West Nile virus: An overview of current information

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Abstract

West Nile Virus (WNV) is a vector-borne flavivirus primarily infecting mosquitoes, birds, horses and humans. WNV is responsible for asymptomatic infections or a variety of clinical manifestations ranging from mild febrile illness to neuroinvasive disease. The frequency and severity of WNV-related disease have lately increased in the European Union and in the neighbouring countries, with particular concern for the Mediterranean area. This trend is probably connected to a raise in both average temperatures and rainfall, favourable factors for WNV spread. Due to the marked and expanding geographical distribution of the vector and the high endemic potential of WNV, this virus is worldwide considered an increasing public health apprehension. An augmented burden of WNV severe illness has been reported. Alarmingly, no vaccine or specific antiviral treatments are currently available for WNV infection. Hereafter, we will review the available information summarizing molecular biology, pathogenesis, clinical manifestations, epidemiology, diagnosis and therapy for WNV infection in humans.

Introduction

West Nile Virus (WNV) is a neurotropic mosquito-borne virus belonging to the *Flaviviridae* family which includes other human pathogens such as dengue, yellow fever, and Japanese encephalitis viruses.

It represents a serious danger for the world population, as a geographic expansion of its arthropod vector empowered a dramatic multifactorial spread of WNV infection amongst humans, birds and horses.[1] WNV was first identified in Uganda, in 1937, from a patient with a viral syndrome.[2] Following the first isolation, the virus was systematically studied only in the 50s around the endemic Nile delta region, from which it takes its name. At that time, WNV detection and high seroprevalence rates were astonishingly common in both people and animals of the area, which was an important source of initial information about the virus.[2-4] In 1957, the first outbreak of WNV neuroinvasive disease (WNND) was reported in Israel, India and Egypt, considered as endemic areas.[5] Later, since the 1990s, the geographic distribution of WNV also included Africa, Western Asia, the Middle East and some parts of Europe.[6]

Today, according to data from the European Centre for Disease Prevention and Control (ECDC), WNV is possibly in a new phase of expansion, in comparison to the past four years. With regard to the 2083 reported human cases in the European Union and the European Economic Area (EU/EEA) countries, as of December 13th 2018, Italy is the most affected country (576 cases) followed by Serbia (415), Greece (311), Romania (277) and Hungary (215).[7]

WNV is transmitted through the bite of infected mosquitoes and is maintained in nature through an enzootic cycle, in which birds represent amplifying hosts, while humans and horses are dead-end hosts.[8] The main vector of WNV is represented by *Culex* genus mosquitoes. Specifically, the dominating vectors differ from place to place and are represented by *Culex pipiens var. pipiens* is the United States, *Cx. univittatus* in Africa and in the Middle East, *Cx. pipiens* and *modestus* in the European continent.[9,10] WNV transmission can also occur through blood transfusions, organ transplantation, occupational exposure and, possibly, sexual contact.[11-13]

A determining driver of WNV spread is represented by the climatic conditions;[14,15] in fact, warmer temperatures and increased (but not extreme) rainfall widen vector abundance and viral transmission.[16,17] Infections are generally asymptomatic (approximately 80%); only an estimated 20% of subjects present symptoms of West Nile fever (WNF), a febrile illness that often includes headache, nausea, myalgia or arthralgia, gastrointestinal problems and a rash.[18] Less than 1% of infected patients develop neuroinvasive disease such as meningitis, encephalitis and flaccid paralysis.[19,20] The incubation period in humans ranges from 3 to 15 days; viremia occurs within 1-3 days and can last up to 11 days.[21] Recovery from WNND may be slow and the mortality rate, approximately 10%, is influenced by patients' age and immunological conditions. Currently, no specific therapies or vaccines are available for either treatment or prevention of WNV infection.[22]

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Virion structure and genomic architecture

WNV is an enveloped virus with an icosahedral symmetry.[23] This virus has a multilayer organization: the outermost layer contains the envelope proteins, while the nucleocapsid core consists of viral genome and capsid protein.

WNV genome is a single-stranded positive RNA [(+) ssRNA] molecule of about 11 Kb.[24] The viral genome includes a single open reading frame and two terminal non-coding regions at the 3' and 5' ends (631 and 96 nucleotides, respectively), which determine the formation of stem-loop structures favouring viral replication.[25] The genome encodes three structural proteins [capsid (C), pre-membrane (prM) and envelope (E)] and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5)[26,27] (Figure 1). Structural proteins allow viral entry (E) and fusion (prM, E), while NS proteins play an important role in viral replication and modulate host immune responses.[28,29]

The capsid protein (C), in association with the viral RNA, forms the nucleocapsid

core; it also participates into virion assembly.[30] The prM gene product codes for a trans-membrane protein protecting the envelope glycoproteins from a misplaced early fusion with host membranes and is later cleaved by furin and furin-like proteases in the acidic trans-Golgi compartment (TGN).[31,32]

The envelope glycoprotein (E) is the primary determinant of WNV external appearance and a major mediator for both attachment and penetration into host-cells.[26] Its ectodomain folds into three structural domains: DI, DII, DIII. The central DI domain is responsible for the structural organization of the E protein;[33] DII promotes the fusion between host and viral membranes;[34] DIII forms an immunoglobulin (Ig)-like fold and rules receptor binding. As a class II fusion protein, its fusion loop is shielded by a cap protein, represented by pr peptide.[33] The NS proteins are fundamental regulators of WNV life cycle, particularly with respect to viral replication and immune evasion. The main functions of both structural and NS proteins are listed in Table 1.[26,30,31,35-42]

Viral replication

WNV is characterized by a notable cell tropism, though it is schematically classified amongst neurotropic flaviviruses. It replicates in various cell types, from a wide range of vertebrate and invertebrate species encompassing birds, amphibians, mammals and insects.[43,44] The virus is capable of infecting numerous human cells including dendritic cells (DCs), monocytes/macrophages, B and T lymphocytes, endothelial cells, hepatocytes and neurons.[45]

WNV life cycle starts after a *Culex* mosquito consumes a blood meal and injects the virus into host tissues.[46] As a first step, the virus binds to cell-surface attachment molecules and receptors to enter in a permissive host cell.[47] Different molecules have been involved as cellular receptors for WNV, such as DC-SIGN and DC-SIGN-R,[48] mannose receptor and glycosaminoglycans.[49] Furthermore, WNV also binds to αvβ3 integrin through DIII RGD/RGE sequence.[50,51] After viral attachment, WNV enters the cell mainly via clathrin-mediated endocytosis,[50] as confirmed in experiments with the antipsychotic drug chlorpromazine, used as an inhibitor of clathrin-coated pit formation.[50,52] After internalization into the host cell, WNV particles are carried within endosomal vesicles. The acidic pH inside the endosomes triggers rapid conformational rearrangements of the E glycoprotein, which results in a dimer-to-trimer shift with the exposure of a membrane-insertable anchor leading to the fusion of the endosomal membrane with the viral envelope.[33]

This step allows viral genome release into the host-cell cytoplasm where the RNA is translated into a polyprotein that is subsequently cleaved by the NS3 and other proteases to form structural (C-prM-E) and non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).[53]

The NS proteins play an important role in the formation of the viral replication complex, a multi-protein structure interacting with virus-manipulated host ER membranes.[54] These proteins assemble and synergistically operate to allow viral replication.[35,54] Virion assembly occurs at the ER membranes: at first, capsid proteins bind and wrap around replicated (+)ssRNA to form a weakly defined nucleocapsid. Then, the nucleocapsid is coated by host ER membranes and the viral E and prM proteins to produce immature virions.[22]

Immature particles are transported through the secretory pathway in the TGN,

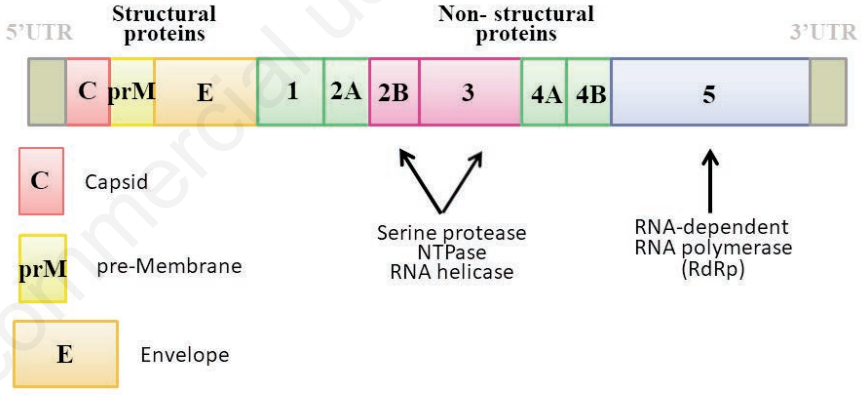

Figure 1. West Nile Virus (+)ssRNA genome, with 3' and 5' untranslated regions (UTRs) and the viral polyprotein encoding structural (capsid, C; pre-membrane, prM; envelope, E) and non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins.

Table 1. Main functions of West Nile virus structural and non-structural proteins.

Protein	Function	Source
C	Formation of nucleocapsid core; Participation into virion assembly	Markoff *et al.* (1997)[30]
prM	Prevention of early-fusion between envelope and host membranes	Mukherjee *et al.* (2011)[31]
E	Attachment, entry and fusion	Mukhopadhyay *et al.* (2003)[26]
NS1	Viral RNA synthesis; Replication complex formation; Immunomodulator	Youn *et al.* (2013)[35]
NS2A	Involved in viral replication and assembly	Leung *et al.* (2008)[36]
NS2B	Co-factor of NS3 protease	Kaufusi *et al.* (2016)[37]
NS3	Viral protease	Wengler *et al.* (1991)[38]
NS4A	Co-factor for the ATPase activity of NS3-helicase	Shiryaev *et al.* (2009),39 Ambrose *et al.* (2011)[40]
NS4B	Inhibition of host interferon responses to West Nile virus	Wicker *et al.* (2012)[41]
NS5	RNA-dependent RNA polymerase	Zhou *et al.* (2007)[42]

C, Capsid protein; prM, Pre-membrane protein; E, Envelope protein; NS1, Non-structural protein 1; NS2A, Non-structural protein 2A; NS2B, Non-structural protein 2B; NS3, Non-structural protein 3; NS4A, Non-structural protein 4A; NS4B, Non-structural

where their maturation is regulated by furin and furin-like proteases cleaving prM into pr peptide and mature M protein, allowing E protein fusion-favouring conformational changes. Meanwhile, the virions contained in the vescicles are transported towards the plasma membrane and released via exocytosis.[55]

Clinical features and pathogenesis in humans

Clinical manifestations

The clinical spectrum of symptomatic WNV infection in humans is wide. About 80% of infected people are asymptomatic; approximately 20% manifest a nonspecific febrile illness referred to as WNF that can range from mild to severe, while a small subset of patients (<1%) develop a potentially lethal neuroinvasive disease.[56,57]

Complicating differential diagnosis, WNF presents as a dengue-like illness, often associated with malaise, headache, myalgia, nausea, vomiting, chills and lymphadenopathy;[58] occasionally, it can be characterized by the appearance of a rash on trunk and extremities.[59-61]

Symptoms may last a few days or weeks, depending on the age of the patient. In children, mild fever is common; in young people, medium to high fever, accompanied by headache and muscle pain, are frequently observed. Symptoms in the elderly tend to be more severe.[62]

Progression to severe neurological illness may include encephalitis or meningitis, sometimes resulting in an acute flaccid paralysis, similar to that seen with poliomyelitis.[20,63]

Several surveillance studies coordinated by the Centres for Disease Control and Prevention (CDC), from 2005 to 2009, report about 12,975 cases of WNV infection of which 35% affected by WNND and 496 fatalities.[63] Patients with WNND may manifest altered mental status, fatigue, stiff neck, movement disorders including Parkinsonism and tremors (Guillain-Barré syndrome).[64,65]

Patients older than 50 years of age and immune-compromised subjects are at increased risk of developing severe neurologic disease.[66,67]

West Nile virus transmission and vector

WNV is maintained through an enzootic cycle, between birds and ornitophilic *Culex* spp. mosquitoes, with occasional spillover involving horses and humans.[68,69]

The virus has been isolated in several mosquitos species, with some geographical variability. In North America, particularly the Northern-Central part of the United States, the most important vector is *Culex pipiens pipiens*, with some western spots dominated by *Cx. tarsalis*;[70-72] *Cx. univittatus* is the main vector in Africa and the Middle East;[73] *Cx. quinquefasciatus, Cx. tritaeniorhynchus* and *Cx. vishnui* are the main vectors in Asia,[8] *Cx. pipiens*, *Cx. modestus* and *Coquillettidia richiardii* are the primary vectors in Europe.[74,75] In Italy, Mancini *et al*. confirmed *Cx. pipiens* relevance and enderpin local vector characterization for WNV.[76] Other mosquito genera such as *Aedes albopictus*, *Aedes vexans*, *Ochlerotatus japonicus* and *Ochlerotatus triseriatus*, represent additional vectors for WNV transmission.[77] It is worth noticing that WNV is an intensely host-versatile virus, whose error-prone NS5/RdRp protein probably contributes to its adaptability.[69]

As for other arthropod-borne viruses, the vector can directly transmit WNV in a vertical transovarian manner to mosquito prole and, in a horizontal way, across different species.[78]

After a blood meal from a viremic animal, infected mosquitoes transmit WNV to vertebrate hosts; specifically, the vector-injected saliva contains active proteins functioning as immune-modulatory and antimicrobial factors.[79,80]

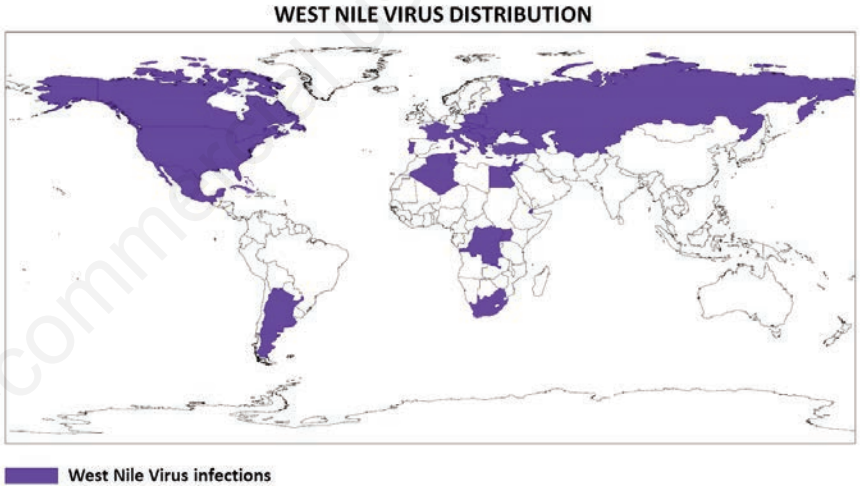

Figure 2. Worldwide distribution of West Nile virus from September 2012 to December 13th 2018 according to data reported by the European Centre for Disease Prevention and Control and World Health Organization.

Table 2. Number of West Nile virus confirmed cases in European Union and European Economic Area, European Union, European Economic Area countries and Italy (September 2012-December 13th 2018).

Period	N. of confirmed cases			
	EU/EEA	EU	EEA	Italy
2012 (Sep-Dec)	907	237	670	28
2013	783	226	557	40*
2014	210	74	136	21*
2015	301	108	193	38°
2016	469	206	263	71°
2017	280	198	82	55°
2018 (Jan-Dec 13th)	2083*°	1503	580	576

EU, European Union; EEA, European Economic Area. *N. of deaths: 2013 (7); 2014 (1); 2018 (181). °N. of patients infected through blood transfusion: 2015 (13); 2016 (22); 2017 (16); 2018 (680).

The distribution of WNV is strongly influenced by the routes of migratory birds, from North to South.[81,82] The natural reservoir of the virus is represented by several bird species. Birds are considered the major vertebrate hosts and the most important amplification hosts.[83] WNV was isolated in several avian species embracing *Passeriformes* (*i.e.* songbirds), *Charadriiformes* (*i.e.* shorebirds), *Strigiformes* (owls), and *Falconiformes* (hawks) that showed adequate viremia levels to infect mosquitoes.[84,85] Blue jay, common grackle, house finch, American crow and house sparrow represent the most important WNV amplifying birds. Moreover, epidemiological data show that house sparrows have an important role in WNV transmission in urban areas.[86]

Merging these findings with ecosystem analyses, two more types of transmission modalities have been outlined: sylvatic cycle and urban cycle. The first is based on ornithophilic mosquitoes spreading WNV across birds species, while the second involves domestic birds and *bridge* mosquitoes as *Cx. pipiens* and *Cx. molestus*.[87]

Although the main vertebrate hosts are represented by birds, equines and humans, WNV can occasionally infect various species amongst felines, canids, rodents, chiropterans, ungulates, bears, alligators and sea mammals.[84,88]

Importantly, iatrogenic infection is also possible through blood and blood components transfusion,[89] cells, tissues and organ transplants, with several reported cases in the United States and Europe.[89-91] Furthermore, WNV transmission can occur due to trans-placental passage, occupational exposure and, possibly, sexual contact.[11-13]

Epidemiology

WNV epidemiology is highly fluctuating. The virus has a nearly global distribution, due to its ability to infect several vertebrate and invertebrate species.[92] WNV was first isolated from a pyretic woman in Uganda, in 1937,[3] while the first occurrence of WNND in humans was documented in Israel, in 1957, during one of the local serial outbreaks of the 50s.[5,93] Since the middle of the 20th century, a number of outbreaks have been reported in humans, horses and birds in all the continents, including Europe.[94]

Following 1990, Algeria, Morocco, Tunisia, Egypt, Israel, Romania, Russia, Poland, Czech Republic, Hungary, Croatia, Serbia, France, Portugal, Spain and Italy experienced human outbreaks, including multiple cases with neurological complications and deaths.[7,95-97]

A WNV imported from Israel or Tunisia, caused a delayed but aggressive spread in North America, at the end of the 90s;[94] it was identified through CDC surveillance, in the New York City area, with a report of 62 infected persons, 25 equine cases and several birds' deaths (corvids). A vast proportion of patients had severe neurological involvement. In the same region, during December 2012, 5387 human cases, with 243 deaths, were reported.[81] These episodes underline the significance of the threat represented by vector-borne pathogens importation.

Today, the virus is largely present across the Americas, Africa, Asia and Oceania.[56,98]

Indeed, WNV massive transport by migratory birds[74,86] contributed to the occasional outbreaks in various previously untouched regions, including Europe[98] (Figure 2).

The first documented European outbreak of WNV infection occurred in 1962-63, in Southern France, in the delta region of the Rhone river, causing several WNND cases amongst humans and horses.[95,99] However, the largest human European outbreaks occurred in Bucharest, in 1996, and in Russia, in 1999.[6,96] respectively causing 393 cases (17 deaths)[100] and 183 cases (40 deaths) due to meningoencephalitis.[6] Later, the virus was also isolated in Portugal, Slovak, Hungary and Moldavia.[9]

The reason for the increase in both severity and frequency of WNV outbreaks after 1996 remains unclear.[2] The spread into regions with higher average age and immunologically *naïve* populations and the circulation of a more virulent strain of the virus, may partially explain this remark.[101]

In recent years, Europe has endured recurrent outbreaks of WNV infection, with an important emergence between June and November.[102] With the outbreaks becoming more frequent and sporadic cases all over Europe, several surveillance programs were established in different countries,[103] allowing an initial gain in WNV control through a careful monitoring across the EU/EEA and neighbouring countries, as WNV outbreaks are still sporadic and unpredictable.

Below, we particularly focus on ECDC data about the distribution of WNV-related disease in EU/EEA countries, with peculiar attention to Italy, highlighting the period from September 2012 to December 2018.[7,102] From 2014 to 2018, with the exception of 2017, ECDC data show an inconstant magnification of WNV diffusion, especially in Central and Southern Europe, with a sharp peak in 2018. In fact, the total number of confirmed cases/year in EU/EAA countries is: 2012 (907), 2013 (783), 2014 (210), 2015 (301), 2016 (469), 2017 (280) and 2018 (2083).[7]

Last updated on December 13th 2018, the ECDC data reported 2083 cases of WNV infection in EU/EEA countries. The highest number of WNV confirmed human cases was observed in Italy (576) followed by Serbia (415), Greece (311), Romania (277) and Hungary (215).[7]

All the human cases across EU/EEA, with particular regard to Italy, are summarized in Table 2.

Of notice: in 2012, outbreaks in Western and Eastern Europe were mainly dominated by WNV-1 and -2 lineages, respectively[104,105] in Italy WNV-1 was the main responsible. In 2017, in Italy, 16 patients were recipient of WNV infected blood donations.

Recent analyses point out a significant increase of WNV distribution in Southern Italy and on Italian major islands, in the last years. According to ECDC data, several cases were identified in Sardinia, Sicily, Basilicata, Puglia and Molise. Additionally, data reported by the National Reference Centre for the Study of Exotic Diseases (CESME) showed about 190 outbreaks of WNV disease amongst *equidae* in Italy.

Today, the situation is complicated by the intrinsic differences between the surveillance systems adopted by distinct countries, which are not completely favourable to data integration and comparisons. Moreover, circulation data are probably underestimated due to misdiagnosis and underreporting.

The current frequency of WNV outbreaks and the worries about an additional expansion lead to an increase in financial support to WNV research from the European Commission (EC) under the 7th Framework Programme.[106]

Laboratory diagnostics, therapy and vaccines

The recognition of WNV outbreaks requires a comprehensive evaluation of different factors such as environmental conditions and clinical symptoms.[94]

Today, different techniques are available for the identification of WNV infection in humans.[106]

Laboratory diagnosis requires both serological and virological methods, respectively based on indirect and direct virus detection.

The suspect of WNV infection, due to

the onset of symptoms including fever, encephalitis or meningitis, can be confirmed by laboratory tests (Enzyme-Linked Immunosorbent Assay – ELISA – or immunofluorescence) performed on patients' serum or cerebrospinal fluid (CSF).[107]

ELISA method allows the detection of anti-WNV IgM immunoglobulins, in serum or CSF samples, as a serological confirmation of recent infection.[58] Many advantages are connected to ELISA testing, compared to other techniques: high accuracy, sensitivity and specificity, rapidity, reproducibility and better cost-efficiency.[22]

After exposure to WNV, IgM and, subsequently, IgG antibodies are produced.[4] In patients with WNND, specific IgM can be found in serum and CSF following the onset of neurological symptoms. IgM antibodies are detected within 4 to 7 days after the initial exposure and may persist more than one year,[108] while anti-WNV IgG are detected 8 days after the onset of clinical manifestations and progressively increase in avidity with time.[109] Consequently, anti-WNV IgM positivity or IgG avidity tests are necessary to distinguish between acute and previous infections.[108] The detection of a specific IgG response is a fundamental tool in the epidemiological studies aiming at WNV diffusion monitoring. Moreover, in case of positive IgG results by ELISA, a further confirmation is needed and requires a more specific test represented by plaque reduction and neutralization test;[100] the latter evaluates the ability of the patient's antibodies to actually neutralize the virus.[110]

In addition to serological tests, WNV can also be detected through molecular biology techniques in various samples such as CSF, serum, plasma, urine or tissues. Methods include virus isolation followed by reverse transcriptase polymerase chain reaction, or immunofluorescence assay.[111] Nucleic acids amplification test for WNV RNA is routinely used as a screening for blood donations.[112] However, viral identification in blood samples is complicated by a short time of viremia. According to different studies, WNV has a longer persistence and detectability in urine samples than in plasma, serum or CSF.[113]

Unfortunately, there is no specific treatment for WNV disease; in fact, clinical management is mainly supportive. The potential use of antiviral agents has been extensively inquired in scientific literature, through various preclinical and clinical studies exploring the potential of novel or repurposed drugs.[114-116] High-dose ribavirin, interferon-α 2b, anti-WNV immunoglobulins and antisense gene-target compound appeared to be promising against WNV *in vitro*; however, dedicated clinical trials are still incomplete.[117,118]

Considering the therapeutic lacks for WNV, prevention remains a cornerstone. To date, no human vaccine is available for clinical use, but there are several vaccines licensed for being used in horses.[22]

The first vaccine was developed by Fort Dodge Animal Health through formalin inactivation of the virus.[94] At present, this vaccine is commercialized in the USA as West Nile-Innovator™; it seems effective and secure, as no adverse responses to vaccination in horses have been reported.[119]

The second vaccine (Recombitek™), commercialized in United States for equine use, efficiently elicits the production of anti-WNV antigens using a heterologous virus backbone.[94] This vaccine uses different vectors as canarypox (Recombitek™), Yellow fever virus (Chimerivax™), and Dengue virus 4 (WN-DEN4).[120,121]

In a phase II clinical trial conducted in healthy and adult patients, it has been demonstrated that an administration of a single dose of ChimeriVax-West Nile induce neutralizing antibodies 4 weeks after vaccination.[122]

The chimeric WN-DEN4 vaccine using attenuated dengue virus as a backbone for WNV prM-E genes is currently being tested in adults patients in a clinical trial at the John Hopkins School of Public Health.[55]

In 2005, a novel DNA plasmid-based vaccine has been developed by the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases. Several clinical trials confirmed its safety, tolerability and effectiveness to evoke anti-WNV neutralizing antibodies.[22]

To date, no WNV vaccines are licensed for human use. Therefore, prevention of WNV infection depends on adequate mosquito control programs to reduce vector density, individual strategies to prevent mosquito bites and screening of blood and organ donors. Personal protective measures rely on mosquito repellents mainly based on N, N-diethyl-m-toluamide and permethrin, along with mosquito nets and behaviors limiting skin exposures, starting from appropriate clothing.[123,124]

Conclusions

Worldwide, WNV infection is a raising concern, due to the virus propensity to expand to new geographical areas and to cause large outbreaks and severe neurological disease, currently lacking specific antiviral drugs or vaccines.

The global emergence of WNV threat, also in previously uninvolved areas, is caused by changed environmental conditions and human activities favouring the enlargement of vector population. For this reason and in the absence of vaccines, prevention still depends on personal protection and vector control.

An increase of national and international surveillance programs and social awareness is required.

A constant vector control would be beneficial for all mosquito-borne viruses and needs to be established across the world, but empowering the existing knowledge is crucial to obtain specific therapies and prevention.

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