

## WEST NILE VIRUS INFECTION IN HUMANS AND OTHER VERTEBRATES

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**Abstract** - The West Nile virus is an arthropod borne or ARBO virus from the Flaviviridae family, which is maintained in nature in the transmission cycle between hosting birds and ornithophilic mosquito vectors. The virus is capable of infecting different vertebrate species and 60 mosquito species. The infection in humans can be asymptomatic or it can have different clinical manifestations ranging from light febrile diseases to fatal meningoencephalitis. This paper presents recent findings on the activity of the West Nile virus in Europe, the USA and Serbia. Presented are the results of serological testing of human populations and animals in Serbia, and the methods of molecular diagnostics to prove the existence of the virus.

**Key words:** West Nile virus, clinical manifestations, epidemiology, diagnostics, prophylaxis

### HISTORICAL BACKGROUND

West Nile Virus was isolated in the West Nile region of Uganda in 1937 from the blood of a febrile woman involved in a project investigating malaria. Infections were registered throughout Africa, southern Europe, Russia, the Middle East, India and Australia. Virus neurotropism was registered for the first time during the 1950s in an epidemic in Israel. The first epidemic and equine epizootics in Europe was registered in the Rhone valley in France in 1962. During the 1990s, the virus virulence changed, and in 1996, an epidemic was registered in Romania with 398 confirmed cases and 17 fatalities (Tsai et al., 1998). Since 1999, when it caused an epidemic in New York, the virus has spread to nearly all Ameri-

can states and has caused hundreds of neuroinvasive cases with lasting consequences and fatal outcomes. Through numerous studies, prevention and control programs have been developed in the USA in order to control the infections in mosquitoes, humans and animals, especially birds (CDC, 2003). Large-scale epidemics and enzootics in the USA have contributed to an improvement in the West Nile virus infection diagnostics of both serological and direct testing for the virus in human and animal samples. During 2010, numerous epidemics caused by the West Nile virus with a considerable number of neuroinvasive cases were registered in Greece, Romania, Turkey and Russia. Sporadic cases were registered in 2010 in Hungary, Spain, Portugal, Italy and France (ECDC, 2011, ECDC/WHO, 2011).

## MORPHOLOGY AND CLASSIFICATION OF THE VIRUS

The West Nile virus is a small spherical virus from the family of Flaviviridae, which contains three genera – *Flavivirus*, *Pestivirus* and *Hepacivirus*. Around 70 viruses of the *Flavivirus* genus, to which the West Nile virus belongs, have been classified into 12 serological groups. West Nile virus was classified into the Japanese encephalitis serogroup, which is related to viruses causing serious encephalitis in humans: Japanese encephalitis virus, Saint-Louis encephalitis virus endemic to America, Murray Valley encephalitis virus in Australia, and Usutu virus, which was isolated in Africa. The Kunjin virus endemic to Australia and Asia was classified according to sequence homology into the subcategory of the West Nile virus (Heinz et al., 2000). The West Nile virus has only one serotype but it demonstrates significant genetic variations and differs from at least seven genetic lines. Lineage 1 includes isolates from Africa, the Mediterranean, southern Europe, India, Australia and America, and Lineage 2 includes isolates from sub-Saharan Africa and Madagascar, which are predominantly enzootic and have not demonstrated pathogenic features before (Burt et al., 2002). Isolates from the epidemics recently registered in Hungary, Austria and Greece belong to Lineage 2. Lineage 2 was isolated in northern Greece in 2010 during an epidemic that caused 191 neuroinvasive cases and 35 fatalities. The isolate from Greece contains proline instead of histidine on locus 249, which is marked as a mutation H249P. This mutation is probably responsible for its neuroinvasiveness (Papa et al., 2011).

Flaviviruses are small spherical viruses with icosahedral nucleocapsid and lipoprotein envelope. The West Nile virus has a positive RNA genome, 11 kb in length, which codes one polyprotein. The open reading frame of the genome (ORF) has a length of 10 029 nt. The virus has 10 proteins that are formed in the cytoplasm of the infected cell by proteolysis of the virus-coded polyprotein, by the action of viral serine esterases and cellular proteases (Brinton, 2002). The structural proteins of the virus are capsid C, premembrane or membrane prM/M and the

protein E, coded by genes located toward the 5' end of the genome. Seven nonstructured proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NSS) are coded by genes located toward the 3' end. The genome is flanked by a short 5' noncoding region and 3' noncoding region. Numerous copies of the C proteins build the spherical nucleocapsid of the 25 nm diameter. The viral envelope has superficial glycoproteins M and E. The E glycoprotein is a dimer, with its distal end in the outer lipid envelope of the virus. The E glycoprotein secures the viral attachment to the host cellular receptor, participates in the processes of pH-dependant fusions and has three domains (I, II and III). The mutations in domain III change the virulence of the virus (Heinz and Allison, 2000). The M and E proteins stimulate cellular and humoral immune response to the virus. Domain III of the E glycoprotein is immunogenic and stimulates the production of neutralizing antibodies.

## TRANSMISSION CYCLE OF THE VIRUS

In nature, the virus is maintained in a transmission cycle between hosting birds and ornithophilic mosquitoes, which are vectors. Approximately 60 types of mosquitoes, belonging to 11 different genera, are responsible for the transmission of the virus. The most significant of them are those belonging to the *Culex* genus. The presence of the virus in the USA in 1999 was largely due to the survival of the virus during the winter within the type *Culex pipiens*, which hibernates during winter in the form of adults (Granwehr et al., 2004). In nature, hundreds of bird species are reservoirs of the virus. These birds belong to the Passeriformes order and are divided into 20 families. The most sensitive are those belonging to Corvidae, especially crows and blue jays. The birds are infected by mosquitoes, which carry the virus in their salivary glands while sucking blood. A wide range of vertebrates (mammals, amphibians and reptiles) carry antibodies for the West Nile virus. In some reptiles and amphibians (snakes, crocodiles, alligators, iguanas and frogs) a high viremia is observed, although the significance of these animals for the maintenance of the virus in nature has not been clarified so far. Domestic and wild animals such as dogs, cats, sheep,

pigs, cows, rabbits, llamas, deer, raccoons, bears, wolves, squirrels and bats are also susceptible to the virus (Beasley, 2005). Humans, horses and some other mammals are accidental hosts whose virus titers are low and insufficient for mosquito infection. There have been cases of virus transmission in humans through organ transplantation, transfusion, transplacental or breast-feeding and accidentally in laboratory work with the live virus (Granwehr et al., 2004).

#### VIRUS ACTIVITY IN EUROPE

The first epidemic registered in Europe was in 1962 in the Camargue region of France with 14 human cases and a large number of diseased horses. The West Nile virus did not seem to pose a public health problem for Europe, although in many countries antibodies have been proven to exist in humans and different animals (Hubalek and Halouzka, 1999). In 1996 it became clear that the West Nile virus is a serious public health issue when an epidemic with serious neuroinvasive diseases was registered in Romania. Nucleotide sequences of the Romanian isolate from the *Culex pipiens* RO97-50 mosquito were identical to isolates from the *Culex neavei* mosquito from Senegal and *Culex univittatus* from Kenya. It is assumed that the virus was carried by migratory birds from Saharan Africa and South Africa into southern Europe (Savage et al., 1999). Up to 2004, European isolates belonged to Lineage 1. In 2004 in Hungary, Lineage 2 was isolated from the goshawk and in 2005 from several birds of prey (Erdelyi et al., 2007). The Greek lineage isolated in 2010 has the greatest level of gene homology with the isolates from Hungary from 2004 and those from South America, and it belongs to Lineage 2 (Papa et al., 2011). However, while neuroinvasive cases were rare and with no fatal outcomes in Hungary, in Greece in 2010 there were 72.9 % of neuroinvasive cases (out of the total of 262 cases of suspected or confirmed infection) with 35 fatalities (ECDC, 2011). In Romania, the virus was active even after 1996, and caused occasional cases. The virus in Romania is endemic in horses, humans and birds. In October 2010, an epidemic was registered in Romania with 46 neuroinvasive cases caused by

Lineage 2 virus (ECDC/WHO, 2011). An increased virus activity was observed in horses in Europe – in northeastern Italy in 2008 (Monaco et al., 2009) and in Spain in 2010 (Garcia-Bocanegra et al., 2011).

#### VIRUS ACTIVITY IN AMERICA

The first epidemic in the USA was in New York in 1999 (Nash et al., 2001). There were 62 registered cases in humans (with 7 fatalities), 25 cases of infection in horses (9 fatal) and a great mortality among birds mostly from the Corvidae family. In this epidemic, the virus NY-99, belonging to the Lineage 1, was isolated and displayed a great resemblance to isolates from Israel and Tunisia isolated in 1998 (Lanciotti et al., 2002). Since then, the virus has spread rapidly in many states. It caused hundreds of neuroinvasive cases with grave consequences every year. During 2002, the virus reached the eastern shore and peaked to 2 956 neuroinvasive cases with 284 fatalities. Since 2003, when there were 9 862 cases of infection among humans, a declining trend has been observed. However, there are still hundreds of cases registered annually, including neuroinvasive ones. The assumption is that the virus was carried into the USA by migratory birds. It is possible that imported infected birds or hazard mosquito transport also had an impact. The existence of the virus in the warm southern parts of America enabled mosquitoes such as *Culex quinquefasciatus* to feed throughout the year, thereby enabling a constant presence of the virus (Granwehr et al., 2004). The epidemiology of infection in Africa, where there are also preconditions for constant transmission, is different. In Africa, people became infected in childhood and neurological disease is rare. In South Africa, epidemics affected 18 000 people with a single registered case of neuroinvasive disease (McIntosh and Jupp, 1976). In the USA, there is one case of encephalitis per 150 infected cases.

#### VIRUS ACTIVITY IN SERBIA

The first data on the activity of the virus in Serbia were gathered by serological research conducted in the 1970s in humans, when the presence of antibodies against the West Nile virus of 2.6-4.7% (Borđoški

et al., 1972) was observed by the reaction of inhibition of hemagglutination in Vojvodina. More recent research also pointed to the activity of the virus in Serbia. The frequency of the IgG antibodies for the West Nile virus was 7.5% on the ELISA test, calculated over a five-year period on a sample of people from Vojvodina treated for viral encephalitis or meningoencephalitis of undetermined etiology at the Clinic for Infective Diseases in Novi Sad (Hrnjaković Cvjetković et al., 2007). In a sample of 105 patients from Serbia professionally exposed to mosquito bites, in 4.76% the presence of IgG antibodies was determined by the indirect immunofluorescence test (Tasić et al., 2008).

Viral RNA was determined by real-time polymerase chain reaction (RT PCR) in *Culex pipiens* mosquitoes collected in Novi Sad in 2010. Detection of viral RNA points to the activity of the virus in mosquitoes in Novi Sad (Petrić et al., 2012). Recent research shows that the virus circulates among humans (Hrnjaković Cvjetković et al., 2012), horses (Lupulović et al., 2011) and birds. In 92 wild bird sera, antibodies have been observed in 7.76%. Viral RNA was found in 9 out of 82 tissue pools of 134 wild birds (Petrović et al., in print). These results show that the virus circulates in Vojvodina and Serbia and that it is necessary to implement research, prevention and infection control programs for this virus.

#### CLINICAL MANIFESTATION

Most infections in humans are asymptomatic. After the incubation period of 3 to 14 days, 20% of the infected develop West Nile fever symptoms, half of which seek medical aid. The West Nile fever starts abruptly and is unspecific, resembling the influenza syndrome. The symptoms include high temperature, fever, weakness and exhaustion, headache, pain in the neck and back, muscles, joints and retro-orbitally. Other unspecific symptoms are loss of appetite, nausea, vomiting, diarrhea and cough. In some epidemics, rash and swollen lymph glands were presented. Maculopapular rash was observed in 50% of the infected during certain epidemics (Petersen and Marfin, 2002). Neurological diseases occur in less

than 1% of the patients. In recent epidemics in the USA, one third of the hospitalized patients presented encephalitis and one third meningitis. In addition, there have been cases of acute flaccid paralysis. The death rate of hospitalized patients in the USA ranged from 4 to 14%. In older patients, there was a significant risk of grave forms of the disease, especially in patients older than 50 years of age. Other risk factors for grave forms of the disease and fatal outcome include prostration, coma, immunological deficiency, hypertension and diabetes mellitus. It has recently been shown that defective alleles in chemokine receptor CCR5 are significantly related to symptomatic infection by the West Nile virus. In addition, it has been observed that gene polymorphisms in the interferon-induced gene 2'-5'oligoadenylate synthetase 1 can be connected with different outcomes of the infection in experimental animals (Frederickson et al., 2004). In humans, the virus enters the CNS through the vascular endothelium by passive transfer or propagation in endothelial cells (Solomon and Vaughn, 2002).

The infection is frequently asymptomatic in horses. Only 10% of infected horses had symptoms such as fever, ataxia and muscle weakness. The mortality in horses in the pre-vaccination period ranged from 25% to 45%. In birds, the typical signs are neurological – ataxia and paralysis. Infection by West Nile virus causes multiple damages to the brain, kidneys, heart, lungs, liver, gonads, spleen, intestines and skin. Since crows are exceptionally susceptible, their fatalities can be a reliable indicator of the increased risk for human infection.

#### DIAGNOSTICS OF WEST NILE VIRUS INFECTION

Serological tests are the main method in diagnosing West Nile virus infection. They are based on the determination of antibodies against viral glycoprotein E in the blood and cerebrospinal fluid. The golden standard in serological testing is a neutralization test of plaque reduction that is the confirmatory test. Diagnosis of acute infection can be made by demonstration of a significant increase in the antibody

titer in the second serum sample. As cross reactions between flaviviruses are possible, it is advisable to perform testing using a battery of flavivirus antigens. Some laboratories use the hemagglutination inhibition test or the immunofluorescence test. The test more widely used in recent years is the ELISA test, which enables the diagnosis of acute infection based on IgM and IgG antibody presence. IgM antibodies are found 2 to 8 days after the clinical signs appear and remain for weeks or months to follow (Roehrid et al., 2002). The presence of IgM antibodies in the cerebrospinal fluid point to CNS infection, since IgM antibodies do not cross the hematoencephalic barrier. Caution is necessary in interpreting the ELISA test results, as it is possible for these antibodies to persist for more than a year (Roehrig et al., 2003). In order to prepare the ELISA test, cell antigens and recombinant antigen culture are used.

Diagnosis can also be obtained after the isolation of the virus from blood, cerebrospinal fluid or tissue in cell culture. It can be performed in a Vero/RK-13/AP61 cell culture; bio safety level III (BSL III) is required. The material is inoculated in cell culture and the cytopathogenic effect is monitored daily. If the cytopathogenic effect does not appear, the immunofluorescent test can be applied to prove West Nile virus infection. The viremia phase commonly precedes the clinical signs of the disease, and can be proven 4 days after the disease occurs.

Real-time PCR (rPCR) has been used since 2003 as a screening test in humans as well as in mosquito and bird pools. Determining the virus antibodies is also used as a pre-screening test of mosquitoes and dead birds in the USA.

### PROPHYLAXIS

So far, a vaccine has not been developed and the therapy is symptomatic.

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