

Orsolya Kutasi: West Nile Virus in Europe

West Nile virus (WNV) is a mosquito-borne zoonotic arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. This flavivirus is found in temperate and tropical regions of the world. WNV has spread globally, with the first case in the Western Hemisphere being identified in New York City in 1999; over the next 5 years, the virus spread across the continental United States, north into Canada, and southward into the Caribbean Islands and Latin America. WNV is now considered to be an endemic pathogen in Africa, Asia, Australia, the Middle East, Europe and in the United States, which in 2012 has experienced one of its worst epidemics, when WNV was killing 286 people in Texas.

Phylogenetic studies have identified 2 main lineages of WNV strains. In the early 2000-s strains from lineage 1 were present in Africa, India, and Australia and were responsible for outbreaks in Europe, in the Mediterranean Basin, and in North America, whereas lineage 2 strains had been reported only in sub-Saharan Africa and Madagascar. The Hungarian equine WNV outbreak reported in 2008 was the first to be caused by a lineage 2 sub-Saharan strain in Europe. The pathogenicity of this lineage 2 strain resembled that of lineage 1 strains, and its sudden spread was unpredictable.

The main mode of WNV transmission is via various species of mosquitoes which are the prime vector, with birds being the most commonly infected animal and serving as the prime reservoir host. Birds develop sufficient viral levels after being infected, to transmit the infection to other biting mosquitoes which in turn go on to infect other birds. In some species of birds, the infection is fatal. When the mosquitoes become less selective, -during late summer and early autumn, when migrating birds leave their previous living area-, begin feeding more readily on other animal types such as humans and horses which are considered incidental hosts. In mammals, the virus does not multiply as readily (i.e. does not develop high viremia during infection), and mosquitoes biting infected mammals are not believed to ingest sufficient virus to become infected, making mammals like humans and equines, so-called dead-end hosts. In our climatic area (Middle Europe) equine outbreaks of the disease occur according to strict seasonality between August and October.

Approximately 80% of WNV infections in humans are subclinical, and this is very similar to horses where most infections are asymptomatic. In humans West Nile fever (WNF), which occurs in 20 percent of cases, is a febrile syndrome that causes flu-like symptoms, like fever, headaches, fatigue and muscle pain. Whether this type of syndrome exist in horses is not

clear, because we only identify cases with neurologic signs and might miss these non-typical transient conditions. In humans less than 1% of the cases is severe and result in neurological disease when the central nervous system is affected. Horses are particularly sensitive to WNV with approximately 10% infected animals presenting neurological disorders.

In horses the disease might start with non-specific signs of general depression, loss of appetite, low-grade fever, colic-like symptoms, lameness, stiffness or poor performance. Weakness, ataxia, and paralysis, -sometimes assymetrical-, are typical features of nervous system involvement in WNV infections and these usually develop primarily in the hindquarters in previously described lineage 1 outbreaks. In many cases caused by lineage 2 viruses, these signs are more prominent in the forelimbs at the onset of the disease. Limb paralysis can affect one or two limbs or all four, the latter usually progressing into recumbency and result in poor prognosis. Skin fasciculations and muscle tremors on the face, neck, pectoral and triceps area are common. Cranial nerve abnormalities, like facial paralysis, dysphagia or vestibular symptoms can be observed less frequently. In a lineage 2 outbreak we have described head posture abnormalities, hyperexcitability, but abnormal behaviour was less common than in lineage 1 north-american outbreaks. Factors other than the strain alone may account for the clinical manifestation of disease. Individual factors of receptivity also are thought to be of major importance for the evolution and clinical expression of WNV infection. Indeed, several authors have demonstrated that variations in certain loci of the host's genome influenced susceptibility and clinical presentation. Furthermore, the presence of antibodies against other flaviviruses is thought to play a role in determining the clinical presentation in certain areas.

Laboratory results are non-specific and can be completely negative. In severe cases we find pronounced increases in WBC counts with neutrophilia. In most cases cerebrospinal fluid sampling (CSF) is performed in the lumbosacral region. The CSF is abnormal in most cases showing mononuclear pleocytosis with lymphocytic predominance, but in some cases, only a high protein concentration can be found. Neutrophil predominance in CSF is also a sign of poor prognosis.

Following peripheral inoculation, initial WNV replication is thought to occur in skin Langerhans dendritic cells. These cells migrate to and seed draining lymph nodes, resulting in a primary viremia and subsequent infection of peripheral tissues such as the spleen and kidney. By the end of the first week, WNV is largely cleared from the serum and peripheral

organs and infection of the central nervous system (CNS) is observed in a subset of immunocompetent animals. The mechanisms by which WNV and other neurotropic flaviviruses cross the blood-brain barrier remain largely unknown, although tumor necrosis factor alpha (TNF- α)-mediated changes in endothelial cell permeability may facilitate CNS entry. It is likely that WNV infects the CNS at least in part via hematogenous spread, as an increased viral burden in serum correlates with earlier viral entry into the brain.

The virus causes polioencephalomyelitis in horses and men. There is usually relatively mild gliosis and perivascular cuffing, lymphocytic and neutrophilic infiltration, neuronophagia, necrosis of neurons and a few scattered perivascular haemorrhages in the cerebrum, with the most severe disease located in the thalamus and hindbrain. The most severe sites of the WN neuroinvasive disease lesions can be focally distributed, but usually there is generalized inflammation. The grey matter of the spinal cord can be severely affected as well.

The differential diagnosis in Europe mainly includes the neurological form of rhinopneumonia of Equine Herpes Virus 1, Borna disease, and rabies. We have to exclude CNS trauma, plant and other toxicoses, bacterial meningitis, Wobbler syndrome and some degenerative disorders. In the Western Hemisphere arboviral encephalitis (e.g., Venezuelan, Eastern, and Western encephalomyelitis) and equine protozoal myeloencephalitis (*Sarcosystis neurona* or *Neospora hughesi*) have to be included in the differentials.

The antemortem diagnosis of acute WNV infection is based on the detection of specific IgM antibodies in serum, CSF or both by an ELISA test or an increase in IgG titers between acute-phase and convalescent sera. Virus-neutralizing antibody responses persist for longer than WNV-specific IgM levels in serum. The duration of IgM appears to be short-lived in horses and this is the reason why it is useful for identifying and differentiating recent infections from previously exposed animals. It is well known that there is an extensive cross-reactive antibody response to members of the *Flavivirus* genus, thus molecular tests should be performed to confirm the clinical diagnosis and identify the causative virus. Horses infected with either WNV lineage 1 or lineage 2 elicit a similar antibody profile in serum samples and there are not any notable differences in the antibody profile.

Most data show that tests to detect WNV RNA in serum or plasma may give false-negative results due to the short duration of viraemia. Consistent with the fact that the kidney is a well-established site of active WNV replication in animals, urine samples may be more appropriate when looking for the presence of WNV, because of longer shedding and higher viral load.

Whole WNV genome reconstruction is also easily achieved from the urine sample in humans. Reliability of testing urine for viral RNA in horses has not been established yet.

What is going on in European countries? In Hungary WNV became endemic causing equine and human disease each year. According to previous isolations we suppose that both lineage 1 and lineage 2 strains are circulating in Hungary but only lineage 2 strains were isolated from neuroinvasive animal cases in recent years. A human outbreak of WNV infections occurred in 2010 in central Macedonia, northern Greece. WNV lineage 2 sequences were obtained from mosquitoes in sites where encephalitis cases occurred a few days before the trapping. The Greek strain showed the highest homology to Hungarian and South African strains, differing from the Russian WNV lineage 2 strain, which suggests that at least two lineage 2 strains have been introduced and established in Europe, causing severe disease to humans and horses. Notably, cases of WNV infection in Greece in 2011 occurred in areas that had not been affected in 2010. WNV infections have been reported in both humans and horses since the summer of 2008 in north-eastern Italy as well. Until 2011 only WNV lineage 1 infections have been described in the country. In 2011 Bagnarelly et al. described a human case of WNF which was confirmed to be caused by a lineage 2 virus. It also showed 99% identity to the complete genome of isolate goshawk-Hungary/04 and to the more recent Nea Santa-Greece-2010. Between July and September 2012, Barzon et al. described 13 confirmed human cases of WNV infection diagnosed in north-eastern Italy. Cases occurred one month earlier than previously reported, and this could be attributable to the ongoing very hot summer season, probably responsible for the very high mosquito density, that has been observed in the affected areas. The outbreak was caused by the lineage 1 Livenza strain which is classified within the Mediterranean cluster, but has several novel amino acid substitutions in non-structural proteins that are not present in other European and non-European strains, that could be relevant for virus transmissibility and neuroinvasive potential.

Although much remains to be learned about the specific mechanisms that define immunity against WNV, both humoral and cellular responses likely are essential for protection. Specific neutralizing antibodies are generated at late times after primary WNV infection, and the development of high-titer neutralizing antibodies after vaccination correlates with protection against challenge. Nevertheless, it remains unknown how the epitope specificity of the antibody repertoire contributes to protective memory responses. T-cell-mediated immunity is also likely necessary to resist a second WNV challenge. Immunization with different WNV vaccine preparations can induce memory T cells, although the relative contribution of

humoral versus cellular memory to vaccine efficacy remains largely undefined. The emergence of lineage 2 strains of WNV in Europe as a cause of clinical disease and mortality in horses raised the question whether the existing WNV vaccines, all based on lineage 1 strains, protect against circulating lineage 2 strains of WNV. The lineage 1 vaccines tested so far against lineage 2 strains protected against the disease, and so would provide veterinarians with an effective tool to control infections caused by lineage 1 and 2 strains of WNV.

References:

Bagnarelli P, Marinelli K, Trotta D, Monachetti A, Tavio M, Del Gobbo R, Capobianchi M, Menzo S, Nicoletti L, Magurano F, Varaldo P. Human case of autochthonous West Nile virus lineage 2 infection in Italy, September 2011. *Euro Surveill*; 2011;16(43).

Bakonyi T, Ferenczi E, Erdélyi K, Kutasi O, Csörgő T, Seidel B, Weissenböck H, Brugger K, Bán E, Nowotny N. Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. *Vet Microbiol*; 2013 Jul 26;165(1-2):61-70.

Barzon L, Pacenti M, Franchin E, Martello T, Lavezzo E, Squarzon L, Toppo S, Fiorin F, Marchiori G, Scotton GP, Russo F, Cattai M, Cusinato R, Palù G. Clinical and virological findings in the ongoing outbreak of West Nile virus Livenza strain in northern Italy, July to September, *Eurosurveillance*, 2012. Sept 17(36)

Castillo-Olivares J, Mansfield KL, Phipps LP, Johnson N, Tearle J, Fooks AR. Antibody response in horses following experimental infection with West Nile Virus lineages 1 and 2. *Transbound Emerg Dis*; 2011 Jun;58(3):206-12.

Kutasi O, Bakonyi T, Lecollinet S, Biksi I, Ferenczi E, Bahuon C, Sardi S, Zientara S, Szenci O. Equine encephalomyelitis outbreak caused by a genetic lineage 2 West Nile virus in Hungary. *J Vet Intern Med*; 2011 May-Jun;25(3):586-91.

Magurano F, Remoli ME, Baggieri M, Fortuna C, Marchi A, Fiorentini C, Bucci P, Benedetti E, Ciufolini MG, Rizzo C, Piga S, Salcuni P, Rezza G, Nicoletti L. Circulation of West Nile virus lineage 1 and 2 during an outbreak in Italy. *Clin Microbiol Infect*; 2012 Dec;18(12):E545-7.

McMullen AR, Albayrak H, May FJ, Davis CT, Beasley DW, Barrett AD. Molecular evolution of lineage 2 West Nile virus. *J Gen Virol*; 2013 Feb;94(Pt 2):318-25.

Minke JM, Siger L, Cupillard L, Powers B, Bakonyi T, Boyum S, Nowotny N, Bowen R. Protection provided by a recombinant ALVAC(®)-WNV vaccine expressing the prM/E genes of a lineage 1 strain of WNV against a virulent challenge with a lineage 2 strain. *Vaccine*; 2011 Jun 20;29(28):4608-12.

Papa A, Xanthopoulou K, Gewehr S, Mourelatos S. Detection of West Nile virus lineage 2 in mosquitoes during a human outbreak in Greece. *Clin Microbiol Infect*; 2011 Aug;17(8):1176-80.

Porter RS, Leblond A, Lecollinet S, Tritz P, Cantile C, Kutasi O, Zientara S, Pradier S, van Galen G, Speybroek N, Saegerman C. Clinical Diagnosis of West Nile Fever in Equids by Classification and Regression Tree (CART) Analysis and Comparative Study of Clinical Appearance in Three European Countries. *Transbound Emerg Dis* 2011 58(3) 197-205.

Samuel MA, Diamond MS. Pathogenesis of West Nile Virus Infection: a Balance between Virulence, Innate and Adaptive Immunity, and Viral Evasion, *J. Virol.* 2006 Oct 80(19) 9349-9360.

Sanchini A; Donoso-Mantke O; Papa A; Sambri V; Teichmann A; Niedrig M. Second international diagnostic accuracy study for the serological detection of west nile virus infection. *Negl Trop Dis* 2013 7, e2184.

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Ziegler U, Angenvoort J, Fischer D, Fast C, Eiden M, Rodriguez AV, Revilla-Fernández S, Nowotny N, de la Fuente JG, Lierz M, Groschup MH. Pathogenesis of West Nile virus lineage 1 and 2 in experimentally infected large falcons. *Vet Microbiol*; 2013 Jan 25;161(3-4):263-73.