West Nile Virus: Potential Risk Factors and the likelihood for introduction into the United Kingdom

Qualitative Risk Assessment

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Table of contents

1 Document History ................................................................. 3
2 Acknowledgements ............................................................... 3
3 Summary ................................................................. 3
4 Background ...................................................................... 5
5 Hazard Identification .......................................................... 5
  5.1 West Nile virus - disease reports ........................................ 6
    5.1.1 European Union ......................................................... 8
6 Risk Question .................................................................. 9
7 Risk assessment .............................................................. 9
  7.1 Terminology related to the assessed level of risk ................. 10
  7.2 Definitions .................................................................. 10
  7.3 Exceptions .................................................................... 10
    7.3.1 Key starting assumptions .......................................... 11
    7.3.2 Uncertainties ............................................................. 11
  7.4 Key criteria .................................................................. 11
    7.4.1 Broad considerations on epidemiology of WNV ........... 11
  7.5 Entry assessment ............................................................ 14
    7.5.1 Legal Trade ............................................................... 14
    7.5.2 Migratory Wild Birds .................................................. 14
    7.5.3 Movements of live equidae .......................................... 17
    7.5.4 Equine germplasm ...................................................... 21
    7.5.5 Authorized biologicals not known to be contaminated ...... 23
    7.5.6 Research samples (including equine sera) ....................... 24
    7.5.7 Poultry and captive birds ............................................. 24
    7.5.8 Non-avian/non-equidae species (e.g. exotic ungulates, reptiles) ........................................................................ 27
    7.5.9 Illegal movements ....................................................... 28
    7.5.10 Accidental import of biological vectors .......................... 28
    7.5.11 Wind borne introduction of infected vectors ................. 29
  7.6 Exposure assessment ......................................................... 30
  7.7 Consequence assessment .................................................. 35
8 Control and risk management options ...................................... 36
  8.1 Vaccines ....................................................................... 36
  8.2 Diagnostic tests ............................................................... 37
9 Conclusions ..................................................................... 38
10 References ...................................................................... 39
1 Document History

<table>
<thead>
<tr>
<th>Version</th>
<th>Updates</th>
<th>Date</th>
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2 Acknowledgements

3 Summary

This qualitative risk assessment specifically addresses the likelihood of the introduction of West Nile Virus (WNV) from abroad to the UK via various pathways and its potential to become established in the UK.

With regard to the introduction of WNV from abroad, we currently consider that:

a) There is a continuous very low likelihood of WNV being introduced by migrating birds. This may change if the disease becomes established in other northern regions of the EU.

b) The likelihood of the introduction of WNV to the UK via legal trade in horses and other equidae is very low;

c) The likelihood of the introduction of WNV to the UK via legal trade of equine semen, ova and embryos, equine meat, specified biologicals and research samples is negligible;

d) The likelihood of the introduction of WNV to the UK via legal trade in live poultry and captive birds, hatching eggs and poultry meat is negligible;

e) The likelihood of the introduction of WNV to the UK via legal trade in live non-avian/non-equidae species, including ungulates, is negligible;
f) Although possible, the likelihood of the introduction of the virus via illegal or non-compliant movements of equidae/poultry is difficult to quantify. It is also impossible to quantify the likelihood of introduction of virus by as yet unrecognised routes;

g) There is a very low risk of WNV being accidentally introduced in infected mosquitoes with imported plants or by means of transport - sea or air cargo;

h) There is a negligible risk of WNV being introduced in infected mosquitoes being blown across to the UK from currently affected regions. This may change if the disease becomes established in other northern regions of the EU.

Should the disease be introduced to the UK, we currently consider that:

a) Horses and poultry exhibit very low, short-lived viraemia and therefore would not contribute to the transmission cycle.

b) Local dissemination would depend upon the abundance of competent vectors; their feeding patterns; bird density and their migratory/local dispersal patterns; environmental considerations, including adequate temperatures that would favour mosquito activity and replication of the virus in mosquitoes;

c) The potential for establishing an enzootic cycle between potentially WNV infected hosts (mainly wild birds) and potentially competent local mosquito populations in the UK is very low, as this is subject to fulfilment of optimal epidemiological, entomological and ecological conditions occurring in combination.

d) There would be some impact on the UK horse industry as the infection may result in disease and mortality in some horses, in which case movements of horses from affected holdings would be subject to official control in line with EU rules.

With regard to availability of vaccines for WNV, we note that:

a) A number of vaccines have been deployed in North America, which are considered to be effective in protecting horses against WNV and have helped reduce the number of cases in horses.

b) A WNV vaccine (Duvaxyn WNV) has recently been given marketing authorisation for use in horses in the EU.
With regard to availability of diagnostic tests WNV, we consider that:

a) At present, testing is offered in the UK to private vets for the differential diagnosis of neurological disease in horses (the Plaque Reduction Neutralisation Test) where the vet considers it low down the list of differentials and AHVLA agree to the submission. For general surveillance in wild birds, and diagnosis of WNV in horse CNS tissue, a PCR technique is used which is the National Reference Method and will detect both WNV lineages. Therefore the UK currently has sufficiently sensitive tests to diagnose WNV in both suspect cases and surveillance programmes.

4 Background

This qualitative risk assessment considers the likelihood of the introduction of West Nile virus (WNV) via various pathways to the United Kingdom. It builds on our previous assessments (http://www.defra.gov.uk/animalh/diseases/monitoring/index.htm) following official reports of confirmed cases of WNV in horses, wild birds and humans in Southern and Eastern Europe and new information relating to vector populations across the UK.

Unless otherwise stated, this document uses official information received from the World Organisation for Animal Health, Paris, France (http://www.oie.int/eng/info/hebdo/A_INFO.HTM) and the European Commission, Brussels, Belgium (Animal Disease Notification System, Weekly Reports, CVO Emergency Notifications, SANCO Documents). Maps were produced using ESRI Data and maps CD - 2002. (Note: All maps in this document are for visual purposes only).

This document primarily addresses the likelihood of the introduction of WNV from abroad to the UK animal and a potentially competent vector population.

We acknowledge that the introduction of the disease into the UK may have some consequences for our equine industry. Potential for further dissemination in the UK has also been briefly discussed.

5 Hazard Identification

The hazard is identified as: West Nile virus
West Nile virus is a notifiable equine encephalomyelitides transmitted by mosquitoes. It is widespread across the EU in wild birds and mosquito vectors and gives rise to seasonal outbreaks in wild birds and spillover into humans and horses. Recent reports suggest the disease could be considered endemic in certain regions in Europe, including Northern Italy.

As one of the equine encephalomyelitides, WNV is notifiable in the EU. According to domestic legislation in the UK, WNV is subject to official notification and control. That is, every case with suspected relevant clinical signs must be reported to official authorities and subjected to investigation. Since June 2008, Veterinary Surgeons wishing to rule out WNV, as part of a differential diagnosis, can do so in consultation with the local Animal Health Divisional Office (Drummond, 2008).

5.1 West Nile virus - disease reports

WNV is a single-stranded enveloped RNA virus which belongs to the family Flaviviridae. In nature, the virus circulates between wild birds, as an amplifying host, and competent biological vectors (i.e. mosquitoes of the genus Culex). The vectors also act as a bridge for the transmission of the virus to other susceptible species (i.e. many species of mammals, domestic poultry, amphibians and reptiles). The role of these species as reservoirs of the virus for a local competent vector population remains largely uncertain.

WNV appears to be widely distributed throughout the world (see map below based on data from the OIE disease reports and country status).
For large areas of the world, comprehensive data on the incidence of WNV are not readily available. In the temperate zones of North America, Europe and the Mediterranean Basin, the incidence of WNV disease appears to be seasonal with peak activity from July through October (Hayes and others, 2005).

Introduction and spread of WNV in non-affected areas is usually attributed to movement of infected wild birds or wind dispersion of infected competent vectors.

Worldwide, two broad groupings (lineages) of WNV have been recognised:

a) WNV lineage I is found in all continents except Antarctica (e.g. in Africa, Europe, the Middle East, North America, India and Australia) (Dauphin and others, 2004). Bakonyi and others (2006) suggest that possibly two distinct strains of Lineage I may be circulating in Central Europe. The first strain is more closely related to the Israel and North American strains and the second is more distantly related. The first strain appears to be more virulent not only in North America, but also in Central Europe;

b) WNV lineage II has historically been restricted to sub-Saharan African and Madagascar (Dauphin and others, 2004). However, for the first time outside Africa a WNV strain belonging to this lineage was identified in
Hungary in 2004 and 2005 in non-migratory birds of prey (Bakonyi and others, 2006). This lineage is now endemic in parts of southern Europe.

5.1.1 European Union

The disease situation across Europe has been developing significantly in recent years. In 2009 Italy reported West Nile virus across the Po river valley in the North East and went on to suggest that virus had successfully overwintered in the mosquito population. In 2010 there were reports of disease in Italy as well as in wild birds in Hungary and Austria. The outbreaks were attributed to WNV lineage II. In 2011 West Nile virus has re-emerged in Southern Europe once more. Two lineages are currently circulating: lineage 1 in the West and lineage 2 in the East. In 2010/11 disease in either humans or horses caused by lineage II was reported from Greece, Romania, Russia, Italy, Israel, Albania, Macedonia, Hungary, Turkey, Bulgaria and Ukraine while Spain, Portugal, Tunisia and Morocco reported WNV lineage I in humans and horses (see Map below).

The first clinical case of WNV in Spain was detected in a horse in September 2010 in Andalusia (Southern Spain). In 2010 Andalusia had the highest rainfall during spring and the hottest summer in the past decade, the area also contains a high number of wild bird nesting areas, providing ideal conditions for Culex Spp. of mosquitoes. This outbreak prompted a surveillance programme in humans, equidae and wild birds to be initiated, during 2011, 5 outbreaks were reported in Andalucía (García-Bocanegra et al., 2011).
The first case of WNV in Greece was detected in a horse in Thessaloniki in August 2010. During June 2011 44 outbreaks in horses were reported across Attiki, Central Macedonia and Thessaly, at the end of June 2011 WNV circulation was also detected in sentinel chickens in central Macedonia, suggesting that WNV may be actively circulating in central Macedonia and that it may have overwintered in Northern Greece (Chaskopoulou et al. 2011).

Italy reported that clinical suspicion of WNV in a horse was confirmed on 8 September 2008 in Ferrara Province. Following confirmation, surveillance has been taking place in the risk areas. In 2011 nearly 100 outbreaks were reported in horses across Umbria, Veneto, Basilicata, Calabria and Sicily, with a large concentration of outbreaks occurring in Friuli-Venezia Giulia, where disease is now considered endemic.

The European situation remains at present quite different to that in North America, where disease is more pathogenic for birds, humans and horses. This may be explained by the different climate conditions and particularly by the mosquitoes in North America where more species are able to act as bridge species over a far wider environmental range.

6 Risk Question

This risk assessment considers the risk posed by West Nile virus to the UK indigenous wildlife and horses. The specific risk question addressed is:

What is the risk of introducing West Nile Virus to the UK through various pathways and what is the consequent risk of spread through UK wildlife and to horses?

To answer the above question, the risk assessment follows the OIE framework of release (or entry), exposure and consequence assessment. Specifically, it is divided into three key areas:

1. What is the probability of introducing West Nile virus in an infected host (mammal, avian or insect) into the UK from current affected countries?
2. What is the probability of West Nile virus becoming established in the UK in the local mosquito and avian population?
3. What is the probability of a UK resident horse becoming infected with West Nile virus given contact with an infected host?

7 Risk assessment
7.1 Terminology related to the assessed level of risk

For the purpose of the risk assessment, the following terminology will apply (OIE, 2004; EFSA, 2006):

<table>
<thead>
<tr>
<th>Level</th>
<th>Definition</th>
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<tr>
<td>Negligible</td>
<td>So rare that it does not merit to be considered</td>
</tr>
<tr>
<td>Very low</td>
<td>Very rare but cannot be excluded</td>
</tr>
<tr>
<td>Low</td>
<td>Rare but does occur</td>
</tr>
<tr>
<td>Medium</td>
<td>Occurs regularly</td>
</tr>
<tr>
<td>High</td>
<td>Occurs very often</td>
</tr>
<tr>
<td>Very high</td>
<td>Events occur almost certainly</td>
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7.2 Definitions

For the purpose of the risk assessment, the following definitions will apply:

<table>
<thead>
<tr>
<th>Term</th>
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<tbody>
<tr>
<td>Equidae</td>
<td>Means wild or domesticated soliped mammals of all species within the genus Equus of the family Equidae, and their crosses (European Commission, 2008)</td>
</tr>
<tr>
<td>Poultry</td>
<td>Means fowl, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants, partridges and ratites (Ratitae) reared or kept in captivity for breeding, the production of meat or eggs for consumption, or for re-stocking supplies of Game (European Commission, 2008)</td>
</tr>
<tr>
<td>Captive birds</td>
<td>In general terms, means birds other than poultry as defined above, which are subjected to commerce.</td>
</tr>
<tr>
<td>Trade</td>
<td>Means intra-Community trade between EU Member States</td>
</tr>
<tr>
<td>Importation</td>
<td>Means temporary admission, re-entry after temporary export, and imports from Third Countries to the EU.</td>
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7.3 Exceptions

This risk assessment is mainly concerned with the risk of disease from the EU and neighbouring countries, rather than other affected countries such as USA and Canada. However third country trade is mentioned briefly to reflect the possibility of trade via another EU Member State.
Additionally, the risk to humans is outside the remit of Defra and will not be covered here. Please refer to the Health Protection Agency website [www.hpa.org.uk](http://www.hpa.org.uk) for further information.

Other non-vector transmission routes once the virus has been introduced into susceptible species in the UK will not be covered here.

### 7.3.1 Key starting assumptions

The key assumptions in this risk assessment are that:

a) Undisclosed infection may be present in live equidae or their products that may be imported to the UK via legal trade or illegal movements from the currently known affected areas;

b) The virus may also enter the UK via migratory wild birds or by infected vectors being brought in by prevailing winds or some other means;

c) Favourable ecological conditions may exist in the UK and would support the establishment and further spread of the virus should it be introduced.

### 7.3.2 Uncertainties

This risk assessment acknowledges that our current understanding of worldwide distribution, the epidemiology of WNV and potential pathways for the introduction and further dissemination has a number of limitations. Therefore, any inferences made have a degree of uncertainty.

### 7.4 Key criteria

#### 7.4.1 Broad considerations on epidemiology of WNV

In natural conditions, WNV is maintained in enzootic cycles of transmission between mosquitoes and wild birds. Certain limiting factors involving either the vector, the host or the environment will mean the transmission cycle cannot be completed. These will therefore limit the spread of the disease in certain areas.

The proportion of infected susceptible bird species does not necessarily indicate the importance of that species in the ecology of the disease. Unless an infected species develops sufficient level of viraemia to effectively infect competent vectors, such species (horses and other mammals) are considered dead end hosts and are highly unlikely to play a role in further propagation of the virus or act as a source of virus for further transmission (Miles, 1960) (see Fig.1).
The intensity of transmission and distribution of WNV would primarily depend on the interaction of infected hosts, vectors, WNV genetics, time and temperature. The minimum temperature for WNV transmission is considered to be $14^\circ$C (Kilpatrick and others, 2008).

Vector capacity is paramount in the transmission of vector-borne diseases. The term is used to describe contributing, interdependent factors such as vector competence (ability to transmit the infectious agent), biting rate (which is host and vector density dependent) and incubation period (which is temperature dependent). All of these are limiting factors in the ability of WNV to infect and spread through a population (Fig 2). Under optimal conditions several factors must be fulfilled to complete the
transmission cycle.
• Infected mosquitoes must survive long enough to develop sufficient
  levels of virus in the salivary gland to be able infect a vertebrate host
  while feeding.
• Infected birds must develop a sufficient level of viraemia (e.g. because
  of a low host immune response) to be infectious and live long enough to
  be available to transmit the virus to mosquitoes.
• Contact between an infected bird and the competent vector must occur.
• Temperature and climatic conditions must allow development of the
  virus in both vector and host.

According to various reports (Savage and others, 2007, and references therein),
WNV has been detected in about sixty species of mosquitoes, of which
approximately 20 species have been demonstrated to be competent vectors in
laboratory conditions. Nevertheless, not all of them would play an important
role in natural transmission cycles.

If a competent vector is to play an important role in the transmission of the
virus, it must be present in large numbers at the relevant period of time and
must feed freely on the species which are most infected by the virus (Miles,
1960). Further transmission and spread would depend on sufficiently high
densities of infected competent vectors and susceptible birds in the area where
virus was introduced. In temperate countries this would usually occur during
the summer months and could be associated with prolonged periods of
increased temperatures and drought.

WNV may be detected in salivary secretions of infected mosquitoes within 10-
12 hours post infection (Kilpatrick and others, 2008). In vivo studies also
demonstrated that infected mosquitoes inoculate approximately $10^4$ to $10^6$
plaque forming units (PFU) of WNV extravascularly and approximately $10^2$ PFU
of the virus intravascularly while probing and feeding on a live host (Styer and
others, 2007). These doses are estimated to be some 600 times greater than
estimated by in vitro capillary tube transmission assay. The amount of virus
inoculated by infected mosquitoes increases with longer probing times reaching
a maximum dose after approximately 4-6 minutes of probing. WNV has also the
potential to severely affect salivary gland function which could extend the time
taken for a mosquito to detect blood. This, in turn could lead to interrupted
feeding and increase the possibility of infecting multiple hosts due to extended
probing time (Styer and others, 2007).

According to Hayes and others (2005) vertical transmission of WNV has been
experimentally demonstrated in Culex pipiens, Cx. quinquefasciatus and Cx.
tarsalis. The virus has also been isolated from hibernating mosquitoes. This
could explain the maintenance of infection from one year to the next, when
continuing transmission to birds has ceased. The same paper also reports the
levels of viraemia required for infection of mosquitoes (at least $10^5$ PFU/ml
blood) compared to the amount circulating in humans (approximately $10^3$ PFU/ml). Hence humans are not involved in the natural transmission cycle, but are considered dead end hosts.

It should also be noted that potential variations in WNV virulence (i.e. the ability to cause a clinical disease) may be vector species specific (Foral and others, 2007).

7.5 Entry assessment

7.5.1 Legal Trade

This assessment recognises the following major groupings of pathways by which WNV may be introduced to equidae in the UK by trade or non-illegal routes (Fig.3).

**Conclusion:** There is a very low risk of a migratory wild bird arriving in this country infected with WNV, and of infection then being transmitted to a susceptible animal by a competent vector.
Key assumptions:

a) Many wild bird species are susceptible to WNV infection and remain asymptomatic. Only certain infected wild birds can be considered competent hosts for WNV and could be involved in establishment of the disease in the UK;

b) Certain species of wild birds may be migratory between affected regions in Europe and UK. However, most migratory birds which overwinter in the UK will arrive from September onwards. This is reaching the end of the vector season. This could reduce the level of contact between UK mosquito and wild migratory bird populations.

c) A competent vector population is only present in certain areas and at certain times of the year in the UK. However, stability and persistence of the virus is influenced by a number of different biological, physical and environmental factors.

Supporting evidence

Generally, WNV viraemia in wild birds is short lived (Dauphin and Zientara, 2007). Birds remain viraemic for up to about a week (Jarvi and others, 2008; Langevin and others, 2001). There have been reports of longer persistence of viral RNA in experimentally infected birds in North America, which may explain the over-wintering process for disease (Reisen and others, 2006). However, the presence of viral RNA does not necessarily indicate active viraemia.

There is a considerable wealth of evidence suggesting a large variety of birds are susceptible to WNV (see http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm for details). Over 300 species of bird have been reported as being infected. Some remain asymptomatic while in others infection has serious effects and can lead to large scale die-offs.

In laboratory studies, Passeriformes (song birds), Charadriiformes (shorebirds), Strigiformes (owls) and Falconiformes (hawks) developed high enough levels of viraemia to infect most feeding mosquitoes, whereas Columbiformes (pigeons), Piciformes (woodpeckers) and Anseriformes (ducks) did not. Certain birds, including houses sparrows, house finches and various corvids were more susceptible to infection and experienced high mortality rates (>40%). These experimental infections were carried out using the NY99 strain, responsible for the outbreaks in the USA and closely related to that isolated from the Israel outbreaks (Hayes and others, 2005). Of all these species, Passeriformes have the longest duration of viraemia (Komar and others, 2003).
Migration of susceptible birds from Europe to UK will occur at certain periods during the year. The most common species involved are some of the dabbling ducks, egrets, hawks and falcons, gulls and terns, pigeons, some finches, some owls, a small number of rooks and large numbers of starlings. The UK sits within the east Atlantic flyway which crosses Spain and the West coast of Africa (Fig 4), this also connects with the Black Sea and Mediterranean flyway and the West Asia and East Africa flyway.

Migratory flyways (the most common routes of long distance bird migrations) are shown in map below. This would suggest that the majority of migratory birds arriving in the UK would be part of the East Atlantic Flyway, which overlaps with the areas where both WNV Lineage I has been detected (North Africa and the Iberian Peninsula) and Lineage II (Austria, Northern Italy etc). Therefore the level of mixing between flyways at certain common wild bird breeding grounds could lead to further mixing between birds and therefore between lineages.

Figure 4: Migration flyways for wild birds

We cannot exclude a possibility that the UK bird populations, both migratory and sedentary, may be capable of maintaining West Nile Virus infection under favourable conditions. According to expert opinion (Var. Pers. Comm., [British Trust for Ornithology, Wildfowl and Wetlands Trust, Joint Nature Conservation Committee]) among the groups of birds known to be susceptible, the passerines are the most numerous in the UK, including starlings, crows, sparrows, finches
and thrushes. Other orders of birds which contain susceptible species are the owls, pigeons and doves, parrots, game birds, swans, ducks and geese, divers, herons, rails and falcons. The absence of susceptible species in the other orders may be merely lack of evidence rather than confirmation of non-susceptibility.

It is, however, important to note that since 2001 the Animal Health & Veterinary Laboratory Agency (AHVLA) in the UK has been carrying out surveillance of wild birds in Great Britain for WNV (Phipps et al., 2008; Brugman et al. 2012). Carcasses of dead wild birds have been submitted to AHVLA regional laboratories in England and Wales and the Scottish Agricultural Colleges (SAC). Between 2003 and 2009 over 2000 migrant and non-migrant birds were tested for WNV using histopathology, virus isolation and viral RNA detection. Samples are taken from all over Great Britain during peak vector season (April to October). Surveillance was targeted at species associated with WNV mortality, including corvids, sparrows and other small passerine species, raptors and water birds and has included samples from mass mortalities of wild birds and those showing neurological signs. Most of the species were wild birds with the majority (92%) as resident species, but also tested were domestic and captive birds. No WNV infection has been detected by PCR or virus isolation since surveillance began in 2001 indicating that widespread avian mortality is not generally a reported feature of WNV in Europe.

Equally in Ireland, nearly 2,500 wild bird, poultry and captive bird samples and nearly 500 equine samples taken between 2005 and 2010 have all tested negative for WNV (Raleigh et al. 2012).

7.5.3 Movements of live equidae

7.5.3.1 Intra-Community trade

**Conclusion:** There is a very low likelihood that WNV may be introduced to the UK via legal trade in live equidae from the currently affected areas in the EU. This conclusion also applies to imports of certain equidae that may have arrived to another EU Member State before entering the UK.

**Key assumptions:**

a) Currently, WNV has been declared endemic in certain parts of northern Italy and infection has also been reported from other EU regions, in horses, wild birds and humans.

b) Intra-Community movement of horses is subject to EU certification rules, however, some exceptions may also apply (i.e. under the Tripartite Agreement)
c) Only a proportion of WNV infected horses will exhibit clinical signs. Nevertheless, infected horses are dead-end hosts with very low levels of circulating virus. Therefore, infected horses are unlikely to contribute to the transmission cycle of the virus in the UK, should it be introduced.

Supporting evidence

WNV is a non-contagious viral disease of equidae. Horses are susceptible to all WNV lineages. During the epizootic in Italy in 2009, only 33 (8.5%) horses developed clinical signs out of a total of 379 testing positive. Five (15.1%) of these 33 horses died. (CESME, 2008). While in 2010 of 128 horses testing positive, only 11 developed clinical signs and five died. These data broadly agree with other published reports of clinical case rates of ~10% (Dauphin & Zientara, 2007). Therefore, this strain does not appear to be causing the high levels of clinical cases and mortality as observed in North America (Figuerola and others, 2008). The reduction in case detection towards the end of December 2008 would suggest the mosquito population declines with the onset of winter (CESME, 2008).

Very little data are available about longevity and level of viraemia in horses. Experimentally infected horses (using the North America strain of the virus, NY-99) developed low viraemia within 1-3 days, of short duration (less than 7 - 10 days), even when an animal developed clinical signs (Bunning and others, 2002). These authors also demonstrated that the low levels of viraemia (<$10^{2.7}$ PFU/ml) in the horses did not give rise to infection in mosquitoes fed on the animals. It is therefore reasonable to assume that naturally acquired infection leads to a short-lived viraemia, as demonstrated in other mammals. For example, experimental infection in rhesus macaques resulted in viraemia lasting up to five days (Ratterree and others, 2004). In humans viraemia appears 1-3 days post infection and lasts 1-11 days, at which time seroconversion occurs (EMEA 2003).

Some infected horses may exhibit neuroinvasive clinical signs. While equidae (i.e. horses, mules, donkeys and zebras) may become infected, they are not considered to play a significant role in the epidemiology of the disease because they do not develop sufficient levels of virus that would result in effective transmission to a competent vector (Hayes and others, 2005).

Movements of equidae within the EU (and Norway) must comply with EU rules. That is, all horses must be accompanied by a passport and comply with movement and import conditions with regard to equine encephalitides. Equidae must come from holdings which meet requirements for freedom from dourine, glanders, equine encephalomyelitis of all types (including WNV), vesicular stomatitis, equine infectious anaemia, rabies, anthrax and African Horse
Sickness. The rules require that if a holding was to confirm a case of WNV, the holding is not considered free until six months have elapsed following the slaughter of the affected equidae. The same rules apply for trade in equidae from Third Countries.

Equidae that are travelling between UK, Ireland and France under the terms of the Tripartite Agreement are not required to be issued with a health certificate. This agreement is currently under review and some horses which are not registered with an approved society may be required to travel with a health certificate in the future.

7.5.3.2 Third Country trade

**Conclusion:** There is a very low likelihood that WNV may be introduced to the UK via legal trade in live equidae from an EU approved Third Country where the virus is present. This conclusion also applies to imports of certain equidae that may have arrived to another EU Member State before entering the UK.

**Key assumptions:**

a) Currently, WNV is present in many countries worldwide;

b) WNV has been present in North America since 1999, and has been responsible for a significant number of equine (and human) cases. There is a significant movement of equidae (mostly vaccinated against WNV) from North America to the UK and the EU, and no case of WNV in the EU has been linked to such imports;

c) The infection of certain equidae with WNV due to exposure to infected mosquitoes while in transit is considered as a chance event subject to fulfillment of optimal conditions for infection to occur;

d) Imported equidae from outside the EU are subject to veterinary checks at Veterinary Border Inspection Posts approved for the species in the EU;

e) Only a proportion of WNV infected horses will exhibit clinical signs.

f) Infected horses are dead-end hosts with very low levels of circulating virus. Therefore, infected horses are unlikely to contribute to the transmission cycle of the virus in the UK, should it be introduced.
EU rules permit the import of live equidae from approved Third Countries or their territories (European Commission, 2004). Current EU import rules distribute Third Countries, authorized for imports of certain categories of equidae, into different sanitary groups on the basis of the potential animal health risk they pose. Furthermore, live equidae are also categorised in accordance to their final import purpose (re-entry, temporary admission, slaughter, and breeding and production) (European Commission, 1992a and 1993, 1993a & 1993b). As an example, the map below shows the different groups of countries authorized for imports to the EU of registered horses and horses for breeding and production.

![Map of EU import rules for equidae](image)

**Note:** Legend indicates that each of these groups will have slightly different risk management requirements for WNV for the purpose of entry to the EU.

Equidae from the approved countries must come from holdings which meet requirements for freedom from dourine, glanders, equine encephalomyelitis (including WNV), vesicular stomatitis, equine infectious anaemia, rabies, anthrax and African Horse Sickness. For full details, please refer to the appropriate legislation.

If a holding in the country of origin was to confirm WNV, and the other animals are not slaughtered, then the holding is not considered free until six months have elapsed following the slaughter of the affected animal. Furthermore, USA and Canada (which belong to Group C in the map above) have to certify
whether or not the horse has been vaccinated against WNV at least 30 days prior to dispatch. A large number of equidae move between the EU and USA and Canada. Despite the WNV situation in the USA and Canada, no outbreaks of WNV in the EU have been linked to such movement.

EU rules require that all imported equidae from Third Countries are checked (i.e. documentary, physical and identity checks) at the port of entry to the EU (Border Inspection Post - BIP). Post-import testing of equidae from all approved Third Countries is subject to a risk assessment by the BIP veterinary inspector at the time of inspection.

EU rules also allow for triangulation. That is, equidae from a non-approved Third Country may be imported into the EU by spending a certain period of time in an approved Third Country to allow official certification for import into the EU. It would be possible that some of those horses, if infected, would show clinical signs of the disease during the compulsory residency period prior to entry into the EU. In such cases, the import would not occur as certificates could not be officially signed off.

EU rules provide a list of approved Third Countries from which EU Member States may import equidae (European Commission, 2004). In accordance with these rules, the UK does not approve transit through a non-approved Third Country or transhipment with unloading at a sea/airport located in a non-approved country prior to import. However, because no legal provision is made for verification of what takes place during the transhipment, the UK only approve such practice where no alternative routing is feasible and information is available to ascertain that the risk is minimal (such as airports located away from vector risk areas). It should also be taken into account that the use of modern, fully vector-proof jet stalls completely closed with mesh and sprayed with insecticide effective against competent vectors would mitigate the risk. Furthermore, these horses normally travel with a veterinarian or a specifically trained groom who can be asked to verify that animal health was not jeopardised.

### 7.5.4 Equine germplasm

#### 7.5.4.1 Intra-Community trade

**Conclusion:** There is a negligible likelihood that WNV may be introduced to the UK via legal trade in equine semen, ova or embryos from currently affected areas in the EU.

**Key assumptions:**
a) The existing risk management measures are considered proportionate to mitigate against possible exposure of live equidae to WNV via these pathways;

b) While it could be expected that virus may be present in blood circulation during viraemic phase, we are not aware of any publication that actually contain data on the presence (or detection) of the virus in semen, ova or embryos.

c) Only a proportion of WNV-infected horses will exhibit clinical signs and therefore not be eligible for collection of germplasm.

Supporting evidence

EU rules require donor animals to come from holdings which are free of equine encephalomyelitis of all types (including WNV). If WNV was confirmed at a holding, and animals are not slaughtered, then the holding is not considered free until six months have elapsed following the slaughter of the affected animal. Furthermore, EU legislation requires semen to be held frozen for at least 30 days prior to trade. Considering that the incubation period of WNV is 15 days, this gives ample time for clinical disease to manifest in the donor animal, the holding or other animals in the area around the collection centre, so that blood samples could be taken to ensure that the donor animal was not viraemic at the time of collection.

7.5.4.2 Third Country trade

Conclusion: There is a negligible likelihood that WNV virus may be introduced to the UK via legal trade in equine semen, ova or embryos from EU approved third countries.

Key assumptions:

a) The existing risk management measures are considered proportionate to mitigate against possible exposure of live equidae to WNV via these pathways;

b) While virus may be present in blood circulation during viraemic phase, we are not aware of any publication that actually contain data on the presence (or detection) of the virus in semen, ova or embryos.
c) Only a proportion of WNV-infected horse will exhibit clinical signs and therefore not be eligible for collection of germplasm.

Supporting evidence

As for EU trade, EU rules for imports from EU approved Third Countries require donor animals to come from holdings which are free of equine encephalomyelitides (including WNV) for 6 months since the slaughter of the last infected animal.

Semen must be held frozen for at least 30 days prior to trade. This period would provide sufficient time either for clinical disease to manifest in the donor animal, the holding or other animals in the area around the collection centre, so that blood samples could be taken to ensure that the donor animal was not viraemic at the time of collection.

7.5.5 Authorized biologicals not known to be contaminated

7.5.5.1 Intra-community trade and imports from Third countries

**Conclusion:** There is a negligible likelihood that WNV virus may be introduced to the UK via legal trade in specified biologicals from affected areas of the EU or from Third Countries where WNV is known to be present.

**Key assumptions:**

- **a)** Import of such products are permitted from EU and from approved Third Countries and are subject to official certification;
- **b)** The virus may be present in specified biologicals if raw material is sourced from equidae incubating the disease, however, the levels of the virus would be low;
- **c)** Processing of such products is considered sufficient to destroy the virus.

**Supporting evidence**

Limited data suggest that WNV can be transmitted between horses by parenteral inoculation of infected blood or organ suspensions (Henning, 1956). Also, there has been confirmed transmission of WNV in humans by blood transfusion, organ transfer and breast milk (OIE Terrestrial Manual, 2008).
According to available data, infection with WNV in equidae results in short periods of viraemia (1-11 days). All plasma derived authorised medicinal products are subject to standard risk reduction measures (e.g. pasteurisation, solvent/detergent treatment, vapour heating and filtration with 15nm filters) and these are considered effective against WNV (EMEA, 2003).

EU rules permit imports of the blood of equidae from approved Third Countries subject to specified conditions (European Commission, 2002) similar to the conditions for intra-Community trade. According to the same EU rules, raw sera from equidae may be imported from approved Third Countries under specified conditions.

7.5.6 Research samples (including equine sera)

7.5.6.1 Intra-Community trade and imports from Third Countries

**Conclusion:** There is a negligible likelihood that WNV virus may be introduced to the EU (and the UK) via research samples.

**Key assumptions:**

a) Such import is subject to licence or authorisation; the existing risk management measures are considered adequate to destroy the virus.

**Supporting evidence**

Such samples may originate from susceptible animals that develop very low and short lived viraemia. EU rules require samples to be treated and handled in laboratory conditions subject to licensing or authorisation conditions that require handling according to good laboratory practices.

7.5.7 Poultry and captive birds

7.5.7.1 Intra-Community trade

**Conclusion:** There is a negligible likelihood that WNV virus may be introduced to the EU (and the UK) via certain species of poultry or captive birds.
Key assumptions:

a) Most species of birds can become infected with WNV. Viraemia in birds is of relatively short duration and the clinical outcome of infection is variable.

b) WNV in domestic poultry is not notifiable under EU rules. Chickens and turkeys are susceptible to infection but appear to be resistant to disease. They are highly unlikely to act as a source of the virus for competent vectors;

c) It remains uncertain whether young ducks and geese may be competent reservoir hosts. Nevertheless, given that viraemia in these species is short lived (i.e. a few days) commercial consignments of these species (i.e. hatching eggs; day-old-chicks) would be highly unlikely to act as a source of the virus for competent vectors in an importing country due to the existing EU rules related to management and trade practices;

Supporting evidence

WNV in domestic poultry is not notifiable under EU rules. Therefore, intra-community trade in poultry and captive birds does not require compliance with any specific WNV requirements.

In experimental conditions many domestic poultry species have been shown to be susceptible to infection via the bite of infected competent vectors or by exposure to the virus orally or by needle injection. Nevertheless, these experiments have also demonstrated that infection caused by infected competent vectors caused different responses compared to infection caused by needle inoculation (Langevin and others, 2001). Therefore, this assessment will review literature data that relate to potential infection by infected mosquitoes in experimental or natural conditions.

While experimental infections of newly hatched chicks resulted in viraemias which may be high enough to infect susceptible mosquitoes (Ciota and others, 2008; van de Meulen and others, 2005; Senne and others, 2000), the viraemia appears to be short lived (up to 6 days). This was also demonstrated by experimental infection with a North American strain of WNV (Langevin and others, 2001). Infection of chickens over three weeks of age result in insufficient levels of viraemia to infect competent mosquitoes (Swayne and others, 2001). It also appears that infected chickens are not able transmit the virus to cohorts or human handlers which justifies their use as sentinels, not only for WNV but also other vector-borne viral infections. It has also been suggested that backyard and free-range flocks are more exposed to infection than housed (intensively reared) flocks. However the latter are more likely to be commercially traded than the former. (P. Calistri, pers. comm.).
In experimental conditions turkeys have been demonstrated to develop a low level of viraemia which was considered insufficient to transmit the virus to competent biological vectors (van de Meulen and others, 2005). While the virus was detected in faeces on day 4 and 7 post infection, no transmission to contact cohorts was demonstrated (Swayne and others, 2000). Therefore, turkeys are not considered to play a significant role as amplifying hosts for the virus or potential source of the virus to local populations of potentially competent biological vectors.

In natural conditions, WNV infection was reported in 17-weeks old farmed domestic ducks kept permanently outdoors in Canada. The infection resulted in 38.7% mortality. However, no disease was observed in young geese kept in the neighbouring pen (Wojnarowicz and others, 2007).

In natural conditions, WNV infection of geese has been reported in Israel, Hungary and Canada (various authors cited in Meece and others, 2006) resulting in significant mortalities. Geese are therefore believed to play a role in local WNV ecology in certain situations (Swayne and others, 2001). In experimental conditions, domestic geese had viraemias lasting up to 5 days and increases in antibody responses were evident at day 14 after infection (Jarvi and others, 2008; Swayne and others, 2001). Nevertheless, geese (and ducks and pigeons) are considered to have a far lower competency index than corvids and other competent reservoir species (Komar and others, 2003). This is supported by experimental infections of wild birds which indicated that commercial mallards, Canada geese and pigeons are only weakly competent hosts comparatively speaking (Komar and others, 2003). Pigeons may be susceptible to infection but are unlikely to act as amplifying hosts (Hayes and others, 2005, Komar and others, 2003).

EU rules ensure that the potential risk of exposure to infected competent vectors is minimised by management practices related to transport of hatching eggs and day-old chicks of domestic poultry (European Commission, 1990).

### 7.5.7.2 Third Country trade

**Conclusion:** There is a negligible likelihood that WNV virus may be introduced to the EU (and the UK) via certain poultry or captive birds.

**Key assumptions:**

a) EU rules allow imports of poultry from approved countries only;
b) Poultry may become infected with the virus, however, they are unlikely to act as a source of infection for local mosquito population.

Supporting evidence

EU rules allow poultry imports from EU approved Third Countries (European Commission 1990). Other supporting evidence as stated in the section 6.4.2.5.1 applies.

7.5.8 Non-avian/non-equidae species (e.g. exotic ungulates, reptiles)

7.5.8.1 Intra-Community trade

**Conclusion:** There is a negligible likelihood that WNV may be introduced to the UK via legal trade in live non-avian and non-equidae species, such as ungulates from the currently affected areas in the EU.

**Key assumptions:**

a) Intra-Community movement of exotic ungulates (not equidae) is subject to EU rules (i.e. the Balai directive) which do not specifically mention WNV. While WNV is not one of the diseases listed under these rules, it is expected that official clinical inspection would detect any signs of disease, which would then have to be further investigated.

b) Not all infected ungulates will exhibit clinical signs. Infected ungulates are dead end hosts with very low levels of circulating virus, and therefore would not contribute to the transmission cycle of the virus in the UK, should it be introduced.

c) Currently, there are no EU rules that regulate animal health requirements for reptiles.

Supporting evidence

Live ungulates that are traded within the EU must come from an approved premises and comply with certain conditions, including certification for disease freedom, registration and identification. They are also subject to pre-movement clinical inspection (European Commission 2004c) and must undergo a veterinary check at the point of entry (Border Inspection Post) to the EU.
There are no animal health requirements for the intra-Community trade in reptiles. Although certain other reptile species are not competent amplifying hosts, there is some evidence for experimental infection of alligators leading to viraemias sufficiently high to infect feeding mosquitoes (Hayes and others, 2005). Transmission through close contact has been confirmed in birds and alligators in laboratory conditions, but not reported in wild populations. Therefore the role of reptiles in the transmission of WNV remains uncertain.

7.5.9 Illegal movements

**Conclusion:** The likelihood that WNV virus may be introduced to the EU via illegal movements of equidae and/or their products is difficult to determine.

**Key assumptions:**

a) Illegal activities may pose the risk of the introduction of WNV virus into any non-affected country;

b) While horses are considered to be a dead-end host with very low viraemia, the likelihood of introduction of WNV via equine products is considered to be negligible.

**Supporting evidence**

We are not aware of any evidence to suggest introduction of the WNV virus to non-affected countries due to illegal activities. However, as demonstrated with some other disease introductions, this possibility cannot be excluded.

This again emphasises the importance of maintaining appropriate enforcement measures at the border and raising awareness among horse owners and industry of potential risks that may be associated with illegal activities, particularly the use of unauthorised biologicals that could potentially be contaminated with WNV.

7.5.10 Accidental import of biological vectors

**Conclusion:** There is a very low likelihood that infected mosquitoes may inadvertently be introduced with the import of plants or through other transport media from countries where WNV is present.
Key assumptions:

a) Some insect (mosquito) species are associated with plants where they lay eggs in the water of wet-footed plants and consequently may be transported with them;

b) Additionally there have been reports of mosquito eggs introduced in car tyres as desiccation-resistant ovipositions.

c) This is not known to be the case for the Culex vectors of WNV, which generally do not withstand desiccation;

d) The proximity of WNV infectious birds, therefore potentially infected mosquitoes, to crops of plants intended for imports into the EU would have to be considered in the context of existing EU rules.

Supporting evidence

Countries exporting plants to the EU have to comply with EU rules for such imports. From within the EU, all rooted plants require a phytosanitary certificate issued by the plant protection service of the exporting country, or can only imported under licence issued by the importing country.

Certificates for imports from EU-approved Third Countries must state that any plant and plant material are free from pests, diseases and any other organisms. Such imports are subject to checks on arrival into the EU at the BIP of entry.

There is well-known evidence that mosquito populations have become established over the past few years in parts of Europe that were traditionally outside their geographical distribution. In particular the Asian tiger mosquito, *Aedes albopictus*, which transmits chikungunya virus, seems to have become established in northern and central Italy, France and as far north as Germany (CDC, 2009; Friedrich Loeffler Institute, 2012). It seems that this mosquito has rapidly spread around the world due to trade in second-hand car tyres (CIEH, 2008). It was also introduced into the Netherlands through the trade in imported *Dracaena* (lucky bamboo) (J. Medlock, pers. Comm.).

7.5.11 Wind borne introduction of infected vectors

Conclusion: There is a negligible likelihood that infected mosquitoes may be introduced via wind borne introduction from countries where WNV is present.
Key assumptions:

a) Disease is currently limited in Europe to the southern and eastern regions.

b) Wind borne introduction of mosquitoes is possible but dependent on local conditions and flight behavior.

Supporting evidence

Countries reporting WNV in the EU have to date been restricted to the southern and eastern European countries. The map in section 5.1 shows the recent outbreaks. The UK would be at greater risk if WNV were identified in mosquitoes in the Low Countries due to their proximity.

Smaller biting midges such as *Culicoides* are known to be blown considerable distances and therefore can be introduced from Europe in the wind plumes, as evidenced by Bluetongue BTV-8 and more recently Schmallenberg virus introductions. The larger and heavier mosquito species, such as *Anopheles* or *Culex* can also be blown long distances under exceptional circumstances but this is a less frequent occurrence. Active flight of certain mosquito vector is approximately 1 km (EFSA, 2005). Wind dispersal on the other hand is less easy to predict and would be influenced by local topography and environment as well as the flight behavior of the species involved, such as whether day flying or crepuscular (EFSA, 2005).

7.6 Exposure assessment

As already discussed, horses are dead-end hosts with very low short lived viraemia and therefore even if an infected horse were to arrive in the UK, it would not be involved in onward transmission. Should an infected wild bird arrive in the UK, the potential for WNV transmission to a competent vector in the UK would be determined by potential for contact with, and the abundance of, competent vectors (also see Fig. 2 and description on limiting factors).

The *Culex pipiens* life cycle describes adult mosquitoes generally hibernating from October until March (J. Medlock, pers comm.). Any later hibernation or earlier emergence leads to severe mortality rates. Nevertheless, there is an estimated 10% survival rate. However, this means populations of mosquitoes would be decreasing at around the time of peak bird migration. It has also been
recognised that the disease has a seasonal pattern in the temperate parts of North America.

The Minimum Infection Rate (MIR) which would be needed to produce an epidemic of WNV in the UK remains uncertain. In the USA, the MIR was estimated to be at anywhere between 5% to 0.5% (i.e. one infected mosquito out of 20 to 1 in 200), however, an even lower MIR could give rise to an epizootic (Hayes and others, 2005).

Recognised WNV competent vectors are known to be present in the UK. *Culex pipiens s.l.* and *Ochlerotatus* spp. Were considered by far the most important in terms of UK distribution, however in 2010 *Culex modestus*, a bridge species (that bites birds, horses and humans), were identified in marshland southeast of England. *Culex modestus* was previously reported in the UK (Portsmouth area) during the 1940s in very low numbers (<10), but had not been found since. It is possible that *Cx. modestus* could have been continually present in southern England between 1945 and 2010 without being detected. *Culex modestus* is a principle vector of West Nile virus in parts of Europe, the occurrence of this species in North Kent marshes in habitats frequented by migratory birds and grazing horses could be a consideration when conducting surveillance for West Nile virus (Golding et al., 2012). Figure 4 indicates the areas of the UK where *Culex pipiens s.l.* (Fig. 4a) and *Ochlerotatus cantans* (Fig. 4b) populations have been found in the UK, according to a study conducted in 1998 (Snow and others, 1998). *Culex pipiens s.l.* represents a species complex, which includes *Cx. pipiens pipiens* which only feeds on birds and the more catholic *Cx. pipiens molestus* (which bites mammals and also occasionally birds in the UK).

Fig 5. Vector distribution in the UK, according to Snow and others, 1998
Medlock and others (2007 and 2005) reviewed the potential for the UK mosquitoes to act as enzootic (i.e. between birds) and bridge vector species (i.e. from birds to mammals) from an ecological and entomological perspective. *Culex pipiens sensu stricto* and *Cx p. molestus* are the main *Culex* species endemic in the British Isles. In the UK the ubiquitous *Cx. pipiens pipiens* form (i.e. *Cx pipiens s.s.*) is considered to exclusively bite birds (ornithophilic), whereas the more geographically restricted *Cx. p. molestus* form is predominantly mammalophilic, but also bites birds. This characteristic would make *Cx. p. molestus* the main bridging species for the UK where it occurs. In certain habitats, or adjoining them, other mosquito species might act as bridge vectors - eg adjacent to wetlands and woodlands - see Medlock and others, (2005), Medlock & Vaux (2011).

Populations of the potential West Nile virus (WNV) vector *Culex modestus* were identified in 2010 in marshland in southeast England (Medlock & Vaux, 2011). Morphological identification was confirmed by DNA barcoding. The abundance of adults peaked in early August.

Mosquito surveys were carried out during 2010 in the North Kent Marshes, south-east England. Larval surveys were carried out at two sites - Cliffe marshes and Elmley National Nature Reserve - each visited in June, July and August. In larval surveys 850 *Cx. modestus* of all stages were collected, along with *Anopheles maculipennis* s.l., *Culex pipiens* s.l. and *Culiseta annulata*. 
**Culex modestus** was the second most abundant species after *Cx. pipiens* s.l. in larval sampling at both sites, making up 44 and 23% of the overall larval population sampled at Cliffe and Elmley respectively.

A total of 649 adult female *Cx. modestus* were captured at Northward Hill between 12 July and 10 September, with a peak of 325 adults in the second week of August. *Culex modestus* comprised 75% of the mosquitoes collected at Northward Hill. Morphological identification of *Cx. modestus* was confirmed by DNA barcoding and phylogenetic analyses.

*Culex modestus* was previously reported in the UK (Portsmouth area) during 1940s in very low numbers (<10), but has not been found since. It seems unlikely that *Cx. modestus* could have been continually present in southern England between 1945 and 2010 without being detected. The mosquito fauna of the North Kent Marshes has been well sampled over this period, with the Elmley site having been the focus of an extensive survey in 2003. It is therefore plausible that these UK *Cx. modestus* populations have become established only recently. The reasons for this are unknown, but international shipping terminals in the area of the North Kent Marshes could be a possible route of introduction for *Cx. modestus*.

In 2003 a population of *Culex pipiens molestus* was reported in Clackmannanshire, Scotland. The colony was believed to have arisen as a result of movement of transport to a local factory and suggested that the mosquito was capable of surviving in the UK, albeit in this semi-underground factory. Genetic analysis using microsatellite “DNA fingerprinting” techniques suggested the population originated in Africa and had been reported in Marseille spreading north across Europe. The colony was destroyed and no further reports have been made.

*Culex modestus* is a principle vector of West Nile virus in parts of Europe, the occurrence of this species in North Kent marshes in habitats frequented by migratory birds and grazing horses could be a consideration when conducting surveillance for West Nile virus. Migration flyways are a useful tool to assess the movement of potential bird reservoirs around Europe. A Defra funded research project on the Mapping of West Nile risk in the UK and Europe did not predict areas of the UK as being at significant risk under present environmental conditions, and this is mirrored by the distribution of certain suitable vector species such as *Culex pipiens molestus* and *Cx p. sensu.sticto*. This situation is likely to change with changing climate and may already be doing so in areas of South East England. The report pointed out the importance of continuing surveillance of European mosquito species along with investigations into their feeding behaviour and ecology. Satellite data such as
used in this project, are important to guide field studies but cannot replace field data.

Mean risk map where environmental conditions are suitable for *Culex pipiens molestus* establishment (From Rogers, 2010)

Predictive map of WNV suitability according to environmental conditions across Europe (blue dots represent WNV positive sites)
7.7 Consequence assessment

Horses are dead end hosts and only a percentage of them appear to be susceptible to WNV. For example, in 2010 in Italy, out of a total of 415 horses on affected farms, only 128 tested positive for WNV and of those, only 11 were clinical cases, however of those 11, 5 died so mortality is high among clinical cases (http://sorveglianza.izs.it/emergenze/west_nile/pdf/bollettino_2010.pdf).

The horse population of the UK is widely distributed but poorly registered. A recent Defra funded project carried out by Glasgow University estimated the population from various data sources and suggested the majority of horses are located in the South, Central and West regions of England. The East Anglia region where the highest risk areas for wild bird and vector incursion from the Continent and vector distribution has one of the lower horse populations.

Therefore a combination of small area of the UK which is suitable for WNV establishment in competent vectors, the low horse population in the area of
greatest risk and low viraemia in any infected horses suggests the consequence of disease introduction may be considered relatively low.

8 Control and risk management options

There are several risk management options available for West Nile virus to prevent infection of a horse. Avoiding the area of highest risk; vaccinating the horse; avoiding being bitten by an infected vector by not going out at dusk and dawn.

8.1 Vaccines

**Conclusion:** Highly effective vaccines have been available for horses in the USA for a while. This has reduced the prevalence of the disease in the USA. One of these has recently been authorized for use in the EU.

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<th>Key assumptions:</th>
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<tr>
<td>a) Vaccines are available in the USA for WNV;</td>
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<tr>
<td>b) At present, one vaccine is licensed for use in horses in Europe.</td>
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</tbody>
</table>

**Supporting evidence**

According the OIE manual (2008), the United States Department of Agriculture (USDA) has licensed a formalin-inactivated WNV vaccine and a live canarypoxvirus vectored WNV vaccine for use in horses. An inactivated human cell line-derived WNV vaccine developed by Crucell NV (the Netherlands) and Kimron Veterinary Institute (Israel) is licensed in Israel as a veterinary vaccine for geese. The USDA has also licensed a WNV DNA vaccine for animals, which contains genes for two WNV proteins, and therefore, does not contain any whole WNV, live or killed. Furthermore, a chimeric vaccine, based on a yellow fever virus vector, was licensed by USDA for use in horses. These vaccines are widely used in the US, are efficacious and safe and give protection against neurological signs of WNV infection.

All recombinant vaccines are produced against NY99 WNV strain (North American strain) (Dauphin & Zientara, 2007). The inactivated WNV vaccine has been used to protect domestic geese in Israel, but there are limited reports of good protection. Other vaccines are currently being tested but are not available at present.
In November 2008, a WNV Vaccine was licensed for use in the EU (EMEA, 2008). The vaccine is an inactivated WNV strain VM-2 vaccine and is licensed for use in horses over 6 months of age. The VM-2 strain is a North American isolate with a high phylogenetic relationship to European strains and strong cross reactivity with strains isolated from France and Romania. Use of the Duvaxyn WNV vaccine in the UK is currently under discussion.

8.2 Diagnostic tests

**Conclusion:** The UK has sufficiently sensitive tests to diagnose WNV in both suspect cases and surveillance programmes.

**Key assumptions:**

a) Testing is offered in the UK to private vets for the differential diagnosis of neurological disease in horses (the Plaque Reduction Neutralisation Test) where the vet considers it low down the list of differentials and AHVLA agree to the submission.

b) For general surveillance in wild birds, and diagnosis of WNV in horse CNS tissue, a PCR technique is used which is the National Reference Method and will detect both WNV lineages.

There are a number of tests deployed to diagnose WNV. However, there does not seem to be agreement on which test and which tissues should be used in surveillance (various authors cited in Foral and others, 2007). However, Foral and others (2007) evaluated the immunofluorescence test (IFA) by comparison with polymerase-chain reaction (PCR) test and demonstrated that IFA has excellent sensitivity for the spleen and high specificity when the spleens or kidneys are used as a test tissue. Therefore, IFA performed on tissue impressions of spleen and kidney should be considered important for selection of tissues for surveillance purposes.

A WNV strain belonging to lineage II was detected for the first time outside Africa in Hungary in 2004 and 2005 - in non-migratory birds of prey. While most routine tests in Europe are designed to detect WNV lineage I strains, inter-laboratory comparison studies suggested that <40% of the participating laboratories were able to detect WNV lineage II strains of WNV (Bakonyi and others, 2006). While a certain level of serological cross-reactivity could be expected between the two lineages, the question remains to what extent the
tests, currently used in Europe, would be effective in detecting infection with lineage II strains of WNV virus.

The AHVLA offer the Plaque Reduction Neutralisation Test (serology) for WNV to private vets for the differential diagnosis of neurological disease in horses. They are also in the process of developing an antibody capture ELISA for WNV which will detect both Lineage I and Lineage II, although it has not undergone full validation yet.

For the national surveillance programme, a nested RT-PCR method is used to detect WNV in wild bird tissues and for diagnosis of WNV in equine CNS tissue from horses which have died from neurological disease. This method is a National Reference Method and has been shown to be able to detect both lineages although it does detect lineage II less sensitively than would be expected. The VLA have also developed a pan flavivirus real time PCR which detects both lineages of WNV as well as other flaviviruses, including Louping Ill, Usutu virus, Tick borne encephalitis virus, dengue virus and Japanese encephalitis virus which could be used to screen in any outbreak situation in wild birds or horses.

9 Conclusions

The possibility that WNV infected wild birds may arrive in the UK cannot be excluded. However, we consider that they would pose a very low risk of introducing the disease, such that it becomes established in the UK because of the requirement for there to be a convergence of competent vector populations and infected migratory bird. This would change if disease became established in the Low Countries during the next vector season because the proximity may mean more infected birds or even infected vectors may enter the UK. Mosquito populations of the most common WNV vector species in the UK, Culex pipiens pipiens, have a wide geographic distribution and are generally ornithophilic (i.e. feed on birds), however the newly identified population of Culex modestus located in marshland in the East of England may act as a bridge species as they bite horses, birds and humans. Although this small population of C. modestus has now been identified in East Anglia and is thought to be established, this constitutes a low vector density and therefore a low risk.

Therefore we consider that the potential for establishing an enzootic cycle between potentially WNV infected wild birds and potentially competent local mosquito population in the UK would be very low, requiring the convergence of optimal epidemiological, entomological and ecological conditions.

The introduction of infected vectors via long range transport either on the wind, in sea cargo, air cargo or in live plant material cannot be excluded, but
the likelihood of establishment of these mosquitoes is low, as the environmental conditions in many areas of the UK are not suitable at present. That may change in the future, according to changes in land management practices (such as establishing wetlands) or climate, but is difficult to predict.

Horses entering the UK from a WNV affected region may have been at risk of contracting WNV. Nevertheless, even if horses were infected, they would have exhibited low viraemia for short periods of time and would have posed a negligible risk of introducing the virus and aiding establishment of the disease in the UK.

Domestic poultry, including ducks and geese, and racing pigeons are considered to have low, short lived viraemias and therefore pose a very low risk of introduction of disease. On the basis of the epidemiology of WNV and the existing import and trade regulations for live poultry and hatching eggs which require additional biosecurity measures to protect against avian influenza and salmonella, we consider that the majority of commercial consignments would pose a negligible risk of the introduction of the virus to the UK. Therefore, any additional risk management measures would be considered restrictive to trade.

While ungulates may have the potential to become infected, there has been no indication of this occurring in Europe during the current situation, or worldwide. Although in Italy in 2009 cattle were detected with neutralising antibody, there was no definitive proof that the animals were infected during that outbreak. On the basis of the epidemiology of WNV we consider that commercial consignments of ungulates would pose a negligible risk of the introduction of the virus to the UK. Therefore, any additional risk management measures would be considered restrictive to trade.

We also consider that meat and other products of animal origin, germplasm and biological samples would pose a negligible risk of the introduction of the virus to the UK.

10 References


