

REVIEW ARTICLE

Implications for Northern Europe of the emergence of West Nile virus in the USA

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INTRODUCTION

The unexpected appearance of fatal encephalitis in six elderly people living in New York in 1999, heralded the re-birth of arbovirology in the United States of America. The subsequent rapid spread through North America and impact of the disease on humans, birds, horses and a wide range of other species including alligators and frogs, has brought *West Nile virus* (WNV) to the attention of governments and the media, worldwide. The response of the public in the United Kingdom has not been hysterical, despite being fuelled by press reports that scientists have demonstrated the presence of WNV antibodies in birds in the UK. Nevertheless, concern has been expressed by government bodies either directly or indirectly connected with the potential health problems that could arise if WNV was introduced and caused the same degree of morbidity and mortality as that seen in the USA. Is the concern justified and are we likely to see significant health problems associated with WNV if this virus is confirmed to be present and circulating amongst birds in the UK? In this review I shall try to put the virus in its true context and assess the risks that WNV might pose both to animals and humans in the United Kingdom.

WEST NILE VIRUS IN THE OLD WORLD

West Nile virus (WNV) is a mosquito-transmitted member of the genus *Flavivirus* in the family

Flaviviridae but it has also been found in ticks in the natural environment. *West Nile virus* is a member of the *Japanese encephalitis virus* serocomplex, a subgroup of the mosquito-borne flaviviruses containing both Old World and New World viruses, all sharing similar ‘lifestyles’, i.e. in the natural environment they circulate between birds and mosquitoes. *Japanese encephalitis virus* (JEV) is found in Asia and is responsible for many cases of human encephalitis. Other well studied members of the JEV serocomplex include *St Louis encephalitis virus* (SLEV) which is widespread throughout both North and South America, causing spasmodic outbreaks of human encephalitis, *Murray Valley encephalitis virus* (MVEV) which causes outbreaks of encephalitis in Australia and Kunjin virus (KUNV), an Australian virus that is sufficiently closely related to WNV to be considered a subtype.

The virus was first isolated in 1937 in the West Nile Delta of Uganda from a woman with a febrile illness [1]. Subsequent studies have revealed that the virus is widespread throughout Africa [2, 3], in all regions where migratory birds and *Culex* spp. mosquitoes share the same habitats and the temperature and rainfall support high mosquito feeding activity. It is now believed that the virus most probably originated (emerged) in Africa only a few centuries ago [4, 5] and then dispersed widely into the Mediterranean region, Western and Central Europe and Asia, finally reaching Australia where it evolved as a subtype of WNV known as Kunjin virus (KUNV). Its wide ranging dispersal in the Old World is the direct result of transport by migratory birds which become infected when ornithophilic mosquitoes carrying the virus excrete it

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into their saliva and then transmit it to the birds as they feed on them. The migrant birds are believed to serve as the reservoir for the virus that is then transmitted to susceptible feeding mosquitoes either at the stopover points on the migratory routes or when the birds reach their destination. The recipient mosquitoes then reproduce the virus and subsequently transmit it to resident (non-migrant) birds when they feed on them. In the Old World, there are comparatively few reports of significant mortality in birds or wild animals infected with WNV, which is taken to indicate that the virus has been circulating amongst both migrant and resident birds for many years. This contrasts with WNV in the USA where, since its introduction, large numbers of infected birds, horses and other wildlife species have died as the result of infection by WNV.

Whilst the virus is endemic in many parts of Africa, epidemics with clinical symptoms, in humans or in animals, are quite rare. Nevertheless, serological surveys show that humans, birds and horses have antibodies to WNV [6], indicating a fairly high incidence of sub-clinical infection. In general this is also true in many parts of Western and Central Europe where there is serological evidence of endemic WNV in birds and horses [7–12]. Historically, WNV has not posed a serious threat to humans in Europe or Asia although outbreaks have been recorded recently in Israel [13, 14], Romania [15], and Volgograd [16]. Whether or not this apparent increase in human epidemicity represents a change in the genetic characteristics of the virus, increased awareness, or the impact of climate change, remains to be seen. Nevertheless, it has been suggested that the lineage 1 New York/Egypt/Israel strains of WNV could be more virulent for birds and possibly horses and humans, than earlier lineage 2 strains of the virus [17]. West Nile virus is also known to be endemic in India but because it overlaps geographically with the closely related and more virulent flavivirus, JEV, it is probably overlooked in diagnostic tests.

An interesting aspect of the survival and geographic dispersal of WNV is its apparent ability to be present in healthy birds as a persistent or latent infection. The length of time that the virus can persist in these healthy birds is not known but when sufficient numbers of birds have been tested, infectious virus has occasionally been isolated [18, 19]. There has been comparatively little research into the mechanisms by which the virus can persist in an apparently non-infectious form and subsequently be re-activated, but

this property appears to be shared with other flaviviruses in the JEV serocomplex.

Human infections with WNV are incidental, i.e. they are not part of the natural virus life cycle. They usually result from the bite of an infected mosquito, in most cases anthropophilic *Culex* spp., although there is some evidence that other mosquito species may play a significant role in transmission to humans. Most human infections (about 80%) are asymptomatic. Symptomatic infections result in a febrile illness, referred to as West Nile fever, from which the patients recover completely. Only about 1 in 150 cases of fever develop neurological complications which may be fatal in older people particularly those over 70 years of age.

WEST NILE VIRUS IN THE NEW WORLD

The appearance of WNV in New York in the summer of 1999 surprised virtually everyone – but it should not have done. In reality, and with the wisdom of hindsight, it was an event that was waiting to happen. Many diseases caused by arboviruses have been introduced into the USA from the Old World. Perhaps the best known are yellow fever, dengue and St Louis encephalitis, the latter caused by a virus closely related to WNV. Apart from WNV, each of these viruses/diseases, and most likely many others, probably first arrived from Africa on the slave boats during the past few centuries [5, 20, 21]. However as the slave trading finally died out it was replaced by large numbers of immigrants and more recently increasing numbers of visitors to the USA. In addition very large numbers of animals, including birds, are introduced into or arrive in the USA daily. There are therefore many opportunities for viruses to be introduced either by infected mosquitoes, birds, or humans travelling from the Old World. Indeed there are now several papers showing that dengue virus is regularly being introduced into Central and Southern America from Asia [22, 23]. Presumably, these viruses are also being introduced into North America on a regular basis from overseas but have so far been unable to become established either due to the lack of sufficiently high numbers of suitable mosquitoes or other factors such as climate. Perhaps it is only a matter of time!

West Nile virus was apparently introduced into New York, and became established as a North American virus, in the summer of 1999. The precise circumstances of its introduction will probably never be

known but there are several possibilities:

- (1) an infected human or animal with viraemia arrived from the Middle East at an airport in New York, and was then bitten by a local mosquito which became infected and subsequently fed on a bird in New York, thus starting the cycle in the USA;
- (2) an infected mosquito arrived in New York on an aeroplane flying from the Middle East, which then fed on a local bird,
- (3) an infected bird was introduced into New York, either legally or illegally, was then bitten by a local mosquito which subsequently fed on a local bird,
- (4) an infected migrating bird was blown off course from the Old World and landed in the New York area where it was bitten by a local mosquito.

In view of the focality of the outbreak, it seems most unlikely that the virus was introduced deliberately. Regardless of the mode of introduction, it rapidly became established in wild birds and *Culex* spp. mosquitoes, and began to infect humans, causing 62 cases of encephalitis and six fatalities in four local States before the first winter. The virus survived the winter months either in over-wintering mosquitoes, or perhaps in sub-clinically infected birds, and became only slightly more widespread in 2000 [24]. Because of the sporadic nature of arboviral diseases in the USA, it had proven difficult to mobilize political and public support for funding to maintain effective arbovirus surveillance and control programmes. The loss of this infrastructure meant that much of the USA was potentially vulnerable to new mosquito-borne disease outbreaks. It is interesting to note that temperature sensitivity of virus transmission efficiency has been demonstrated for WNV and this may be one of the most important factors in the establishment and spread of newly introduced arboviruses, such as WNV, when considering the risks to humans in Northern Europe. The year 1999 was particularly warm in North America and this may have contributed significantly to the successful establishment of the virus in the wild-life species.

By the end of the 1999 mosquito-transmission season, WNV had been found in birds in 28 counties. The year 2000 was a little cooler and although the virus did spread it was still located mostly in the North Western counties around the New York area although moving gradually down the East Coast. By the end of 2001, 319 counties had recorded the presence of WNV in birds. The virus first appeared between January and April in birds in Florida in 2002 and gradually spread

northwards and westwards as the birds dispersed to become widespread in that state (information available from <http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm#map1>). By the middle of August, there had been a small number of fatal human cases but then the numbers started to increase rapidly and by the middle of December over 3852 cases of encephalitis had been recorded with 232 fatalities (information available from <http://www.cdc.gov/od/oc/media/wncount.htm>). It is important to note that the number of fatal infections in birds and horses had also increased markedly before the sudden increase in human encephalitis. By the beginning of October the virus had been detected in birds in 1456 counties. The most important factors considered responsible for this sudden increase are the climate and the mosquito density that is particularly dependent on warm weather and increased rainfall. Undoubtedly, birds (and possibly bats but this is not yet known) were largely responsible for the efficient dispersal of the virus.

A potentially extremely important and not yet fully researched aspect of the spread of WNV through the human population in North America has been its observed transmissibility through blood transfusions, organ transplants, human breast milk and transplacentally. Under experimental conditions the virus has also been shown to be transmissible orally but there is no evidence of this route of infection in humans. Surprisingly, there have never been any reports of other flaviviruses being spread through humans by these routes despite their known similarity to WNV. Perhaps the most surprising example of this failure is JEV which is very closely related to WNV and causes large human epidemics in Asia. Until more detailed information is available, the most likely explanation seems to be that JEV is capable of spreading by these routes but nobody has yet recognized this. The fact that WNV seems to spread through blood transfusions and organ transplants is already having a significant impact on the development of rapid diagnostic reagents. Moreover, because WNV has also been transmitted accidentally to laboratory personnel on several occasions, steps are being taken to develop harmless chimaeric viruses that carry the important diagnostic genes of WNV.

CLINICAL FEATURES OF WNV INFECTION

Only about 20% of infections result in the development of West Nile fever which has an incubation

period of 2–6 days. Many patients experience a sudden onset of high fever with chills, malaise, headache, backache, arthralgia, myalgia and retro-orbital pain which is aggravated by eye movement [25, 28]. A variety of other non-specific features such as anorexia, nausea, diarrhoea, coughing and sore throat may also occur. In some cases patients have a flushed face, conjunctival injection and a generalized lymphadenopathy [25, 28]. A rash which appears from day 2–5 post-onset is seen in about 50% of cases and is more common in children [25]. A range of exanthemas has also been described in WN fever, including a rubelliform, scarlatiniform, discrete form roseolar, papular or mottling rash on the chest back and arms [25, 29]. In some cases hepatomegaly and splenomegaly have been reported and myocarditis, pancreatitis and hepatitis have also been observed in severe WNV infections. [18].

The symptoms of West Nile encephalitis, which is seen in less than 0.1% of all infections, are similar to those described for JE and include aseptic meningitis, encephalitis, myelitis or combinations of the three. Following 1–7 days of a febrile prodrome with headache, weakness, and gastrointestinal symptoms, patients become drowsy, confused and disoriented. Although the prodrome is often non-specific, up to 15% of patients may have symptoms similar to those of West Nile fever, including eye pain, facial congestion, pharyngeal or conjunctival hyperaemia, lymphadenopathy, arthralgia or cutaneous eruptions [30, 31]. A bi-phasic illness is not uncommon. Signs of meningeal inflammation, such as neck rigidity and a positive Kernig's sign (back pain when the knee is hyper-extended) are often observed. With encephalitis, patients may be quiet and withdrawn, confused, drowsy or deeply comatose [31, 32].

Electroencephalograms show diffuse high amplitude theta or delta waves [31, 32]. Focal neurological signs include upper motor neurone weakness, lower cranial nerve palsies, tremor and ataxia. *West Nile virus* can cause a flaccid paralysis of the limbs and respiratory muscles which may require ventilation. In some of the American cases of encephalitis, a provisional diagnosis of Guillian-Barré syndrome was given. Indeed, JEV and WNV have been associated with cases of Guillian-Barré in India and a causal relationship was implied [33, 34]. In the American cases, nerve conduction studies revealed reduced motor amplitudes with normal sensory potentials [30], possibly indicating anterior horn cell damage. Either urinary incontinence or retention may be observed in West

Nile encephalitis. Convulsions have been significant in some WNV epidemics but were not a major feature of the New York outbreak. Of 400 patients in the 1996 Romania outbreak, 40% had meningitis, 16% had encephalitis and 44% had meningoencephalitis [15]. In the American outbreak, to October 2002 the case fatality rate has been approximately 10%, corresponding to less than 0.1% of total infections. As of early October 2002, the average age of patients with fatal encephalitis in 2002 was 79, with a range of 27–99 years. Fatal encephalitis in children or young adults is rare.

The gross pathological and histopathological features of West Nile encephalitis are similar to those resulting from Japanese encephalitis. The leptomeninges are normal or slightly hazy and histological examination shows an inflammatory infiltrate. There is perivascular cuffing of the brain parenchyma, together with infiltrates of microglia and polymorphonuclear leucocytes, neuronal degeneration and neurophagia leading to microglial nodules [35, 36]. These lesions are prominent in the brainstem and spinal cord but also occur in the thalamus, cortex and cerebellum. They are found in white and grey matter. In some cases, inflammation also involves the lower cranial nerve roots. Immunohistochemical analysis of the lymphocyte populations showed numerous CD8⁺ T cells and fewer CD4⁺ T cells. CD20⁺ B cells were scattered and most prominent around blood vessels [35].

In the laboratory, mild leucopaenia is often seen in West Nile encephalitis [30, 31]. Lumbar puncture usually reveals clear or slightly opalescent CSF with a moderate lymphocytic pleocytosis (<100 cells/mm³), normal or mildly elevated protein (150 mg/dl), and normal glucose [30–32, 37]. Polymorphonuclear cells may predominate if the CSF is examined early and in some patients there may be no cells [30].

VIRUS DIAGNOSIS, ISOLATION AND IDENTIFICATION

The most reliable indicator of infection by WNV is virus isolation and subsequent identification which can be achieved either using a WNV-specific monoclonal antibody [38] or by reverse-transcription and polymerase chain reaction (RT-PCR) nucleotide sequencing. Both tests are highly sensitive and specific, providing an absolute identification of the virus. Diagnosis by serological methods on the other hand has to be carried out cautiously because many of

the tests detect cross-reactive antibodies to related flaviviruses. Cross-reactivity is particularly evident in haemagglutination-inhibition and ELISA tests. Nevertheless, depending on the geographical location, and immunization history of the patient, most of these cross-reactive flaviviruses can readily be ruled out. For example, in primary infections, the presence of WNV-specific IgM antibody in a capture ELISA is strongly indicative of a WNV infection, although cross-reactivity due to other related flaviviruses such as JEV, MVEV, SLEV has to be ruled out. This is easy in Europe where the only closely related flavivirus yet identified is Usutu virus (USUV) which to date has been found only in Vienna. Therefore, an IgM positive competitive-blocking ELISA assay in a European with no known history of immunization against *Yellow fever virus* (YFV), *Tick-borne encephalitis virus* (TBEV) or JEV is likely to indicate infection by WNV. In general the plaque reduction neutralization test (PRNT) is less cross-reactive and a positive result in an acute serum, with evidence of increasing antibody titre in the convalescent serum, would be considered to be a good indicator of exposure to the virus.

TREATMENT AND CONTROL

There is no specific antiviral treatment for infection by WNV. High quality supportive medicine to reduce and control the side effects of the illness is effective. Several potential antiviral products are currently being tested and may prove to be useful but they are several years away from being fully developed. Engineered chimaeric vaccines based on the highly successful yellow fever 17D vaccine, with the immunologically important viral envelope gene of WNV replacing the equivalent YFV gene have been produced and current trials are producing encouraging results. If this approach is acceptable to the public, it may become the method of choice for preventing infection against WNV and indeed other arboviruses that will undoubtedly be shown to be emerging in Europe and the United States of America in the future.

The simplest and most practical form of control is avoidance of being bitten by an infected mosquito. This can most effectively be achieved by remaining indoors in air conditioned buildings at the times of the day when the mosquitoes feed. In the case of most *Culex* spp. this is during dusk and dawn. The application of suitable repellents, and wearing clothing that covers as much of the skin as possible are also

recommended in all areas where there is a known risk of exposure to mosquitoes.

DOES WNV POSE A RISK TO HUMANS AND ANIMALS IN THE UK OR NORTHERN EUROPE?

As indicated earlier, there is now doubt that WNV is present in Northern Europe, at least in birds. Since there have been no reports of human West Nile fever or encephalitis in Northern Europe it seems unlikely that the virus poses a risk to human health. However, unpublished work presented at a meeting held in New Orleans by CDC in February 2003 strongly inferred that WNV in the USA is more virulent for birds than an African strain of WNV. Therefore, if more virulent strains of WNV are replacing earlier strains the risks of WNV to human health in the future could be more serious than they are currently. The same arguments apply to the UK – however, the presence of WNV in the UK has not yet been formally demonstrated.

During the early autumn of 2002 there were several unsubstantiated media reports of the presence of WNV in resident and migrant birds in the UK. These reports were based on the apparent detection of WNV-specific neutralizing antibody and WNV-specific RNA in the serum and brains of healthy birds. This work has now been submitted for publication. There have been no reports of birds dying off or encephalitis in horses that might represent infection by WNV but this is not surprising since this virus rarely causes fatal illness in birds in the Old World. However, there is currently no evidence of infectious WNV in humans and tests for the presence of WNV-specific RNA in brain samples from cases of undiagnosed fatal encephalitis have thus far proven to be negative. Nevertheless, a systematic programme of investigation for evidence of the presence of WNV in the UK has been initiated by teams of virologists with the appropriate knowledge, skills and facilities.

It is worth noting that even if WNV is found to be present and circulating amongst birds in the UK, the potential risk of human infections resulting in fatal encephalitis is likely to be extremely small, for the following reasons. First, epidemics of WNV in Europe and Asia have occurred only rarely and they have been in regions that are generally warmer than those normally encountered in the UK. Second, mosquito population densities in the South East of England, the warmest area of the UK, are significantly lower than those recorded in Central and Southern Europe.

Third, virus transmission efficiency increases with temperature, i.e. it is likely to be much less efficient in the UK than in the warmer Central and Southern European countries. Fourth, there is no reason to believe that WNV has suddenly started to be introduced into the UK. If indeed WNV is present in resident birds it is highly likely that it has been arriving annually from Africa for decades or even one or two centuries but has so far gone unnoticed by the scientific and medical establishments. Therefore WNV is unlikely to be present in sufficient density amongst the UK mosquito and bird populations to present a significant risk factor to the general human population. On theoretical grounds, the human risk groups most likely to be exposed to the bites of ornithophilic mosquitoes would be farm workers, forestry workers and people closely associated with wild birds. There have been no records of increased signs of fever or encephalitis in these risk groups.

It is also worth noting that there are several other related and even some unrelated arboviruses that circulate in similar environments to WNV in Africa and Asia – for example, *Koutango virus*, *Yaounde virus* [39], *Usutu virus* which has recently been reported to have caused bird die-off in Vienna [40], *Japanese encephalitis virus* which circulates widely in Asia annually causing thousands of cases of fatal encephalitis [41] and *Sindbis virus* which is a significant cause of arthritis and polyarthritis in humans in Scandinavia [42] and co-circulates with WNV in Africa. It can easily be argued that any of these viruses presents at least an equivalent threat to human, bird and horse populations in Europe and the UK. However, as with WNV they have not made their presence felt, at least in the UK or in any of the more northern parts of Europe.

In summary, unless there are significant and sustained increases in average temperature in the UK which would most likely be accompanied by an increase in mosquito densities or unless a more virulent strain of WNV emerges and becomes established in the Old World, WNV does not appear to pose a major threat to humans, birds or horses in the UK or in Northern Europe.

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