West Nile Virus Infection

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West Nile Virus Infection

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Advanced article

Article Contents

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West Nile virus (WNV) is an arthropod-borne virus transmitted mainly by mosquito bites. Wild avifauna constitutes its natural reservoir. Mammals are dead-end hosts as they generally do not develop a viraemia high enough to trigger a new infection cycle. WNV belongs to the *Flavivirus* genus and can be responsible for a disease known as *WN* fever. Humans and horses are the most sensitive hosts as they may develop severe neurological signs. WNV has a worldwide distribution and caused numerous outbreaks since the late 1990s, most notably in North America and Europe. Lineage 1 strains have caused the most severe outbreaks to date. However, the situation has recently evolved with the emergence in the late 2000s of pathogenic lineage 2 strains in South Africa, Russia and Eastern and Southern Europe. Many developments in the fields of WN diagnosis and vaccination have been described in the recent years and may help control WNV spread.

Introduction

West Nile virus (WNV) is a mosquito-borne neurotropic *Flavivirus* native to Africa, Europe, the Middle East and Western Asia that primarily cycles between mosquitoes and birds. It can infect a wide range of vertebrate animals, including mammals, among which humans and horses were found to be the most susceptible. WNV infection remains generally subclinical in these latter hosts but can also cause disease with symptoms ranging from mild fever to severe meningoencephalitis. WNV is a tremendous example of how rapidly a zoonotic pathogen can leap out of its historical geographical barriers to emerge and spread into new territories, as was the case in 1999 in the United States. This article aims to gather recent insights into WNV epidemiology, biology, diagnosis and prevention.

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WNV Transmission Cycle

WNV is maintained in a mosquito–bird–mosquito transmission cycle (**Figure 1**). WNV is transmitted to naïve hosts by the bite of a mosquito primarily contaminated following a blood meal on an infected bird, after the extrinsic incubation period, that is, the time lapse between the blood meal and the presence of the virus in the mosquito's saliva (a minimum of 5–9 days post infection in *Culex pipiens* mosquitoes). Many mosquito genera have been found infected with WNV including *Culex*, *Aedes*, *Anopheles*, and *Mansonia*, although only a few species, mainly from the *Culex* genus, have been identified as competent vectors. Ticks are also WNV vectors, but to a lesser extent. As the virus is transmitted mainly by mosquitoes, the transmission period falls between June and September in the Northern hemisphere when weather conditions for insect growth and reproduction are optimal. **See also**: **Arthropod-borne Viruses**

WNV epidemiological cycle is characterised by a wide variety of hosts. Wild birds constitute its natural reservoir and amplifier and the competence of avian hosts in WNV amplification (depending on the duration and intensity of WNV viraemia) varies by bird species. Passeriformes (the largest and most diverse clade of birds, with more than 6000 recognised species, such as the American crow (*Corvus brachyrhynchos*), the Common magpie (*Pica pica*) or the house sparrow (*Passer domesticus*)), Charadriiformes (aquatic birds such as the Herring gull (*Larus argentatus*)), falconiformes, diurnal birds of prey (such as the Goshawk (*Accipiter gentilis*)) and strigiformes or owls (such as the Barn owl (*Tyto alba*)) have viraemia levels high enough to infect most competent mosquitoes. On the other hand, Columbiformes (doves and pigeons), Piciformes

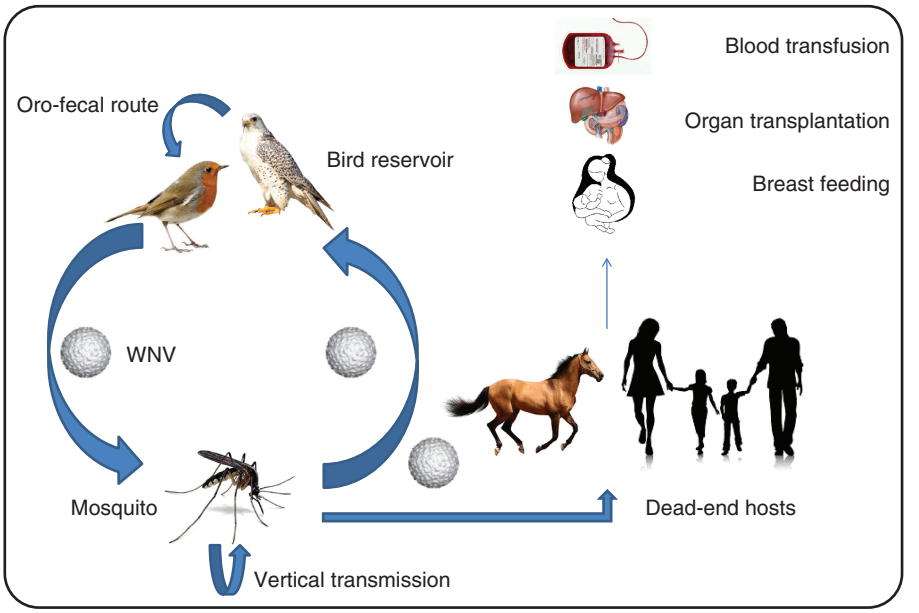


Figure 1 WNV transmission cycle. WNV is an arbovirus transmitted by mosquitoes, mainly from the genus *Culex*, and their reservoirs are wild birds. Transovarial transmission in *Culex* mosquitoes has been infrequently reported and could explain WNV overwintering in certain endemic areas (USA, Italy, etc.). Direct transmission *via* the oral route or direct contacts between birds can participate in WNV amplification, albeit generally at a low level. When virus circulation between reservoir birds and vectors is intense, humans and horses can get incidentally infected by bridge vectors (e.g. mosquitoes feeding both on birds and mammals). Humans and horses are dead-end hosts for WNV as they do not develop viraemia high and long enough to infect naive mosquitoes. Human-to-human transmission can be observed through blood transfusion, organ transplantation and breast feeding upon recent infection of donors or mothers respectively.

and Anseriformes (ducks, geese) are generally resistant to the infection and develop low viraemia. Long-distance migratory birds are probably the main drivers of WNV introduction into Europe from sub-Saharan Africa. Initially, WNV was thought to cause only asymptomatic infections in birds. In 1998, in Israel, high mortality rates were reported for the first time in birds, and specifically in young storks and geese. Shortly afterwards, the WNV epidemics and epizootics in New York city in 1999 and more commonly in the United States were shown to be associated with high mortality rates in diverse bird species (LaDeau *et al*., 2007): the population dynamics of passerines, and particularly corvids, such as the common grackle (*Quiscalus quiscula*), the American crow (*C. brachyrhynchos*), the blue jay (*Cyanocitta cristata*), the magpie (*P. pica*), the house finch (*Carpodacus mexicanus*) and the house sparrow (*P. domesticus*) have been heavily affected over the past two decades.

Spill-over infections in mammals and other vertebrates are caused by bridge vectors with a mixed host diet (e.g. feeding on both birds and mammals). Many animals, including reptiles, amphibians and mammals, can be infected, but most are generally considered as dead-end hosts due to low-level and short-term viraemia (with the exception of reptiles, squirrels and white-tailed rabbits, identified as potentially low WNV amplifiers). Humans and equids are the most susceptible mammals to WNV infection. After an incubation period of 3–15 days, most WNV infections in humans and equids will remain asymptomatic, and about 20% of infected animals or patients will develop mild fever or neuroinvasive forms of West Nile disease. Clinical signs in humans include fever, headache, malaise, lymphadenopathy, myalgia, fatigue, skin rash, diarrhoea and vomiting and in horses include fever, anorexia weakness and muscle fasciculation. These can progress to severe meningoencephalitis (tremors, ataxia, paresis, paralysis, mainly of the hind legs in horses, cranial nerve deficits and cognitive impairment) in both humans and horses or to flaccid paralysis resembling poliomyelitis reported in humans only and, less frequently, death (in approximately 10% of neuroinvasive cases in humans; lethality rates up to 20–57% have been recorded in horses in America and Europe). The risks of developing neuroinvasive forms of the disease are higher in the elderly and in immunocompromised patients. Long-term sequelae (lasting for more than 1 year) are common in patients recovering from WNV encephalitis or flaccid paralysis. The severity of the clinical signs reported in horses depends on individual factors (age, genetic susceptibility and WNV virulence) as well as on breeding and housing methods and prior exposure to close flaviviruses. **See also**: **Flavivirus Infections in Humans**

WNV Epidemiology

The causative agent of West Nile fever, WNV, was discovered in 1937 in Uganda, in the West Nile district. It was isolated from the blood of a febrile woman (Smithburn *et al*., 1940). WNV belongs to the family *Flaviridae*, genus *Flavivirus*. It is a member of the Japanese Encephalitis (JE) serocomplex, which also comprises the JE virus, present in South-East Asia, Saint-Louis Encephalitis Virus (SLEV), mainly described in the United States and Murray

Valley Encephalitis Virus (MVEV) reported in Australia. **See also**: **Arthropod-borne Viruses**

In the recent past, two mosquito-borne flaviviruses pathogenic for birds have been identified in Europe. First, Usutu virus (USUV), belonging to the JE serocomplex, formerly found only in Africa, was isolated in Austria and neighbouring countries, Belgium and Spain mainly from mosquitoes or black birds (*Turdus merula*) (Ashraf *et al*., 2015). Second, Bagaza virus (BAGV), belonging to the Ntaya complex, was identified in Spain in 2010 in partridges (*Alectoris rufa*) and pheasants (*Phasianus colchicus*) (Aguero *et al*., 2011).

Up to the mid-1990s, WNV was essentially maintained in endemic cycles in Africa, West Asia, India, the Middle East and Europe and only affected humans and horses sporadically. Epidemics with encephalitic forms were first observed in Algeria in 1994 and in Romania in 1996. Since then, human epidemics and equine epizootics with a high incidence of WN neuroinvasive disease and deaths have occurred in Eastern and Southern Europe: in the Mediterranean area (Israel since 1998, Tunisia since 1997, Morocco since 1996, etc.), in South Africa in 2008–2010 (Venter and Swanepoel, 2010), and more regularly and with a considerable impact on human and veterinary public health in North America; for this latter region, since its introduction in 1999, WNV has caused more than 40 000 human cases associated with more than 1700 deaths in the United States alone (**Figure 2**). The identification of moribund or dead birds is a specific feature of Israeli and North American infections with lineage 1a strains belonging to the American/Israeli group (see section 'WNV Phylogeny' for more details on WNV classification), as well as of European infections with recently introduced lineage 2 strains (these latter strains mainly affected falconiformes).

Interestingly, in Europe, the epidemiological scenario is quite different from the one in North America, with epidemics that have been limited in both time and space; even in the context of exceptional WNV transmission seasons in Europe, such as was the case in 2010, 2012 or 2013, at most 1200 life-threatening human cases have been reported in Europe since 1999, mainly in the Balkans (Greece, Serbia, etc.) and in Italy. Several hypotheses have been proposed to account for such a discrepancy, including differential virulence of WNV strains and differences in WNV ecology (variety, competence of hosts and vectors, influence of environmental parameters …). In the recent past, European and North American strains were shown to be equally virulent for mammals, at least in the mouse model. However, virulence determinants present in North American strains and some European strains could explain the high mortality rates observed in birds, and in particular a proline residue at position 249 in the helicase domain of NS3, associated with increased virogenesis and pathogenesis in American corvids. **See also**: **Animal Viruses Pathogenic for Humans**

Bird species and populations differ between the two continents. It has been hypothesized that Palearctic bird populations in Europe and Africa have co-evolved with WNV, rendering them less susceptible to WNV infection than American species naïve to WNV. Also, reservoir biodiversity or species richness could potentially act as protective factors via a dilution effect or as WNV transmission facilitators. Moreover, WNV vectors in North America and in Europe may differ in their competence and in their feeding behaviour, as such in their ability to infect avian and/or mammalian hosts. Finally, past exposure to various mosquito and tick-borne flaviviruses circulating in Europe might confer partial or complete cross-protection to WNV. **See also**: **Virus Evolution**

WN Virion Structure and Biology

The ultrastructure of WNV particles has been elucidated in detail. Electron cryomicroscopy revealed that WNV particles are

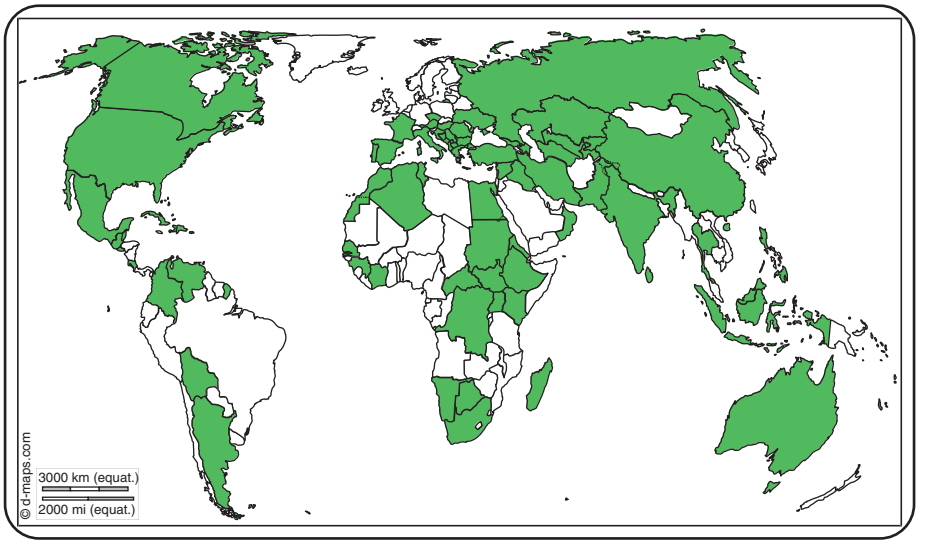


Figure 2 Worldwide distribution of WNV, established with data collected on the pro-Med website up to September 2014. Countries are coloured when WNV or WN fever/encephalitis has been detected in humans, birds, horses and other animals or vectors.

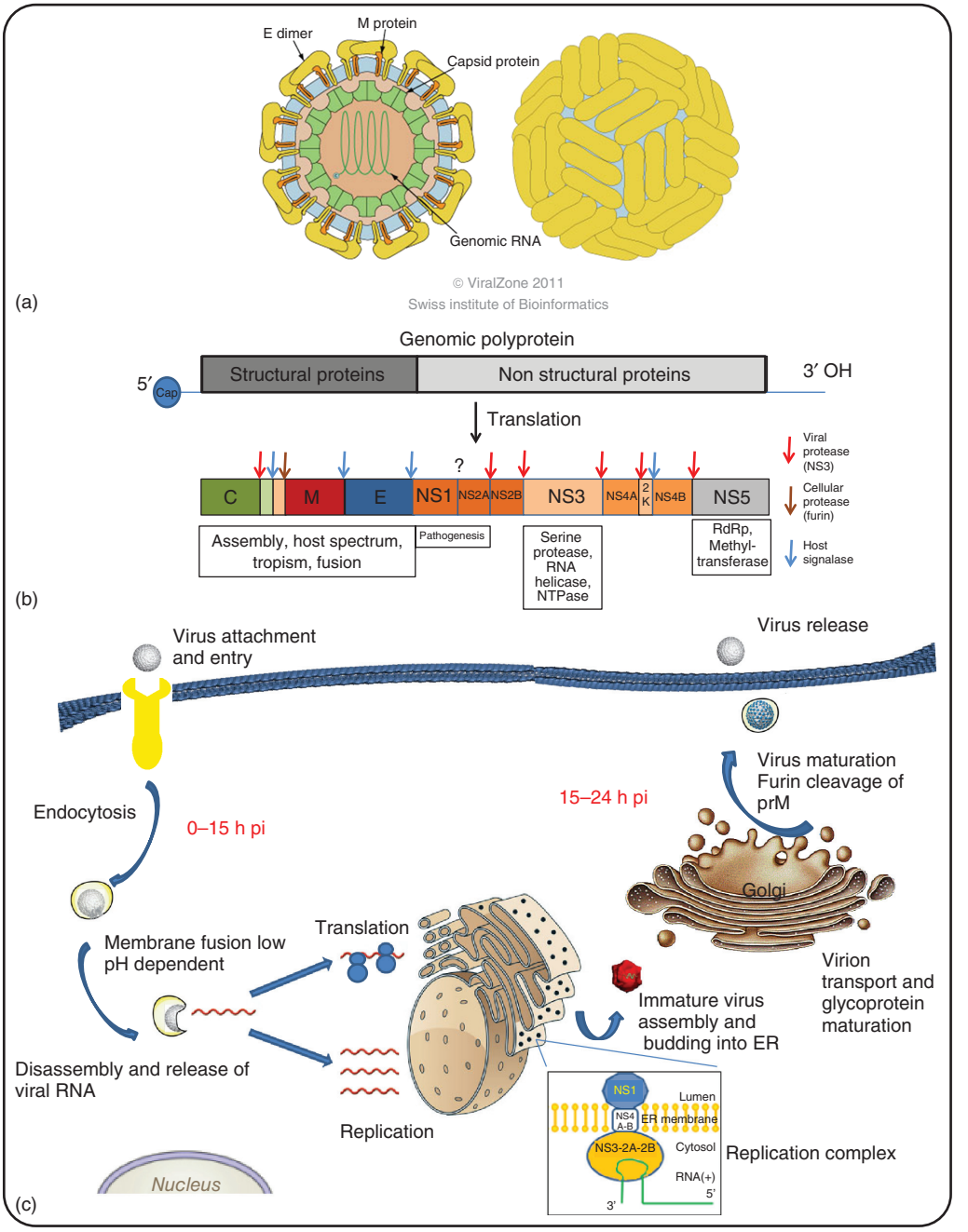


Figure 3 (a) Schematic representation of WNV viral particles, courtesy of ViralZone, SIB Swiss Institute of Bioinformatics. Left, representation of envelope (E) and membrane proteins at the surface of the virion. Capsid proteins form a shell protecting the viral RNA. Right, organisation of surface E dimers. Reproduced with permission from ViralZone, SIB Swiss Institute of Bioinformatics. (b) Schematic representation of WNV genomic organisation and viral proteins. Genomic RNA is capped at its 5′ end and non-polyadenylated at its 3′ end. WNV RNA encodes for a polyprotein of 3300 amino acids, subsequently cleaved into 10 viral proteins: three structural proteins (C, prM and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Structural proteins are involved in viral assembly, host tropism, binding and fusion to host cell. Non-structural proteins play key roles in RNA replication, control of host immune reactions and viral morphogenesis. Cleavages at the C–prM, prM–E, E–NS1, NS4A–NS4B and probably also the NS1–NS2A junctions are performed by the host signal peptidase located within the lumen of the ER. The remaining cleavage bonds are processed by the viral NS3 protease. RdRp, RNA-dependent RNA polymerase. (c) Schematic representation of WNV replication cycle. WNV interacts with its host target cell through the envelope protein that contains the receptor binding domain. The viral particle is then endocytosed. Acidic pH of the endosome induces conformation changes in the envelope protein, leading to the exposure of its fusion peptide. The viral capsid is then freed from the endosome, a step followed by decapsidation of the genomic RNA. The positive-sense RNA is immediately translated and synthesised NS viral proteins form a replication complex at endoplasmic reticulum membranes. Newly synthesised genomic RNAs are encapsidated, bud into the ER lumen and are transported in fusion vesicles. New virions are released through the exocytic pathway. ER, endoplasmic reticulum; pi, post infection.

spherical with a 50-nm diameter (**Figure 3a**). Mature WNV particles consist of a smooth lipid envelope containing two viral proteins, the envelope E and the membrane M, surrounding an icosahedral capsid made up of a single viral protein, the capsid protein C. Viral particles protect a 11-kb single-stranded positive-sense RNA (RNA(+)) genome. WNV is capped in its 5′ extremity (7-methylguanosine), and not polyadenylated in its 3′ end. Both 5′ and 3′ non-coding regions (NC) are well conserved and form secondary hairpin structures, necessary for translation, replication and viral RNA packaging. The WNV genome encodes for 10 genes: C, prM and E within the 5′ portion of the ORF (open reading frame) are structural proteins, and non-structural protein (NS) NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 are encoded within the 3′ portion (**Figure 3b**). **See also**: **Flavivirus Infections in Humans**; **RNA Virus Genomes**

Structural proteins are essential to the packaging of viral RNA (C), and to primary interactions (binding) and fusion with the host cell (E). E and M were shown to stimulate B- and T-cell responses, although E appears to be the most immunogenic and to induce most of the neutralising antibody response. Non-structural proteins ensure viral replication and virion assembly, and counteract the host antiviral response. Intracellular NS1 acts as a cofactor for viral replication and a TLR (toll-like receptor)-signalling antagonist. NS1 can also be expressed at the cell surface or be secreted and was shown to antagonise complement activation. NS2A participates to the formation of replication complexes at endoplasmic reticulum (ER) membranes, and interacts with NS3, NS5 and the 3′ non-coding part of the genome for viral particles assembly. NS2A also inhibits the innate α/β interferon (IFN) response. NS2B forms a stable complex with the serine protease region of NS3 and is a cofactor of NS3 for NS2A/NS2B, NS2B/NS3, NS3/NS4A and NS4B/NS5 cleavages. The serine protease domain is located on the *N*-terminal end of the NS3 protein and is also involved in the maturation of the *C*-terminal domain of C and NS4A. The *C*-terminal domain of NS3 displays RNA-stimulated nucleoside triphosphatase (NTPase), ATPase/helicase and 5′-triphosphatase (RTPase activity needed for the dephosphorylation of the 5′ end of the viral RNA before cap addition) activities. NS3 also induces cell death by apoptosis consequently to WNV infection, through the activation of caspase 8. NS4A is localised at replication complexes, supporting its role in viral RNA replication. NS4A was also shown to be pivotal for the rearrangement of cytoplasmic membranes and participates in virion budding. Finally, NS4B and NS5 block the activation of the IFN signalling pathway avoiding the induction of antiviral responses in infected cells. NS5 is the RNA-dependent RNA polymerase and is the major component of replication complexes in association with NS3. NS5 also displays a methyltransferase activity (MTase).

The WNV replication cycle (**Figure 3c**) first involves the binding of the E protein to receptors on the cell membrane. Receptors are not formally identified but DC-SIGN, alphaVbeta3 integrin and laminin-binding protein are potential candidates. Subsequently, the virus enters the cell through endocytosis in clathrin-coated pits. Acidification of the endosome compartments induces major conformational changes of E allowing the fusion of viral and cellular membranes. After viral RNA is released into the cytoplasm, it is immediately translated into a polyprotein that will be cleaved by cellular and viral proteases (**Figure 3b**). Viral proteins form a replication complex in which an antisense negative strand RNA is produced by the viral NS5 protein. During virus replication, antisense RNA, used as a template for generation of the infectious sense RNA genome, is produced by NS5. WNV newly synthesised virions assemble at virus-induced membranes derived from the ER and immature virions bud into the ER lumen. Virion maturation is ensured by its transit through the mildly acidic compartments of the trans-Golgi network, inducing E rearrangements and allowing the generation of mature M proteins through proteolytic processing of prM by furin proteases. Finally, mature virions are released by the host cell through exocytosis. **See also**: **Virus Replication**; **Virus Host Cell Receptors**; **RNA Plant and Animal Virus Replication**

WNV Phylogeny

WNV can be subdivided into at least six lineages (Gray and Webb, 2014). Lineages 1 and 2 are the most widespread and are at the origin of most major epidemics encountered so far. Lineage 1 contains isolates from Europe, North America, the Middle East, Africa (clade 1-a), Australia (Kunjin isolates) (clade 1-b) and India (lineage 1-c). Lineage 2 was only described in sub-Saharan Africa and Madagascar up to 2004 and was considered of low pathogenicity. However, in 2004, a lineage 2 WNV strain was identified for the first time in Europe in Hungary and found to be virulent for birds of prey. During the same period, lineage 2 strains caused neuroinvasive infections in humans and horses in South Africa. Since then, European lineage 2 WNV spread in Greece, having caused more than 600 human cases and 40 human fatalities since 2010 (Papa *et al*., 2010), and also in Austria, Czech Republic, Serbia, Croatia, Italy, Romania and Russia (Barzon *et al*., 2013; Hernandez-Triana *et al*., 2014; Kurolt *et al*., 2014; Petrovic *et al*., 2013; Platonov *et al*., 2011; Sirbu *et al*., 2011; Wodak *et al*., 2011). Unexpectedly, a change in WNV ecology has occurred since 2010 in Europe, with lineage 2 strains being responsible for most human WNV cases, in particular, in the Balkans. Lineage 3 consists of a unique virus strain isolated from *C. pipiens* mosquitoes in the Czech Republic (Rabensburg virus) (Bakonyi *et al*., 2005). Lineage 4 was isolated from *Dermacentor marginatus* ticks and *Uranotaenia unguiculata* mosquitoes in Russia (Leiv virus) and is closely related to the proposed lineage 6, aggregating WNV strains identified in Spain (2006) and Austria (2013) from *C. pipiens* and *U. unguiculata* mosquitoes, respectively (Pachler *et al*., 2014; Vazquez *et al*., 2010). 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 clade 1-c. Lineages 7 and 8, respectively, the Koutango virus isolated in Senegal (Traore-Lamizana *et al*., 2001) and the Sarawak strain isolated in Malaysia (Ching *et al*., 1970), correspond to divergent WNV strains (lineage 7 is currently considered as a separate virus species). Interestingly, even if Koutango disease has never been observed in patients or animals in Africa, Koutango virus was shown to be the most virulent isolate among WNV and WNV-like strains to date, at least for model mammals,

for example, mice (Prow *et al*., 2014). **See also**: **RNA Viruses: Control, Mutagenesis and Extinction**

WNV Infection in Insects, Birds and Mammals

After a blood meal has been ingested by a mosquito on a heavily viraemic bird (viraemia > 5 log10 PFU (plaque forming unit) of virus per millilitre to infect *C. pipiens* mosquitoes) and processed, WNV infects and replicates in the insect's midgut cells. Afterwards, the virus enters the haemolymph and finally reaches the salivary glands where it replicates and generates high viral loads in its saliva. After a silent extrinsic incubation period of 1–3 weeks, the mosquito can excrete the virus in the saliva and contaminate mammalian or avian hosts upon blood feeding. Resistance to WNV infection in the mosquito is conferred by physical barriers due to chitins and other surface proteins hindering WNV interactions with the midgut epithelium as well as induction of RNA-interfering responses through the sensing of viral RNA or components by TLR or RIG I-like receptor (RLR) (Lazear and Diamond, 2014).

In mammals (**Figure 4a**), after the bite of an infected mosquito, WNV is believed to initially replicate in keratinocytes, newly recruited neutrophils and skin dendritic cells such as Langerhans cells (LCs). Infected LCs rapidly become fully functional antigen-presenting cells, migrate to regional lymph nodes and reach the bloodstream. Early virus spread and the infection of peripheral tissues such as the spleen, liver and kidneys are the result of this primary viraemia (3–4 days post infection in mammals). WNV is cleared from the blood and peripheral organs by day 7 post infection.

Infection of the central nervous system (CNS) can be observed starting at day 5 post infection, provided that sufficient peripheral replication of WNV has occurred. Three major routes of CNS entry have been proposed: (1) entry through the blood–brain barrier (BBB) via infected leukocytes, (2) direct crossing of the BBB after its integrity has been compromised subsequent to tumour necrosis alpha (TNF-α) secretion or to direct infection of brain endothelial cells, and (3) entry by retrograde axonal transport after the peripheral nervous system has become infected. Infection of neurons is associated with degeneration, a loss of cell architecture and cell death. Mononuclear cell infiltrates can be observed throughout infected regions and help destroy WNV infected cells while contributing to pathogenesis by releasing pro-inflammatory cytokines that are toxic for CNS cells. **See also**: **Flavivirus Infections in Humans**

In peripheral tissues, early innate immune responses (antiviral cytokine and chemokine secretion, complement activation), and especially the IFN type I response, limit virus infection and dissemination. **See also**: **Immune Evasion by Viruses**

WNV infection has mainly been studied in mammals, so there is little information about the early stages of infection in birds. It is supposed that, as for mammals, WNV replicates at the inoculation site and is then distributed to all organs. However, the virus can be detected in the blood as early as 30–45 min (generally 1 day) after a mosquito bite, suggesting that primary viraemia does not necessarily depend on local replication. The viraemia peak and organ distribution vary widely in bird species. WNV can infect all major organ systems (**Figure 4a**) and a wide variety of individual cell types. The organs most frequently infected are the spleen, kidneys, skin and eyes, while the liver is the least likely to harbour infectious virus particles. WNV also reaches the CNS via the bloodstream and was shown to infect endothelial cells or invade the CNS through infected immune cells. Even though little is known about innate avian host defences against WNV infection, it is speculated that immune responses in birds are different from those observed in mammals and could promote viral persistence.

Adaptive Immune Responses in Vertebrate Hosts

As for all viral infections, both arms of adaptive immunity, humoral and cellular responses, are induced. As regards humoral immunity, neutralising antibodies (NAbs) are critical for the control of viral dissemination. IgM antibodies are detectable in the blood and the cerebrospinal fluid (CSF) peaking 2–8 days after symptom onset but can be detected up to over a year in humans (Murray *et al*., 2013) (**Figure 4b**). Because IgMs do not cross the BBB, their presence in CSF indicates CNS infection. IgGs appear in the blood with a short delay, as soon as 5–12 days at the latest after neurological symptom onset. IgGs can persist for several years and confer long-term sterilising protection (Murray *et al*., 2013) (**Figure 4b**).

Most NAbs are directed against epitopes in E and M proteins that are generally less accessible in mature virions (Diamond *et al*., 2008). The E protein is organised into three structural domains that are targeted by Nabs, even though domain III (E-DIII) generates the most potent antibodies. Anti-E-DIII antibodies do indeed inhibit WNV entry through the blocking of receptor binding sites or at later stages of WNV–cell interactions. Furthermore, NS1 is the only flavivirus glycoprotein that is secreted or exposed at the surface of infected cells and is consequently also targeted by protective Abs (Chung *et al*., 2006). The binding of antibodies to cell-surface-bound NS1 facilitates the phagocytosis of infected cells through activated Fc-γ receptors and their clearance.

As regards innate cellular responses, limited data suggest that macrophages and dendritic cells may directly clear WNV. However, some data also suggest that subversion of the antiviral activities of macrophages by WNV may facilitate viral replication and spread, enhance the intensity of immune responses and lead to severe immune-mediated disease. **See also**: **Immune Evasion by Viruses**

Adaptive cell responses are characterised by T-cell proliferation resulting from the recognition of infected cells expressing class-I MHC (major histocompatibility complex)-associated antigens at their surface and secreting pro-inflammatory cytokines. CD4+ T cells play an important role in the control of WNV infection through CD8+ T-cell priming and killing of infected cells, cytokine production, B-cell activation and direct cytotoxic activity (Samuel and Diamond, 2006).

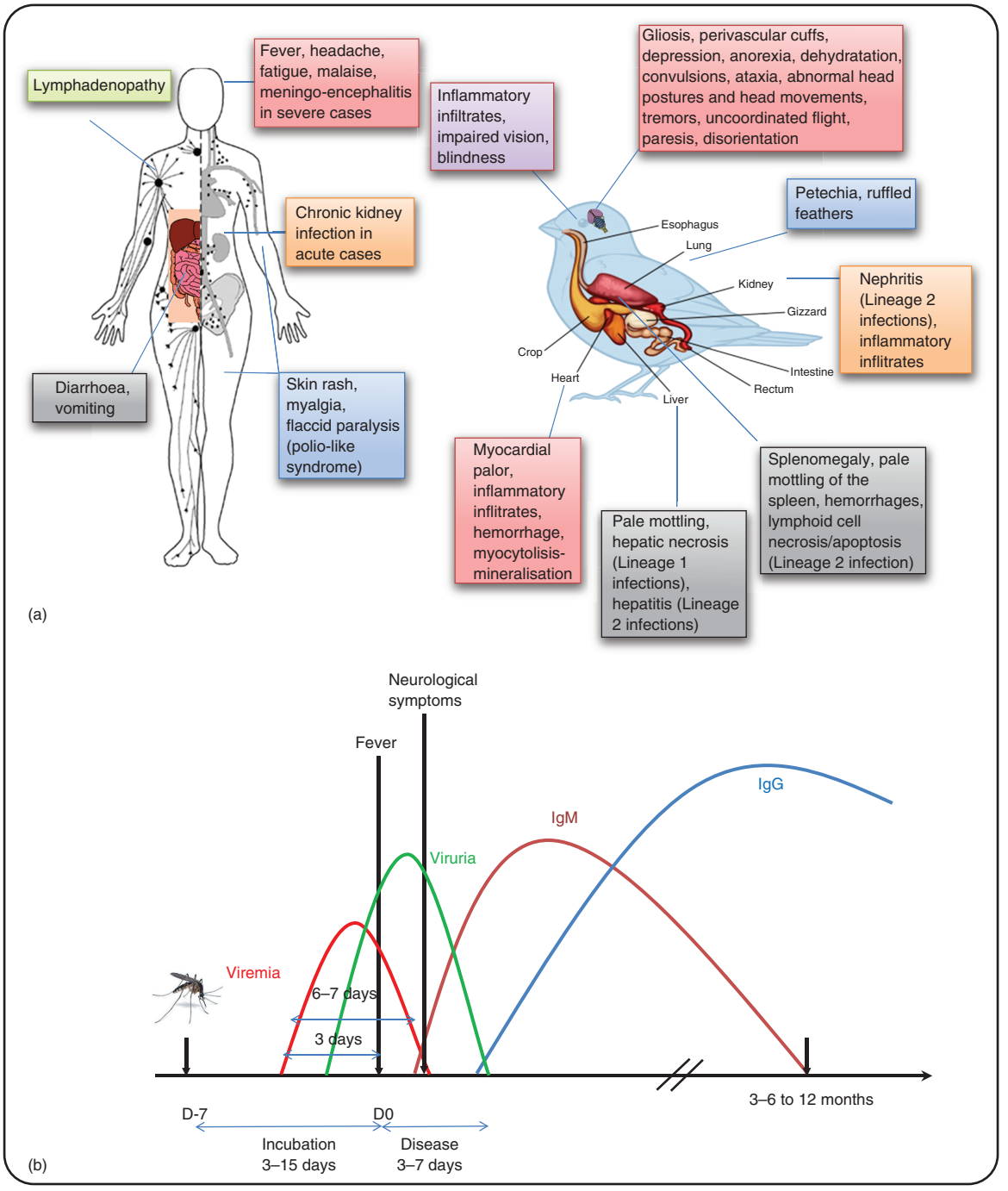


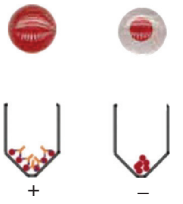
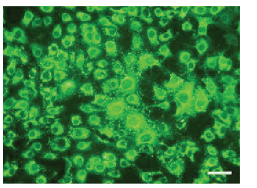
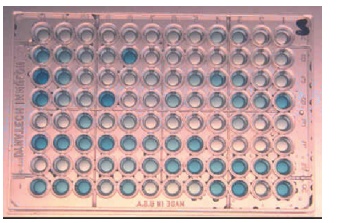
Figure 4 (a) Cartography of WNV infection sites and pathology in humans and birds. Note: Pathology displayed for birds depends on bird species and/or WNV lineage (Gamino and Höfle, 2013). (b) Kinetics of infection markers after WNV inoculation into the skin. Primary viraemia can be detected 3–4 days post infection in mammals. Shortly afterwards, WNV is detected in the urine of patients. IgM antibodies are detectable in the blood and the cerebrospinal fluid approximately 4–7 days after infection with a peak 2–8 days after the onset of neurological symptoms and can last from 3–4 months to up to 1 year. IgG immunoglobulins are produced in the blood from day 5–12 post infection and can persist for several years in the blood.

WNV Diagnostic Tools

Neurological symptoms and lesions are not specific to WNV infections. Consequently, laboratory tests are essential to confirm or exclude WNV infection. Indirect or serological tests that aim to detect WNV antibodies, such as enzyme-linked immunosorbent assays (ELISAs), haemagglutination-inhibition tests (HITs) or immunofluorescence assays (IFAs) are the preferred diagnostic tools (see **Table 1**). Most serological tests rely on the detection of anti-prM, anti-E and/or anti-NS1 antibodies. ELISAs offer rapid, sensitive, reproducible, multispecies (in case of competition or inhibition formats) and affordable screening. They can help differentiate IgG and IgM antibody response (in-house and commercial IgM antibody capture assays have been developed). Moreover, ready-to-use diagnostic kits for the surveillance of animal and human West Nile cases are commercially available. Because of the low specificity of rapid WNV serological assays, comparative virus neutralisation tests (VNTs) should be performed to confirm WNV diagnosis, considering flaviviruses circulating in the area under investigation (Dauphin and Zientara, 2007). Improvements of ELISA specificity have been sought using a recombinant version of the E-DIII antigen, as E-DIII contain virus-specific epitopes (Diamond *et al*., 2008), or by substituting NS1 (Chao *et al*., 2015) for the whole virus or prM-E antigens. NS1 ELISAs confer another advantage as they do not react with sera from animals vaccinated with most of the DNA or recombinant WNV vaccines (differentiating infected from vaccinated animals – DIVA – strategies) (Kitai *et al*., 2007). More recently, microsphere multiplex immunoassays have been developed, coupling different flavivirus antigens to fluorescent and differentiable beads (Houser, 2012), but their specificity still needs to be improved.

NS1 protein is detected in sera of infected animals during acute infection, as early as 1 day after WNV infection, with a sensitivity significantly higher than that of real-time PCR from samples of WNV-infected mice (Ding *et al*., 2014). Virus detection in humans and horses is tricky once neurological symptoms have developed because of the low-level and short-term viraemia in addition to the late appearance of clinical signs, when the viraemic phase is over. However, viraemic patients can usually be detected when WNV fever is present, during the first 4 days of illness. Viral isolation is useful for WNV detection in vertebrate and avian samples (CSF), blood, urine or the brain of infected mammals, as well as oral and cloacal swabs, feather follicles and multiple peripheral organs (liver, kidneys, intestine, etc.) in addition to the brain, in birds, as well as mosquito homogenates. It can be performed through isolation in cell culture (mammalian Vero, RK-13 or mosquito C6/36 or AP61 cell lines in particular) or in *ex* and *in vivo* systems (embryonated chicken eggs, new-born mice) (Bahuon *et al*., 2012; Barzon *et al*., 2014; Castillo-Olivares and Wood, 2004; Ostlund *et al*., 2001). WNV can also be identified by reverse-transcription polymerase chain

Table 1 Main serological tests used for WNV diagnosis

	VNT	HIT	IFA	ELISA
Principle	Neutralisation of virus attachment to the cells or of post-binding steps	Inhibition of virus-induced erythrocyte aggregation	Antibody binding to virus-infected cells coated on microscope slides, revealed by fluorophore-conjugated antibodies	Antibody binding on plates coated with a viral antigen and revealed by enzyme-conjugated antibodies
Antibody target	Neutralising epitopes in the E protein	E protein	Whole virus	Whole virus, viral cell lysate or recombinant proteins (prM-E, NS1, E-DIII, etc.)
Interpretation	 Protection from viral lysis in the presence of neutralising antibodies	 Haemagglutination inhibition in the presence of antibodies in the serum	 Fluorescence of virus-infected cells in the presence of antibodies in the serum	 Colorimetric reaction intensity correlating with the amount of antibodies
Field applications	Confirmation of past infections	Screening tool	Investigation of clinical cases	Investigation of clinical cases, screening tool and trade certification

VNT, virus neutralisation test; HIT, haemagglutination-inhibition test; IFA, immunofluorescence assay; ELISA, enzyme-linked immunosorbent assay.

reaction (RT-PCR, classical or in real-time) (Lim *et al*., 2013), antigen detection by immunofluorescence and immunohistochemistry (Dauphin and Zientara, 2007). Virus or RNA detection methods remain costly and time consuming and require the use of biosafety level 3 facilities.

Vaccines and Treatments

Only veterinary vaccines are currently used in Europe for vaccinating horses. An inactivated vaccine (EQUIP® WNV, formerly Duvaxyn® WNV, Zoetis) and a canarypox recombinant vaccine (PROTEQ WEST NILE®, Merial/Sanofi Aventis) have been commercialised in Europe since 2009 and 2011, respectively. They have proven to be very effective in protecting horses from meningoencephalitis in North America (Epp *et al*., 2007) where four vaccines are available (two inactivated, one DNA and one canarypox recombinant vaccine). Minke *et al*. (2011) and Bowen *et al*. (2014) also demonstrated their efficacy in protecting horses against heterologous strains (with lineage 1 vaccines conferring protection against lineage 2 strains). Human vaccines are under development. One of them, the Chimerivax-West Nile virus vaccine (Acambis, Sanofi-Pasteur) based on the attenuated Yellow Fever Virus (YFV) vaccine strain (17D) incorporating the WNV prM and E genes, is currently undergoing clinical trials (Biedenbender *et al*., 2011; Iyer and Kousoulas, 2013). A recombinant live attenuated measles virus (MV) vaccine expressing the soluble ectodomain of WNV E elicited protective immunity in mice and non-human primates as early as 2 weeks after immunisation (Brandler and Tangy, 2013).

Several therapies are under investigation, including immune γ-globulins, WNV-specific neutralising monoclonal antibodies, corticosteroids, ribavirin, interferon α-2β and antisense oligomers (Beasley, 2011; Diamond, 2009; Long, 2014).

Conclusion

WNV remains a serious threat to the public and animal health, especially to very young, elderly and immunocompromised individuals. The emergence of WNV in the United States and the appearance of new pathogenic strains belonging to lineages 1 and 2 in Europe reveal the intensity of WNV strains introduction and spread over the past few years. These outbreaks are associated with WNV neuroinvasive disease and West Nile fever. Thus, sustainable surveillance and vector management programs are critical. Diagnostic tests are also important for identifying outbreaks and have improved considerably, enabling rapid detection of WNV. Progress continues in the development of WNV vaccines and therapies for humans and horses.

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