

## ETIOLOGY, EPIDEMIOLOGY, CLINICAL FEATURES AND LABORATORY DIAGNOSTICS OF WEST NILE FEVER – A REVIEW

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### Summary

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West Nile fever is a non-contagious viral disease affecting birds, horses and humans. The disease is transmitted by blood-feeding insects (mosquitoes), mainly of *Culex* genus, but representatives of genera *Aedes*, *Anopheles*, *Coquillettidia*, *Culiseta*, *Deinocerites*, *Mansonia*, *Orthopodomyia*, *Psorophora* and *Uranotaenia* may also have an important role in the spreading of the disease. Wild birds and particularly members of Corvidae, Passeridae and Charadriidae families are reservoir hosts of the infection. Mammals, including humans and equidae, are considered as “dead-end” or incidental hosts. The disease is characterized with fever, encephalitis, encephalomyelitis, paresis, paralysis, general weakness, prolonged recumbency, depression. West Nile fever is endemic in many regions in Africa, the Mediterranean basin and the Middle East from where the infection could be introduced into new areas by migratory birds. Due to the expanding geographical distribution of the disease, accurate and fast diagnosis becomes essential, especially in areas settled with highly susceptible human and animal populations where the virus is introduced for the first time.

**Key words:** West Nile Fever, West Nile virus

### INTRODUCTION

West Nile fever (Latin: *Encephalitis Nili occidentalis*) is a non-contagious, transmissible, arboviral disease affecting a large range of hosts, the most susceptible being birds, horses and humans. It is one of the most widespread arthropod-borne diseases registered in all inhabited continents. West Nile fever is endemic in many regions in Africa, the Mediterranean basin and the Middle East from where the infection could be introduced into new areas by migratory birds. In most instances, the infection is asymptomatic but when clinical signs appear, the outcome is

often fatal. Annually, numerous West Nile fever outbreaks are documented around the world involving thousands of people and horses, causing death of hundreds. Considering the constantly increasing area of spreading of the infection, the disease is becoming more and more significant.

### ETIOLOGY

The West Nile virus is a member of the Flaviviridae family, genus *Flavivirus* (Calisher *et al.*, 1989; Murphy *et al.*, 1995; OIE, 2009; Anonymous, 2010). West Nile

fever belongs to the Japanese encephalitis virus antigen group that includes also the Murray Valley virus, Saint Louis virus, Usutu virus, Cacipacore, Koutango, Yaounde (Calisher *et al.*, 1989; Murphy *et al.*, 1995; Burke & Monath, 2001; OIE, 2009).

Virions are approximately 50 nm in diameter, with spherical shape (Anonymous, 2010). They consist of a nucleocapsid, surrounded by a lipid membrane. The nucleocapsid is spherical, with icosahedral symmetry and a size of 20–25 nm. WNV contains a single-stranded, positive-polarity infectious RNA. The full genome of the West Nile virus consists of 11,029 nucleotides. At the 5' end is situated a methylated cap whereas at the 3' end the polyadenosine tail is missing. The genome codes the synthesis of three structural proteins: capsid (C), premembrane (prM/M) and envelope (E) and seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Brinton, 2002; Anonymous, 2010). The content of lipids is 17 %. Embedded in the membrane are two of the structural proteins: E- and M-proteins (the latter could be under his precursor form – prM) (Brinton, 2002). These proteins are responsible for many properties of the virus, including host range, tissue tropism, replication, assembly, and the stimulation of B and T cell immune response (Brinton, 2002).

Virions enter host cells via a receptor-mediated endocytosis, followed by fusion of endosomal membrane with the viral one and release of the nucleocapsid into the cytoplasm. After the translation of the genomic RNA a polyprotein is synthesized and then it is cleaved by some of the non-structural viral proteins and cell proteases forming the mature viral proteins. The RNA-dependent viral RNA-

polymerase (NS5) copies complementary negative-polarity RNA strands that serve as templates for the synthesis of new genomic RNAs. The assembly of the virions occurs in association with the endoplasmatic reticulum membrane (Brinton, 2002).

Phylogenetic analyses segregate the isolates in two main lineages (Lanciotti *et al.*, 2002; Zeller & Schuffenecker, 2004). Strains from lineage 1 are distributed in Europe, North, Central and South America, Israel, India, Australia (subtype Kunjin/Kunjin virus). Those in lineage 2 are endemic strains from Central and South Africa, and Madagascar (Berthet *et al.*, 1997; Burt *et al.*, 2002; Lanciotti *et al.*, 2002; Morales *et al.*, 2006; OIE, 2009).

Strains from both lineages induce disease in animals and humans. However, the most recent outbreaks involving humans and horses are caused by strains clustered into lineage 1 (OIE, 2009).

## EPIDEMIOLOGY

West Nile fever affects mainly birds, horses and humans. There are sporadic cases of disease in other animal species as chimpanzees, dogs, cats, squirrels, bats, deers, sheep, llamas, alligators, seals. Reservoir hosts of the infection are wild birds (Berthet *et al.*, 1997), especially the members of the Corvidae, Passeridae and Charadriidae families. Mammals, including humans and equidae are considered “dead-end” or incidental hosts.

West Nile virus was isolated for the first time from a woman with a fever, living in the West Nile province of Uganda (Smithburn *et al.*, 1940). The initial serological experiments classified West Nile virus within the Japanese encephalitis virus antigenic complex (Smithburn *et al.*, 1940). Up to the 1950s, infec-

tions related to encephalitis in humans were rarely notified but since then numerous outbreaks including fatal cases, have been reported in Romania, Russia, Israel, France, Italy, Tunisia, Morocco, Egypt, South Africa, India and North America (Melnick *et al.*, 1951; Taylor *et al.*, 1956; Tsai *et al.*, 1998; Center for Disease Control and Prevention, 1999; Hubalek & Halouzka, 1999; Savage *et al.*, 1999; Bin *et al.*, 2001; Hayes, 2001; Lanciotti *et al.*, 2002; Del Giudice *et al.*, 2004; Zeller & Schuffenecker, 2004). In 1996–1997, 500 cases of meningoencephalitis with a high mortality rate of 10% were registered in Bucharest and the nearby rural areas (Tsai *et al.*, 1998; Savage *et al.*, 1999). According to Bárdoš *et al.*, (1959) in Europe the disease was detected for the first time in 1958 in Albania. In Bulgaria, seroconversion in humans, domestic animals and migratory birds was demonstrated during the 1960s, while the virus was isolated from mosquitoes during the 1970s (Hubalek & Halouzka, 1999).

In the 1960s, cases of encephalomyelitis in horses were reported in Egypt and France (Schmidt & El Mansoury, 1963; Panthier *et al.*, 1966). Since 1996 outbreaks involving disease in horses were registered in Morocco (1996 and 2003), Italy (1998), Israel (2000), France (2000–2004), Canada, USA (Cantile *et al.*, 2000; Ostlund *et al.*, 2000; Murgue *et al.*, 2001; Zeller & Schuffenecker, 2004; Hayes *et al.*, 2005; Schuffenecker *et al.*, 2005). After the introduction of the virus in the western hemisphere, where it was first detected in 1999 in New York, the disease extended its range and spread dramatically in many geographical regions – almost the entire territory of the USA as well as in regions in Canada, Mexico, the Caribbean islands, Central America, Venezuela, Colombia and Argentina (Center

for Disease Control and Prevention, 1999; Ostlund *et al.*, 2000; Davis *et al.*, 2001; Hayes, 2001; Davis *et al.*, 2005; Morales *et al.*, 2006).

Most bird species can become infected with WNV. The disease was discovered in zoo birds in the USA and domestic geese in Israel and Canada (Steele *et al.*, 2000; Austin *et al.*, 2004; Zeller & Schuffenecker, 2004). Cases of West Nile infection in wild synanthropic and migratory birds were reported in Poland, The Czech Republic, Germany and Israel (Bin *et al.*, 2001; Malkinson & Banet, 2002). Some species of wild birds are especially susceptible to infection. The members of the Corvidae family, namely American crows (*Corvus brachyrhynchos*) and blue jays (*Cyanocitta cristata*) are among species with high mortality rate, caused by West Nile fever (Komar *et al.*, 2003; OIE, 2009).

Due to the extended and high-titre viraemia some bird species might transmit the disease not only by the predominant mosquito-borne mode, but also via alimentary or airborne routes. Therefore, birds are considered as “amplifying” hosts. After the survey of the infection the persistence and shedding of the virus can last up to 13, respectively 10 days (Komar *et al.*, 2003; Zeller & Schuffenecker, 2004; Hayes *et al.*, 2005).

Mammals, including human beings and equidae are “dead-end” or incidental hosts because they do not produce significant viraemia, and do not contribute to the transmission cycle (Davis *et al.*, 2001).

The principal vector of West Nile virus are mosquitoes of the genus *Culex*, but members of the genera *Aedes*, *Anopheles*, *Coquillettidia*, *Culiseta*, *Deinocerites*, *Mansonia*, *Orthopodomyia*, *Psorophora* and *Uranotaenia* could also be involved in the transmission cycle with a different epidemiological importance in

the various geographical areas. Although the West Nile virus was isolated from other blood-feeding arthropods such as *Culicoides sonorensis* (USA) and ticks from the genera *Argas*, *Ornithodoros*, *Amblyomma*, *Dermacentor*, *Hyalomma*, and *Rhipicephalus*, their role in the virus maintenance is still unknown (Toma *et al.*, 2008).

In the temperate regions the disease is characterized with an established seasonal dynamics. A high mortality rate among wild birds is observed in the beginning of the spring (April–May). In the early summer antibodies against West Nile fever could be detected in blood samples from sentinel animals. During that period the causative agent could be isolated from mosquito pools. Most of the human and equidae infections with the virus occur in the late summer with higher rates in the early autumn.

Until recently the only existing way of human-to-human virus transmission was thought to be through the bite of an infected mosquito. However, cases of infection via blood transfusion, organ transplantation, breast-feeding, as well as intrauterine and percutaneous infections were reported in 2002 in the USA (Center for Disease Control and Prevention, 2002a, b, c, d, e; 2004; Hayes & O’Leary, 2004; Zeller & Schuffenecker, 2004).

#### CLINICAL FEATURES

The clinical signs of the disease are various, ranging in severity from asymptomatic to severe neuroinvasive disease with a lethal outcome.

The incubation period in humans is 3 to 6 days. In most cases (80%) the infection is asymptomatic. In 20% of the cases the infection occurs with non-specific febrile illness – a sudden onset of

intermittent fever accompanied by headache, somnolence, back pain, fatigue, conjunctivitis, myalgia and anorexia, nausea, abdominal pain, sore throat that could last for 3 to 5 days. A roseolar or maculopapular rash involving primarily the face and trunk occurs in about half of the patients and can last up to a week. Generalized lymphadenopathy is common (Hubalek & Halouzka, 1999; Azad & Thomas, 2004). Approximately 1 in 150 infected persons (<1% of cases) develops a severe form of the disease involving the central nervous system (meningitis or meningoencephalitis). The incidence of severe neuroinvasive disease with a fatal outcome is higher in people over 50 years. The mortality rate is approximately 15%.

In the temperate regions the infection in horses has an established seasonal dynamics with a peak of the cases in the early autumn. The viral incubation period lasts from 3 to 15 days. Most infections are clinically inapparent or manifested with symptoms such as fever (sometimes absent), weakness, recumbency, muscle fasciculation, mild to severe ataxia that could last up to 10 days. The neuroinvasive form is characterized with hind limb weakness, paralysis, seizures and coma. The outcome of that form is usually lethal. Occasionally hyperaesthesia and violent reaction to any stimulus may occur (Cantile *et al.*, 2000; Ostlund *et al.*, 2000; 2001; Snook *et al.*, 2001; Weese *et al.*, 2003; Abutarbush *et al.*, 2004; OIE, 2009). The clinical presentation of the disease in horses is similar to this one in humans – 70% of the infections are asymptomatic, <20 % are mild infections and 10% – neuro-invasive form. The mortality rate in horses is approximately 30% (OIE, 2009).

Most of the bird species infected with West Nile fever do not exhibit clinical

symptoms. In general, the infection is asymptomatic. Most of the infected birds are found singly and not as a part of a mass die-off at a single time and place, with no lesions pathognomonic for WNF infection. External haemorrhage, either from the mouth or from the cloaca could be observed in American crows (Komar *et al.*, 2003; Gibbs *et al.*, 2005). Occasionally, in some bird species or after experimental infection, signs of illness as generalized lethargy, depression, ruffled feathers, unusual posture, appetite and weight loss, somnolence, feather peaking, tremor, blindness, ataxia and leg weakness might occur (Hubalek & Halouzka, 1999; Steele *et al.*, 2000; Komar *et al.*, 2003; Austin *et al.*, 2004).

#### LABORATORY DIAGNOSTICS

Due to the diverse non-specific clinical symptoms, the absence of pathognomonic lesions during necropsy and the high rate of asymptomatic form, the diagnosis, based on clinical features, pathological findings and epizootological investigation could not be definitive. The presence of high mortality rate among wild birds, increased vector population, influenza-like illness or meningoencephalitis among humans could be indicative for West Nile fever.

The definitive diagnosis is based on laboratory tests. Multiple laboratory methods for West Nile fever are developed. Enzyme-linked immunosorbent assay (ELISA) tests are often used due to the facility and readiness in their application. There are different types of commercial ELISA kits based on the detection of the presence of IgM antibodies (IgM capture ELISA) or IgG antibodies in serum samples from horses, birds and humans. Positive results obtained with this

method should be confirmed due to the cross-reactions to other members of the Japanese encephalitis antigenic complex which can be misleading for the diagnosis. The haemagglutination inhibition test is commonly used for identifying West Nile virus antibodies in avian samples (Beaty *et al.*, 1989; Hayes, 1989; OIE, 2009).

The most sensitive serological assay for detection of antibodies is the plaque reduction neutralization test, which is used for confirmation of WNV specific neutralizing antibodies (OIE, 2009).

An alternative method is the virus neutralization test. It has the advantage of requiring low cost of both volumes of tested sample and necessary reagents.

The viral load in avian organ samples is higher than in equine ones. Specimens for viral isolation from birds include kidney, heart, brain, liver or intestines. In horses, most appropriate specimens are obtained from the brain and the vertebral column. The virus may be propagated in susceptible cell cultures, such as RK-13 or Vero. More than one cell culture passage may be required to observe a cytopathic effect. Immunohistochemical staining of formalin-fixed avian tissues is a reliable method for identification of West Nile virus in bird samples.

The reverse transcriptase polymerase chain reaction (RT-PCR) is often used as a virus detection assay from a wide variety of samples. Real-time RT-PCR has also been developed (Tewari *et al.*, 2004). It is a rapid and reliable method which is characterized with high specificity and sensibility (Lanciotti *et al.*, 2000; Murgue *et al.*, 2001). Identification of the antigen is enhanced when a nested PCR approach is applied (Lanciotti *et al.*, 2000; Johnson *et al.*, 2001).

Other neurological diseases can be confused with West Nile Fever. Diseases

that can induce identical clinical signs are Eastern, Western, Venezuelan and Japanese equine encephalomyelitis, equine protozoal myeloencephalitis (*Sarcocystis neurona*), equine herpes virus-1, Borna disease, rabies, babesiasis and botulism.

In the past decade extensive field studies have been carried out on West Nile virus spreading and ecology. Apparently in the last years the geographical distribution of the disease has been extended and the formation of endemic zones which could be active over the whole year might occur. Considering the proximity of Bulgaria to regions endemic for West Nile fever (especially Romania) and the migration routes of wild birds passing through the territory of our country, the accurate and fast diagnosis and the preparedness of the country official veterinarians to take effective actions in case of outbreaks are crucial for public and animal health protection and for preserving the biological diversity of endangered bird species.

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