little importance in viral transmission cycles. Both hard ticks (*Hyalomma marginatum*) and soft ticks (*Ornithodorus maritimus, Argus hermanni*) may harbor WNV, and swallow bugs (*Oeciacus hirundinis*) may be acting as vectors in Austria.[6]



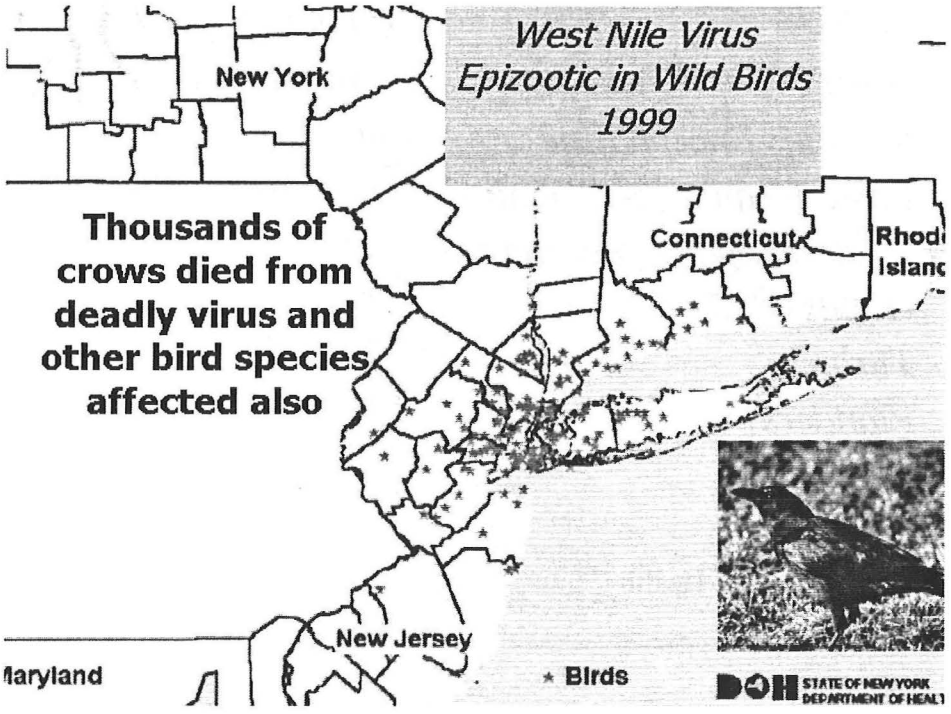
WEST NILE VIRUS ENTOMOLOGY

West Nile virus has been isolated from > 40 mosquito species, although the potential for all of these to serve as competent vectors has not been confirmed.

***Culex* species:** *Cx.univittatus, Cx. Perixiguus, Cx. Pipiens, Cx. Modestus, Cx. Quinquefasciatus, Cx. Tritaeniorhyncus, Cx, vishnui*
Other genera of mosquitoes: *Aedes, Aedeomyia, Anopheles, Coquillettidia, Mansonia, Mimomyia;*
Isolated from ticks:
Hard tick *genera:* *Argas, Ornithodora*
Soft tick *genera:* *Amblyomma, Dermacentor, Hyalomma, Rhipicephalus*
(Source: CDC)

At least 16 species of mosquitoes have been confirmed to be competent vectors of WNV in experimental transmission studies. Five of these have been investigated for their potential to transmit the NY1999 strain of WNV – *Culex pipiens, Aedes japonicus, Ae. Sollicitans, Ae. Taeniorhyncus and Ae. Vexans* – and all were competent vectors. [6] Recent work by Sardelis, Turell and colleagues at the U.S. Army Medical Research Institute of Infectious Diseases demonstrated that container-breeding species such as *Aedes albopictus* were highly efficient laboratory vectors of the NY1999 strain of WNV. Multiple *Culex* species were tested and were intermediate in their susceptibility to the NY1999 strain; however, once disseminated infection had developed, all species tested were found to be highly capable of transmitting WNV through a bite. [14,16] The same group also reported their findings that vertical transmission of WNV could be detected in female *Culex pipiens* mosquitoes, thus providing one mechanism by which the WNV could overwinter and re-initiate an epidemic cycle the following spring. [15]

WEST NILE VIRUS AND HOST SUSCEPTIBILITY: THE AVIAN EPIZOOTIC OF 1999



West Nile Virus and Host Susceptibility: Birds

The majority of vertebrate hosts are susceptible to WNV infection in experimental settings, including a wide array of birds as well as mammals, amphibians and reptiles. Among avian species, both wetland and terrestrial species can participate in transmission cycles. In addition, experimental WNV infection has been demonstrated in crows, falcons, chickens, doves, pigeons, ducks, herons, sparrows and others. Levels of viremia sufficient to sustain WNV in transmission cycles are generally found only in birds, and not in other vertebrate "dead-end" hosts. Therefore, birds are the primary amplifying hosts, and their status as a reservoir between transmission cycles is under investigation.[6]

The table below lists the arboviruses of importance in the U.S., along with the expected mortality among native and exotic birds. Note that WNV is unique in its proclivity to cause fatal infections of both native and exotic birds, although the extent of the avian die-off in the 1999 and 2000 epizootics is unusual as compared to previous WNV avian outbreaks in the Old World.

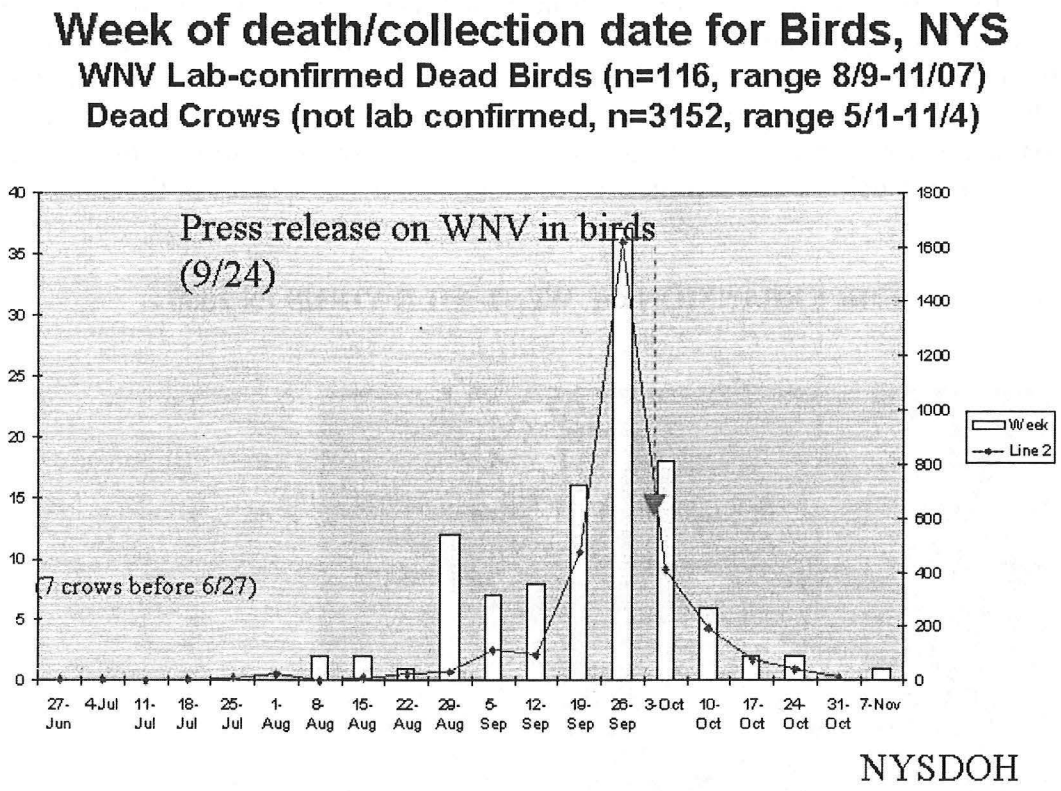
Mortality from Mosquito-Borne Viruses of Birds in U.S.

VIRUS	*BIRD SPECIES*
West Nile Virus	***Native & Exotic***
St. Louis Encephalitis	*None*
Eastern equine encephalitis	*Exotic & a few native*
Western equine encephalitis	*Exotic*
Highlands J	*None*
Turlock	*None*
Flanders	*None*

(Source: National Wildlife Health Center)

The 1999 Epizootic

As early as July, 1999, veterinary health officials began receiving reports of a massive avian die-off throughout the city, primarily affecting crows (*Corvus* spp.). By one estimate, two thirds of the crow population may have been extinguished.[17] Dozens of ill and dead crows (*Corvus* spp.) were observed around the Bronx Zoo precinct as well. By September, exotic species at the zoo were also dying unexpectedly – species such as South American flamingos, cormorants and a bald eagle that manifested head tremors prior to its death.[4] Dr. Tracy McNamara, the veterinary pathologist at the Bronx Zoo, determined that the epizootic was not likely to be due to either SLE or EEE (see Introduction), but she was frustrated by the inability to find an appropriate veterinary testing facility where she could submit avian specimens to determine the true etiology of the outbreak. Ultimately, she submitted samples to the CDC and to a U.S. Army Lab.[18] West Nile virus (WNV) infection was confirmed by reverse transcriptase-PCR (RT-PCR) and genome sequencing of neural tissue from dead crows and exotic zoo birds submitted to the CDC and the National Veterinary Services Laboratory in Ames, Iowa.[4,5]



(Source: New York State Dept. of Health)

Clinical and Pathological Findings in West Nile Virus -Infected Birds

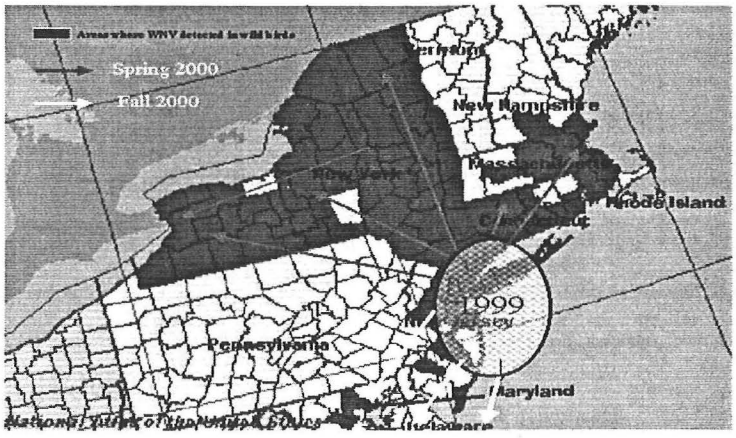
Among crows, manifestations of WNV include lethargy and general weakness, failure to respond to danger in the environment, and the inability to perch, walk, fly or maintain

wing position. Dr. McNamara and colleagues performed necropsy examinations in 27 WNV-infected birds (14 species). The presence of WNV in various tissues was confirmed by RT-PCR, viral isolation, immunohistochemistry, in situ hybridization and standard histology. Results show that WNV could be detected in 88% of brains, 96% of hearts, 83% of spleens, 70% of livers, 100% of kidneys and in adrenals, lungs, ovaries, intestines and the pancreas as well. [19] The virus appeared to target cells of the CNS and peripheral ganglia, in addition to the myocardium, renal tubular epithelium and monocytes, among others. With the exception of crows and magpies, WNV was especially virulent for Purkinje cells on pathological examination. Upon gross inspection of necropsy specimens, brain hemorrhage, meningoencephalitis, myocarditis with necrosis and splenomegaly were the most notable lesions.[19]

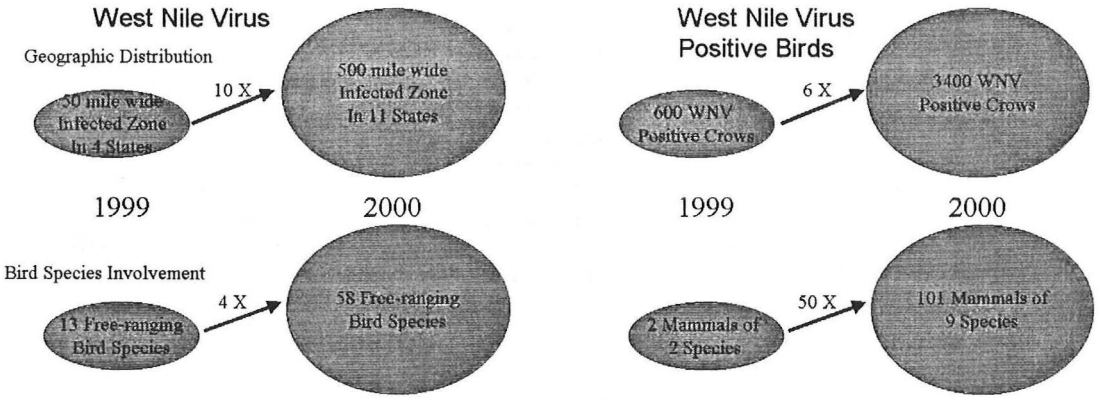
The Ideal Avian Reservoir Host for West Nile Virus

Although *Corvidae* or crows were the most dramatically affected bird species in the 1999 epizootic, their short survival after infection renders them less useful in the transmission cycle of WNV. On the other hand, a common species with a lower rate of morbidity and mortality would provide a more effective host reservoir. Investigations into the level of WNV viremia sustained by various species by N. Komar of the CDC may point to the sparrow as the prime reservoir host for WNV in the New World. For example crows (and blue jays) manifested viral loads in the trillions per milliliter of blood, and this finding appears to correlate with the high lethality of WNV in this species. On the other hand, the viral load detected in house sparrow, while still markedly elevated, was approximately 1000 times lower than that seen in blue jays and crows. Studies such as these may help elucidate the significance of individual avian species in the transmission cycle of WNV. [18]

THE EXPANSION OF WEST NILE VIRUS IN 2000



Proof of the overwintering capability of WNV was evident in the marked expansion of WNV between the 1999 and 2000 outbreaks. From the original 50 mile wide infected zone involving four states (CT, MD, NJ, NY), the virus was now detectable in a 500 mile wide infected zone involving twelve states (CT, DE, MD, MA, NH, NJ, NY, NC, PA, RI, VT, VA & D.C.) . With regard to the epizootic, the initial 13 free-ranging infected bird species had expanded to 58 free-ranging species over the 500 mile wide zone in 2000. The initial count of 600 WNV-infected crows in 1999 was now at 3400+ WNV-infected crows in 2000. [18,20]



(Source: National Wildlife Health Center)

Although an overwhelming majority of WNV+ birds collected in 2000 were still of the *Corvidae* or crow family, additional species were being represented with increasing frequency (see below).

WNV Positive Birds 2000

Bird Group	Number	% Total
Corvidae Total	3579	91.0
Crows	3408	86.5
Blue jay	170	4.3
Other species	287	9.0
Songbirds	62	1.6
Waterbirds	44	1.1
Raptors	42	1.1
Peridomestic sp.	29	0.7
Total Birds	3942	

Moreover, the number of distinct North American bird species shown to be susceptible to WNV infection grew dramatically in the 2000 season, as seen in the list below:

North American Bird Species Positive for West Nile Virus in 2000

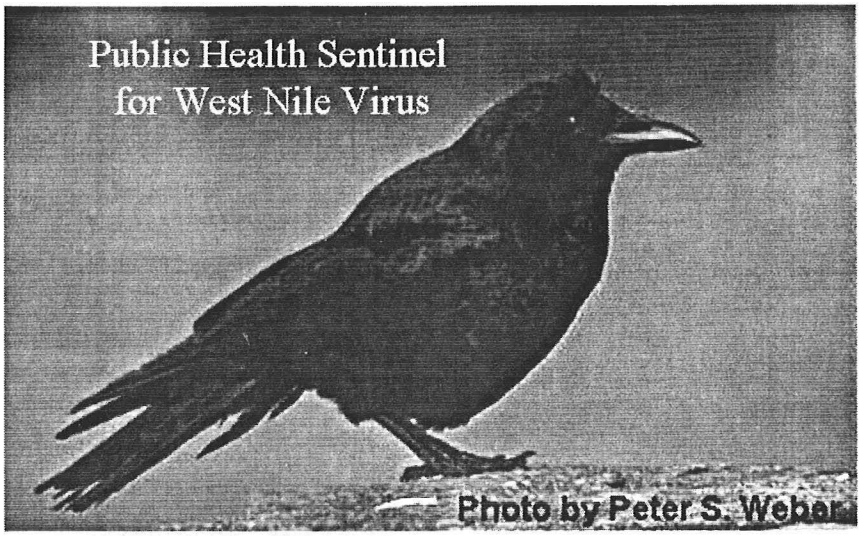
- *Bittern, Least*
- *Blackbird, Red-winged*
- *Bluebird, Eastern*
- *Cardinal, Northern*
- *Catbird, Gray*
- *Chickadee, Black-capped*
- *Cormorant, Double-crested*
- *Crow, American*
- *Crow, Fish*
- *Dove, Mourning*
- *Duck, Mallard*
- *Finch, House*
- *Goldfinch, American*
- *Goose, Canada*
- *Gull, Great Black-backed*
- *Gull, Herring*
- *Gull, Ringed-billed*
- *Grackle, Common*
- *Grouse, Ruffed*
- *Hawk, Broad-winged*
- *Hawk, Cooper's*
- *Hawk, Red-tailed*
- *Hawk, Sharp-shinned*
- *Heron, Great Blue*
- *Heron, Green*
- *Hummingbird, Ruby-throated*
- *Jay, Blue*
- *Kestrel, American*
- *Killdeer*
- *Kingfisher, Belted*
- *Merlin*
- *Mockingbird, Northern*
- *Nighthawk, Common*
- *Ovenbird*
- *Owl, Great Horned*
- *Raven, Common Robin, American*
- *Skimmer, Black*
- *Sparrow, Song*
- *Titmouse, Tufted*
- *Thrush, Wood*
- *Turkey, Wild*
- *Turnstone, Ruddy*
- *Veery*
- *Vulture, Black*
- *Warbler, Black-throated Blue*
- *Warbler, Canada*
- *Warbler, Yellow-rumped*
- *Waxwing, Cedar*

Source: National Wildlife Health Center

CROWS AS THE IDEAL SURVEILLANCE MARKER FOR WEST NILE VIRUS

As a direct consequence of the high mortality of WNV in crows, this species can be employed as a sentinel marker for the presence of WNV in a particular community. Features which favor the use of crows in national WNV surveillance initiatives include the following:

- Wide distribution throughout the geographic area of interest; in this case, the Eastern Seaboard of the U.S.
- Species exists in a variety of habitats, including urban, suburban, rural and wilderness areas
- Species remains fairly local, especially while nesting
- Dead crows are fairly conspicuous, and the public can be educated in their collection and submission for testing
- Sensitive indicator of local viral replication due to high susceptibility to WNV infection



Therefore, dead bird surveillance has emerged as a critical part of WNV control programs, and infrastructure for collection and testing is being developed in states deemed to be at high risk for the spread of WNV; including Alabama, Connecticut, Delaware, Florida, Georgia, Louisiana, Mississippi, Maryland, Massachusetts, North Carolina, New Jersey, New York, Pennsylvania, Rhode Island, South Carolina Texas, Virginia, and Washington, D.C.

WEST NILE VIRUS IN MAMMALIAN SPECIES

Similar to the situation with avian species, a wide variety of mammalian species are susceptible to WNV infection. However, unlike birds, mammals harbor relatively low levels of viremia; insufficient to sustain transmission cycles, rendering mammals "dead-end" hosts as noted above.[6] Successful experimental WNV infection has been reported for the following mammals: pigs, donkeys, mules, sheep, water buffalo, cattle, dogs, lemurs and other primates, various rodents, hedgehogs, rabbits, frogs and humans. [6]

Investigation of the 1999 WNV outbreak in New York City included reports of two non-human mammalian species. On Long Island, 25 horses developed symptomatic WNV infection, and 9 died.[3] In addition, one domestic cat became ill with WNV in New Jersey and was euthanized. [3] After the geographic expansion of the virus during the winter of 1999-2000, infection of 101 mammals of at least nine different species was reported by the National Wildlife Health Center. The nine species proven to harbor WNV in the 2000 season are below:

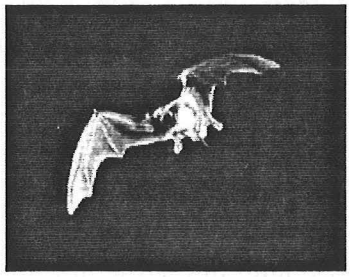
Mammal species positive for WNV

Wild Mammals

- Big brown bat
- Little brown bat
- Keen's bat
- Eastern chipmunk
- Raccoon
- Eastern gray squirrel

Domestic Mammals

- Horse
- Cat
- Rabbit



(Source: National Wildlife Health Center, USGS)

West Nile Virus Infection of Horses

Of the domestic mammals, equine species appear to be uniquely susceptible to arboviral encephalitides. Known as Near Eastern equine encephalitis in Egypt and *lourdige* in France, WNV infection has been associated with a high mortality rate in Egypt, France, Italy, Portugal and Morocco.[21] Horses manifest WNV infection as fever, hind limb weakness, paralysis of the lower lip, decreased vision, ataxia, disorientation, seizures, dysphagia, depression or excitability, anorexia, listlessness, paresis, coma or death.[4] Necropsy studies reveal CNS edema, petechial hemorrhages and meningoencephalitis, along with pulmonary edema. The clinical and pathological features of an equine outbreak involving 14 horses in Tuscany, Italy in 1998 were reported by Cantile et al. [22] The horses demonstrated ataxia, hindlimb paresis, and in six horses, tetraplegia developed within two to nine days. Eight animals recovered without sequelae, and necropsy was performed on six animals. Results showed a nonsuppurative polioencephalomyelitis picture with universal involvement of the ventral horns of the thoracic and lumbar spinal cord. Focal gliosis and hemorrhage of the brain stem and spinal cord were also seen in some cases.[22]

WEST NILE VIRUS IN HUMANS: CLINICAL MAN IFESTATIONS

In humans, the majority of WNV infections remain asymptomatic, especially in children. As noted above, the clinical to subclinical ration of infections in the Romanian outbreak was estimated to be between 1:140 and 1:320, with a corresponding case fatality rate (CFR) of 4-7%. [6,8] The CFR was 6% in the Russian outbreak of 1999, and 11 % of 62 cases in the NCY 1999 epidemic. Mortality across all of the recent epidemics remains highest for individuals > 50 years of age. [6]

The normal incubation period for WNV is 5-15 days, but it may be as short as two days. The most common symptoms include abrupt onset of fever (up to 103F), myalgia, headache and backache, which persist for three to six days. Less common manifestations include pharyngitis, conjunctival injection and gastrointestinal symptoms (nausea, vomiting, diarrhea and abdominal pain). Generalized tender lymphadenopathy may occur in as many as 75% of patients, and may provide a helpful clue. In addition, approximately 50% of patients develop a maculopapular rash, affecting primarily the thorax and upper extremities, which persists for approximately one week. [3,23] Evidence of widely disseminated infection is supported by the concomitant findings of clinically-apparent pancreatitis, hepatitis and myocarditis (as noted in birds and horses) in some cases.

Neurological manifestations, while relatively rare overall, may consist of aseptic meningitis, meningoencephalitis, myelitis (similar to poliomyelitis), optic neuritis and polyradiculopathy. Extensive neurologic disease tends to affect the elderly and sometimes children. [3]

Laboratory Features of West Nile Virus Infection

Leukopenia is common, and CSF pleocytosis with an elevated CSF total protein is found in patients with CNS disease. In immunocompetent patients, viral isolation from blood is likely to be successful only during the first 10 days after the onset of fever. Viremia usually peaks between days 4 and 8 at approximately 10^3 particles/mL blood (considered to be a very low level as compared to viremic birds). However, in immunocompromised patients, culture positivity may be detectable up to 28 days post-infection. [3]

DIAGNOSIS OF WEST NILE VIRUS INFECTION

Suspected recent WNV infection may confirmed by the following:

1) **A four-fold rise in serum antibody titer**, which may be demonstrated by ELISA, complement fixation (CF), neutralization, or hemagglutination inhibition (HI);
2) **Isolation of the virus from blood, CSF, or other fluid**(which can be performed by inoculating the virus into suckling mice or on mammalian or mosquito cell lines); or by detection of viral genomic sequences (PCR) or viral antigen (by immunohistochemistry-IHC) in the above fluids or tissue. It should be noted that

IHC may identify cross-reacting flaviviral antigens of the Japanese encephalitis serocomplex;

3) **IgM detection in serum or CSF by antibody capture ELISA.** Positive CSF IgM tests are diagnostic and reflect an intrathecal antibody response. On the other hand, positive serum IgM tests should be confirmed by repeat analysis for the presence of IgG by HI or plaque-reduction neutralization antibody (PRNT) assay to exclude other cross-reacting flaviviruses. [3] In addition, serum IgM tests require confirmation because the results may be misleading – IgM in serum can sometimes persist beyond a single transmission season in certain infections.

Regarding the various serological methods, the most broadly reactive test is the HI assay (> 1:320 in a single serum specimen is significant), since many arboviruses agglutinate goose erythrocytes. Therefore, heterologous reactions among the flaviviruses occur with increased frequency. In addition, HI antibodies are long-lived in many patients, making accurate diagnosis of recent infection difficult. CF tests have greater specificity (> 128 is significant), but the CF antibody response may not occur until later in the course of infection (>4 weeks) or not at all in many individuals. However, CF antibodies, if present, have a shorter half-life of only 2-3 years and therefore indicate relatively recent infection. Neutralizing antibody tests have the highest degree of specificity, but they may not be able to differentiate among closely related viruses in the case of superinfection. For plaque-reduction neutralization assay, a significant antibody titer in a single serum specimen is >160. Enzyme-linked immunosorbent assays (ELISA) are sensitive tests for the measurement of IgG and IgM antibodies, but significant cross-reactivity is observed among the flaviviruses. IgM capture ELISA, listed above, has greater specificity for IgM measurement.[23-24]

The longevity of anti-WNV IgM antibodies has recently been studied in the context of the Romanian outbreak.[24,25] It appears that human anti-WNV IgM can persist for 6 to 9 months, but it is unclear at this point whether the IgM can persist from one transmission season to the next. Therefore, testing of acute and convalescent serum samples should still be undertaken.[24,25] Guidelines for epidemiologic investigations into WNV encephalitis cases are summarized below.

Case Definitions of West Nile Virus Encephalitis

Confirmed Case: Defined as a febrile illness associated with neurological manifestations such as headache, aseptic meningitis or encephalitis, plus at least one of the following:

1) Isolation of WNV from, or demonstration of WN viral antigen or genomic sequences in, tissue, blood, CSF or other bodily fluid;
2) Demonstration of IgM Ab to WNV in CSF by IgM-capture enzyme immunoassay (EIA)
3) A > 4 fold serial change in plaque-reduction neutralizing (PRNT) antibody titer to WNV in paired, appropriately timed serum or CSF specimens;

4) Demonstration of both WNV-specific IgM (by EIA) and IgG (screened by EIA or HI and confirmed by PRNT) antibody in a single serum specimen.

Probable Case: Defined as a compatible illness (as in confirmed case) that does not meet any of the above laboratory criteria, plus at least one of the following:

1) Demonstration of serum IgM Ab against WNV (by EIA);
2) Demonstration of an elevated titer of WNV-specific IgG Ab in convalescent-phase serum (screened by EIA or HI and confirmed by PRNT).

Non-Case: Defined as an illness that does not meet any of the above laboratory criteria, plus:

1) A negative test for IgM Ab to WNV (by EIA) in serum or CSF collected 8-21 days after the onset of illness; and/or
2) A negative test for IgG antibody to WNV (by EIA, HI, or PRNT) in serum collected > 22 days after onset of illness.[26]

Molecular Diagnostic Techniques for West Nile Virus

Selected state public health and veterinary facilities and reference laboratories are working to update their capabilities for molecular diagnostic techniques to detect arboviral outbreaks in the U.S. Real-time PCR is rapidly emerging as a quick and sensitive alternative to RT-PCR methodology. [25] Lanciotti et al have reported excellent results in applying a rapid TaqMan reverse transcriptase-PCR assay to detect WNV in human (serum, CSF and tissue specimens), avian and mosquito specimens.[27] The investigators compared their TaqMan assay to a traditional RT-PCR assay and to viral isolation in Vero cells. The TaqMan assay showed overall greater sensitivity than RT-PCR, and correctly identified WNV in 98% of culture-positive avian tissue specimens and in 100% of culture-positive mosquito pools. The results with human specimens are less encouraging, however. Although the TaqMan assay detected WNV in all human brain specimens tested, it only detected WNV in 16 of 28 CSF specimens (none of which were positive by traditional RT-PCR). It has been suggested that the limited utility of the TaqMan assay as applied to human CSF specimens is related to the short-lived nature of West Nile viremia in humans. [27]

The overall improved sensitivity of the TaqMan assay over traditional RT-PCR is felt to be due to the amplification of much smaller DNA fragments (< 100bp), as well as to the use of a 5' exonuclease probe cleavage mechanism, which does not require the synthesis of full-length DNA products. Both of these attributes appear to augment the sensitivity of the assay, and the three hour processing time make this technique an attractive alternative for epidemiological investigations into mosquito pools in particular. Of note, no false-positive results were seen with any of the antigenically-related flaviviruses tested, and the assay appears to be most specific for the NY1999 strain of WNV.[27]

CHARACTERISTICS OF THE HUMAN WEST NILE VIRUS CASES FROM THE 1999 NYC OUTBREAK

59 of the 62 recognized cases in the 1999 NYC human WNV outbreak were hospitalized, and their clinical and epidemiological characteristics were recently described by Nash et al in the *New England Journal of Medicine.*[2]

Hospitalized patients tended to be older individuals (median age 71; range 5 to 90 years old). In addition, the attack rate for clinically-apparent WNV infection in patients greater than 50 years old was 20- fold greater than that of younger individuals. Only 32% of individuals interviewed recalled any mosquito bites within the month prior to the onset of disease. 63% of patients developed an encephalitic picture, while 29% manifested meningitis without encephalitis. The illness was of short duration prior to the hospital admission – mean duration of symptoms was 5.3 days. 90% of patients had fever, 56% noted weakness, 53% experienced nausea, 51% had episodes of emesis, 47% had cephalgia, 46% presented with altered mental status, and 19% noted a stiff neck. Of interest, only 19% presented with a maculopapular or morbilliform rash in this series, in contrast to approximately 50% of patients in reports of WNV outbreaks in the Old World.[2-3,23]

On examination, one third of patients had loss of muscle strength, and one third demonstrated hyporeflexia (especially if encephalitis had been diagnosed). 10% developed a diffuse, flaccid paralysis. Electrophysiologic testing was conducted in ten individuals, eight were found to have evidence of axonal polyneuropathy (decreased nerve conduction velocity of sensory nerves, motor nerves, or both; along with decreased compound muscle action potentials and fibrillation potentials by electromyography).[2]

The most common underlying chronic medical condition in this series was hypertension in 42%. Diabetes and coronary artery disease were each present in 20% of patients. Interestingly, only 14% had known histories of immunosuppressing conditions, including malignancy in 5; and HIV, alcoholism and prednisone use in one patient each.[2]

Laboratory Abnormalities in the Human West Nile Virus Infection Cases from 1999

The typical leukopenia noted in prior reports of WNV infection was not observed in the NYC cohort of patients. However, the mean CSF cell count was elevated at 38/mm^3, (range 0 to 525), with an elevated mean CSF total protein concentration of 104 mg/dL (range 38 to 899). Computerized tomography (CT) scans were performed in almost three quarters of patients and were unrevealing. A subset (27%) of individuals underwent magnetic resonance imaging of the brain. Of these 16 patients, 31% had scans demonstrating leptomeningeal enhancement, periventricular enhancement, or both.[2]

Autopsy Results in Fatal Cases of West Nile Virus Infections

Autopsies were conducted on four of the NYC patients, revealing only mild CNS inflammation. The brain stem was more likely to demonstrate pathological evidence of WNV infection than other areas of the brain. In one patient, examination of the medulla and the cranial nerve roots showed perivascular and perineuronal inflammation and a scattered microglial nodules. One case of hemorrhagic pancreatitis was seen in this series, but hepatitis and myocarditis were not reported. [2,28-29]

Prognostic Factors in the West Nile Virus Outbreak of 1999

The highest risk for developing encephalitis with muscle weakness was associated with an age of greater than or equal to 75 (RR 2.7; 95% CI 1.3 to 5.8). The risk of death was also associated with age > or = to 75 (RR 8.5; 95% CI 1.2 to 59.1). The only other significant risk factor for death identified in this study was the presence of diabetes (age-adjusted RR 5.1; 95% CI 1.5 to 17.3). [2]

Characteristics of the Human West Nile Virus Cases from the 2000 Outbreak

Although not as much detailed clinical information is available regarding the individuals diagnosed with WNV infection during the New York/New Jersey 2000 outbreak, there have been 19 recognized cases with one fatality. 62% of patients developed encephalitis and 38% presented with aseptic/viral meningitis.[2]

NEUROPATHOGENESIS AND IMMUNOLOGY OF WEST NILE VIRUS INFECTION

Viral Neuropathogenesis Determinants and the Importance of the Envelope E Glycoprotein

Flaviviruses enter susceptible host cells by receptor-mediated endocytosis, so viral envelope determinants play a critical role in pathogenic potential of WNV strains. As discussed above, West Nile virus taxonomy is based on the flaviviral envelope (E) protein, which is a primary target of the humoral immune response. ***Therefore, changes in neuroinvasiveness of WNV strains have been attributed to alterations in the E protein.*** Subtypes of WNV are determined by antigenic variation in the E protein, encoded by the E gene. Two lineages of WNV have been described based upon phylogenetic analysis of E glycoprotein nucleic acid sequence data. **Critical areas of differentiation include specific signature amino acid motifs in the E glycoprotein, along with the presence or absence of a 12 nucleotide deletion site which codes for an N-glycosylation site** (Asn-Tyr-Ser) at amino acid positions 154-156. [11,12,30]

Lineage 1 encompasses West Nile and Kunjin viruses from Europe, the Middle East, Africa, Australia and southern Asia, and **are strongly associated with neuroinvasive**

potential and acute human disease. Lineage 2 consists of WNV isolates from overlapping areas in Africa as well as Madagascar. In general, **lineage 2 isolates are not associated with human disease outbreaks**, but are instead associated with endemic, enzootic cycles. [6,11,30]

A murine model of WNV encephalitis has been utilized to determine correlates of neuroinvasiveness. Prior studies suggested that in mice, the loss of neuroinvasiveness was associated with N-linked glycosylation of the E protein. [31,32] Therefore, glycosylation state was assumed to correlate with attenuation of WNV strains. However, Chambers et al investigated E protein genomic sequences for their neuroinvasiveness potential, and their results challenge that assumption. Their group found that although *prolonged* passage of WNV strains in mosquito cell lines does lead to both attenuation and acquisition of an N-linked glycosylation site; *limited passage* also leads to glycosylation *but not to loss of neuroinvasiveness potential in mice.* Therefore, glycosylation of the E protein alone may not wholly responsible for attenuation status. They investigated a second E gene locus and found that a single mutation (203 T→C) led to substitution of proline for leucine at residue 68 (L68→P), and that this change correlated with decreased neuropathogenic potential.[31] In addition, the investigators hypothesized that glycosylation may be required to stabilize the effects of the amino acid change at residue 68 on the E protein, as carbohydrates have been shown to help maintain the antigenic properties of E protein epitopes. [31,33] Finally, the group also determined that there are apparent neuropathogenesis determinants outside of the E protein, and therefore additional mechanisms of neuropathogenesis remain to be elucidated. [31]

Host Determinants Important for the Immune Response to West Nile Virus

Elements of the host immune system have been shown to affect neuroinvasiveness potential, as well as the viral determinants discussed above. One of the most important defenses against neuroinvasion appears to be the macrophage response. Studies investigating the non-neuroinvasive strain of WNV, known as WN-25, in a murine model have revealed that macrophage depletion allowed the WN-25 strain to penetrate the CNS and cause fatal encephalitis in a murine model. [34] Macrophage depletion, achieved by administration of dichloromethylene diphosphonate, also led to a higher viral load and prolonged viremia in mice. A dramatic difference was noted in mortality rates for intact versus macrophage-depleted mice: control mice had 0 % mortality after infection with the attenuated WN-25 strain, while the macrophage-depleted mice had up to 75% mortality. [34]

In addition to the role played by macrophages, immunity to WNV also relies on the cytotoxic T lymphocyte response. WNV infection has been shown to increase MHC-I and MHC-II expression on multiple cell types, thus facilitating cytotoxic CD8+ T cell binding and presentation to CD4+ T-helper cells, respectively. [35] Additional investigations performed by Shen et al have looked at the ability of flaviviruses to induce ICAM-1 expression in fibroblasts as an important feature of flavivirus-host interactions. Their group found that WNV induces increased expression of ICAM-1 in fibroblasts by

two distinct mechanisms: an early, cytokine-independent mechanism; and a delayed, indirect mechanism which is mediated by the release of interferon type 1 (alpha & beta). Interestingly, the potential for WNV to induce ICAM-1 expression was dependent on the cell cycle – only cells in G_0 arrest were susceptible. [35] This finding may be related to the observation that WNV-infected cells in G_0 express much higher levels of MHC-I molecules than actively cycling cells, and that these cells are 10 fold more susceptible to lysis by WNV-specific cytotoxic T cells than are actively dividing cells.[35] WNV is unusual in that the virus induces high levels of MHC-I, MHC-II and ICAM-1 expression. It has been hypothesized that these events are critical to viral pathogenesis as mechanisms to increase cell-cell adhesion and therefore the potential for cell-cell viral transmission and disseminated infection. [35]

POTENTIAL THERAPY FOR WEST NILE VIRUS INFECTION

Up to the present, treatment of WNV encephalitis has been supportive, including fluids, nutrition, ventilatory support as needed, antibiotics for secondary infection and rehabilitation for neurologic manifestations. However, there are suggestions that antiviral therapy for WNV infection may not be as distant as originally thought.

Nucleoside analogues have been evaluated as potential antiviral agents for WNV. ***Pyrazofurin***, which alters the activity of orotate monophosphate decarboxylase in pyrimidine synthesis, was shown to have activity against WNV in an oligodendrocyte cell line. However, this compound proved to have unacceptable cytotoxicity, and is not being investigated further at this time.[11,40] Jordan, Briese and Lipkin have also investigated ***ribavirin***, a guanosine analogue known to have broad-spectrum activity against DNA and RNA viruses. Ribavirin has been utilized as therapy for respiratory syncytial virus infection, Lassa fever, Argentine hemorrhagic fever, Hantaan virus infection, LaCrosse encephalitis virus infection, and was investigated in HIV-1 infection. At present, ribavirin is currently FDA-approved for the treatment of hepatitis C infection (in conjunction with interferon).

Ribavirin is phosphorylated after entry into the host cell. It has activity against some viral polymerases, and it has been shown to alter with host cell inosine monophosphate dehydrogenase activity. This interference leads to decreased levels of intracellular GTP levels. In an *in vitro* model, ribavirin inhibited WNV replication in oligodendrocytes. [11,40] However, in a non-neural primate cell line (Vero cells), higher concentrations of ribavirin were required for inhibition (> 200 μM), and higher drug levels would be expected to lead to the development of ribavirin-induced hemolytic anemia.[11,40] Ribavirin was evaluated in the summer of 2000 in 35 WNV-infected Israeli patients. Although there was no control group, ribavirin was not felt to have impacted clinical outcome in that setting, and further studies are clearly needed. [18] Other compounds investigated for their anti-WNV activity include ***melatonin***, which protects against encephalitis in a murine model, and ***dehydroepiandrosterone (DHEA).***[41,42]

WEST NILE VIRUS VACCINE INITIATIVES

The cross-reactivity among flaviviruses has been exploited in the efforts to find a safe and effective vaccine for WNV. In animal models, prior immunization with JEV vaccine, SLE vaccine, or Yellow Fever vaccine (YF) can attenuate the severity of WNV-related disease. Tesh et al recently reported their results utilizing the inactivated JE-VAX preparation; a live attenuated JEV strain (SA14-2-8); an attenuated field strain of SLE virus and the 17D live attenuated YF vaccine in a hamster model. Vaccinated animals were protected against encephalitis and death in that study.[36] In another study, variants of the attenuated WN25 strain (specifically WNI-25A) was investigated as a potential veterinary vaccine and found to be genetically stable (i.e. did not revert to pathogenicity) and effective in protecting geese from WNV. Of note, this WNI-25A strain closely resembles the NY1999 strain of WNV.[37]

Two studies by Kurane et al characterize the CD4+ T lymphocyte responses in humans vaccinated with the JEV vaccine. Two out of five CD4+ T cell clones isolated responded to WNV antigen as well as to JEV antigen, indicating the theoretical potential for cross-protection in JEV-vaccinated individuals to WNV infection.[38,39]

The most promising vaccine candidate to date appears to be the Orovax product, which is based on a technique developed by Thomas Chambers of St. Louis University. Utilizing the live attenuated YF 17D vaccine as a background, the YF envelope gene is deleted and replaced with the gene for the WNV envelope protein. The NIH is supporting the Orovax effort, and human trials are anticipated within the next few years.[18]

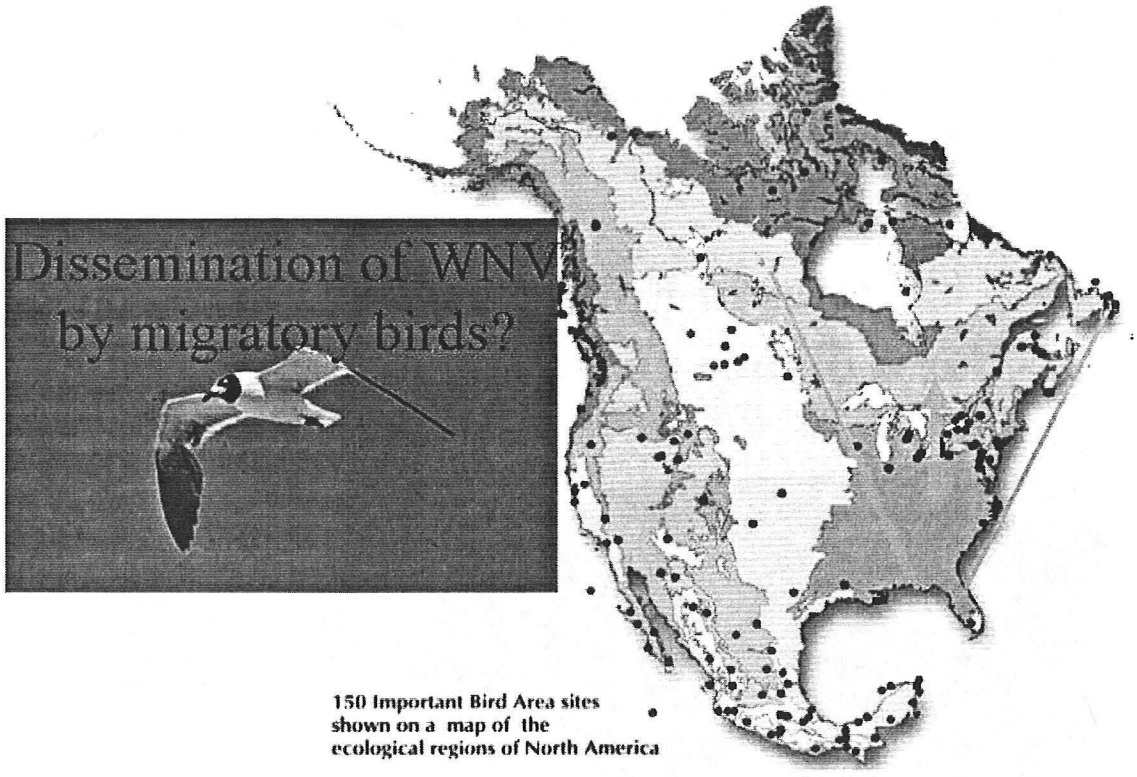
SURVEILLANCE AND CONTROL MEASURES TO MINIMIZE THE IMPACT OF WEST NILE VIRUS IN NORTH AMERICA

Limiting the spread of WNV throughout the Americas hinges on the implementation of effective surveillance and control measures. The New York State West Nile Virus Response Plan includes all of the following: intense active and passive surveillance for human cases; enhanced laboratory capability for the identification of WNV in mosquito pools, birds and other mammals; improved mechanisms for data collection and sharing among public health, veterinary and state and federal regulatory agencies; and aggressive education programs for both the public and the medical community.[43] Specific activities targeted for improvement are described below:

1) Human Case Surveillance – the NYC Dept. of Health (NYCDOH) is working with physicians, hospitals and laboratories to facilitate early reporting f suspected cases, with periodic updates to be sent to physicians to keep up a heightened sense f awareness of WNV;
2) Prevention, Response and Control – the NYCDOH implemented a broad Integrated Pest Management (IPM) program for mosquito control, including mosquito surveillance, education, source reduction (elimination of breeding sites), and larval and adult mosquito spraying. 300 mosquito surveillance sites have been

set up in the greater metropolitan area as well as the peripheral areas at risk, and an extensive network of scientists have been trained to provide comprehensive, sensitive and standardized arbovirus surveillance;

3) Bird and Mammal Surveillance – the NYCDOH has improved the sentinel early bird warning system to detect WNV appearance in the community, with increased public health lab capacity for specimen testing. Dead bird surveillance will continue as a sensitive indicator of the presence of WNV in the crow population. Veterinary cooperation has been enhanced to achieve early reporting of WNV in domestic pets and in horses;
4) Public Communication – an aggressive public awareness campaign has been mounted by the NYCDOH called "Fight the Bite"; designed to educate the public on personal protective measures (PPM), elimination of breeding sites in standing water, and monitoring for dead birds.[43]



THE TEXAS DEPARTMENT OF HEALTH WEST NILE VIRUS MONITORING EFFORTS

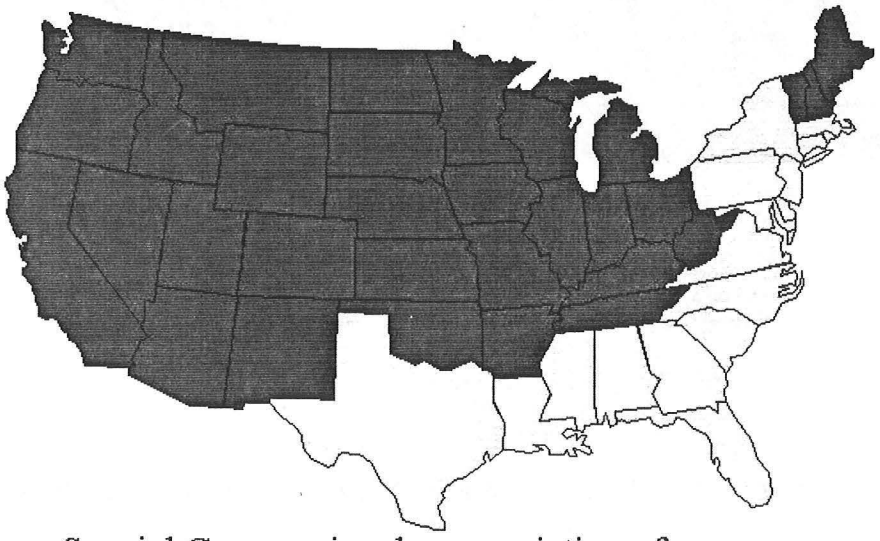
As seen by the rapid spread of WNV down the eastern seaboard to Florida, where a current human case of suspected WNV is under investigation, the eventual introduction of WNV to Texas via bird migration seems inevitable. Texas was identified early on in the national planning strategy sessions for WNV control efforts, as it lies within bird

migration patterns. Disease-carrying mosquitoes found in Texas include *Culex quinquefasciatus* (common house mosquito), *Aedes albopictus* (Asian tiger mosquito), and *Aedes egypti*. These mosquitoes are known as "backyard biters" and may reproduce in peridomestic water containers. Currently, state regulatory agencies are actively engaged in the national WNV prevention and control efforts. The Texas Department of Health (TDH) issued a list of recommendations for the public, which can be found on the TDH website: www.tdh.state.tx.us/news. The recommendations include the following:

- Empty cans, buckets, old tires and any other water containers in the vicinity
- Keep gutters clear of debris and standing water
- Change water in pet dishes, wading pools and bird baths several times weekly
- Fill in low areas in the yard and tree holes that catch water
- Pool and hot tub maintenance
- Stock ornamental ponds with fish that eat mosquito larvae
- Cover trash containers so that they do not collect water
- Water lawns and gardens carefully so as to avoid standing water
- Repair any leaking plumbing and outside faucets
- Screen rain barrels and openings to water tanks or cisterns
- Keep drains and ditches clear of weed and trash so that water will not collect

Source: TDH Website

States in Enhanced WNV Surveillance and Control Project



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Special Congressional appropriation of
$2.9 million, Fiscal Year 2000

CONCLUSIONS

Lessons learned from the West Nile Virus epidemics in 1999 and 2000 have been incorporated into recommendations for improving the public health infrastructure to respond to bioterrorism, as the introduction of WNV was seen as a possible terrorist threat at one point. Areas targeted for improvement include the following: enhanced awareness and training of clinicians; the augmentation of public health resources and expertise; improved communication between human and animal health authorities; strengthening and modernization of laboratory capabilities; and the development of comprehensive public education and media outreach programs.[1] If these recommendations are heeded, and the public health system infrastructure is reconstructed (after many years of decline), then the potential to respond appropriately to future infectious disease (and bioterrorist) threats will be maximized.

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REFERENCES

1. Fine A, Layton M (2001). "Lessons from the West Nile Viral Encephalitis Outbreak in New York City, 1999: Implications for Bioterrorism Preparedness." ***Clin Infectious Dis,*** *32 (2): 277-82.*

2. Nash D, et al (2001). "The Outbreak of West Nile Virus Infection in the New York City Area in 1999." ***N Engl J Med,*** 344 (24): 1807-4.

3. Asnis DS, Conetta R, et al (2000). "The West Nile Virus outbreak of 1999 in New York: the Flushing Hospital experience." ***Clin Infect Dis.***, 30(3):413-8.

4. Studdert MJ (2000). "West Nile Virus finds a new ecological niche in Queens, New York." ***Aust Vet J.***, 78(6): 400-1.

5. Holloway M (2000). "Outbreak not contained. West Nile virus triggers a reevaluation of public health surveillance." ***Sci Am.***, 282(4): 20, 22.

6. Komar N (2000). "West Nile Virus Encephalitis". ***Rev sci tech Off int Epiz.***, 19(1): 166-76.

7. Rappole JH, Derrickson SR, Hubalek Z (2000). "Migratory birds and spread of West Nile Virus in the Western Hemisphere." ***Emerg Infect Dis.***, 6(4): 319-28.

8. Marra CM (2000). "Encephalitis in the 21st century." ***Semin Neurol.***, 20(3): 323-7.

9. Hall RA (2000). "The emergence of West Nile Virus: the Australian connection." ***Viral Immunol.***, 13(4): 447-61.

10. Calisher CH (2000). "West Nile Virus in the New World: appearance, persistence, and adaptation to a new econiche – an opportunity taken." ***Viral Immunol.***, 13(4): 411-4.

11. Jordan I, Briese T, Lipkin WI (2000). "Discovery and molecular characterization of West Nile Virus NY 1999." ***Viral Immunol.***, 13(4): 435-46.

12. Rappole JH, Derrickson SR, Hubalek Z (2000). "Migratory birds and spread of West Nile Virus in the Western Hemisphere." ***Emerg Infect Dis.***, 6(4): 319-28.

13. Lanciotti RS, Roehrig JT, et al (1999). "Origin of the West Nile Virus responsible for an outbreak of encephalitis in the northeastern United States."***Science,*** 286(5448):2333-7.

14. Sardelis MR, et al (2000). "Potential for North American mosquitoes to transmit West Nile Virus". In: ***Program and Abstracts of the 49th Annual Meeting of The American Society of Tropical Medicine and Hygiene***, Houston, Texas Oct. 29-Nov. 2, 2000.

15. Dohm DJ, et al. " Experimental vertical transmission of West Nile Virus by *Culex pipiens* and Aedes albopictus."In: ***Program and Abstracts of the 49th Annual Meeting of The American Society of Tropical Medicine and Hygiene***, Houston, Texas October 29-November 2, 2000.

16. Turell MJ, O'Guinn M, Oliver J (2000). "Potential for New York mosquitoes to transmit West Nile Virus." ***Am J Trop Med Hyg.***, 62(3): 413-4.

17. Enserink M (1999). "New York's lethal virus came from Middle East, DNA suggests." ***Science,*** 286(5444): 1450-1.

18. Enserink M (2000). "Infectious disease. The enigma of West Nile." ***Science***, 290(5496):1482-4.

19. Steele KE, Linn MJ, et al (2000). "Pathology of fatal West Nile Virus infections in native and exotic birds during the 1999 outbreak in New York City , New York." ***Vet Pathol.***, 37(3): 208-24.

20. Anonymous (2000). "Update: West Nile Virus activity – eastern United States, 2000." ***Can Commun Dis Rep***, 26(24): 214-6.

21. Hubalek Z (2000). "European experience with the West Nile virus ecology and epidemiology: could it be relevant for the New World?" ***Viral Immunol.***, 13(4): 415-26.

22. Cantile C, Di Guardo G, et al (2000). "Clinical and neuropathological features of West Nile Virus equine encephalomyelitis in Italy." ***Equine Vet J.***, 32(1): 31-5.

23. Tsai TF . "Viral Infections: Arboviruses and Related Zoonotic Viruses:. In: ***Principles and Practive of Pediatrics, Second Edition***, ed. Frank A Oski, et al. J.B. Lippincott Company, Philadelphia, 1994.

24. Tardei G, Ruta S, et al (2000). "Evaluation of immunoglobulin M (IgM) and IgG enzyme immunoassays in serologic diagnosis of West Nile Virus infection." ***J Clin Microbiol.***, 38(6): 2232-9.

25. Gubler DJ, Campbell GL et al (2000). "West Nile virus in the United States: guidelines for detection, prevention, and control." ***Viral Immunol.***, 13(4): 469-75.

26. Anonymous (2000). "West Nile Virus in the Americas." ***Epidemiol Bull.***, 21(4): 9-11.

27. Lanciotti RS, Kerst AJ, et al (2000). "Rapid detection of West Nile Virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay." ***J. Clin. Microbiol.***, 38(11): 4066-71.

28. Sampson BA, Ambrosi C, et al (2000). "The pathology of human West Nile Virus infection." ***Hum Pathol.***, 31(5): 527-31.

29. Shieh WJ, Guarner J, et al. "The role of pathology in an investigation of an outbreak of West Nile encephalitis in New York, 1999." ***Emerg Infect Dis.***, 6(4): 370-2.

30. Berthet FX, Zeller HG, et al (1997). "Extensive nucleotide changes and deletions within the envelope glycoprotein gene of Euro-African West Nile viruses." ***J Gen Virol.***, 78 (Pt 9): 2293-7.

31. Chambers TJ, Halevy M, et al (1998). "West Nile Virus envelope proteins: nucleotide sequence analysis of strains differing in mouse neuroinvasiveness." ***J Gen Virol.***, 79(Pt10): 2375-80.

32. Halevy M, et al (1994). "Loss of active neuroinvasiveness in attenuated strains of West Nile virus: pathogenicity in immunocompetent and SCID mice." ***Arch Virol.***, 137:355-70.

33. Guirakhoo F, et al (1989). " Epitope model of tick-borne encephalitis virus envelope glycoprotein E: analysis of structural properties, role of carbohydrate side chain and conformational changes occurring at acidic pH. ***Virology*** 169: 90-99.

34. Ben-Nathan D, Huitinga I, et al (1996). "West Nile virus neuroinvasion and encephalitis induced by macrophage depletion in mice." ***Arch Virol.***, 141(3-4): 459-69.

35. Shen J, Devery JM, King NJ (1995). "Early induction of interferon-independent virus-specific ICAM-1 (CD54) expression by flavivirus in quiescent but not proliferating fibroblasts – implications for virus-host interactions." ***Virology***, 208(2): 437-49.

36. Tesh RB, et al. "Immunization with heterologous flaviviruses protects against fatal West Nile virus encephalitis." In: *Program and Abstracts of the 49th Annual Meeting of The American Society of Tropical Medicine and Hygiene*, Houston, Texas October 29-November 2, 2000.

37. Lustig S, Olshevsky U, et al (2000). "A live attenuated West Nile Virus strain as a potential veterinary vaccine." ***Viral Immunol.***, 13(4): 401-10.

38. Kurane I, Okamoto Y, et al (1995). "Flavivirus-cross-reactive, HLA-DR15-restricted epitope on NS3 recognized by human CD4+ CD8- cytotoxic T lymphocyte clones." ***J Gen Virol.***, 76 (Pt 9): 2243-9.

39. Aihara H, Takasaki T, et al (1998). "Establishment and characterization of Japanese encephalitis virus-specific, human CD4 (+) T-cell clones: flavivirus cross-reactivity, protein recognition, and cytotoxic activity." ***J Virol.***, 72(10): 8032-6.

40. Jordan I, Briese T et al (2000). "Ribavirin inhibits West Nile Virus replication and cytopathic effect in neural cells." ***J Infect Dis.***, 182(4): 1214-7.

41. Ben-Nathan D, Maestroni GJ, et al (1995). "Protective effects of melatonin in mice infected with encephalitis viruses." ***Arch Virol.***, 140(2): 223-30.

42. Fine A, et al (2000). "Testing for West Nile Virus". ***Lancet*** 356:1110

43. Novello AC (2000). "West Nile virus in New York State: the 1999 outbreak and response plan for 2000." ***Viral Immunol.***, 13(4): 463-7.

44. Anonymous. (2001). "Serosurveys for West Nile Virus Infection – New York and Connecticut Counties, 2000." ***MMWR*** 50 (3): 37-9.