West Nile virus and North America: an unfolding story

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Summary

Before the introduction of the West Nile virus (WNV) into the United States of America (USA) in 1999, conditions in North America were ideal for an arboviral epidemic. Such factors as the large, susceptible and non-immune animal and human populations, the presence of competent vectors, increasing international travel and commerce, existing methods for rapid dissemination and an ill-prepared animal and public health infrastructure all combined to create the essential elements for a severe animal and public health crisis – the ‘perfect microbial storm’. The introduction of WNV into New York City was the final factor, serving as the catalyst to initiate one of the most significant epidemics in the USA. The spread of WNV across the country resulted in very large populations of wildlife, equines and people being exposed and infected. The epidemic is still not fully understood and its character continues to change and adapt. The recent recognition of a number of non-vector modes of transmission has revealed the disease as a greater threat and more difficult to control than first thought. West Nile virus gives every indication that it will become a permanent part of the ‘medical landscape’ of the USA, continuing to threaten wildlife, domestic animals and humans as a now endemic disease. This paper discusses the features of this extraordinary epidemic, and emphasises the need for an integrated surveillance system, greater diagnostic capacity and improved control strategies.

Keywords


Introduction

The introduction of West Nile virus (WNV) into the New York metropolitan area in 1999 has provided a unique opportunity to follow the establishment of an introduced flavivirus into a new geographic location. The ‘perfect storm’ metaphor which is the foundation of this issue of the Review is a fitting description for the history of WNV. North America has large, pristine, non-immune populations of human and animal hosts, multiple efficient vectors and a favourable environment for disease transmission and spread. The introduction of West Nile virus became the catalyst and final element to initiate this microbial ‘storm’ for which scientists know the beginning, but not the end of the story. The aggressiveness with which WNV became integrated into the diverse ecosystems within the Americas and the severity of the epizootics in terms of human and animal morbidity and mortality continually surprised, dismayed and frustrated public and animal health communities as they worked to educate the public, detect virus emergence, diagnose clinical cases and try to slow epizootic transmission. Each year has led to new information about the host range, modes of transmission and clinical manifestations of infection. Old paradigms were continually discarded as new information became available through the co-ordinated efforts of public health agencies, the medical community, wildlife officials, veterinarians, animal health agencies, basic researchers and industry in a broad coalition of disease surveillance,
reporting and research initiatives established in the wake of the virus. While the effort to establish a surveillance infrastructure was huge, nothing prepared the human and animal health community for the rapidity of the geographic migration of the virus or the escalating severity of the epizootic as it occurred at the leading edge of this migration.

This article will review aspects of the North American encounter with and response to WNV emergence. It is not intended to be a comprehensive review of WNV biology and pathogenesis. However, it will attempt to provide an epidemiological perspective that focuses on the key factors driving this emerging zoonotic disease, which continues to change and develop. Several comprehensive and informative reviews dealing with the epidemiology and ecology of WNV, and the human illness caused by it, have recently been published (5, 8, 13, 14, 25, 27, 28, 38) and the reader is directed to these for additional information. This paper helps to describe WNV on the North American continent and serves to complement other papers in this issue of the *Review*, which observe WNV in other geographic settings.

### The first year

In an era of heightened awareness of bioterrorism, WNV exposed significant weaknesses in the surveillance mechanisms for zoonotic infectious disease emergence within the United States of America (USA). As the epizootic unfolded in the summer and autumn of 1999, two parallel investigations were being conducted, which would eventually converge (64). One was the investigation of a cluster of meningoencephalitis cases in humans with associated neuromuscular weakness, originally diagnosed as St Louis encephalitis on the basis of serological assays. The other was an investigation of avian mortality by wildlife officials and a veterinary pathologist at the Bronx Zoo (New York). The combined efforts of multiple participants in the medical, wildlife and veterinary fields, as well as in government and private laboratories, led to the isolation and identification of WNV as the cause of both human and avian illness and mortality (9, 45). A later investigation of a cluster of neurological illnesses among equines on Long Island revealed additional WNV activity, which occurred at the same time as the morbidity and mortality in humans (66, 82).

The mechanism by which WNV was introduced into the New York metropolitan area is not known. The fact that the virus was nearly identical to the viruses widely circulating in Israel from 1997 to 2000 provides circumstantial evidence that the virus identified in New York City originated in that area (10, 34, 45). The extensive travel and commercial activity associated with New York City provides a plausible means for its delivery.

### Establishing surveillance

Programmes for arbovirus surveillance in many areas of the country had disappeared or were on the brink of dissolution. The re-establishment of surveillance capabilities required the rebuilding of mosquito surveillance programmes and laboratory infrastructures within the public and animal health arenas. One of the most challenging aspects of the epizootic was providing the testing services required to support massive surveillance programmes and real-time diagnostic testing for clinical cases, as well as efficiently integrating all animal and human surveillance data. The surveillance programmes established after 1999 have led to the accumulation of data that document the movement of WNV across the continent. Integrated surveillance activities involving the testing of mosquitoes, dead birds, equines and humans were instituted regionally from the East to the West between 1999 and 2004. A year-to-year summary of WNV movement in the USA and Canada is depicted in Figures 1 and 2. Within the USA, data from surveillance activities are cooperatively collected in a system called ArboNET (57).

In collaboration with the Centers for Disease Control and Prevention (CDC), the United States Geologic Survey maintains interactive maps of WNV activity, with information available for most areas at the county level (http://westnilemaps.usgs.gov/index.html). In Canada, Health Canada maintains a website with updated information about WNV activity in humans and submitted wild birds (http://www.hc-sc.gc.ca/english/westnile/).

Survey activities include the following:
- dead bird sightings reported by the general public
- testing subsets of dead birds
- mosquito trapping and testing
- sentinel bird testing
- equine testing
- human testing.

Dead bird sightings were generally the most sensitive indicator of the presence of the virus in a geographic area, but equine cases were the first indication of virus transmission in several regions in the Mid-western and Central West USA in 2002 and 2003. In general, surveillance of dead birds gave the earliest warning of WNV, followed by seroconversion of sentinel hosts, mosquito surveillance and the identification of veterinary cases, with human cases being the least sensitive indicator of the presence of the virus.
Fig. 1
The geographical spread of West Nile virus in the United States of America

Source: website of the United States Centers for Disease Control and Prevention
Host range and clinical signs

West Nile virus has an extremely broad host range, replicating in birds, reptiles, amphibians, mammals, mosquitoes and ticks. Mortality in avians has been documented in 198 species (38), with mortality approaching 100% in American crows (Corvus brachyrhynchos) in some locations. Among mammals, clinical illness following WNV infection has been detected most frequently in humans and horses. Morbidity in humans is evenly distributed, irrespective of age group or sex, but increasing age is associated with a higher likelihood of developing encephalitis or meningitis (12, 17, 64, 72). A comprehensive review of clinical illness in humans has recently been published (28) and will not be dealt with further here.
Clinical signs in equines have included the following:
- acute onset ataxia
- muscle fasciculation
- weakness
- flaccid paralysis of one or more limbs
- recumbency
- fever
- blindness (66, 67, 82).

Animals presenting with more severe neurological abnormalities generally have the gravest prognosis. A mortality rate of approximately 30% has been consistent from year to year in horses with clinical signs following infection. The availability of WNV vaccines for horses (60, 65) has probably substantially contributed to the decreases in equine morbidity and mortality during 2003 and 2004.

While bird mortality is a useful surveillance tool, most public health laboratories accepted only corvid species for testing. This, combined with the strain on testing capacity in wildlife and veterinary laboratories, has limited the information available on wildlife clinical illness and mortality due to WNV. In 1999, an investigation conducted on diverse avian and mammalian species in a captive population at the epicentre of the initial epizootic revealed broad susceptibility to infection, with 34% of birds and 8% of the mammals tested found to have antibodies against WNV (53). Of the mammalian species tested, one species – two Indian rhinoceroses (Rhinoceros unicornis) – had clinical illness, but only one rhinoceros demonstrated seroconversion. Signs of the disease consisted of the following:
- partial anorexia
- depression
- lip droop
- lethargy.

Among the avian species tested, 22% of birds also had clinical signs. These signs were often non-specific and included the following:
- anorexia
- weakness
- depression
- weight loss
- recumbency
- being found dead with no preceding abnormal signs noted.

Some birds exhibited neurological abnormalities, such as:
- ataxia
- tremors
- disorientation
- circling
- impaired vision
- abnormal head and neck posture.

Similar signs were reported in a group of eleven birds identified as WNV cases in 2002 (18). The most common clinical signs reported were anorexia, depression, the sudden onset of recumbency, mild ataxia and tetraparesis and, less frequently noted, signs of tremors, nystagmus, seizures and sudden death. More than 280 species of birds were reported to the CDC as WNV casualties from 1999 to November 2004, and a comprehensive review of the avian species experiencing morbidity or mortality due to WNV infection has recently been published (38).

In addition to diverse avian species, WNV has caused morbidity and mortality in alligators and crocodiles (59, 80), squirrels (30, 37, 41, 42), reindeer (Rangifer tarandus) (68), canids (50, 51), sheep (Ovis sp.) (88), alpacas (Lama pacos) (43, 88), a rabbit (Oryctolagus cuniculus), an eastern chipmunk (Tamias striatus) (42) and a cat (39), among others.

The effects of WNV on wildlife populations remain largely unquantified. Birds of the Corvid family have demonstrated high morbidity and mortality (42, 54, 87, 89). In one instance, where a population of crows was tracked, a confirmed death rate of 68% due to WNV infection was identified (89), and other studies suggest significant short-term impacts on crow populations (20, 56). Collective data from surveys of breeding birds and an annual winter bird census of a variety of passerine species indicate local declines in areas documented as having a high level of WNV epizootic activity, but no wide-ranging declines can be attributed to the virus (58). A serosurvey of black bears (Ursus americanus) in New Jersey has confirmed exposure to and survival of WNV infection in this species (23). There is little question that WNV is capable of infecting a large variety of bird and mammal populations. The lack of systematically collected data on wildlife morbidity and mortality and the scarcity of diagnostic resources to determine causality ensure that drawing any empirical conclusion about the effects of WNV on wildlife populations is a challenging proposition. The long-term effect of WNV on wildlife species and ecosystems has yet to be determined.

Modes of transmission

The most common exposure to WNV is through the bite of an infected mosquito. The virus has been identified in 43 species of mosquito throughout the USA (28), although not all of these are competent vectors (26, 75, 76, 83, 84, 85). The predominant species involved in WNV transmission vary according to geographical
location. In the northeast, *Culex pipiens*, *C. restuans* and *C. salinarius* were the species most frequently found to test positive for virus (42, 63). Of these, *C. restuans* and *C. salinarius* have demonstrated vector competence (75). The recent discovery of hybrid *Culex* species feeding on both birds and mammals may indicate that these play a significant role as bridge vectors between animal and insect hosts in the northeast USA (Fig. 3) (24).

Several new modes of transmission in humans were identified and described, including the following:
- infection through contaminated blood products (29, 71)
- organ transplantation (33)
- maternal transmission through breast milk (16) and intrauterine transmission (27).

Occupational exposure was also described through percutaneous exposure to WNV-contaminated laboratory ‘sharps’ (1). Within the human health field, identification of asymptomatic infected individuals has become a crucial issue for blood banks after 23 cases of WNV infection were linked to transfusion with donated blood products (70). Routine screening of blood donors for WNV by nucleic acid amplification assays (reverse-transcription polymerase chain reaction or RT-PCR) in small pools was rapidly instituted after 2002. As a result of this more rigorous screening in 2003, more than 800 WNV-positive blood donations were removed from the blood supply, but six cases of transfusion-associated WNV were identified, each associated with a single donor with low viraemia (16). Screening of individual samples was begun in areas with active transmission in 2004. A total of 193 virus-positive blood donors were identified and one case of transfusion-associated transmission was reported in Arizona (15).

Several experiments in mammals and birds have demonstrated the establishment of infection after oral exposure to the virus (2, 40). Cats were productively infected after ingesting WNV-infected mice (2) and 5 avian species out of 16 which were challenged orally with the virus became infected (40). Oral exposure to horse meat from WNV-infected animals was the hypothesised route of infection in a group of farmed alligators in 2002 (59).

The possibility of faecal shedding of WNV from infected birds was considered after careful histopathological studies and immuno-histochemical localisation of the virus in the gastro-intestinal and renal epithelium, performed in the first year of the epizootic (79). The amount of antigen detected in these tissues suggested the likelihood of significant faecal shedding of the virus and a potential alternative exposure route for other animals in the environment through aerosol or oral exposure. Direct transmission of the virus from infected birds to others in the same cage (or ‘cage mates’) has now been experimentally demonstrated for five species out of the 16 tested (40, 49, 55). Experimental infections in crows demonstrated direct transmission from the infected birds to their control cage mates (40, 54). Horizontal transmission was relatively efficient, with five of seven controls becoming infected in one study (54), and five out of five contact controls becoming infected in the other (40). Experimental infection in chickens indicated that non-insect-mediated transmission was much less efficient in this species, with no detectable transmission in one study (77) and only one out of 16 contact birds becoming infected in another (49). Horizontal transmission of WNV has also been demonstrated in blue jays (*Cyanocitta cristata*), black-billed magpies (*Pica pica*) and ring-bill gulls (*Larus delawarensis*) (40). Both experimental and field evidence indicate that horizontal transmission also occurs among geese (3, 81). Rough estimates of the quantities of cloacal shedding of WNV in several species of experimentally infected birds were obtained from measuring the virus on cloacal swabs (40). Most birds shed some virus at a low titre, but the passerines, owls and corvids that were tested excreted more than six logs of

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**Fig. 3**

A schematic illustration of the West Nile virus transmission cycle

*Adapted from (71)*
Molecular epidemiology

West Nile virus was introduced into a setting that permitted widespread and efficient replication in an astonishingly large variety of host species. In that kind of environment, it might be expected that selective adaptation would occur. Nucleotide sequence analysis of early isolates from the East Coast indicated that they were nearly identical (48). Analysis of additional isolates from New York, collected from 2002 to 2003, revealed the presence of a virus segregating to a different clade emerging in 2002 (22). Strains belonging to this more recently identified clade have a shorter extrinsic incubation period in mosquito hosts and appear to have a selective advantage, as they have become the dominant variants in 2003 in New York State. Additional isolates analysed in Texas show phenotypic differences in plaque morphology and neuro-invasiveness in mice, but not in neurovirulence (19). The emergence of a new dominant clade of viruses in at least one geographic region suggests that adaptation of WNV will be a dynamic and unpredictable process, and that molecular and phenotypic evaluation of isolates must continue.

Diagnostic testing

Diagnostic evaluation of samples involves identification of both the antibody and the antigen. Many recent advances have been made in the commercial production of rapid testing methodologies. Rapid assays for the detection of antigen include RT-PCR assays (10, 35, 44, 46, 47, 62, 69, 78) and a commercially available antigen-capture assay (32, 74). The RT-PCR assays are all relatively sensitive, although it is difficult to compare them directly. For assays with defined copy number controls, detection limits of 20 to 40 copies of ribonucleic acid can routinely be obtained. Virus can be detected in the tissues, especially in avian kidneys, brains and hearts (42). Virus can also be detected on oropharyngeal or cloacal swabs from WNV-infected birds and this has become a valuable surveillance tool (52, 89).

Standard antibody detection tests include the haemagglutination inhibition test (4) and plaque reduction neutralisation test (PRNT). The former assay suffers from non-specific reactivity in some samples and will generally detect antibodies against related flaviviruses. The PRNT is more specific and, in most instances, can differentiate among antibody reactivity to closely related viruses. Exposure to more than one related flavivirus, or distance in time from the initial infection, generally results in a broader serum neutralising response with a loss of the ability to identify the infecting virus. The PRNT requires biosafety level 3 facilities in the USA, which limits the availability of the test. Immunoglobulin M (IgM) capture enzyme-linked immunosorbent assays (ELISAs) have been developed for humans, equines, canines and chickens, and are useful for determining recent exposure to the virus. The fact that IgM persists in humans complicates test interpretation (11, 12), but it is more transient in equines (66, 67) and canines (A. Glaser, unpublished findings). Samples that test positive for the presence of antibody by IgM-capture ELISA must then have that antibody confirmed as being specific to either WNV or St Louis encephalitis virus, with a labour-intensive PRNT. Fortunately, a blocking ELISA test has been developed that offers species-independent use and the ability to discern the origin of antibody reactivity (6, 7, 36). A broadly reactive IgG-capture ELISA for bird serum has also been developed. This assay performed well when tested with serum from 23 species (12 orders) of birds (21), but specificity needs to be determined using the PRNT. Finally, the production of expressed protein antigens has led to the development of a growing array of antibody test platforms, which should greatly facilitate rapid serological diagnosis of flavivirus infections and/or exposure (31, 61, 73, 86).

The epidemiology and biological characteristics of WNV in North America continue to be explored and elucidated. The sustained level of WNV activity since 1999, the complexity of the epidemiology of transmission, the high levels of viraemia in wildlife reservoirs, the large number of hosts and species of mosquitoes found infected, and the observation of new modes of transmission all suggest that WNV is a greater public and animal health threat in North America, and particularly in the USA, than scientists initially appreciated. The scope, scale, intensity and consequences of this epidemic in the USA are unprecedented for an arbovirus.

West Nile virus is a remarkable example of an emerging zoonosis that involves the dynamic interface of wildlife, domestic animals and humans. North America, with its non-immune hosts, competent vectors and opportunities for rapid transmission, created an ideal setting for the ‘perfect microbial storm’ to occur. Today, the storm ‘continues to rage’ and the prospect of controlling it in the near future is unlikely. West Nile virus will remain in North America and, with continuous ecological changes, adaptation of the virus and the potential expansion of its host range, the WNV story will continue to unfold, often in unpredictable ways.
Le virus West Nile et l’Amérique du Nord : une histoire qui se dévoile

A. Glaser

Résumé
Avant l’introduction du virus West Nile aux États-Unis d’Amérique en 1999, l’Amérique du Nord réunissait toutes les conditions pour qu’une épidémie à arbovirus y fasse son apparition. L’existence de populations animales et humaines nombreuses, susceptibles et non immunisées, la présence de vecteurs compétents, l’accroissement des échanges et des déplacements internationaux, la facilité des méthodes favorisant une dissémination rapide et la piétre préparation des infrastructures de santé animale et publique sont autant de facteurs qui ont conflué vers une situation de crise zoosanitaire et de santé publique d’une grande gravité, véritable « cyclone microbien ». L’introduction du virus West Nile dans la ville de New York a constitué le dernier facteur catalyseur en déclenchant l’une des plus importantes épidémies ayant jamais affecté les États-Unis d’Amérique. La propagation du virus dans tout le pays s’est traduite par l’exposition et l’infection de vastes segments des populations d’animaux sauvages, d’équidés et d’êtres humains.
À l’heure actuelle, l’épidémie n’a pas encore été entièrement élucidée, tout en continuant à évoluer et à s’adapter. La reconnaissance récente de certains modes de transmission non vectoriels a mis en évidence que la maladie représente une menace plus grave et difficile à maîtriser qu’on ne l’avait supposé dans un premier temps. Le virus West Nile semble voué à jouer un rôle permanent dans le « paysage médical » des États-Unis d’Amérique, en s’installant durablement, et désormais sous forme endémique, aussi bien dans la faune sauvage que chez les animaux domestiques et la population humaine.
L'auteur examine les caractéristiques de cette extraordinaire maladie et met l’accent sur la nécessité de disposer d’un système de surveillance intégré, d’une capacité diagnostique renforcée et de stratégies de prophylaxie améliorées.

Mots-clés

La historia del virus West Nile y América del Norte sale a la luz

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Resumen
Antes de que el virus West Nile (VWN) penetrara en los Estados Unidos de América, cosa que sucedió en 1999, América del Norte ofrecía condiciones idóneas para que surgiera una epidemia arbovírica. La combinación de factores tales como la existencia de grandes poblaciones animales y humanas sensibles y no inmunizadas, la presencia de vectores competentes, la intensificación de los viajes y el comercio internacionales, los métodos existentes para una rápida propagación y la poca preparación de las infraestructuras sanitarias y zoosanitarias generó la coyuntura necesaria para que se desatará una grave crisis zoosanitaria y de salud pública: el perfecto ‘‘huracán microbiano’. La introducción del VWN en la ciudad de Nueva York fue el último factor, la espoleta
References


