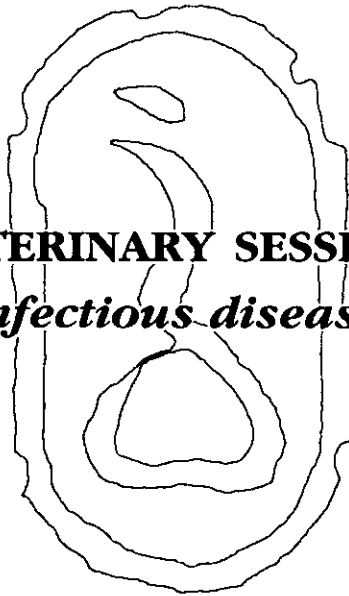




**VETERINARY SESSION:**  
*Infectious disease*



## WEST NILE VIRUS: THE EMERGENCE OF A NEW DISEASE AT BELMONT PARK AND THE WESTERN HEMISPHERE

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### ABSTRACT

In September 1999, West Nile Virus was diagnosed as the pathogen causing simultaneous disease outbreaks among people, birds and horses in the greater New York area. Although the virus has an almost worldwide distribution, it had not previously been isolated in North America. A flavivirus, West Nile Virus is an arbovirus transmitted by mosquitoes. Birds serve as reservoir hosts. Belmont Park became part of the equation when 2 racehorses developed signs of encephalitis and were confirmed positive to West Nile Virus. Veterinarians of the New York Racing Association, in a cooperative effort with several state and local authorities, instituted programmes to contain the outbreak, study the disease and serve as a source of medical and technical information. By 2002, West Nile Virus had been documented halfway across the USA, with predictions that by 2004 the entire nation will play host. In August 2001, the Federal Drug Administration (FDA) granted a conditional licence to the world's first West Nile Virus vaccine, a killed virus product developed by Fort Dodge Animal Health for use in horses.

### INTRODUCTION

In 1999, a phenomenon took place that had not been witnessed since Yellow Fever 2 centuries earlier, an arbovirus epidemic in New York.

### DISEASE OUTBREAKS

In the late summer of 1999, 3 disease outbreaks simultaneously plagued New York City and the surrounding area. People began noticing dead birds littering the sidewalks and parks. The numbers ultimately reached 17,000, most of which were crows. During the same time period, a score of North and South American birds died at the Bronx Zoo showing central nervous system signs. In all of 1998, New York City had recorded 9 reports of encephalitis. In the third week of August 1999, 5 cases sprung up in the same neighbourhood. By September, over 30 cases were logged. Seventy miles east of the city in the Riverhead area of Long Island, an equine practitioner recognised a cluster of neurologic illness within a 4-mile radius. Details of incidence are presented in Figure 1.

On 3rd September, Mayor Rudolf Giuliani announced that The Centers for Disease Control (CDC) had confirmed that blood and spinal fluid from the initial encephalitis patients tested positive for St. Louis Encephalitis, a flavivirus transmitted by mosquitoes. This was the most widely reported mosquito-borne disease in the United States but had never before been diagnosed in New York City. Within hours, trucks, aeroplanes and helicopters blanketed the city with malathion. The health department began public service announcements via television, radio and newspapers. They built a

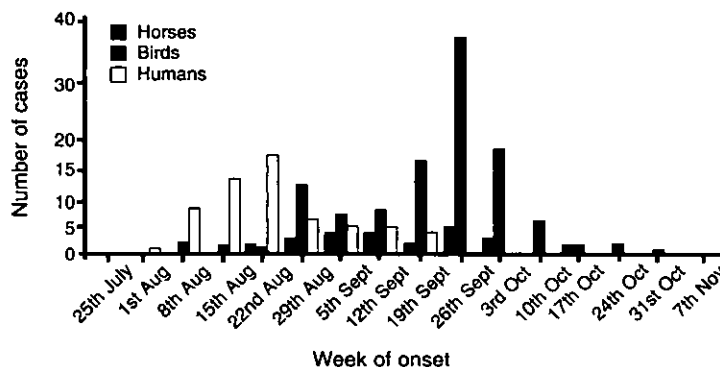


Fig 1: West Nile Virus, New York, 1999 by species and week of onset.

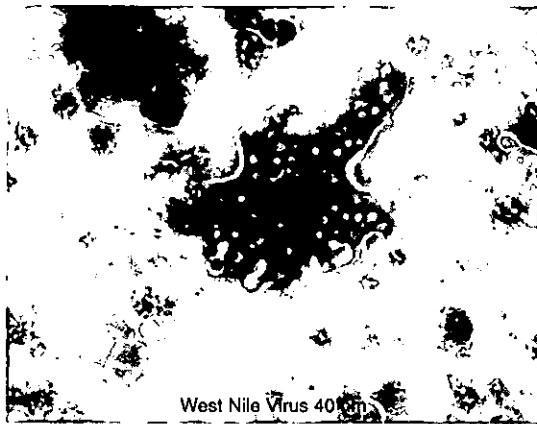


Fig 2: Electronmicrograph of West Nile Virus, 40 nm.

website, set up a 24-hour public hotline and distributed flyers in 8 languages.

## ARBOVIRUSES

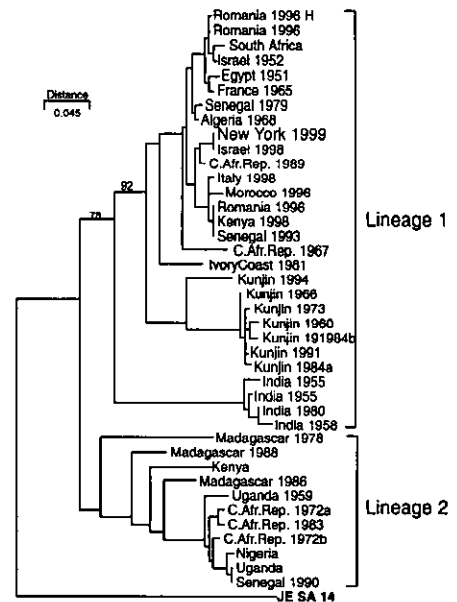
From the 17th to the early 20th centuries, infections carried by insects – malaria, yellow fever, plague and typhus – killed more people and sowed more disease than all other causes combined. What those diseases have in common, along with the infection that struck New Yorkers in 1999, is that the agents that cause them swim in the blood of arthropods. The largest phylum in the animal kingdom, arthropods include lobsters, crabs, cockroaches and scorpions. With regard to public health, the deadliest members of the clan are fleas, ticks and mosquitoes. Viruses transmitted by these blood-feeding arthropods are called arboviruses. Mosquitoes have survived for 30 million years. They locate us by exhaled carbon dioxide up to 100 yards away. Lactic acid and the chemicals in our sweat attract them as well. Their visual sensors can discriminate when clothing contrasts with a background.

Disease transmission begins when the female mosquito, who needs proteins for her eggs, bites a warm-blooded animal. If the bitten animal is a viraemic reservoir host with high levels of virus in their bloodstream, the virus will enter the female mosquito's gut where it replicates. The mosquito becomes infected and remains so for life. The virus then travels to other organs, including the salivary glands. Within a few days of her original blood meal, the mosquito 'inoculates' her next victim.

In a kind of viral chain letter, mosquitoes bite viraemic hosts, which in turn infect more mosquitoes, which bite more animals (Drexler 2002).

## IDENTIFICATION

Dr Tracey McNamara, of the Wildlife Conservation Society at the Bronx Zoo, heard the news reports



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

Fig 3: Phylogenetic tree based on envelope glycoprotein sequence data.

of St. Louis Encephalitis but knew that birds don't die as a result of infection from that virus. Besides the death of many exotic birds, she had begun noticing dead crows on the zoo's grounds. Because wildlife disease archives had never recorded a crow die-off in the USA, she feared a virulent strain assaulting a naïve population. Head of the Pathology Department, Dr McNamara was accustomed to being on the alert for diseases that might accidentally be introduced to the zoo animals by indigenous wildlife. She launched her own laboratory investigation, performing a full post mortem examination on every bird that died (Nolan 1999).

Avian Influenza and Newcastle's Disease were ruled out because the farm birds, including chickens and turkeys, were unaffected. When she discovered brain lesions, a viral encephalitis was suspected. Eastern Equine Encephalitis, a mosquito-borne illness that strikes people and is fatal to emus, was eliminated as a differential because the zoo's population of emus were healthy. Dr McNamara, who studied at The Plum Island Animal Disease Center and had a background in comparative pathology, made the intuitive leap that there existed a common pathogen between species.

Dr McNamara sent the specimens to the National Veterinary Services Laboratory (NVSL) who isolated an unknown flavivirus from brain tissue. This finding supported the suspicion that what was

**TABLE 1: Classification of arboviruses**

Group A arboviruses, now classified as Alphaviruses of the family *Togaviridae*

- Western Equine Encephalitis (WEE) Virus
- Eastern Equine Encephalitis (EEE) Virus
- Venezuelan Equine Encephalitis (VEE) Virus

Group B arboviruses, now classified as Flaviviruses of the family *Flaviviridae*

- West Nile Virus
- St Louis Encephalitis Virus
- Japanese B Encephalitis Virus
- Murray Valley Encephalitis Virus
- Kunjin Virus

killing New Yorkers was also killing the birds. Since the NVSL did not have the technology to pinpoint the virus further, they sent the Bronx Zoo isolates to the CDC for identification and characterisation.

Originally, when the human cases were recognised, the CDC ran IgM and IgG ELISAs. Both samples came back positive for St. Louis Encephalitis. The gold standard for identifying a human virus, however, is to grow it in other mammalian cells such as mice, monkey cells or in a chicken egg – a technique known as virus isolation. The CDC was never able to grow the virus from the human clinical samples it had received and unequivocally diagnose the pathogen because, once the symptoms had begun, there were too few circulating virus particles to detect.

When the CDC received the bird isolates, genetic tests ruled in flavivirus, but were negative for St. Louis Encephalitis. A patch of the virus's nucleic acid sequence was submitted against the sequences of all known flaviviruses. The DNA strands were sequenced, then the strings of genetic code were submitted via the Internet to the National Library of Medicine's GenBank's database (Lanciotti *et al.* 1999). The genetic sequence confirmed West Nile Virus (Fig 2). Flaviviruses contain nearly identical proteins on their surfaces. As a result, they cross-react to antibodies targeted for other family members, meaning that serum containing one flavivirus could easily come up positive in tests for other flaviviruses (Table 1). Thus, the initial presumptive diagnosis of St. Louis Encephalitis.

## AETIOLOGY

The branch of the flavivirus family to which West Nile belongs is known as the Japanese Encephalitis Virus Antigenic Complex, and includes the Japanese Encephalitis Virus in Asia, Murray Valley Encephalitis Virus, Kunjin Virus in Australia, and St. Louis Encephalitis in the Americas (Fig 3). First

discovered in the blood of a fever-ridden woman in the West Nile district of Uganda in 1937, West Nile Virus had been tracked in Africa, the Middle East and western Asia where, because the disease is endemic, most people become immune in childhood (Smithburn *et al.* 1940). The genomic sequences found in the strain that hit New York were virtually identical to a 1998 strain from a farm goose in Israel.

## PATHOGENESIS

There are many possible ways that the virus could have been introduced into the United States. If an infected traveller from overseas landed in New York during the virus's incubation period, when the virus in the blood peaks, that person theoretically could have infected a mosquito. A bird smuggled through customs could have imported the virus. A migratory bird or an exotic bird blown off course may have flown to the wetlands around Kennedy Airport, a prime stop for migrating birds. A mosquito could attach to a person or crate of produce. Well documented is the phenomenon of malaria-infected mosquitoes riding in the cargo holds and wheel wells of jets travelling from tropical to temperate countries. Ticks can withstand travel and have been shown to carry West Nile Virus in Europe.

There was speculation that the disease outbreak was a biological attack by terrorists. Federal officials discounted the theory because West Nile Virus does not wipe out enough people to be a credible weapon and it depends on the tenuous life cycle of mosquitoes to spread – not a reliable way to sow panic. Just how and when the deadly virus entered the USA will probably remain an enigma. What is known is that at least one infected mosquito bit a suitable reservoir host (Drexler 2002).

Climatic changes allowed for an over-abundance of mosquitoes. The mild winter of 1998/1999 enabled many mosquitoes to survive into the spring. Drought in spring and summer concentrated nourishing organic matter in their breeding areas and simultaneously killed off mosquito predators, such as ladybugs, that would have kept the mosquito numbers in check. Drought forced birds to share limited watering holes with mosquitoes. Torrential rains in late summer provided abundant water sources for mosquito breeding habitats for an already record population.

## EPIDEMIOLOGY

Only birds meet the criteria for a reservoir since they have a high titre and prolonged viraemia that

**TABLE 2: Frequency of clinical symptoms in humans (from Weiss *et al.* 2001)**

Fever	17 (90%)
Fatigue	12 (63%)
Altered mental state	11 (58%)
Headache	11 (58%)
Muscle weakness	8 (42%)
Nausea	8 (42%)
Myalgia	6 (32%)
Abdominal pain	4 (21%)
Seizures	3 (16%)
Cerebellar abnormality	2 (11%)
Cranial nerve palsy	2 (11%)
Arthralgia	2 (11%)

enables them to serve as a source of infection for an arthropod vector. Some birds have such high viraemia that they may be able to pass the virus amongst themselves. Mosquitoes feeding on infected birds become infected themselves and pass the virus on to other birds or warm-blooded animals. Horses and people become infected when infected mosquitoes bite them. They are considered dead end or incidental hosts because they cannot transmit the disease.

Combined results of studies at Plum Island, Colorado State and the CDC strongly suggest that horses are not involved in West Nile Virus transmission. Their study describes how mosquitoes that fed upon viraemic horses were negative for the virus, and horses infected with West Nile Virus developed low viraemia levels of short duration. These findings support the theory that horses are unlikely to serve as amplifying hosts for West Nile Virus in nature (Bunning *et al.* 2002).

### CLINICAL SIGNS

West Nile Virus causes encephalitis, or inflammation of the brain. Manifestations in people can be subclinical or vary in severity from temporary fever to serious encephalitis. Onset of the disease is sudden with fever, cephalalgia, lymphadenopathy, cutaneous lesions, muscular and articular pain and, often, gastrointestinal upset. Less frequently seen are myocarditis and meningitis (Table 2). The most striking symptom in the early cases was muscle weakness, which has never been ascribed to St. Louis Encephalitis. The clinical signs in horses mimic several CNS diseases and include depression, stumbling, incoordination, weakness of limbs, ataxia, convulsions, circling, hyperexcitability, partial paralysis and death. Fever may or not be present (Table 3).

Case fatality rate suggests against Equine Protozoal Myelitis (EPM). Rabies must always be considered first because of its zoonotic potential.

**TABLE 3: Frequency of clinical symptoms in horses (New York State Department of Agriculture and Markets)**

Neurologic signs: ataxia, stupor	86%
Depression	51%
Hindlimb weakness	49%
Difficulty/inability to rise	46%
Muscle tremors	41%
Fever	24%
Death	38%

Other possible diagnoses include equine herpesvirus (EHV-1), eastern equine encephalitis (EEE), western equine encephalitis (WEE) and botulism. The cases at Belmont exhibited non-specific neurologic involvement, including ataxia, proprioceptive defects, circling and head tilt. Muscle involvement was witnessed as in people.

### WEST NILE VIRUS AT BELMONT PARK

Because of the public health significance, human testing superceded animal testing. Laboratory capability was severely strained due to limited resources for antigen, so blood from neurologic horses suspected to be infected was collected, the serum frozen and saved. Eventually, 2 neurologic horses at the racetrack were confirmed positive for West Nile Virus, along with subclinical stablemates, one-third of the chickens bled, and positive mosquito pools. We were at Ground Zero.

Until the New York outbreak, West Nile Virus had never been diagnosed in the Western Hemisphere. Except for sporadic outbreaks in southern Europe, the disease existed primarily in Africa, the Middle East, and southwestern Asia. Although there were no restrictions on the transportation of horses within this country, the Hong Kong Jockey Club announced a temporary suspension on the importation of horses from North America. In addition, the United Arab Emirates required a supplementary health certificate to accompany any horse entering their country stating that the horse had not been in New York within 14 days prior to export.

### PREVENTION

A cooperative effort was established with the Nassau County Department of Health and The New York Racing Association which operates Belmont Park, Aqueduct and Saratoga Racetracks. Preventing West Nile Virus is best achieved by minimising exposure to mosquitoes and eliminating their breeding habitats. Inspectors evaluated the premises and made recommendations for immediate mosquito control

strategies. Old tyres collect stagnant water and provide ideal conditions for mosquito breeding. These were removed along with water trapping containers. Several of the first human victims of West Nile Virus collected rainwater in their backyard. Mosquitoes can breed in any puddle that lasts longer than 4 days, so low areas were graded to prevent standing water. Sanitation was improved and manure was removed daily.

Ponds were aerated with fountains and stocked with 4,000 *Gambusia affinis*. These are known as 'mosquito fish' and are top-feeding predacious minnows that eat several hundred mosquito larvae per day. Donut-shaped objects called 'dunks' were placed in the ponds, sumps and surrounding vegetation. Dunks are biological larvacides that release the bacteria *Bacillus thuringiensis israelensis*. While harmless to wildlife and fish, mosquito larvae are killed when they ingest it. Other biological larvacides, granules using *Bacillus sphaericus* as their active ingredient, were placed in any body of water in which mosquitoes could potentially breed. Sustained-release growth regulators in the form of briquettes were scattered throughout the vegetation. Growth regulators prevent pupa from becoming adults.

Carbon dioxide light traps were set up by the county within the perimeter of the racetrack. The traps were programmed to have either a steady or flickering light source. Carbon dioxide is delivered either as dry ice or emitted at 400–500 ml/min. The release of carbon dioxide simulates the exhaled respiratory gases of birds or mammals. The light source tempts mosquitoes as well. When close, mosquitoes are drawn in by a suction fan and collected for disease monitoring.

Screens with a 16–18 mesh were used as mechanical barriers in structures housing horses. Fans were recommended to reduce the mosquitoes' ability to fly. Pesticides were encouraged, the most effective of which are the pyrethroids and diethyl phthalate, diethyl carabate, N, N-Diethyl-3-Methylbenzamide (DEET).

Chickens can remain healthy, but carry West Nile Virus and amplify the disease by infecting the mosquitoes that bite them. The racetrack was home to thousands of chickens at risk of amplifying the disease. To decrease the chicken population and eliminate this risk factor, racecourse personnel were offered US\$5 for each chicken brought to the offices at Belmont and Aqueduct. After blood was taken for serological testing, the chickens were transported live to The Berkshire Bird Paradise, a non-profit sanctuary for endangered, disabled or unwanted birds.

The New York State Department of Agriculture and Markets offered horsemen free, confidential

serological screening of their racehorses. The goal was to establish basic titres for West Nile Virus in individual horses for future reference, helping to understand the epidemiology of the disease.

Although 43 species of mosquitoes have been identified as carrying West Nile Virus, the most common culprits in the USA are *Aedes* and *Culex* as evidenced by the positive mosquito pool surveys. There is speculation that different species of mosquito may cause disease in different species of mammals, specifically *Culex* with people, and *Aedes* with horses, because positive *Aedes* were collected on the premises of the initial equine index cases.

Habitat and climate determine which species of mosquito will be present in any area. *Aedes* are day feeders and prefer transient water sources such as tidal floodwater, flooded roadside ditches, tree holes and woodland pools. Their eggs can withstand desiccation and their life cycles require alternating periods of wet and dry. The nocturnal *Culex* prefers permanent or semi-permanent water. Brackish water or salt marshes are their natural habitats. They are found in stagnant water with a high organic content.

There was a great deal of uncertainty surrounding this new disease, and with uncertainty comes fear. The media was a useful tool in educating the public. The veterinarians at Belmont Park, in the epicenter of the outbreak, were interviewed frequently and passed on information about the disease as it became available. The goal was to keep the public informed and concerned, but also have the disease threat remain in perspective – 2,000 people die in the New York City area due to influenza each year; only 7 people died of West Nile Virus in 1999.

## **SURVEILLANCE**

To define the geographic extent of the outbreak, determine vector type, density and breeding sites, to target control management efforts and to evaluate effectiveness of those measures, the New York City and New York State Departments of Health established comprehensive West Nile Virus surveillance programmes. These included wild bird testing, mosquito pool testing, sentinel bird testing, medical hospital cases, veterinary cases and other mammal testing. Investigators drew blood from residents living near the original cases and found that 2.6%, or 8,200 individuals, had West Nile Virus antibodies. Overwintering *Culex* mosquitoes were collected to determine whether West Nile Virus might persist throughout the winter and initiate a zoonotic transmission cycle in the spring. Overwintering of West Nile Virus in adult

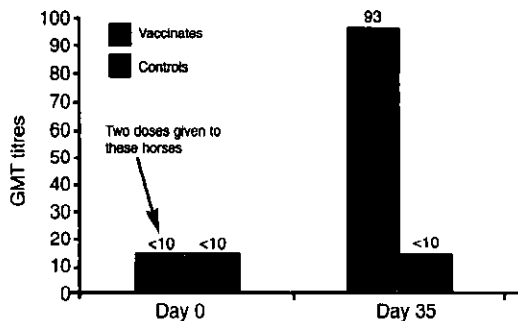


Fig 4: Plaque reduction titres post vaccination.

mosquitoes has been confirmed (Cooper *et al.* 2000). Transmission through mosquito eggs is less certain.

## EQUINE TESTING

The NVSL developed several laboratory tests to detect the presence of viral antigen or virus. IgM capture enzyme-linked immunosorbent assay (ELISA) detects recent infection. Plaque reduction neutralisation (PRNT) detects any past infection. Nested polymerase chain reaction (PCR) is sensitive and quantitative. Virus isolation is the gold standard. Differential diagnosis is by clinical signs. The best specimens are serum from a live horse and brain tissue from a dead horse. Testing for West Nile Virus is available through the NVSL or the Cornell Diagnostic Laboratory, which was remodelled to achieve the Biosafety Level 3 requirement. Samples collected from post mortem examination are processed only after the animal has tested negative for rabies.

## FIRST WEST NILE VIRUS VACCINE

The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) granted a one-year conditional licence in August 2001 to Fort Dodge Animal Health for an equine vaccine intended to aid in the prevention of disease in horses caused by West Nile Virus. The protocol is 1 cc im dose, followed by a second 1 cc dose in 3 weeks and a yearly booster thereafter. It is a killed, whole cell virus product. Vaccinated horses do not develop detectable levels of IgM antibody, so horses found to have IgM are probably infected naturally, and can be distinguished that way.

The conditional licence was granted because there existed a credible threat of disease, and testing showed that there was a reasonable expectation of efficacy and safety. In keeping with federal regulations, the vaccine is restricted to use by a veterinarian in those states where use of the

vaccine has been approved. Laboratory testing demonstrated similar neutralising antibody levels compared to other encephalitis vaccines. The challenge studies are in the final stages of data accumulation, at which time the product's performance will be evaluated.

Up to 2002, more than 1.4 million doses were given with a low reaction rate, usually muscle soreness. However, there are reports of approximately 132 horses that developed West Nile Virus after receiving the vaccine, most after the first dose. Because only 30% of horses seroconvert after the first dose of vaccine, protection from natural infection is not expected until 2 weeks after the second dose. The most likely scenario is that the sick horses had not achieved high enough levels of neutralising antibody to be adequately protected, and acquired the virus naturally (Fig 4).

## EPILOGUE

In 1999, 62 people were hospitalised with West Nile Virus, 7 of whom died. Most patients had become ill during the third and fourth weeks of August. Although the greatest concentration of cases was in lower New York, investigators also found the virus in birds and mosquitoes in eastern Long Island, the lower Hudson Valley, New Jersey and Connecticut, as well as in 25 horses (of which 9 died) in Suffolk County, New York, and a cat in New Jersey. By early 2002, the virus surfaced as far west as Texas and scientists believe that West Nile Virus will soon reach the shores of the Pacific. Most mosquitoes are unable to fly more than 40 miles, so it is believed that birds have, and will be, transporting the disease via the 3 bird migratory flyways in the USA; The East Coast, The Canada Central and The Canada Pacific.

The distinction between national threats and global threats is artificial. West Nile Virus's leap across the continents is a reminder of just how mobile today's pathogens are. Scientists must be kept apprised of pathogens abroad because related organisms may already be living among us awaiting their opportunity. In the case of West Nile Virus, the astute persistence of a veterinary prodigy saw her bird isolates uncover the existence of a new disease in the Western Hemisphere. The underlying message in emerging infections is that they are impossible to predict. In a time of unprecedented travel and transport, West Nile Virus was probably just another tourist (Drexler 2002).

## ACKNOWLEDGEMENTS

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A special debt of gratitude to Dr Tracey McNamara, the veterinary pathologist at Wildlife Conservation Society whose bird specimens from the Bronx Zoo unlocked the mystery of West Nile Virus, and whose perseverance saved not only animals, but human lives as well.

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