DISCONTOOLS

West Nile Virus

Control Tools

Diagnostics availability

Commercial diagnostic kits available worldwide

A range of diagnostic kits are commercially available for use in humans and animals. These are generally for use in laboratories and there is no indication of pen side test availability.

Most are based on ELISA, often competition ELISA (humans, horses, birds) and IgM-capture ELISA (humans, horses). Several CE IVD-marked WNV IgM and IgG serology assays, ELISA or CLIA, are available for use in humans; fully validated WNV IgG and IgM ELISA assays (with batch validation performed by European or international reference laboratories) are available for use in animals. Besides their use on blood sera, some tests can detect antibodies also in plasma and Cerebro-Spinal Fluid (CSF). WNV IgG avidity assays are also available to distinguish recent from past infections in humans.

Several WNV molecular assays are commercially available for use in humans (CE IVD-marked) and others in animals. These tests are based on real-time RT-PCR and can detect WNV only or other targets besides WNV (multiplex real-time RT-PCR). Some tests are quantitative. WNV RNA can be detected by molecular assays in serum, plasma, whole blood, urine, and CSF and some tests are validated for all these different types of samples. These tests have good sensitivity and high specificity. FDA-approved and CE IVD-marked Nucleic Acid Tests (NAT) for the qualitative detection of WNV RNA in individual living blood donors and other donors of substances of human origin (SoHO) are available in Europe and the USA. Suitable sample is plasma. These tests have high sensitivity and show cross-reactivity with other flaviviruses such as Usutu virus.

WNV serology and molecular tests widely used in many EU countries for WNV surveillance in humans.

List of commercial diagnostic tests (Diagnostics for Animals)

GAPS :

Sensitivity and specificity of WNV IgM and IgG serology assays for use in humans and animals are variable among tests. Cross-reactivity with closely related flaviviruses (such as Usutu and Tick-Borne Encephalitis viruses prevalent in Europe) is common. Specificity of serology kits needs improvement.

Diagnostic kits validated by International, European or National Standards

Competition and IgM capture ELISA validated by the EU-RL for WNV (plus other RT-PCR and ELISA kits in the future).

Diagnostic method(s) described by International, European or National standards

See chapter 3.1.25 of the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. (1).

Commercial potential for diagnostic kits in Europe

Both serological and molecular diagnostic tests in horses are currently of commercial interest in Europe due to the recent EU outbreaks. The market in Europe is limited (in particular for real-time RT-PCR kits; serological screening is more widely used). The opportunities for the development of diagnostic kits are increasing with the increasing number of outbreaks; nonetheless there is still little incentive to develop new diagnostic kits for the screening of WNV infections in animals.

Tests for use in humans are of commercial interest. Increasing market potential in Europe for both molecular and serology tests due to the expansion of WNV with associated human outbreaks. WNV NAT tests for screening of SoHO donors have also a large commercial potential.

GAPS :

Cross-reactions with other flaviviruses reported in Europe (and specifically Usutu, Tick-Borne Encephalitis, Louping ill and Bagaza viruses) are frequently identified in WNV circulation areas.

The use of target antigens that are less cross reactive – prM, Domain III of E, NS1 and NS5 – has to be promoted. While NS1 ELISAs in animals are interesting for the differentiation of flavivirus infections, NS1 ELISAs in humans only reduce the occurrence of cross-reactions (in particular in humans with a history of Dengue virus infection). The development of binding domain assays as for SARS-CoV-2 serology in humans is essential.

DIVA tests required and/or available

A differential diagnostic assay, such as an ELISA assay against non-structural (NS) protein, can be used to distinguish immunity derived from vaccination vs. natural infection whether marker vaccines are used.

A DIVA test in horses would be useful for monitoring the virus circulation in areas with surveillance and control activities. If countries or events require WNV as a CORE vaccine in horses, a DIVA vaccine would be important (provided that a marker vaccine – recombinant vaccine deleted for NS protein(s), highly purified inactivated vaccine for example- is used against WNV).

GAPS :

One needs to consider what is measured by serological tests. If an unvaccinated horse developed neutralizing antibodies, one could deduce that horse, at some time in its past, had been exposed to WNV. It is likely protected from infection for its life.

Competitive and indirect assays targeting NS1 should be effective at differentiating between naturally infected and vaccinated animals. DIVA tests are accordingly available (for example, inhibition NS1 ELISA developed and performances assessed on experimental sera), but not fully validated and tested in field conditions.

Eradication of arboviral infections in wild reservoirs is not practical nor realistic.

Vaccines availability

Commercial vaccines availability (globally)

Only vaccines for use in animals have been registered.
A number of vaccines have been authorised in North America.
In 2003 a monovalent inactivated whole-virus vaccine for horses (West Nile-Innovator) was fully licensed in the USA.
In 2004 a modified live canarypox virus (CNPV)-vectored WNV vaccine expressing prM/E genes (Recombitek Equine West Nile Virus) was licensed for use in horses. In the same year an inactivated human cell line vaccine developed by Crucell NV (the Netherlands) and Kimron Veterinary Institute (Israel) obtained a market authorisation in Israel as a veterinary vaccine for geese.
In July 2005, the USDA issued the first fully licensed WNV DNA vaccine for animals in the USA (West Nile Innovator DNA). This vaccine, expressing two WNV proteins prM/E, has been discontinued.
A life chimeric vaccine (PreveNile), based on a yellow fever virus (YFV) vector expressing prM/E envelope proteins, was licensed by USDA for use in horses in 2006 but was discontinued in 2010.
In 2009, a killed virus vaccine (Vetera WNV) was licensed by the USDA.
An inactivated chimeric vaccine (EquiNile) was licensed in 2011.

A number of multivalent vaccines to control WNV, Eastern and Western Equine encephalomyelitis and in some cases Venezuelan encephalomyelitis are available in the USA.

In the EU, the West Nile-Innovator vaccine (based on the lineage 1 VM-2 strain) was licensed in November 2008, under the name Duvaxyn WNV, comprising the first WNV vaccine to be used in Europe. Since 2012 this vaccine has been marketed as Equip WNV.
In August 2011 Proteq West Nile, a modified live CNPV-vectored WNV vaccine containing the prM and E genes, was approved for use in horses. Equilis West Nile, an inactivated chimeric vaccine, based on a yellow fever virus vector and containing the envelope proteins E and M was licensed in June 2013. All recombinant vaccines are produced based on the NY99 WNV strain (North American strain). Although all the vaccines licensed in Europe are based on WNV lineage 1 strains, protection against infection with WNV lineage 2 has been demonstrated either through experimental challenges (Equip WNV and Proteq West Nile), based on serological data (Equilis West Nile), or under field conditions based on clinical and laboratory investigation (Equip WNV).

GAPS :

No WNV vaccines have been approved for use in humans.

Marker vaccines available worldwide

A differential diagnostic assay, such as an ELISA assay against NS protein(s), can be used to distinguish immunity derived from vaccination vs. natural infection whether conventional, DNA or vectored vaccines not including NS proteins or NS-encoding genes are used. The WNV DNA, canarypox virus and chimeric YFV-vectored vaccines do not contain non-structural proteins (NS1 to NS5) and are expected not to induce anti-NS antibodies. Conversely, WNV-Innovator conventional killed vaccine has been shown to elicit anti-NS immunity (anti-NS1 antibodies) after vaccination, indicative of the presence of NS1 proteins in the vaccine suspension.

At least the CNPV- and chimeric YFV-vectored vaccines could be used as marker vaccines in Europe.

GAPS :

The definition and validation of DIVA assays is needed prior to the characterization and validation of marker vaccines.

Effectiveness of vaccines / Main shortcomings of current vaccines

WNV lineages 1 and 2 are the most widespread and caused most of the major epidemics and epizootics encountered so far. In Europe, both lineages are reported, while lineage 1 strains only are reported in the USA.

All horse vaccines licensed in Europe have been shown to reduce viremia following challenge with WNV lineage 1. In addition, the CNPV-vectored and chimera vaccines were demonstrated to reduce clinical signs associated with WNV infection.

Protection against WNV lineage 2 following challenge in horses was demonstrated for the inactivated whole-virus and the CNPV-vectored vaccine. Vaccination with either of these vaccines reduced both viremia and clinical signs in horses following an experimental challenge. The inactivated whole-virus vaccine was also demonstrated to be efficacious against encephalitis due to natural infections with the Greek lineage 2 strain under field conditions. For the chimera vaccine, protection against lineage 2 viruses was suggested based on serology data.

All 4 currently marketed USA horse-licensed vaccines are likely to demonstrate good protection in the field.

Vaccines licensed for use in horses in Europe have shown to be effective at protecting birds against WNV pathology (falcons, geese).

GAPS :

Horse owners are likely to use different registered vaccines for prime-boost and annual booster immunization depending on the availability of vaccines and costs. Thus, duration of immunity and long-term protective efficacy should be established for prime-boost with different combinations of registered vaccines. The protective status of post-vaccination exposure should be established that will allow horse owners to determine if the annual revaccination is essential to protect their horses against re-exposure.

It should be kept in mind that in an endemic area, a vaccinated horse may also be naturally exposed. Only a small percent of exposed horses (even unvaccinated ones) ever demonstrate clinical disease with WNV. In new incursions with non-vaccinated horses, clinical disease will be high as well as death loss. In the USA, there are focal outbreaks that recur annually or every 3-4 years. When it involves small collections of non-vaccinated horses, there is a relatively high number of affected horses with mortality. This also occurs in Canada.

In Europe this may be lower due to circulation of other flaviviruses, possibly providing cross-protection against WNV clinical disease (partial or complete protection offered by USUV immunity not assessed in equids, but demonstrated in mice and magpies).

One of the most important barriers to vaccine effectiveness is market penetration. 1) There is little information in the USA on vaccine use in horses – estimates are as low as 40% vaccine administration across the USA. As regards the EU market, the information is even scarcer. 2) Are annual vaccine campaigns followed up? Updated guidelines with information risk based on geographical information on past outbreaks, in the

region of residence as well as in countries or regions in which horses frequently move should be published.

Commercial potential for vaccines in Europe

In the current epidemiological situation and given the large range of vaccines available in horses, the potential for new vaccines with similar profile as the existing ones could be considered limited. An improved vaccine would include one shot vaccine with early onset of immunity and long duration of immunity.

WNV vaccines are not available for humans but are urgently needed. Requirements for vaccines for humans are safety and high and sustained immunogenicity and efficacy, including in elderly and immunocompromised individuals.

Cross-protective immunity against WNV by Japanese encephalitis virus (JEV) vaccine has been reported. In laboratory settings, personnel that manipulate WNV can be offered a vaccine against JEV.

GAPS :

Regulatory hurdles exist in the USA and likely, the EU for updating vaccines as well as development of new vaccines.

Regulatory and/or policy challenges to approval

Regulatory guidance concerning the use of genetically modified vaccines is available but the use of GMOs is still a sensitive topic in some countries.

Commercial feasibility (e.g manufacturing)

Feasible to manufacture a range of types of vaccines.

Opportunity for barrier protection

In principle if WNV becomes a problem in a country, vaccination can be used as a prevention measure.
Competitive and indirect assays targeting NS1 should be effective at differentiating between naturally infected and vaccinated animals, depending on the vaccine type used (marker vaccines not including NS-expressing genes: DNA, CNPV- and chimeric YFV-vectored vaccines).

GAPS :

Development of one-shot vaccine for earlier onset of immunity in horses is needed.

The role of vaccination as a protective tool while WNV is emerging in an area or country can be questioned. While the detection of WNV clinical cases in horses is generally very rapid after symptoms onset, the onset of neurological signs follows the bite of an infected mosquito by 3-14 days. Can the currently used prime-boost WNV vaccination protocols be effective at limiting epizootics intensity and duration? The induction of protective immune responses takes a long time (at least 4-5 weeks), when WNV epizootics generally last 10 weeks and its acute phase when most neuro-invasive cases in horses last 4-6 weeks.

Pharmaceutical availability

Current therapy (curative and preventive)

No specific antiviral-drugs are available for human patients and animals with WNV infection. Humans and horses are given supportive therapy (fluids, anti-inflammatory drugs, vitamins).

Some immunoglobulins have been used in hamster models and humans and showed beneficial effects also in recovery and chronic lesions. Antibody product(s) have been conditionally licensed in the USA. Anecdotal reports of efficacy.

GAPS :

Biodelivery and bioavailability data are not available for most antiviral drugs found effective at decreasing WNV replication in cellular models.

Moreover, the time window during which antivirals would be effective is essentially unknown.

Achieving sufficient delivery across the blood-brain barrier is a key challenge in the development of drugs to treat central nervous system (CNS) disorders. Should WNV antivirals be developed, what would be the best strategies to ensure their delivery to the CNS (transcytosis, nanoparticles, …)?
What would be the effects of WNV antivirals on WNV-infected cells as well as other CNS cells (neurons, microglia, …)?

Generating antibodies for passive immunization for human use is a promising direction, should there be a real demand.

Therapeutics could be a useful support to ring-vaccination to limit the number of WNV clinical cases before the establishment of vaccine-induced immunity– such a strategy should be tested and evaluated beforehand.

Future therapy

Candidate targets of drugs for WNV infection include viral proteins involved in viral entry and genome replication, such as the NS3 protease and the NS5 polymerase, and, to a lesser degree, the E glycoprotein, C protein, NS4B, NS3 helicase, and NS5 MTase. The most promising anti-WNV drug candidates target conserved enzymatic motifs in viral NS3 protease and NS5 polymerase and are effective against different flaviviruses. Targeting host factors required for viral infection and replication (e.g., cell entry factors, endoplasmic reticulum and Golgi apparatus) and modulation of host innate antiviral response (e.g., autophagy induction) are also promising approaches, which may lead to the development of compounds with broad-spectrum antiviral activity.

A recombinant neutralizing monoclonal antibody against the E protein has been investigated in phase 1 and in a pilot study as a therapeutic antibody in humans. These studies showed a high rate of adverse events and no therapeutic efficacy.

GAPS :

No major funding is available for the development of antiviral drugs since WNV infection in humans and animals is perceived as self-limiting.

Targeting shared motifs and signalling pathways between flaviviruses, or host factors required for viral infection and replication or for the modulation of host innate antiviral response are also promising approaches, which may lead to the development of compounds with broad-spectrum antiviral activity. The development and registering of antiviral peptides and drugs for other conditions than WNV (COVID-19,…) could also benefit WNV therapy.

Commercial potential for pharmaceuticals in Europe

Nil at present.

GAPS :

No major funding is available for the development of antiviral drugs since WNV infection in humans and animals is perceived as self-limiting.

Regulatory and/or policy challenges to approval

None.

Commercial feasibility (e.g manufacturing)

Areas of interest would be in controlling the mosquito through improved methods of prevention and reduction in numbers. Novel, improved methods of surveillance were recently reported based on providing sugar to mosquitoes or on analysing mosquito excreta (2). Innovative control strategies such as the release of transgenic mosquitoes or of mosquitoes infected by bacterial symbionts could be used as in the case of dengue virus control, provided that strategies effective against Aedes albopictus and Ae. aegyptimosquitoes are adapted to Culex mosquitoes.

GAPS :

No major funding is available for the development of antiviral drugs since WNV infection in humans and animals is perceived as self-limiting

New developments for diagnostic tests

Requirements for diagnostics development

A major problem for WNV serological assays is their high degree of cross-reactivity with antibodies produced in response to other simultaneous and/or previous flavivirus infections; and the long-lasting IgM response in humans. New tests and kits are being developed and there remains a need for improved sensitivity, specificity, costs, and practicality.

- Fast and easy to use antigen detection tests that could be used to detect infected animals, preferably not requiring a BSL-3 laboratory for their implementation.
- Molecular tests that can differentiate between WNV lineage 1 and WNV lineage 2 and used to monitor outbreaks.
- Serological tests that can be used in several animal species, and in particular in equids and birds.
- DIVA serological tests (see section 1.5).
- High throughput and fully automated platforms for serology and molecular assays.

Several human and animal WNV diagnostic tests are licensed (see Section "Commercial Diagnostic kits available worldwide").

GAPS :

Penside tests and Point-Of-Care Testing would be interesting to develop, for rapid detection of WNV (human and horse neuroinvasive disease cases, dead birds), e.g., lateral flow, agglutination as well as rapid molecular tests (RT-LAMP, etc.). Point-Of-Care Testing would also help in determining the serological status of animals and humans (lateral flow,…)

Multiplexed assays such as luminex or protein microarrays can use multiple antigens from multiple flavivirus species to improve the sensitivity and specificity of WNV serological assays.

The integration of WNV in syndromic rapid tests for the diagnosis of CNS infections or arbovirus infections in humans and animals would be useful.

No major funding is available for such new developments in diagnostic tools, with most of the tools needed for WNV case confirmation being licensed.

Time to develop new or improved diagnostics

Time and costs depend on the nature of the test. Several years may elapse between research output and the test becoming commercially available.

GAPS :

There is a long wait before the licensing of validated commercial diagnostic tests.

Cost of developing new or improved diagnostics and their validation

Developing and validating diagnostic tests and kits are time consuming and labour intensive which can result in high costs, in consideration of the still limited commercial potential.

Validation of diagnostic tools for use in animals in the EU is performed by the EU-RL (equine diseases, including West Nile disease).

GAPS :

Cost effectiveness in humans demonstrated for the WNV NAT screening of SoHO donors returning from WNV endemic areas.

Research requirements for new or improved diagnostics

Work closely with medical researchers and commercial companies to evaluate new tests developed for use in humans to assess whether they could be adapted to animals.

Identify alternative test methodologies and potential for rapid reliable diagnosis in the field as well as the laboratory. Development of new tests required for reservoir hosts, birds and mosquitoes.

GAPS :

Penside and rapid tests would be interesting to develop, for rapid detection of WNV (human and horse neuroinvasive cases, dead birds).

Multiplexed syndromic tests would be very useful for the differential diagnosis in humans and in animals with neurological symptoms or febrile illness.

No major funding is available for such developments.
A multi-species diagnostic test would be the most useful.

New developments for vaccines

Requirements for vaccines development / main characteristics for improved vaccines

An improved vaccine would include one shot vaccine with early onset and long duration of immunity. Current vaccines that are on the market already have good safety and efficacy profiles.

Eradication of disease is impossible due to extensive wildlife reservoirs (birds) and bird migration.

Diagnostic tests aim at detecting disease for clinical purposes. Although for this purpose no DIVA test would be required, a DIVA assay would still be useful in identifying WNV emergence in horse populations.

Requirements for vaccines for humans are safety and high and sustained immunogenicity, including in elderly and in immunocompromised individuals.

GAPS :

Since West Nile Fever is a WOAH notified disease, the possibility to differentiate an infected from a vaccinated animal is a crucial issue. The availability of a DIVA test would be useful when surveillance activities are carried out in areas with WNV vaccination to determine the extent of the infected area. As a matter of fact, in those areas, vaccination of horses is applied to prevent the clinical form of the disease.

One shot, non-infectious (replicon-based, RNA) vaccines or subunit vaccines could be beneficial to protect animals against West Nile disease. Needle free delivery would be a plus, especially in the wild or captive avifauna.

Time to develop new or improved vaccines

Time consuming (5-10 years), but time to develop dramatically reduced in recent years.

Cost of developing new or improved vaccines and their validation

Depending on product profile and requirement. There is no real need for other or improved vaccines for animals. The currently available vaccines are offering good protection against lineage 1 and 2 strains.

Development of vaccines are expensive but may be spin off from medical research into vaccines for humans. Vaccines in humans are needed to offer clinical protection in individuals at risk of severe neuroinvasive forms of West Nile disease, including the elderly and immunocompromised individuals.

GAPS :

Promoting research to establish the correlates of protection will reduce the cost of developing new and improved vaccines.

The development and validation of new WNV vaccines in animals may make the development of a human vaccine cheaper and more attractive for vaccine companies to take up this need.

Research requirements for new or improved vaccines

Current WNV vaccines licensed in the EU have shown good safety and efficacy profiles. Therefore, there is no immediate need for additional research and development for new vaccines in animals.

At variance, research is needed to develop human vaccines. Several candidate human vaccines, based on different platforms (DNA plasmid vaccines; protein subunit vaccines, hydrogen peroxide and formaldehyde inactivated whole virus vaccines; live, attenuated chimeric vaccines; vectored vaccines), are available and have shown immunogenicity and efficacy in animal models, and safety and immunogenicity in phase 1 and phase 2 clinical studies in humans.

GAPS :

The results achieved so far in vaccine development for humans are promising and support further research to generate an effective vaccine. Issues that need to be addressed in vaccine development are the immunological cross-reactivity between flaviviruses and the associated risk of infection enhancement.

In addition to domestic animals, animals in zoos, wildlife reserves and recovering centres, etc., would be contemplated in vaccination campaigns.

New developments for pharmaceuticals

Requirements for pharmaceuticals development

There is a urgent need for effective antiviral drugs or monoclonal antibodies to treat patients and animals with West Nile Fever and Neuroinvasive Disease. Broad-acting antiviral drugs are preferable.

GAPS :

No major funding is available for the development of antiviral drugs and other pharmaceuticals since WNV infection in humans and animals is perceived as self-limiting.

Generic therapeutic approaches modulating the immune response and targeting WNV immunopathology should be prompted. Drugs targeting host factors involved in viral entry, replication, and neuroinvasion are also of interest.

Time to develop new or improved pharmaceuticals

Time consuming (several years) to investigate the efficacy, bioavailability and toxicity of new pharmaceuticals.

GAPS :

No major funding is available for the development of antiviral drugs and other pharmaceuticals since WNV infection in humans and animals is perceived as self-limiting.

Cost of developing new or improved pharmaceuticals and their validation

Costs are very high (random testing of natural or chemical products, clinical studies,…).

GAPS :

No major funding is available for the development of antiviral drugs and other pharmaceuticals since WNV infection in humans and animals is perceived as self-limiting.

Research requirements for new or improved pharmaceuticals

Generic therapeutic approaches modulating the immune response and targeting WNV immunopathology should be prompted. Drugs targeting host factors involved in viral entry, replication, and neuroinvasion are also of interest.

GAPS :

No major funding is available for the development of antiviral drugs and other pharmaceuticals since WNV infection in humans and animals is perceived as self-limiting.

Disease details

Description and characteristics

Pathogen

West Nile Virus (WNV) is a single-stranded enveloped RNA virus which belongs to the genus Orthoflavivirus in the family Flaviviridae. It is a mosquito-borne virus grouped within the Japanese Encephalitis virus serocomplex.
An approximately 11-kilobase (kb) genome contains a single open reading frame (ORF) that is translated in its entirety and cleaved by both cell and viral proteases into 11 viral proteins containing three structural proteins, including capsid (C), premembrane (prM)/membrane (M), and envelope (E), and seven nonstructural (NS) proteins.
Maturation of the virion through proteolytic cleavage of the prM precursor to its processed 8 kDa form, M, is required to yield a fully functional infectious viral particle. The ORF is flanked by 3? and 5? untranslated regions (UTRs) involved in RNA stability, suppressing immune response, and modulating replication.

The E protein is a dimeric protein composed of three domains and E-DI has been associated with virulence in mice models. The other domains of the E protein display also important functions: DII exhibits the fusion peptide, essential for viral RNA delivery into the cytoplasm, and DIII through which the virus interacts with the cell receptor, holds its main neutralization sites.

Several members of the genus Orthoflavivirus are responsible for neurological disease in horses including Japanese encephalitis virus (JEV), West Nile virus (WNV), Kunjin (KUN), and Murray Valley encephalitis virus (MVEV), although KUN, endemic in Oceania is now considered a strain of WNV. In addition, the Tick-borne encephalitis virus (TBEV) transmitted by Ixodes tick bite and found throughout Europe can cause disease in horses, as shown in Austria and Switzerland.

GAPS :

Recent studies focussed on the identification and role of viral proteins and on the function of the programmed ribosomal frameshifting (able to generate an additional NS protein, NS1’ in the members of the Japanese Encephalitis virus serocomplex) but further research is needed.

There are close to 60 different species of viruses in the genus Orthoflavivirus that are capable of being transmitted by arthropod vectors

(mosquitoes or ticks) to the targeted hosts. Traditionally, they are classified into different serological groups based on serological relatedness among them. Understanding the antigenic structure of the protective antigens encoded by the flaviviruses that may influence the host tropism and immune responses are critical to improve the specificity of serodiagnostics and the development of an efficient marker vaccine.

Molecular studies are needed to embrace the genetic diversity and relatedness among virus strains and clades and to identify which molecular signatures are associated with increased virulence in vertebrate hosts or increased transmission in mosquitoes. Increased full genome sequencing of virus obtained from mosquitoes and virus obtained from brains of humans and horses is needed. How does the virus change and create variants in vivo? What is the viral diversity within the vertebrate host and are there specific ‘quasispecies’ upon infection that enter the CNS?

Entry receptors in humans and other species should be identified further. Although some cell surface molecules (such as the ?v? 3 integrin) have been proposed as WNV cell receptors, a big knowledge gap is still lingering in this specific topic. Lack of detailed knowledge on the interactions that this virus establishes with the surface molecules of the target cells impairs a more in-depth understanding of many essential processes governing its behaviour, e.g. cell tropism and host range; vector competence/capacity; pathogenicity and virulence for certain hosts; molecular basis of virulence, transmissibility and antibody neutralization, in addition to phenotypic differences (in virulence, transmissibility, etc.) between viral strains.

Variability of the disease

Phylogenetic analyses have identified 7 to 9 lineages of WNV, with lineages 1 and 2 distinctly dominant and associated with human disease. Lineage 1 is found in all continents except Antarctica. Lineage 2, which was historically restricted to sub-Saharan Africa and Madagascar, has been detected in Europe since 2004.

Lineage 1 viruses have caused mortality in domestic geese in Israel and in Canada, although numbers are small compared to humans and horses. Lineage 1 viruses have also caused fatal illness in a variety of domestic and wild species of mammals as well as non-mammals such as alligators and some birds. The pathogenicity in birds appears to be related to strain genetics. The role of small mammals such as squirrels in WNV epidemiology is not clear, but most likely minimal, if at all. The recent re-introduction of WNV Lineage 1 in Italy in 2020 has been associated with a significant increase in human diseases.

Lineage 2 viruses have recently been reported as the cause of disease in horses in Africa, as well as in animals (horses, birds, particularly raptors) and/or in humans in Hungary, Austria, Greece, Italy, Serbia, Croatia, Romania, Bulgaria, Slovakia, Czech Republic, France, Germany, Spain and the Netherlands. The list of countries reporting lineage 2 WNV strains is growing fast, see the ECDC website for an update (https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/).

A third lineage has been recently proposed to include the Rabensburg virus, a European strain isolated in the Czech Republic. A fourth independent lineage comprises isolates from Caucasus and is closely related to the proposed lineage 6, aggregating WNV strains identified in Spain (2006) and Austria (2013). It has been suggested that strains isolated in India as early as 1950 form a separate lineage, lineage 5, while initially considered as belonging to lineage 1, clade 1-c. Lineages 7 and 8, respectively, the Koutango virus isolated in Senegal and the Sarawak strain isolated in Malaysia, correspond to divergent WNV strains. A new WNV lineage has been recently isolated in Senegal from Culex mosquitoes, which might be considered the 7^{th} or 9^{th} lineage, depending on the phylogenetic classification chosen (3).

GAPS :

There is a big knowledge gap regarding the forces governing WNV evolution, traits under selective pressure, and how the virus changes and adapts to different environments.

Emergence of distinct clades of WNV in Eurasia and Americas has significantly impacted the welfare and health of humans and animals. Questions remain regarding the origin and introduction routes of such clades, the evolutionary interaction of viruses between vectors (various mosquito species) and amplifying hosts (various avian species) in different environments, and the impact of these interactions related to the derivation of de novovirus strains in the transmissibility of the virus and disease expression or severity of disease-targeted hosts.
The role of recombination in WNV genetic variation and evolution needs a more in-depth evaluation, particularly since we know that many variants, clades and even genetic lineages are co-circulating in wide endemic areas.
Moreover, the impact of the co-infection of vertebrate hosts or vectors by different WNV strains (different lineages) or by closely related flaviviruses (for example WNV and Usutu virus) on immune responses, host and vector competence, virus evolution and pathogenicity should be explored.

Genotype-phenotype correlates of disease in humans and animals should be studied further. The pathogenic potential of WNV lineages other than lineages 1 and 2 for humans and animals deserves attention. Mechanisms of viral entry in the CNS and risk factors for neuroinvasive disease in humans need further investigation. Prognostic markers of severe disease and sequelae should be identified.

Stability of the agent/pathogen in the environment

WNV is inactivated by heat and disinfectants containing detergents or lipid solvents and is susceptible to sunlight and drying. It is also inactivated by sodium hypochlorite and standard laboratory fixatives, such as paraformaldehyde, formalin, and glutaraldehyde.

Understanding inactivation of viruses in the diagnostic laboratory is also important. Not all diagnostic agents are the same. Recent work has indicated that guanidium isothiocyanate, the AVL buffer (RNeasy kit) and the TRIzol® LS Reagent inactivate flaviviruses at 10^6PFU/mL.

GAPS :

Inactivation of WNV in blood supply by UV irradiation is under investigation but without remarkable success. In this regard, methylene blue photoinactivation seems to represent progress in this field but more work is still needed to implement these treatments in blood donations.

Although the role of certain bird hosts and vectors was intensively investigated, further research is needed to fully understand how WNV persists in the environment. The nature of its interaction with bird and vector species circulating in Europe is less well known than in those in the USA. In this regard, persistent infection in wild bird hosts is still poorly understood. The overwintering mechanisms are unknown. The role of alternative arthropod vectors (e.g. ticks) and transovarial transmission has been poorly examined.

Species involved

Animal infected/carrier/disease

Mosquitoes become infected when they feed on an infected bird and ingest the virus in the blood. The mosquitoes act as vectors spreading the virus from infected bird to other birds and to other animals. Mosquitoes and birds (passerines, raptors from Strigiformes and Accipitriformes orders, aquatic birds belonging to the Charadriiform order) serve as WNV amplifying hosts in an endemic mosquito-bird cycle. Spill over to a larger range of animal species occur when infected bridge mosquitoes bite alternative hosts, such as mammals. Equids and humans are the most sensitive mammals to WNV disease; scarce reports also indicate that sheep can develop encephalitis after WNV infection. Other mammals that can be infected are cats, bats, raccoons, chipmunks, skunks, squirrels, cervids, swine, rabbits and dogs but in almost all cases, these animals are dead-end hosts. Most mammals do not develop a sufficiently high and long-lasting viremia to be considered as WNV

carriers/reservoirs. However, WNV infection has also been reported in amphibians and reptiles, and some of these species could serve as amplifying hosts. The alligator work was performed in very young farmed alligators that were raised in warm water to enhance growth. It is questionable whether or not there is sufficient amplification in this host in nature.

WNV has been associated with the death of large populations of wild animals and mainly birds (passerines and raptors in the first place) mostly in association with WNV spread and epizootics in North America; it poses challenge to conservation efforts in endangered wild species (such as the Greater sage-grouse, Japanese quail,…). and could threaten populations of polar bears and wild canids as well The lasting impact of the disease on wildlife populations is difficult to assess.

Certain wild bird species, in particular passerines, play a central role in the amplification of WNV. Many raptors are especially susceptible to WNV infection. Poultry can be infected but do not develop disease unless less than 4 days old when infected. Adult chickens have been used in many countries (USA, Italy, France, Greece) as "sentinels" to detect infection in areas thought to be at risk. Domestic pigeons have also been used similarly.

GAPS :

Bird species dominating transmission in different European endemic circulation areas are essentially unknown. No super-spreader bird species have been identified yet in Europe. The magnitude and duration of viremia, as well as clinical description, has not been assessed extensively in all WNV host species in Europe. In this regard, it is necessary to enhance research to reproduce the effects of WNV infection (using a wide range of strains/lineages) in a wide range of wild bird hosts to assess host competence for transmission and to study genetic and phenotypic adaptations of WNV strains circulating in different bird populations. The factors contributing to the modulation of avian host competence (for example, the simultaneous or serial infection with other flaviviruses, co-infections with other pathogens modifying host immune responses, the microbiome) should be studied further. The development of alternative approaches and in particular of in vitro avian models (cells/organoids/ primary tissue culture, etc.) would be extremely helpful to evaluate host species susceptibility.

Also, non-vector-borne transmission (e.g., direct contact, prey or carrion on infected animals) should be carefully assessed. Duration of infectivity in infected carcasses in field conditions is another gap that needs to be filled.

Moreover, WNV overwintering mechanisms in Europe are poorly investigated and in particular the contribution of the different mechanisms is unknown.

Infections of amphibians have been described; a novel lineage of WNV was described in Uranotaenia unguiculata mosquitoes, which regularly feed on amphibians. Which species are infected and what is the epidemiological relevance of these infections?

Human infected/disease

The majority of people who become infected do not suffer from any illness. Around 20-30 % of infected people develop a ‘flu-like’ disease; a small number (less than 1% of infections) suffer neurologic disease with potentially fatal meningitis, encephalitis, acute flaccid paralysis, or other neurological symptoms.
Around one in ten serious neurological cases can result in death. This rate is even higher in the elderly.
Persistent infections occur in a low percentage of affected individuals, and sequelae of the disease (sometimes severe) may last for long periods (months/years). Urine shedding of the virus may last far beyond the viremic period, and thus urine constitutes a useful source of samples for virus isolation.

GAPS :

WNV has been associated with severe neuroinvasive disease and long-term sequelae in people. Host and viral genetic traits associated with the risk of severe disease and sequelae need further investigation. Mechanisms through which comorbidities (such as hypertension, diabetes or heart condition) enhance the risk of WNV neuroinvasive disease should be investigated more in depth. Are there heart/hypertensive medications that can reduce severity of disease?
The role of host innate and adaptive immune responses in determining WNV infection outcome need further investigation; many factors (co-infection with other viruses such as SARS-CoV-2 or pathogens, microbiome) can modulate host immune responses. Host factors that restrict WNV infection and replication and CNS invasion should be investigated as potential targets for drug development.

Vector cyclical/non-cyclical

According to various reports, 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. The key species involved in virus amplification may differ across Europe, and in urban / peri-urban areas.

GAPS :

Studies investigating host-virus-vector interactions in enzootic and epizootic conditions in Europe are scarce. The involvement of mosquito species in WNV transmission in Europe is difficult to assess, due to very low infection rates (about 1/100,000).

Several questions on vector-borne WNV transmission that are key for a better understanding of WNV epidemiology in Europe remain: what are the most efficient bridge vectors and what are the conditions to drive the shift in host tropism? Are the main vectors/bridge vectors identical for all the WNV lineages, clades or variants? How vector competence is affected by simultaneous or serial infection with other flaviviruses? Do alternative vectors exist and what are their importance for WNV transmission?

Although vertical transmission in some mosquito species has been proven, this seems to occur at very low rates. Its importance is probably low but vertical transmission should be explored more in the European context.

Reservoir (animal, environment)

The reservoir of the virus are birds, and virus persistence may rely on birds and mosquitoes in warmer months and overwintering mosquitoes and chronic infections in birds in colder or dry months. Many wild birds are susceptible to WNV infection and remain asymptomatic. Birds can be chronically infected. They have a limited length (and magnitude) of viremia upon acute infection. Transmission is possible without viremia (e.g. alimentary route).

GAPS :

The identification of bird species involved in the introduction, amplification and spread of WNV strains in Europe should be strengthened, through field surveys and experimental infections. Very few species of wild birds have been subjected to a careful examination in experimental in vivo infection studies (more in North America than in Europe or Africa). This list should be enlarged if we want to gain more knowledge on the ecological processes governing WNV transmission and spread. Are the main reservoir hosts/spreaders identical for all the lineages, clades or variants?

How chronic infections in birds could contribute to virus persistence is poorly known.

Also, non-vector-borne transmission (e.g. eating contaminated food, direct contact, etc) should also be better investigated.

Although mammals are considered dead-end hosts for WNV transmission, some mammal species (e.g. lemurs, tree squirrels…) have shown the capacity to transmit enough infectious virus to a biting mosquito to enable the infectious cycle to occur. However, their role in transmission in

nature is unknown and further studies are needed to clarify this point, particularly experimental infections of suspected species (4).

Description of infection & disease in natural hosts

Transmissibility

The main route of transmission of WNV is through mosquito bites. Direct transmission between birds and reptiles through oral or cloacal shedding or through predation of smaller birds could occur when WNV intensely circulates and highly dense bird populations are present.

Transmission in humans typically occurs through the bite of infected mosquitoes. Transmission may also occur through donations of blood and blood components or transplantation of cells, tissues, and organs. Transplacental mother-to-child transmission and transmission via breastfeeding are extremely rare.

Other than concerns associated with necropsy, there is no indication that infected horses pose a risk for transmitting WNV to other animals including humans.

GAPS :

Direct transmission has been proven in some bird species and in reptiles (crocodiles), but the epidemiological significance of this route is unknown. Due to the low incidence of this type of transmission, it is hard to prove how relevant it could be in nature.

Pathogenic life cycle stages

WNV is maintained in nature by cycling through birds as an amplifying host, and mosquitoes which are competent biological vectors (i.e. mosquitoes of the genus Culex, among others). The virus must multiply in the mosquito and reach the salivary glands before the mosquito can pass on the infection to another vertebrate host. The vectors also act as a bridge for the transmission of the virus to other susceptible species (i.e. many species of mammals, amphibians and reptiles). Numerous avian and mosquito species support virus replication, however, not all species of birds are “equal” in capability to serve as reservoir host. Duration and titer of viremia vary among host species. Humans and horses are considered dead end hosts.

GAPS :

The mechanisms governing the “jump” of WNV from the amplification cycle (mosquitoes-wild birds-mosquitoes) to the outbreak cycle (mosquitoes-residential birds- mosquitoes with the possible involvement of horses and humans as dead-end hosts) are not known.

Signs/Morbidity

Several vertebrate species are very susceptible to infection, but clinical manifestations are very rare.

Although most horses become seropositive without showing clinical signs, some develop severe neurological illness which can be fatal. Horses develop clinical signs when infected with strains belonging to lineages 1 and 2. When clinically apparent, both systemic and neurological abnormalities occur. A mild to moderate increase in rectal temperature (38.6-39.4°C), anorexia and depression are the most common initial systemic signs. Gait abnormalities including overt lameness or dragging of a limb has also been reported before development of an obvious neurologic syndrome. One of the initial signs of motor abnormality is a short, slow stilted gait described by observers as lameness. Many horses have periods of hyperexcitability and apprehension, sometimes to the point of aggression, which can be interspersed with sudden sleep-like activity resembling narcolepsy. Fine and coarse fasciculations of the face and neck muscles are very common and can be quite severe and involve all four limbs and trunk affecting normal activities such as walking, eating, and interaction with handlers and other horses. Spinal abnormalities are characterised by ataxia and paresis that can be highly asymmetrical or involve only one or two of the front limbs or hind limbs. Cranial nerves are frequently abnormal for short periods with weakness of the tongue, muzzle deviation, and head tilt the most common abnormalities reported. Dysphagia has been reported with choking as a sequelae. After initial signs abate, about 1/3 of clinical horses do experience an increase in severity of clinical signs after 3 to 7 days of apparent recovery. Overall, once the horse has demonstrated significant improvement, full recovery within 1 to 6 months can be expected in 90% of the patients. Residual weakness and ataxia appear to be the main problems with long-term loss of the use of one or more limbs infrequently described. In addition, mild to moderate persistent fatigue upon exercise has also been noted.

Humans with WNF present with fever, headache, myalgia, arthralgia, rash and, less frequently, gastrointestinal symptoms. Patients with neuroinvasive disease present generally with high fever, headache, neck stiffness, confusion, coma and paralysis.

GAPS :

Frequency and severity of mid and long-term sequelae should be studied in more details.

Genotype-phenotype correlates of disease in humans and animals should be studied further for WNV lineage 1 and 2 strains. The pathogenic potential of WNV lineages other than lineages 1 and 2 for humans and animals deserves attention.

Vertical transmission in the horse is still debated. One case of vertical transmission in the horse has been reported (Lineage 1, South Africa), yet little information is available regarding its exact incidence. This is largely due to lack of surveillance for vertical transmission. In addition, the peak WNV season usually is associated with later times in gestation. With asymmetrical breeding of show horses, vertical transmission may become apparent.

Incubation period

In humans and horses, the incubation period is typically 3 to 6 days but ranges from 2 to 15 days.

In experimental infection, viremia by virus isolation and PCR-based assays is reported within the first five days of inoculation. The duration of viremia in these models is between 1 to 3 days. Based peripheral (needle and mosquito) and intrathecal challenge studies coupled with case control studies of natural infection demonstrated that horses develop a short duration of viraemia that is below the minimum threshold for transmitting the virus to mosquitoes. Upon infection, virus titres, if detectable, usually range between $10^{1,0}$PFU/ml and$10^{3,0}$PFU/ml of serum between 1 and 3 days after experimental infection.

Mortality

During outbreaks, approximately 10 - 20% of infected horses may develop neurological signs. The reported case fatality rate in horses varies from 23% to 57%, depending on the outbreak; in the USA, it is approximately 30-40%. In very early work, a 1:11 symptomatic to asymptomatic ratio was projected based on small epidemiological studies and peripheral inoculation studies in horses.

In humans with neuroinvasive disease, the case fatality rate ranges from 10% to 20%. Severe sequelae persist in 20 to 40% of survivors.

GAPS :

Several factors are thought to play a role in determining the final morbidity and mortality rates: infectious dose (related also to mosquito abundance), virus strain, host susceptibility (related to breed, genetic susceptibility) and species (horses versus donkeys). Risk factors associated with the development of WNV neuroinvasive disease in horses should be investigated.
Mortality is complicated by euthanasia of recumbent horses. The spontaneous mortality rate may actually be lower.

The mechanisms of pathogenesis in the horse (and likely humans) is unclear. The pathology and virus load of WNV in the brain is comparably (and markedly) low compared to other fatal viruses (Eastern Equine Encephalitis virus, Equine Herpesvirus-1 neurotropic). The virus localizes to nerve cell bodies with limited virus in neuropil. Does this disrupt neuronal function, without marked pathology? Limited studies have been done detecting the virus in situ with more sensitive techniques (RNAscope).

Shedding kinetic patterns

In horses, a fleeting viremia of low virus titre precedes clinical onset.

In humans with WNV infection, WNV RNA can be detected in blood a few days after exposure and for approximately 8-10 days. WNV is excreted in urine, where it is detectable at a higher load and for longer (up to one month after symptom onset) than in plasma. Infectious virus can be isolated from serum and urine samples within the first 3-5 days after symptom onset. In patients with neuroinvasive disease, WNV RNA can be detected in the CSF, although in less than 40% of cases and at low titre. Patients with severe disease may have prolonged viraemia and viral shedding in biological fluids.

Mechanism of pathogenicity

Certainly with Lineage 1 and 2 WNV, lethal disease may be caused by significant immunopathology, i.e., by the immune system in response to WNV infection in the CNS. This may be modified to increase survival without altering sterilising immunity. Importantly, this is evidently in the absence of significant neuronal death, which is in any case very much less than other neurotropic (admittedly DNA) viruses such as e.g., Herpes viruses in encephalitis.

GAPS :

Commonalities and differences in WNV pathogenesis for strains belonging to different genotypes should be studied more precisely. Molecular determinants of pathogenesis and molecular interactions modulating WNV pathogenicity are not fully understood.

Zoonotic potential

Reported incidence in humans

Human and horse cases have been reported from many parts of Europe, including Spain, France, Italy, Greece, Romania, Russia, Bulgaria,

Serbia, Croatia, Albania, Hungary, and Germany. In particular, according to ECDC data, in the period from 2012 to 2022, human cases of WNV infection have been reported in the following EU/EEA countries: Austria, Bulgaria, Croatia, Cyprus, Czechia, France, Germany, Greece, Hungary, Italy, the Netherlands, Portugal, Romania, Slovakia, Slovenia, and Spain. Following the emergence of WNV in the USA and Canada with associated human cases and deaths, and the severe epidemics in humans experienced by South-Eastern European countries (specifically Romania, Serbia, Italy, Greece), there has been an increased public concern.
Remarkably, despite virus presence, only sporadic cases are reported south of the USA.

Low to medium risk of underreporting of neurological signs, depending on testing protocols and regulations followed in Europe. Moreover, mild cases may occur unnoticed. ECDC worked on the harmonization of case definition and has surveillance systems in place in Europe.

GAPS :

WNV incidence in Europe has increased during the last decade, and in particular in 2018 and 2022. All variables that may play a role in the determination of the final incidence in humans and animals are not known. Factors contributing to enhanced incidence (virological, ecological, …) and their contribution to such increase would deserve attention. Identification of the factors underlying decreased incidence of severe cases south of the USA (such as strain virulence, pre-existing cross-reactive antibodies, …) is needed.

What is the rate of under-reporting / undiagnosed cases in endemic, non-endemic areas or in countries with low incidence?

Risk of occurence in humans, populations at risk, specific risk factors

WNV is mainly transmitted to people by mosquitoes that have fed on infected birds.
Other methods of transmission reported include donated blood and organs (suspected in 0.1% of cases in the USA, 2003-2008 upon implementation of blood screening), mother-to child (1.6% in the USA, 2003-2008), and laboratory exposure (0.04% in the USA, 2003-2008). Aerosol inhalation should be borne in mind, since this has been reported in laboratory exposure to Japanese Encephalitis virus.

Risk factors for developing neuro-invasive disease include older age (>60 years old) and a history of solid organ transplantation and might also include other immunocompromising conditions, cancer therapy, diabetes, and hypertension.

This forces Public Health authorities to put in place strict controls on blood and organ donations in case of WNV circulation.

GAPS :

Genetic factors, linked to higher susceptibility to WNV serious disease, must be explored.

Mechanisms underlying enhanced clinical disease in the elderly or in patients with risk factors (hypertension, diabetes, …) deserve additional studies.

Symptoms described in humans

When disease does occur, it is usually a flu-like illness with fever. Humans with WNF present with fever, headache, myalgia, arthralgia, rash and, less frequently, gastrointestinal symptoms. A small proportion of cases (less than 1%) develop meningo-encephalitis which produces nervous signs and may be fatal. Patients with neuroinvasive disease present with high fever, headache, neck stiffness, confusion, coma, paralysis and other neurological symptoms. Polio-like flaccid paralysis has been observed rarely in the most severe cases.

Likelihood of spread in humans

Humans, horses and most infected mammalian species are dead-end hosts, i.e. there is no natural spread from them to other people or animals. WNV does not normally spread between people except under special circumstances (e.g. blood transfusion, organ transplantation, transplacental transmission, breastfeeding).

Impact on animal welfare and biodiversity

Both disease and prevention/control measures related

High impact on bird biodiversity in the USA. Some significant suffering may occur in affected horses but their numbers are low.

GAPS :

The impact of WNV on populations and welfare of the most sensible avian species (i.e. raptors and susceptible passerines) should be studied.

Endangered wild species affected or not (estimation for Europe / worldwide)

Impact is likely to be low in Europe (low bird mortality). Mass fatalities have been described in passerines (corvids in particular) in the USA.

GAPS :

Risk may be higher in some endangered raptors that may consume WNV infected preys, or wild birds with small population size whose death may go unnoticed.

Slaughter necessity according to EU rules or other regions

Horses infected by WNV develop a brief low-level viremia that is not infectious to mosquitoes. Most horses recover from the infection. However, recumbent horses are less likely to recover. Euthanasia of affected horses is usually a decision related to animal welfare and odds of recovery, horse age, value, cost of treatment, etc.

Geographical distribution and spread

Current occurence/distribution

The virus historically occurs in Africa, Europe, the Middle East, West and Central Asia, and a low virulence lineage 1 strain is also common in Australia (Kunjin, KUN). Outbreaks have regularly occurred in the Mediterranean basin (Morocco (1996, 2003, 2010), Israel (1999-2000, 2010, 2015, 2018), Italy (1998, 2008 to date), Greece (2010 to date), Turkey (2010-2011), …) and in Southern Europe (Romania (1996 to date),

Bulgaria (2010), Spain (2010, 2020), Serbia (2012), Croatia (2012, 2013, 2017, 2018), France (2000, 2003, 2015, 2018 to date), …).
Unexpectedly, a change in WNV epidemiology has occurred since 2010 in Europe, with lineage 2 strains being responsible for most human WNV cases, in particular in the Balkans.
To date, human WNV cases have been reported in at least 22 European countries. For details see the website of the ECDC (https://www.ecdc.europa.eu/en/west-nile-virus-infection).

In 2022, it has been observed to spread further north and west in France, well beyond the Mediterranean region. WNV outbreaks and overall activity have increased in Europe in the last decades.
WNV lineages 1, 2, and 8 are co-circulating in the African continent; there is evidence of circulation of WNV among humans, animals and vectors in at least 28 countries the epidemiological situation of WNV is not known for the other 19 countries. Real time RT-PCR-based analysis confirmed WNV circulation in Central African Republic, Guinea, Ghana, Gabon, Nigeria, Senegal, and Sierra Leone between 1983 and 2020, while serological surveys reported WNV circulation in humans in Algeria, Central African Republic, Democratic Republic of Congo, Egypt, Ethiopia, Gabon, Ghana, Kenya, Madagascar, Mali, Morocco, Mozambique, Namibia, Nigeria, Senegal, Sierra Leone, South Africa, South Sudan, Sudan, Tanzania, Tunisia, Uganda, and Zambia.
Several WNV outbreaks in humans were registered in the African continent starting from the 1950s. Neurological disease cases and fatalities related to WNV lineage 2 were reported in South Africa while in the Mediterranean basin, hundreds of cases of encephalitis and deaths related to WNV lineage 1 (clade A) were registered in Tunisia between 1997 and 2018 (1997, 2003, 2007, 2010, 2011, 2012, 2016, 2018). Furthermore, WNV lineage 1 human infections were recorded for the first time in 1994 and 1996 in Algeria and Morocco, respectively.

Since its first appearance in the USA in 1999, the virus (belonging to lineage 1) has spread throughout much of the country where it is now considered to be endemic. In addition, WNV has also been detected in Central and South America, with the disease occurring to a lesser extent than in North America.

GAPS :

Strain monitoring at the genetic (genomic) level is really necessary to clarify many aspects regarding WNV origin, spread and evolution. In particular, genome sequencing (preferably whole genomes) should be implemented systematically, paying more attention to areas of WNV circulation where these data are scarce, such as in Africa. By studying WNV genome diversity in different areas, and establishing phylogenetic relationships, high resolution phylo-geographic and molecular clock analyses would more and more accurately reconstruct the natural history of this virus and point out the main routes of geographic spread, as well as the mechanisms behind its expansion, which currently is only barely sketched.
A recent study, uncovering the origins and dispersal history of African and European WNV lineage 1 and 2 strains between the two continents, shows two diverse geographical patterns of transmissibility. It highlights: i) a complex structure and unpredictable behaviour for WNV lineage 1 strains, with back-and-forth exchanges most between West Africa and Europe, particularly through an ideal corridor connecting Senegal,

Morocco, and Southern-Western Mediterranean countries; and ii) WNV lineage 2 homogeneity, with one main independent introduction from South Africa to Hungary, from where the virus spread and established in Europe.

What are the factors driving long and short distance spread of WNV strains? A better understanding of transmission dynamics and environmental factors that promote the transmission (temperature, drought, winter rainfall) of varied WNV strains among reservoir hosts and vectors in Europe is needed. Need to develop bird tracking to better understand bird migrations across Europe, and between the European and African continents.

As lineage 1 and 2 strains are co-circulating in some European countries (Italy, France,…), genetic evolution of the virus in presence and absence of lineage 1 and 2 virus co-circulation should be compared. Strain replacement dynamics should also be monitored closely.

Epizootic/endemic- if epidemic frequency of outbreaks

The state of endemicity in the USA is characterised by intense recurring focal outbreaks although the risk is not uniform across the USA. The disease has become highly seasonal July-October. Drought has been identified as a potential amplifier of virus transmission. In addition, there are several high-risk areas within the states that cover multiple counties. There is also a directional shifting of risk south to north in the summer and vice versa in the late fall. As expected precipitation enhances risk, but extreme drought results in a sharp increase in risk, likely due to aggregation of vectors and hosts. Nonetheless avian species (as related phylogenetically) is the highest driver over climate and land cover.
WNV has been re-emerging in Europe since the last 15 years, with Southern, Central (see section 12.1) and more recently Northern (Germany) countries reporting recurring outbreaks.
Temperature is one of the most important environmental variable modulating WNV activity in Europe as it affects both mosquito breeding and the extrinsic incubation period for WNV. Above-normal spring and summer temperatures have been shown to influence dispersal into new areas and amplification.

GAPS :

Complex epidemiological cycle. Much work and modelling was undertaken these last years in Europe. Risk maps have been established and risk factors underlined, and ecoregional approaches have been found very promising in drawing predicting models.

The influence of global change, including the effect of climate change in the epidemiological behaviour of WNV is still a big gap in the research of this virus. Observations of WNV outbreaks occurring more and more northwards every year is suggestive of a trend involving WNV expansion to the North, but the ultimate processes determining this behaviour remain essentially unknown.

Speed of spatial spread during an outbreak

Speed of spread depends on a number of interdependent factors including the presence of viremic birds and vectorial capacity, e.g. ability of the vector to transmit the infectious agent, biting rate on competent host (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.

GAPS :

Drivers of WNV spill-over in a given location are still far from being well characterized, at least in a variety of WNV transmission settings.

Transboundary potential of the disease

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

Possible introduction of infected mosquitoes through vehicles and trade of goods (e.g., car tires for Aedes).

GAPS :

Clarify the geographic origin, routes, species involved, distances travelled, etc., of the birds that might be acting as WNV carriers during their migration between continents. Clarify the actual mechanism by which WNV is carried by birds (infection? Carrying infected ticks?)
Clarify if, apart from migratory birds, there are other mechanisms involved in long-distance movements of WNV (e.g., legal or illegal trade of birds or other animals, movements of infected mosquitoes associated with travel and/or trade, etc.).

Route of Transmission

Usual mode of transmission (introduction, means of spread)

Intercontinental migration of birds, with back-and-forth exchanges of virus strains between Africa and Europe, is the most likely mechanism of the infection being introduced. WNV can be transmitted to humans and animals via the bites of infected mosquitoes. Infection of other animals (e.g., horses, and also humans) is incidental to the transmission cycle since most mammals do not develop enough virus in the bloodstream to spread the virus.

GAPS :

Migrating birds did not explain the rapid east to west spread of the virus across the USA.
Bird migration patterns are typically north to south and vice versa. Short distance migration or reservoir host dispersal may play a role in WNV spread. Are there other factors playing a role?

Occasional mode of transmission

Transmission via infected blood, tissues, placenta, breast feeding, needle stick, organ transplantation and laboratory exposure is possible but less common than vector-borne transmission. Gastrointestinal route in birds and potentially wild animals (by eating infected dead birds) has been suspected in North America and may be responsible for infection in raptors in Europe.

GAPS :

Strict controls on blood and organ donations in countries with WNV circulation.

Conditions that favour spread

See Section “Speed of spatial spread during an outbreak”.

Detection and Immune response to infection

Mechanism of host response

Neutralizing antibodies are generated and are protective, but cellular immunity also plays an important role in protection from neuroinvasive disease. The length of detectable IgM antibodies depends on the host species. Regarding humans, IgM antibodies generally persist for over 6 months and may be still detectable after 1 year in about 20% of asymptomatic blood donors and in about 50% of patients with neuroinvasive disease. In horses, the length of detectable IgM antibodies is about 1-2 months.

Innate immunity also plays a role both in control and immunopathology, in particular the myeloid lineage. There is a significant infiltration of leukocytes and a major microglial response to neuronal infection in the brain.

GAPS :

Determinants and duration of humoral and cellular immunity in humans and horses are partly assessed after natural infection. Few data are available about the duration of IgM or neutralising antibody response in horses (and birds or other mammals). Only hypothetical estimates of what constitutes a protective neutralising titer.
The effects of prior flavivirus immunity on the adaptive immune response to WNV need investigation.

Innate and cellular correlates of immunity have not been studied sufficiently in the horse. Most of the data have been derived from mouse models which are not comparable to either human or equine disease.

Immunological basis of diagnosis

Development of antibodies to WNV, confirmed by a neutralization test. Detection of WNV IgM antibodies in the CSF of patients with neuroinvasive disease, in the serum or CSF or horses with WNF or neuroinvasive disease.

GAPS :

Testing cell-mediated immune response could be useful for the diagnosis (and differential diagnosis) of flavivirus infections.

Main means of prevention, detection and control

Sanitary measures

Notification and investigation of suspect disease in horses with implementation of control measures including vector control. Infected horses do not contribute to the transmission cycle of the virus and therefore infected horses could not introduce the infection into a free country. Once WNV is reported in a particular region, enhanced integrated surveillance, information campaigns to describe the disease and give advice on vector reduction and avoidance should be implemented.

GAPS :

Vaccination of horses in WNV endemic areas should be encouraged.

Mechanical and biological control

Limit exposure to the vector using a number of techniques.

- Control of vectors through the elimination of mosquito breeding (stagnant water, rain butts, etc.) with possible use of insecticides.
- Avoid contact with the vector by keeping animals away from vector sites, use of insecticides, insect proof housing and use of insect repellents.
- Destroy the vector with the use of insecticides to kill the larvae and adults and possible biological control of the mosquito
- Biological control might also include the use of bacterial infection of mosquitoes.
- Release of sterile (SIT or sterile Insect Technic, male mosquitoes receive X-rays) or genetically modified mosquitoes, the majority of which are non-feeding males, into areas of dense mosquito populations has shown promise. These mosquitoes breed with the wild type population. The offspring is non-viable (sterile insect technique) or contain a gene that renders them incapable of reaching maturity, thereby decreasing the breeding population and overall mosquito numbers (GMO). Such strategies are not operational yet for Culex mosquitoes. Social acceptance of GMOs varies a lot between countries and regions with legal, social, ecological, cultural, historical, and economic aspects.

GAPS :

The recurrent use of insecticides might cause insecticide resistance in mosquitoes. More effort should be made to develop alternative and sustainable methods to reduce or limit the emerging insecticide resistance. It is unknown what long-term effects may be caused by the release of genetically modified mosquitoes on the environment, thereby reducing the acceptability of such control approaches.

Mosquito control is difficult and a multi-approach strategy seems to be the most effective. More information is needed on the resistance of mosquitos to several insecticides and the capacity of surviving during winter periods.

Evaluation of mosquito surveillance and control methods should be systematically developed, in order to assess the efficiency of vector control technics.

Diagnostic tools

There are a number of tests deployed to diagnose WNV infection.

Identification of the virus:

- Virus isolation on tissue samples, primarily nervous tissue, from dead animals.
- Range of PCR-based tests (RT-PCR, real-time RT-PCR)
- Sequencing

Identification of antibodies :

- IgG/competition ELISA (screening)
- IgG avidity ELISA (For humans only)
- Plaque reduction neutralisation test (Gold standard) and other serum neutralization test variations
- Immunofluorescence assay (IFA)
- IgM-capture ELISA

Commercial competition and IgM capture ELISAs are available in Europe and IgG ELISA may be available in other locations.

GAPS :

Multispecies infection. Specificity/sensitivity of test for species (especially if other than species for which test was developed) may be uncertain – e.g., tests for horses may not be valid for dogs, cats, livestock. Could be due to species-specific reagents in tests or due to lack of data on test performance in species. This does not seem to be a problem if RNA detection methods are used.

Virus isolation: the virus is difficult to isolate, especially from horse samples (difficulty in accessing post-mortem samples). Virus has not been successfully isolated from blood.

Antibody detection using epitope blocking (competitive) assays targeting NS1 protein have been effective for detecting WNV infection in horses and birds. Similar specificity to neutralisation when screening out SLEV and other flaviviruses in the Americas – needs further field evaluation in Europe. Also may be effective for differentiating natural infection from vaccination but need further field trials.

Immunohistochemistry may be used in some cases, but may lack sensitivity or specificity.

Vaccines

Worldwide. Three types of vaccines are available on both the EU and USA market for use in horses:

- Formalin-inactivated WNV vaccine derived from cell culture,
- WNV live canarypox virus-vectored vaccine,
- Inactivated chimeric vaccine, which uses yellow fever virus backbone

In the USA, a DNA vaccine and a live chimeric vaccine (yellow fever virus backbone), were licensed. These two products are currently no longer available on the market.
Replicating, non-infectious, replicon-based WNV vaccines delivered as DNA, RNA, or VLPs and subunit vaccines have also been developed and undergone preclinical trials.

GAPS :

Need for vaccine development and licensing in humans.

Therapeutics

WNV antibody product(s) have conditional licence in the USA – anecdotal reports of efficacy.

GAPS :

Research needed in this area for development and licensing of antivirals in humans and in animals (welfare, animal species conservation).

Biosecurity measures effective as a preventive measure

WNV has to be manipulated in BSL3 facilities. Containment Level 3 facilities, equipment, and operational practices for work involving infectious materials, animals, or cultures are used. Necropsy is associated with higher risks of acquiring WNV infection from infected animals and appropriate biosecurity measures should be used (limited use of sharp objects, precautions when accessing the brain,…).

GAPS :

Development of diagnostic tests and vaccines not based on infectious virus.

Because clinical signs are consistent with rabies, body fluids are handled as potentially infectious for rabies virus, even though there may be limited virus in plasma, until rabies infection is ruled out.

Border/trade/movement control sufficient for control

Trade and movement of horses are not affected since they are dead-end hosts.

Prevention tools

Prevention tools are limited to vaccination and vector control.

Surveillance

Statutory notification of suspected disease in horses is followed by outbreak investigation. Dead and ailing wild birds are submitted to laboratories for WNV diagnostics. Passive surveillance in horses appeared to be the earliest system in several European countries (France, Italy, Spain,...). Active surveillance, aiming at increasing the sensitivity of WNV surveillance, can be implemented: regular sampling and serological screening in domestic birds or horses, in at risk areas and during WNV season. However, a very high number of birds need to be screened for the method to be effective at detecting WNV circulation early in the season. Mosquito surveillance is an important and early tool to reveal WNV activity in a certain area; however, its costs are high. Investigation of human neuroinvasive disease cases may reveal presence of WNV before cases are detected in animals.

GAPS :

Awareness of (owners and) veterinarians and physicians is important for rapid notification and investigation of suspected cases.

The feasibility and effectiveness to use alternative hosts for WNV surveillance (domestic animals, such as pigeons, dogs for example) should be evaluated further.

Low cost detection methods are needed to facilitate the development of active surveillance systems on birds and mosquitoes with high throughput screening of a high number of samples (FTA card samplings, high-throughput screening RT-PCR-based assays,…).

Past experiences on success (and failures) of prevention, control, eradication in regions outside Europe

In the USA the virus has spread throughout the country and is now endemic. In Europe, less of a problem although human WNV cases have been reported in at least 22 European countries and the virus is endemic in Romania, Hungary, Serbia, Greece, Spain, Portugal, France, Germany, Austria, Bulgaria, and Italy.

The effectiveness of mosquito control activities is variable. Even well-structured mosquito control plans are thought to be able to reduce the

vector abundance but not to interrupt the virus transmission chain.

GAPS :

Variable pathogenicity seems to be linked to lineage and strain within the lineage.

Mosquito control is difficult and a multi-approach strategy seems to be the most effective. More effort should be made to develop alternative and sustainable methods to reduce or limit highly abundant mosquitoes and the emerging insecticide resistance.

Costs of above measures

Unspecified.

Disease information from the WOAH

Disease notifiable to the WOAH

West Nile fever is a disease listed in the WOAH Terrestrial animal health code and must be reported by members according to the WOAH Code (1): https://www.woah.org/en/disease/west-nile-fever/
https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.25_WEST_NILE.pdf

Delay between first suspicion / first confirmation and WOAH first emergency notification is variable and depends on the efficiency of the surveillance systems and the emergence of clinical signs in equids. However, the involvement of horses and humans is later in the transmission cycles and it is always preceded by the WNV circulation in bird populations.

ECDC maps human and equine cases:
https://gis.ecdc.europa.eu/portal/apps/experiencebuilder/experience/?id=4876503d343a4c1abf5941557eb071f1

Validated data and key statistics are presented by EFSA and ECDC in a chapter of annual European Union One Health Zoonoses report:
https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7666

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WOAH disease card available

https://www.woah.org/en/disease/west-nile-fever/

WOAH Terrestrial Animal Health Code

https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_wnf.htm

WOAH Terrestrial Manual

https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.25_WEST_NILE.pdf

Socio-economic impact

Zoonosis: impact on affected individuals and/or aggregated DALY figures

High impact on affected individuals.

Outbreaks cause public concern but there is unlikely to be a major impact on society. In the USA approximately 1 in 150 WNV infections will result in severe neurological disease. Among those with severe illness due to WNV, case-fatality rates range from 3% to 15% and are highest among the elderly. Overall deaths occur in approximately 1 in 1,000 infections. A total of 43 states have reported WNV infections in people, horses, birds, or mosquitoes in the USA in 2022. Overall, 1,126 cases of WNV disease in people have been reported to CDC. Of these, 816

(72%) were classified as neuroinvasive disease (www.cdc.gov).

Impact in Europe varies from year to year but in 2022, ECDC reported 1,133 locally acquired human cases in EU countries, including 92 deaths.

Attempts to reduce mosquito populations or human exposure could be mildly disruptive.

GAPS :

It is still unclear exactly why certain individuals are susceptible to neuroinvasive disease. Risk factors include age and underlying health conditions, but the reason for this is not understood.

Zoonosis: cost of treatment and control of the disease in humans

No vaccine is available for use in humans, and there is no specific treatment. Detailed costs for the surveillance and control of West Nile fever are not available. According to the experiences in Europe and in the USA, the incidence of clinical manifestation in humans is so low, that there is no real demand on vaccines.

Efficient monitoring systems were developed and are applied for the testing of blood.

GAPS :

Following the experience of COVID-19 vaccine development, there may be opportunities to develop cost-effective vaccines that could be deployed in the event of an outbreak for at-risk populations. A vaccine should be licensed for reactive use for at risk groups (elderly, underlying health conditions) in the event of local outbreaks. Public health authorities in areas that are at increased risk of emergence should consider sponsoring this.

Direct impact (a) on production

Mortality in horses. Commercial vaccines are available for horse owners, but these vaccines are expensive, need annual boosts and are not widely used in breeding and working horses.

Mortality in geese was also reported in Israel and Canada.

Variable impact, most in the USA where it has become endemic.

GAPS :

As it spreads through Central and South America, greater impact may be observed there, as well. However, this does not seem to have been realised now that WNV has been detected in southern South America. There have been reports of neurological disease in equids in South America although the impact of WNV has not been as dramatic as in North America. The reason for this is unclear. Possibility that the presence of other flaviviruses may provide cross-protection from infection or disease.

Direct impact (b) cost of private and public control measures

Includes costs of vaccination and of the implementation of surveillance systems. The vaccination costs are mainly borne by private horse owners.

GAPS :

Countries in northern Europe are now having to consider increasing surveillance and control for WNV.

Indirect impact

Low impact, however, risk perception by the public may generate perturbation on tourism and people travel. There has been evidence for local impact on tourist sites although no restrictions on equine transportation between continents.

Trade implications

Impact on international trade/exports from the EU

International trade and movements of horses are not affected, since they are dead-end hosts. Possible limitation to the trade of birds, including ornamental species. Specific standards are laid down in the WOAH Terrestrial Animal Health Code.

Good to have a prophylactic for the horses travelling to endemic countries. Vaccination should be encouraged for horses travelling to endemic areas.

GAPS :

Need to consider what is detected by any movement-related testing (e.g. antibody, antigen) as well as disease kinetics for the analyte tested to make science-based recommendations. Also need to consider practical aspects of sampling, sample transport, test availability and performance, test turnaround time, when considering regulations.

Impact on EU intra-community trade

See Section “Impact on international trade/exports from the EU due to existing regulations”.

Impact on national trade

See Section “Impact on international trade/exports from the EU due to existing regulations”.

Links to climate

Seasonal cycle linked to climate

WNV outbreaks are seasonal and related to weather and environmental conditions (including suitable bird and mosquito habitats). Infections are dependent on mosquito abundance and are seasonal in temperate climates, peaking in the late summer/early autumn in the Northern

Hemisphere.

ECDC monitors WNV reports in humans, equids and wild birds.
The emergence and spread of WNV lineage 2 in Europe have occurred during a period of climate change although direct association between this and particular climatic changes have not been established. There appears to be a continuing trend for the WNV season to start earlier in the year.

GAPS :

Pluri-annual studies are needed to monitor the long-term effect of climate change on WNV trends.

In Europe, a greater number of cases were reported on “hot” summer years. Need more detailed analyses of winter temperature / spring rainfalls / summer drought to better assess which factor(s) are most predictive and track how these change temporally and geographically with global climate change.

Little data on WNV transmission are available in the Southern Hemisphere. WNV causes disease outbreaks in South Africa, is present in Australia as Kunjin virus and has been detected in South America.

Distribution of disease or vector linked to climate

Climate change may result in changes in vector distribution, population density and the ability of the virus to develop in new species of mosquitoes. Milder winters and warmer spring seasons will promote mosquito survival. WNV has been reported further north (Germany and the Netherlands), and west (France) but unclear how effective the virus is at persisting long term (overwintering) at these latitudes.

GAPS :

Are there links between climate change and WNV emergence and spread? Difficult to predict (inverse effects of the temperature on mosquito number, survival and virus multiplication/transmission, bird abundance and diversity). Culexspecies are the main vector but other mosquito species may play a role locally and more data on the vectorial capacity for WNV of mosquito assemblages across Europe is needed.

Changes in bird migration timing and patterns may influence long distance movement of WNV between continents.

Outbreaks linked to extreme weather

Heavy rainfall does not seem to be associated with increased WNV infection. Post Hurricane Katrina in the USA and following floods and heavy rainfall in the Balkans in 2013, mild WNV seasons or even disappearance of the virus were observed. Heavy rain may wash away mosquitoes. However, other weather conditions may influence outbreaks – e.g., increased rainfall in spring that supports the mosquito population followed by a hot dry summer; a mild winter which increases the survival of adult mosquitos and eggs/larvae.

GAPS :

Relationships between temperature and precipitation and virus transmission need to be understood better to be predictive. Needs to be done on local scales.

Sensitivity of disease or vectors to the effects of global climate change (climate/environment/land use)

Vectors may be sensitive to climate change. Climate change may increase the opportunity for invasive mosquito species to establish e.g., Culex quinquefasciatus, or the expansion of bridge vector species such as Culex modestus. Increased temperatures are predicted to increase virus replication in the vector and reduce extrinsic incubation period.
Any change that increases mosquito abundance (Culex spp.) and contact rates between mosquitoes and reservoir hosts will increase the risk of WNV transmission.

GAPS :

Clear scientific evidence of the effects of climate changes to mosquito-borne diseases are currently lacking. According to historical data, in the Italian areas during WNV circulation, the vector population (species and abundance) did not show any significant changes compared to the previous years. Other vector-borne viral diseases are spreading to previously unaffected areas, especially in a northward pattern, in the USA and elsewhere. Additionally, the ecological distribution of vectors has been increasing over time.

Main perceived obstacles for effective prevention and control

Main perceived facilitators for effective prevention and control

Global challenges

Antimicrobial resistance (AMR)

Impact of AMR on disease control

Established links with AMR in humans

Digital health

Precision technologies available/needed

At international (Europe -https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/disease-data-ecdc, international - https://www.plateforme-esa.fr/fr/bulletins-hebdomadaires-de-veille-sanitaire-internationale-) and country level (USA - https://www.cdc.gov/westnile/index.html, Italy - https://www.epicentro.iss.it/westnile/), public web sites are available for disseminating information and continuously updated data to have: the current and past epidemiological situations; updated maps on WND cases, entomological, serological and virological surveillance activities. Not every EU countries disseminate the results associated with their surveillance activities.

GAPS :

National informative system recording WND cases, and surveillance activities should be implemented at least at National level.

Data requirements

Data on WND cases and surveillance activities.

Data availability

Surveillance data for WNV are pluri-disciplinary in nature and are available in different institutes and Ministries. Data need to be shared under the One Health guidelines.

Data standardisation

Data verification criteria are highly variable. Data are generally not standardised nor interoperable.

GAPS :

Efforts towards data standardization and interoperability should be implemented.

Climate change

Role of disease control for climate adaptation

WNV is spreading to the north in Europe, reaching new countries (i.e., Germany and The Netherlands). In high-risk areas, vector surveillance for WNV should be recommended to occur early in the spring to predict transmission to humans.

Local mosquito population suppression may be attempted although this may have environmental costs. Public advice should be given more

frequently to reduce exposure to mosquito bites (cover arms/legs, apply mosquito repellent, avoid outdoor activities during mosquito-biting periods).

GAPS :

The role of climate change on vector population dynamics and vector-virus interactions including possible non-linear relationships with temperature should be better investigated and understood.

More research required to predict the impact of climate change on WNV emergence and spread.

Effect of disease (control) on resource use

Little effect. Equine vaccination should be recommended to horse owners.

GAPS :

Public advice and education may by the most effective means (e.g., personal protection from mosquito bite) of preventing transmission to humans.

Effect of disease (control) on emissions and pollution (greenhouse gases, phosphate, nitrate, …)

Minor effects. Control of vectors in response to disease outbreaks can have negative impacts on biodiversity because of the use of biocides with side effects on animals other than mosquitoes.

GAPS :

Environmentally friendly means of vector control at scale will be required.

Preparedness

Syndromic surveillance

Birds represent the first wave of infections in each WNV transmission season, as enzootic transmission takes place in wild birds. Infections can be observed earlier compared to human or equine cases. Some infected Corvidae species (e.g., blue jays and crows) can die from the infections, however, most birds survive. This also depends on the pathogenicity of a given viral strain.
Monitoring equine encephalitis cases can be helpful to notify public health authorities. However, there were cases where the onset of cases in humans was observed earlier compared to the onset of cases in horses.
Syndromic surveillance in horses (identifying abnormal clusters of encephalitis or fatalities in horses) has been shown to trigger earlier signals when integrated in surveillance networks (at an experimental stage).

GAPS :

More active surveillance programs and easier access to mosquito surveillance are needed.

Operational syndromic surveillance should be encouraged.

Syndromic surveillance in horses relies on awareness in owners and veterinarians, which may be improved in some regions (especially in non-endemic areas).

Diagnostic platforms

Protocols for high-throughput and multiplex RT-PCR-based and serological assays have been developed. They allow for easier differentiation between closely related flavivirus infections.

Protocols for whole genome sequencing of lineage 1 and lineage 2 WNV strains via NGS have been developed, for rapid genomic characterization directly on biological specimens from humans, animal hosts and mosquito vectors,, i.e., without prior virus isolation in cell cultures.

GAPS :

Cheap, easy to use and commercially available diagnostic tools are needed, and specifically as far as rapid genome sequencing is concerned.

In order to generalise neutralisation testing in a wider set of diagnostic laboratories, serum neutralisation assays using pseudoviruses or chimeras from insect-specific flaviviruses should be developed.

Mathematical modelling

Studies related to the development of mathematical models to predict WNV transmission and outbreaks have become available in the literature. Such models feed from data from various sources, such as bird populations, vector populations, climate data, death rates of birds, etc.

GAPS :

Models are developed for use in specific areas/countries and adaptation to enable their universal applicability are difficult.
Platforms for Dengue prediction based on recent developments of Dengue models are now available - avenue for developments on WNV?

Intervention platforms

Not applicable

Communication strategies

Several approaches are taken by national and international disease control and prevention organisations to raise awareness about the disease, including mostly virus transmission prevention practices (mosquito control).

GAPS :

Communication strategies should be strengthened through experience sharing between countries and participative approaches.

Main critical gaps

Special attention should be given to further definition of the ecology, epidemiology, prevention (in humans more particularly) and treatment of West Nile disease, and to the identification of factors that predispose to disease outbreaks in the EU or in other endemic regions. Animal and human surveillance has been strengthened in Europe through the ECDC/EFSA joint initiative; development of preparedness plans and response to outbreaks will benefit public and veterinary health. Updated guidelines for the vaccination of horses would also be helpful.

Impact of the co-circulation of Usutu virus and WNV, and of different lineages of WNV on the ecology and evolution of WNV should be assessed.

Development of more cost-effective methods for viral surveillance in mosquitoes and birds and of more specific serological tools is required. DIVA assays would help to assess WNV circulation in Europe, should the vaccination be mostly used.

There is a need for development of vaccines for use in humans and of therapeutics for use in humans and in horses.

Conclusion

WNV incidence in Europe has increased during the last decade, with Southern, Central and more recently Northern countries reporting recurring outbreaks. The reporting of high numbers of outbreaks in humans and animals particularly in 2018 and 2022 is of concern. The causative virological, ecological and environmental factors that predispose to outbreaks are not fully understood; clear scientific evidence of the effects of climate change to WNV are currently lacking. Routine equine vaccination may be necessary in Europe should WNV become sufficiently endemic to present a substantial and persistent seasonal risk.

Outbreaks in North America and Europe are difficult to predict and the long-range epidemiological pattern in Europe remains undefined.

In Europe the ECDC and EFSA initiative improves WNV surveillance for both humans and animals. Integrated One-Health surveillance schemes

are promoted in Europe.

Sources of information

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STAR-IDAZ Research Road Maps