West Nile viral encephalitis

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Summary

West Nile virus (WNV) has emerged in recent years in temperate regions of Europe and North America, presenting a threat to both public and animal health. The most serious manifestation of infection is fatal encephalitis in humans and horses, as well as mortality in certain domestic and wild birds. A recent development in the epizootiology of this mosquito-borne flavivirus was the occurrence of a severe outbreak in New York City and surrounding areas. During this outbreak, mortality was observed in humans, horses, a cat and numerous species of wild birds, particularly members of the family Corvidae (crows). The author reviews basic information and summarises recent developments in the epidemiology and epizootiology of WNV.

Keywords


Introduction

West Nile virus (WNV) has emerged in recent years in temperate regions of Europe and North America, presenting a threat to public health, equine health, and since 1998, the health of bird populations. The most serious manifestation of infection is fatal encephalitis in humans and horses, as well as mortality in certain domestic and wild birds. A comprehensive review of the ecology and epidemiology of WNV has been prepared previously (28). This chapter reviews basic information and summarises recent developments in the epidemiology and epizootiology of WNV.

History

West Nile virus was first isolated from a febrile adult woman in the West Nile District of Uganda in 1937 (78). The ecology as a mosquito-borne virus was characterised in Egypt in the 1950s (83). The virus became recognised as a cause of severe human meningoencephalitis in elderly patients during an outbreak in Israel in 1957 (80). Equine disease was first noted in Egypt and France in the early 1960s (27, 75). The appearance of WNV in North America in 1999, with encephalitis reported in humans (14) and horses (62), may be an important milestone in the evolving history of this virus.

Geographic distribution

West Nile virus has been described in Africa, Europe, south Asia, Oceania (sub-type Kunjin), and most recently, North America. The list of countries affected by WNV has been summarised (31). Recent outbreaks of WNV encephalitis in humans have occurred in Algeria in 1994 (44), Romania in 1996-1997 (31, 67, 86), the Czech Republic in 1997 (32), the Democratic Republic of the Congo in 1998 (59), Russia in 1999 (A. Platonov, personal communication) and the United States of America (USA) in 1999 (14). Epidemics of disease in horses occurred in Morocco in 1996 (84), Italy in 1998 (12) and the USA in 1999 (62). In the USA, WNV has been recovered in the north-eastern States of New York, Connecticut, New Jersey and Maryland (Fig. 1). In humans, disease has only been reported in south-east New York State, including New York City. Equine disease was clustered on Long Island, east of New York City.

Molecular biology

West Nile virus is a positive-stranded ribonucleic acid (RNA) virus belonging to the Japanese encephalitis serocomplex of the family Flaviviridae (38). Structurally, the virus forms a 40 nm-diameter particle composed of an 11.3-kb genomic RNA associated with a core protein (nucleocapsid), surrounded by a host-derived membrane with viral membrane and envelope proteins (71). Two genetic lineages
Transmission cycles

West Nile virus is amplified during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and avian reservoir hosts (Fig. 2). Infectious mosquitoes carry virus particles in the salivary glands and infect susceptible bird species during blood-meal acquisition. Competent bird reservoirs will sustain an infectious viraemia for 1 to 4 days subsequent to exposure, after which these hosts develop life-long immunity. A sufficient number of vectors must feed on an infectious host to ensure that some survive the extrinsic incubation period (approximately two weeks depending on temperature) (17) to feed again on a susceptible reservoir host. People, horses and most other mammals rarely develop the infectious-level of viraemia, and thus are considered 'dead-end' hosts (28). The transmission cycle may amplify when complex ecological and climatological criteria are met, but usually endures only a few months, after which ecological parameters hinder transmission.

Primary vector species and vertebrate reservoir host species vary with geographic location (Table I). Determining the species components of transmission cycles generally requires experimental data for vector and vertebrate host competence, multiple virus isolations from mosquitoes and vertebrates, and serological data. Serological surveys alone which indicate exposure to WNV, are insufficient to incriminate a particular vertebrate host as an amplification reservoir. In general, Culex species mosquitoes serve as vectors and passerine birds are vertebrate reservoirs in enzootic WNV transmission cycles.
Alternative vector-borne transmission cycles for WNV have been proposed. Natural infections in ticks have been observed on multiple occasions in Ornithodoros maritimus and Argas hermanni (soft ticks), and Hyalomma marginatum (hard ticks), as well as single isolations from five other species of hard ticks in Africa, Europe and Asia (31). Swallow bugs (Oeciacus hirundinis) have been implicated as vectors in Austria (77). Infections of non-culicine arthropods are considered incidental or of secondary importance and not essential for virus perpetuation. However, inter-epizootic maintenance of WNV has not been well characterised, and may depend on alternative hosts.

Experimental transmission of WNV in the absence of arthropod vectors has been described for hamsters (70) and mice (56, 60). A preliminary finding of experimental low-level transmission between birds using the New York strain of WNV requires further documentation (N. Komar, unpublished findings).

Mechanisms for WNV maintenance between periods of continuous transmission are unknown but may also depend on alternative vectors and reservoir hosts. Shuttling of WNV between temperate transmission foci and sub-tropical/tropical foci by means of viraemic migrating birds has been proposed (45, 52, 55). Mechanisms for overwintering in temperate zones may include prolonged infection in hibernating adult Culex species mosquitoes that undergo diapause during winter months (83) or low-level transovarial transmission in certain species of mosquitoes (28, 55).

### Experimental infections

Most classes of vertebrate hosts are susceptible to WNV infection, including birds, mammals, amphibians and reptiles (28). Experimental infections of birds have been described for crows, falcons, chickens (63, 83), doves (48, 91), pigeons (48, 76, 83), ducks (48, 76), herons (9, 48, 91), sparrows (48, 91) and other birds (48). Experimental infection studies of other vertebrates are described for pigs (34), donkeys (75), mules, sheep, a water buffalo (83), cattle (81), dogs (8), lemons (72) and other primates (30, 53, 63, 68, 78), African wild rodents (46), laboratory mice (21, 22, 24, 43, 56, 60, 63, 78, 87), rats (21, 22), hamsters (65, 70), guinea-pigs (36, 78), hedgehogs and rabbits (78), frogs (40) and humans (79). These studies confirm that high-titred viraemia is rare in species other than birds. Additional studies employing the New York strain of WNV are in progress with a variety of species. At least sixteen species of mosquitoes are competent vectors in experimental transmission studies of WNV (28, 33, 35, 65), including five species, namely: Culex pipiens, Aedes japonicus, Ae. sollicitans, Ae. taeniorhynchus and Ae. vexans, which were tested with a New York strain of WNV (M.J. Turell, personal communication). Interestingly, C. pipiens complex mosquitoes, although implicated as vectors in urban

### Table I

<table>
<thead>
<tr>
<th>Location</th>
<th>Vertebrate hosts</th>
<th>Vector mosquitoes</th>
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<tbody>
<tr>
<td>Europe</td>
<td>House sparrow</td>
<td>Culex pipiens</td>
</tr>
<tr>
<td></td>
<td>(Passer domesticus)</td>
<td>Cx. modestus</td>
</tr>
<tr>
<td></td>
<td>Hooded crow</td>
<td>Coquiellidio richardi</td>
</tr>
<tr>
<td></td>
<td>(Corvus corone aorandius)</td>
<td>Cx. univittatus</td>
</tr>
<tr>
<td></td>
<td>Other birds</td>
<td>Cx. poicilipes</td>
</tr>
<tr>
<td>Middle East</td>
<td>Turtle dove</td>
<td>Cx. antennatus</td>
</tr>
<tr>
<td></td>
<td>(Streptopelia turtur)</td>
<td>Cx. decens</td>
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<tr>
<td></td>
<td>Other birds</td>
<td>Aedes albopictus</td>
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<tr>
<td>South Asia</td>
<td>Birds</td>
<td>Mimomyia species</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cx. univittatus</td>
</tr>
<tr>
<td>Oceania</td>
<td>Herons (Ardeidae)</td>
<td>Cx. quinquefasciatus</td>
</tr>
<tr>
<td>North America</td>
<td>Passerine birds</td>
<td>Cx. annulirostris</td>
</tr>
</tbody>
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**Fig. 2**

Basic transmission cycle of West Nile virus in birds and Culex mosquitoes

Alternative transmission cycles may exist.
outbreaks, are consistently poor vectors in competence studies (2, 37, 39). Some non-culicine arthropods, including the soft ticks *O. maritimus* and *O. erraticus* (28) and *A. arboraeus, A. hermanni* and *A. persicus* (1) have been evaluated experimentally and were found to be competent hosts for viral replication and, in some cases, for transmission to vertebrates.

**Disease associations**

In humans, WNV infection is usually asymptomatic, but WNV-attributed morbidity occurred in 11% of a population of cancer patients experimentally infected with the EG101 strain (79). The apparent-to-inapparent ratio of infections during the WNV epidemic in Romania was estimated between 1:140 and 1:320 (86). Disease attributed to WNV (and Kunjin virus) is typically associated with fever and, in rare cases, a more severe syndrome that includes meningitis and encephalitis (28, 66). In recent outbreaks, the case fatality rate was 4% of 393 cases in Romania (86), approximately 6% of 500 encephalitis and meningitis cases in Russia (A. Platonov, personal communication), and 11% of 61 cases in New York (15).

In horses, WNV disease is detected rarely. Serological studies in horses suggest that neurological infections represented approximately 20% of all infections in Tuscany, Italy, in 1998 (R. Lelli, personal communication), and less than 40% in Long Island, New York, in 1999 (S. Trock, personal communication). These studies indicated that infection rates in horses residing in close proximity to case premises were approximately 40% in Tuscany and approximately 20% in Long Island. Neurological disease in horses is characterised principally by posterior ataxia, proprioception deficits (Fig. 3) and altered behaviour. The most severe cases evolve to paralysis of the hind legs, recumbency, terminal convulsion and death (84; J. Lubroth, personal communication). The case fatality rate in Morocco in 1996 was 44% of 94 cases (84), 43% of 14 cases in Italy in 1998 (12), and 45% of 20 cases in the USA in 1999 (62). In other mammals, naturally acquired WNV encephalitis is unknown, with the exception of a single case which occurred during the outbreak in North America in a domestic cat (euthanised after the onset of seizures), which was confirmed by virus isolation from the brain (N. Komar, unpublished findings).

Avian morbidity and mortality resulting from natural WNV infection is a novel characteristic of WNV emergence. Prior to 1998, the only evidence for virulence in naturally infected birds was the isolation of WNV from a sick fledgling pigeon (*Columba livia*) in Egypt (90). Experimental evidence of avian mortality is limited to the observation of severe mortality in hooded crows (*Corvus corone sardonius*) (100%), but not doves (*Streptopelia senegalensis*), falcons (*Falco tinnunculus*) or herons (*Bubulcus ibis*), as a result of mosquito-borne infection with the Ar-248 strain of WNV (91). In 1998, WNV was isolated in Israel from the brains of four dead white storks (*Ciconia ciconia*), a lappet-faced vulture (*Torgos tracheliotus*) and domestic geese (*Anser spp.*) (52). An isolate from a goose was 100% homologous, based on limited nucleotide sequence data, to the WNV isolates from North America that also caused severe mortality in certain avian populations (42).

An outbreak of WNV among eight- to ten-week-old domestic geese in Israel in November 1999 resulted in 400 cases with a mortality rate of 40% (61).

The impact of WNV on North American bird populations during the 1999 epizootic has not yet been evaluated fully. Nonetheless, it is clear that corvids (crows and jays) in particular, as well as members of thirteen other families of...
birds, spanning twelve orders, have suffered mortality, as indicated by virus isolation. At the time of writing (January 2000), over 200 avian deaths in the USA have been attributed to WNV by virus isolation from, or detection in, tissues. Approximately 10% of these cases occurred in zoological collections of exotic South American and Asian species, as well as native North American species (82). Approximately 80% of laboratory-confirmed WNV-associated deaths were American crows (Corvus brachyrhynchos) and other corvids (N. Komar, unpublished findings).

**Pathogenesis**

In humans, WNV preferentially targets tissues in which reticulo-endothelial cells predominate (79). However, disease occurs as a result of degeneration of infected cells in the central nervous system (CNS). Similar findings are described for non-human primates (53) and horses (12). One unique finding to WNV pathogenesis compared to that of other flaviviruses is the targeting of Purkinje cells of the cerebellum in both mammals (53, 75) and birds (82). In birds, immunohistochemical analyses indicate that there is a predilection for kidney and heart, although other tissues can be affected (82). Gross pathological findings in fatal cases of birds and horses include meningoencephalitis, and congestion and haemorrhages of brain vessels. Tiber reported pulmonary oedema and congestion, and brain oedema with petechiation and suffusive haemorrhages in horses (75, 84). Cantile et al. reported the most severe lesions of WNV-infected horses in the ventral horns of the thoracic and lumbar spinal cord (12). Steele et al. reported myocarditis and splenomegaly as major findings in birds (82) (Fig. 4).

**Laboratory diagnosis**

Diagnosis of WNV infection cannot be made on clinical findings alone and requires laboratory-based confirmation. Laboratory diagnosis is complicated by the antigenic cross-reactivity within members of the Japanese encephalitis (JE) serocomplex of viruses, including Alfuy, Japanese encephalitis, Kokobera, Koutango, Kunjin, Murray Valley encephalitis, St Louis encephalitis (SLE), Stratford, Usutu and West Nile viruses (11). Definitive diagnosis of WNV infection can be made by detection of viral RNA (69) or by virus isolation from specimens (serum, cerebrospinal fluid [CSF] and tissues) or from serological evidence of recent infection. Virus isolates, amplified in cell culture or in suckling mice, are identified by virus neutralisation assays using specific antisera, by enzyme immunoassay, or by the detection of WNV-specific RNA sequences. Specificity of reagents must be validated against antigenically cross-reacting flaviviruses such as SLE (in North America) and JE (in Asia).

Serological evidence of recent infection is established by demonstrating a four-fold rise in neutralising antibody titre between acute-phase and convalescent-phase serum samples, or by the detection of specific immunoglobulin M (IgM) antibody in serum and/or CSF. The latter was used as a screening test in the outbreaks in the USA and Romania (13, 16). Detection of IgG or virus-neutralising antibodies may indicate previous infection, but cross-reaction with other flaviviruses must always be evaluated in these non-specific tests. A four-fold greater titre for WNV compared with that of other flaviviruses confirms diagnosis of primary infections. However, secondary heterologous flavivirus infections may demonstrate the phenomenon of 'original antigenic sin' in which the titre against the agent of the primary infection is greater than that of the agent of the secondary infection (36).

Complement fixation and haemagglutination inhibition tests have been used for the detection of WNV antibody but these are not as specific as the neutralisation test. Significant caution is encouraged when interpreting serological test results for West Nile virus and other members of the JE serocomplex.

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**Fig. 4**

Gross pathological findings in crows infected with West Nile virus

*a* Splenomegaly

The blue bar indicates maximum size of normal spleen

*b* Cardiac haemorrhage and hepatomegaly

Photographs: courtesy of W.B. Stone, New York State Department of Environmental Conservation
Treatment and control

Currently, there are no proven treatments for clinical WNV infection in humans, horses or any other animal. Experimentally infected animals (usually mice) have been treated with pentoxysphine (3), gentamicin (5), melatonin (6), tilorone hydrochloride (87) or steroids (25). Encephalitis in humans should be treated with supportive therapy. Partial recovery was observed in a patient after treatment with an appetite stimulant (66). Intravenous administration of nutritional fluids for horses and other encephalitic animals may alleviate emaciation that results from the inability to eat during the neurological phase of disease.

No WNV vaccine is available commercially. As is the case with other arboviral encephalitides, human WNV encephalitis is so rare that vaccine development may not be feasible economically. Vaccination of horses may have benefits in protecting valuable animals from a potentially fatal disease, but trade and competition practices may make this undesirable as some countries may use positive antibody tests, which would result from either vaccination or natural infection, as justification for importation restrictions, albeit unwarranted on scientific grounds. In 1999, commercial goose flocks in Israel were vaccinated against Israel turkey meningoencephalitis (ITM) virus, a flavivirus which is distantly related to WNV, with the intent to cross-protect against WNV infection (61). Further studies are required to determine the efficacy of flavivirus vaccines, such as those against ITM and JE viruses for protection against WNV infection.

Control of WNV infection is best achieved by reducing vector populations. Methods of mosquito control have been described (19, 41).

Surveillance

Surveillance for WNV outbreaks generally relies on passive case reporting of human and equine cases of encephalitis. Historically, mosquito-based surveillance for WNV and use of sentinel birds (chickens and pigeons) have been used successfully to monitor WNV transmission in enzootic regions such as South Africa (47, 50) and Australia (20, 73). Recent programmes of active surveillance for WNV in bird and mosquito populations were instituted in Romania (74), the Czech Republic (32) and Italy (G. Ferrari, personal communication). Active surveillance in the USA in 2000 will rely on surveillance systems already in place for SLE, which include virus isolation and antigen detection in *Culex* mosquitoes, and serological detection of specific IgM and haemagglutination-inhibiting (HI) antibodies in sentinel chickens. In some States, house sparrows are sampled as wild bird sentinels (51). In such instances, detection of SLE antibodies may serve as a screen for flavivirus infection. Positive samples should be retested using the plaque-reduction neutralisation test to differentiate between SLE virus and WNV infections. Guidelines have been published to assist States in developing new arbovirus surveillance programmes in preparation for the possible spread of WNV activity in North America (15).

Mosquito- and bird-based surveillance programmes are most useful for monitoring arbovirus activity in known transmission foci. The WNV infection rate of *C. pipiens* mosquitoes collected during the outbreak investigation in Romania was less than 0.1% (74). Seroprevalence in resident birds, on the other hand, is generally high in enzootic transmission foci (83) and in epizootic foci (74). Investigation of avian infection in Romania in 1996 revealed that more than 30% of fowl had developed neutralising antibodies to WNV. Preliminary studies in 1999 have confirmed this observation among resident birds in New York City, but not among migrating birds (N. Komar, unpublished findings).

Active surveillance in the USA in 2000 will rely on surveillance systems already in place for SLE, which include virus isolation and antigen detection in *Culex* mosquitoes, and serological detection of specific IgM and haemagglutination-inhibiting (HI) antibodies in sentinel chickens. In some States, house sparrows are sampled as wild bird sentinels (51). In such instances, detection of SLE antibodies may serve as a screen for flavivirus infection. Positive samples should be retested using the plaque-reduction neutralisation test to differentiate between SLE virus and WNV infections. Guidelines have been published to assist States in developing new arbovirus surveillance programmes in preparation for the possible spread of WNV activity in North America (15).

Economic impact

The economic impact of WNV infection has not been evaluated thoroughly. However, other cost studies of arboviral encephalitis may apply to WNV. The finding that a single case of residual eastern equine encephalitis (EEE) in a human infant may exceed a cost of US$3 million is considered a strong justification for active surveillance programmes for EEE in the USA (89). One estimate of public expenditure attributed to the WNV outbreak in New York State in 1999 exceeds US$15 million (D.J. White, personal communication). Additional costs in the USA outbreak resulted from embargoes on some international shipments of horses, including the exit of horses through New York City ports (N. Faizi, personal communication).

Perspectives

West Nile virus encephalitis appears to be an emerging concern for public health in European and North American cities, and for equine health in horse-farming communities. Several outbreaks of human and equine encephalitis have occurred since 1996, whereas prior to that year, outbreaks were recorded on rare occasions. Global climate change has been speculated as contributing to the increase in WNV disease outbreaks in temperate regions (31). A novel development with WNV in the USA and Israel is the observation of avian mortality, especially among wild crows.
Presumably, the possible spread of WNV in North America could be tracked by mapping WNV-confirmed crow deaths. Crow mortality may become an important surveillance tool and consequently help prevent encephalitis in human and horse populations. The long-term impact of decimated crow populations is unknown at present. Determining the impact on public, veterinary and wildlife health as a consequence of WNV introduction in North America will undoubtedly provide many valuable lessons for the preparation against other emerging zoonotic diseases yet to be recognised.

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Encéphalite due au virus West Nile
N. Komar

Résumé
Le virus West Nile est apparu ces dernières années dans les régions tempérées d'Europe et d'Amérique du Nord, faisant peser une lourde menace sur la santé des animaux et de l'homme. La manifestation la plus grave de l'infection est une encéphalite mortelle pour l'homme comme pour le cheval, ainsi qu'une infection mortelle affectant plusieurs espèces d'oiseaux domestiques et sauvages. Le flavivirus causal, transmis par des moustiques, a récemment connu une nouvelle progression avec l'apparition d'une grave épidémie à New York et dans la région. Celle-ci a entraîné plusieurs décès chez l'homme ainsi que la mort de chevaux, de plusieurs espèces d'oiseaux sauvages (notamment des corvidés) et d'un chat. L'auteur fait le point sur l'état actuel des connaissances ainsi que sur les récentes découvertes en matière d'épidémiologie et d'épizootiologie de l'infection par le virus West Nile.

Mots-clés

Encefalitis causada por el virus West Nile
N. Komar

Resumen
El virus West Nile, surgido en los últimos años en regiones templadas de Europa y Norteamérica, constituye una notable amenaza en términos tanto de salud pública como de sanidad animal. Tanto en el hombre como en el caballo, la manifestación más grave de la infección es una encefalitis de consencuencias
La infección también afecta a varias especies de aves domésticas y silvestres, ocasionando mortalidad entre estos animales. La reciente aparición de un brote de considerable gravedad en la ciudad de Nueva York y áreas circundantes constituye un hecho inédito en la epizootiología de este flavivirus transmitido por mosquitos. Dicho brote provocó casos mortales en seres humanos, caballos, un gato y varias especies de aves silvestres, pertenecientes sobre todo a la familia de los córvidos. El autor repasa los datos fundamentales y sintetiza la evolución reciente de la epidemiología y la epizootiología del virus West Nile.

Palabras clave

References


