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## WEST NILE VIRUS AND USUTU - A THREAT TO POLAND

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### ABSTRACT

In recent years emergence of new infectious diseases and the growing spread of pathogens to new areas is observed. Most of these pathogens are zoonotic viruses and their transmission route is from animals to humans and vice versa. These so called emerging and re-emerging pathogens that were present previously only in Africa and Asia are becoming a threat to European countries. These include, e.g. West Nile virus and USUTU virus.

The aim of the study is to present the clinical course of infections caused by WNV and USUTU, diagnostic and therapeutic possibilities and, above all, the current epidemiological situation of these infections in the world. We also tried to answer the question whether, there is a risk of infection with these viruses in Poland.

After analyzing the available literature we venture a conclusion that in Poland there is a risk of WNV and USUTU infection. Global warming, change of socio-economic conditions, travelling greatly affect the spread of these viruses. In addition there are confirmed human cases of these diseases in countries neighboring Poland, as well as presence of both viruses and the presence of vectors (*Culex pipiens* s.l. and *Culex torrentium* (Diptera: Culicidae)) in our country.

All these facts indicate that there is a necessity of epidemiological studies and consideration of these pathogens in the differential diagnosis of febrile illness and neuroinfection .

**Key words:** *West Nile Virus, WNV, USUTU virus, Poland*

### INTRODUCTION

Emerging and reemerging infectious diseases such as West Nile Fever or disease caused by USUTU virus, previously bound to Africa or South-eastern Asia, are more and more frequently found also in Europe.

The aim of the study was the presentation of current epidemiological situation of WNV and USUTU infections in the world with emphasis on the potential risk of infection in Poland. Additionally clinical picture of infections, diagnostic methods and therapeutic possibilities of these diseases will be discussed.

### WEST NILE VIRUS (WNV)

West Nile Virus (WNV) is a RNA virus which belongs to Flaviridae. At least 2 genetic lineages can be differentiated: Lineage 1, which is found in Europe, Africa, North America, Australia, India and lineage 2, which is found mostly in Africa. In nature, West Nile

virus is transmitted between mosquitoes (e.g. *Culex pipiens* s.l., *Culex torrentium*) and birds. Mosquitoes with WNV bite and infect people, horses and other mammals (1,2). WNV may be also transmitted by blood transfusion, organ transplants, via placenta or breast milk. Although it is theoretically possible, so far there are no proofs of transmission via contact with infected birds, humans, horses (1,2). Humans may act as “dead end” in viral cycle (3). WNV is an etiological pathogen for West Nile Fever.

The first case of human WNV infection was reported in west Uganda in 1937 (West Nile District). The incubation period of WNV infection is 3-15 days after mosquito bite. The majority of cases is asymptomatic (80%). In 20% the disease takes a mild course, and in <1% - severe: meningoencephalitis, muscle weakness, stroke (4).

Mild form of WNV infection is usually self-limiting. Patients complain on fever, headache, joint pain, rash, enlarged lymph nodes, nausea, vomits. Severe form of WNV infection may manifest as encephalitis, meningi-

tis, acute flaccid paralysis, tremors, myoclonus. Patients complain on high fever, headache, confusion, tremors, muscle paresis, stupor. Patients with chronic diseases of kidneys, circulation, with diabetes, in elderly or immunodeficient patients are more predisposed to fatal outcome (4,5).

In lab tests normal or elevated WBC, lymphocytopenia, anemia may be observed, while hyponatremia may be present in patients with encephalitis. In cerebrospinal fluid (csf) lymphocytic pleocytosis <500 cells/ $\mu$ l, increased protein concentration and normal glucose concentration is observed. Brain CT (computer tomography) shows no abnormalities, while in MRI (magnetic resonance) in 1/3 of patients enhancement of meninges and/or periventricular area is observed (1,2).

The best diagnostic method is detection of antibodies in IgM class in serum or/and csf by ELISA method (1, 2, 6).

IgM antibodies do not cross blood-brain barrier and their presence in csf indicates on CNS infection. It has to be taken into consideration that samples collected too early (<7 days) may give false negative results. Samples should be transported in containers keeping low temperature. Polystyren containers and freezing of samples should be avoided.

In countries where other Flaviviridae exist, e.g. tick-borne encephalitis virus (TBEV) every positive result should be confirmed by PRNT (neutralization test) to exclude false positive results due to cross-reactions. Also vaccination against Flaviviridae such as yellow fever, Japanese encephalitis, TBE can cause false positive ELISA test result. PCR (polymerase chain reaction) is not effective because the duration of viremia is short. Although it may be useful in case of immunodeficiency. The limitation of this method is that it has to be performed in csf and tissue samples only (1, 2, 6).

The treatment of WNV infection is symptomatic, hospitalization, fluids iv, superinfections prophylaxis and in case of respiratory insufficiency – artificial ventilation is needed. In some report high doses of ribavirin and interferon alpha-2b show in vitro activity. There are no clinical data concerning GCS, anticonvulsant, osmotherapy (4).

The main prevention is avoidance of mosquito bites. It has to be taken into consideration that mosquitos eggs may hatch in stagnant water after 4 days. Because of that water should be stored properly, rain water should be sheltered to prevent from contact with mosquitos. Also swimming pools should be covered. Additional preventive methods are: moscitieres, avoidance of evening activity of people and usage of repellents with 10-35% DEET, KBR, IR3535 and others (1, 2, 4, 6).

WNV is widespread and is present not only in Africa, Asia and Australia, but also in USA, Canada, Mexico and many European countries. Approximate

number of WNV humans infections in USA in 1999-2014 according to CDC was 41 762 cases (7). However, it is suspected that these data may be underestimated because the results of the serologic survey showed 950 000 cases of WNV infection (2).

As far as Europe is concerned seropositive animals/isolates were confirmed in 1960 in France, Portugal and Cyprus. WNV was then considered less pathogenic for humans than dengue virus (DENV) or yellow fever virus (YFV). More vicious/pathogenic genotypes of WNV were discovered in 1998 in Israel and in 2003 in Hungary. Recently WNV infections were reported in central and south-eastern Europe: in 2010 – Bulgaria and Greece, in 2011 – Albania and Macedonia, in 2012 – in Croatia, Serbia and Kosovo. In 2010 in Greece 261 cases (34 deaths) were confirmed, in Romania 57 cases (5 deaths) and 480 cases (6 deaths) in Russia. Numerous outbreaks of WNV infection have been noted since 2010 in Italy. What is interesting, these outbreaks were caused by genetically different isolates (1).

According to ECDC reports in 2012 the highest incidence on infections caused by WNV was observed in Greece (162 cases), Italy (28 cases), Hungary (17 cases) and Romania (15 cases) (8).

In 2013 *Rudolf* et al. isolated WNV from mosquitoes in Czech Republic. They proved that it was related with strains isolated in Austria, Italy, Serbia in 2008, 2011 and 2012 (9). WNV virus was also found in Ukraine. The first reports of humans and birds infections come from seventieths of the XX<sup>th</sup> century (10). Recently clinical WNV infections were detected in patients in the Ukraine in the summer of 2011 (eight cases of WNV in three different regions) and in 2012 (12 deaths in Poltavaska region) (10). In 2013, the presence of anti-WNV antibodies was stated in 13.5% of 300 tested horses (11).

The possibility of WNV presence in Poland was mentioned for the first time in years 1995-1996, when anti WNV antibodies were detected in 2.8% of examined house sparrows and 12.1% Eurasian tree sparrows in Lomianki (near Kampinos Forest) (12). The results of following studies performer either on animals or on humans varied. *Hermanowska-Szpakowicz* et al. described a confirmed case of WNV infection in a patient hospitalized because fever, headache, muscle pain and diarrhea. In 2006 foresters from 2 voivoidships (Swietokrzyskie and Podlaskie) were tested for the presence of anti WNV IgG antibodies in serum. In 1 of 52 (1.9%) examined foresters in Swietokrzyskie and 4 of 42 in Podlaskie the result was positive (13, 14).

In other two studies no WNV virus was detected. *Niczyporuk* et al. in years 2007-2010 examined brain tissue sample of migratory birds by RT-PCR and serologic methods and did not find proof of WNV infection (15). Also *Czupryna* et al. did not find WNV RNA in any of 24 patients hospitalized because of neuroinfections (16).

In 2015 *Niczyporuk et al.* reported that analysis of serum samples from birds (white storks, common pheasants, common chaffinches, wild ducks, white-tailed eagles passenger pigeons, goshawks, hooded crows, northern goshawks, common swift, common blackbird, common starling, common raven, and common buzzard) confirmed presence of anti WNV antibodies in 63/474 (13.29%) of examined wild birds, in 1/378 (0.26%) horse and 14/42 (33.33%) humans. All positive samples from birds were confirmed with PRNT method (17).

Positive results of samples from birds, horses and humans may indicate that WNV is connected with Polish ecosystem.

Presence of WNV observed in many European countries, including countries near Poland (Czech Republic, Ukraine) and occurrence of mosquitos belonging to *Culex pipiens* s.l. and *Culex torrentium* (Culicidae) in Wrocław area (Poland) (18) support the hypothesis of high probability of occurrence of WNV in Poland. Therefore WNV should be included in differential diagnostic of meningitis.

## USUTU VIRUS

USUTU virus is a Flavivirus that belongs to Japanese Encephalitis serocomplex. It is transmitted by african mosquitos: *Culex pipiens* (holoartic), *Cx. neavei*, *Culex perexiguus*, *Aedes albopictus* (from South-East Asia), *Ochlerotatus caspius* (palearctic), *Anopheles maculipennis* complex (holoartic), *Culex perfuscus*, *Coquillettidia aurites*, *Mansonia Africana* (19, 20). Its natural life cycle involves mosquito-bird-mosquito cycles (19, 20). Also transmission via blood transfusion and transplants is possible (21, 22).

The virus was first isolated from *Culex neavei* mosquito in 1959 in Republic of South Africa. More strains of USUTU were detected in various species of birds and mosquitos in Africa. As far as Europe is concerned, the virus was isolated from bats in Germany (23). Mosquitos transfer the virus to humans, horses or rodents that act as an accidental hosts. Mammals and humans are 'dead-end' hosts.

First case of symptomatic human USUTU infection was reported in Central African Republic in 1981. First European case was confirmed in Italy in 2009 (20). In 2012 in Germany presence of anti-USUTU antibodies was stated in 1/4200 patients (24), and in 2013 in Croatia virus was detected in three patients with encephalome-ningitis (25).

The incubation period is 2-14 days. The disease usually manifests with rash and fever. Infection may present as neuroinvasive disease similar to West Nile fever or hepatitis (21, 22, 26).

So far the data concerning diagnostic methods are scarce. IgM antibodies appear 5 days after fever onset. However no tests for IgM detection are yet available. In 2012 first test for IgG detection were developed. Because USUTU belongs to Flaviviridae, in case of positive result, confirmation with PRNT test is necessary. Cerebrospinal fluid and blood may be tested with RT-PCR (20, 26).

The treatment of USUTU infection is symptomatic.

In 2001 in Austria few birds (mostly sparrows) died because of USUTU infection. In following years there were some reports of USUTU virus presence in dead birds or mosquitos in Hungary (2005), Italy (2009), Spain (2006 and 2009), Switzerland (2006) (19). Serological features of USUTU infection were reported in wild birds in Czech Republic (2005), UK (2001 and 2002), Germany (2007), Italy (2007), Spain (2003 and 20006), Switzerland (2006). Authors concluded, that one-time introduction of virus from Africa to Europe (Vienna) is highly probable, especially that the same serotype is wide spread in Central Europe (19, 20).

Comparison of USUTU virus genetic sequence detected in various animal species in various counties, show, that in Italy in 2008 and 2009 probably 2 various serotypes were circulating in horses, chickens, wild birds and mosquitos (serology, virusology) (19, 20).

In Poland USUTU antibodies were found in 2008 in a seagull (*Larus ridibundus*) (27).

## SUMMARY

To sum up after analyzing the available literature we venture a conclusion that in Poland there is a risk of WNV and USUTU infection. Factors such as have influence on circulation of these viruses: global warming, travelling all around the world. What is more, confirmed human WNV cases in neighboring countries (Czech Republic, Ukraine), presence of both viruses and the presence of vectors (*Culex pipiens* sl and *Culex torrentium*) in our country indicate on necessity of further epidemiological studies and including these pathogens in differential diagnosis of fever and neuroinfection.

## REFERENCES

1. Beck C, Jimenez-Clavero MA, Leblond A, et al. Flaviviruses in Europe: complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. *Int J Environ Res Public Health* 2013;10(11):6049-83.
2. Chancey C, Grinev A, Volkova E, et al. The global ecology and epidemiology of West Nile virus. *Biomed Res Int* 2015;2015:376230.

3. Pfeffer M, Dobler G. Emergence of zoonotic arboviruses by animal trade and migration. *Parasites & Vectors* 2010;3:35
4. Sejvar JJ. Clinical manifestations and outcomes of West Nile virus infection. *Viruses* 2014;6(2):606-23
5. Lindsey NP, Staples JE, Lehman JA, et al. Medical risk factors for severe West Nile Virus disease, United States, 2008-2010. *Am J Trop Med Hyg* 2012;87(1):179-84
6. De Filette M, Ulbert S, Diamond M, et al. Recent progress in West Nile virus diagnosis and vaccination. *Vet Res* 2012; 43:16.
7. CDC, Centers for Disease Prevention and Control: West Nile virus disease cases and deaths reported to CDC by year and clinical presentation, 1999–2014; <http://www.cdc.gov> (accessed on February 2016).
8. ECDC, European Centers for Disease Prevention and Control: West Nile Fever Map Available online: <http://ecdc.europa.eu> (accessed on February 2016).
9. Rudolf I, Bakonyi T, Sebesta O, et al. West Nile virus lineage 2 isolated from *Culex modestus* mosquitoes in the Czech Republic, 2013: expansion of the European WNV endemic area to the North? *Euro Surveill* 2014;19(31):2-5.
10. Sidenko VP, Stepankovskaia LD, Solomko RM, et al. Results of a study of West Nile fever in the South of the European part of the USSR. *Zh Mikrobiol Epidemiol Immunobiol* 1974; 00: 129.
11. Ziegler U, Skrypnyk A, Keller M, et al. West Nile virus antibody prevalence in horses of Ukraine. *Viruses* 2013; 4;5(10):2469-82.
12. Juricová Z, Pinowski J, Literák I, et al. Antibodies to alphavirus, flavivirus, and bunyavirus arboviruses in house sparrows (*Passer domesticus*) and tree sparrows (*P. montanus*) in Poland. *Avian Dis* 1998; 42(1):182-5.
13. Hermanowska-Szpakowicz T, Grygorczuk S, Kondrusik M, et al. Infections caused by West Nile virus. *Przegl Epidemiol* 2006;60(1):93-8.
14. Kondrusik M, Ferenczi E, Zajkowska J, et al. The evaluation of serum presence of antibodies reacting with West Nile Fever virus (WNV) antigens among inhabitants from Podlaskie and Swietokrzyskie region. *Przegl Epidemiol* 2007;61(2):409-16.
15. Niczyporuk JS, Samorek-Salamonowicz E, Kozdrun W, et al. Attempts to detect West Nile virus in wild birds in Poland. *Acta Vet Hung* 2011;59(3):405-8.
16. Czupryna P, Niczyporuk J, Samorek-Salamonowicz E, et al. Detection of West Nile Virus RNA in patients with meningitis in Podlaskie Province. *Przegl Epidemiol* 2014;68(1):17-20, 109-11.
17. Niczyporuk JS, Samorek-Salamonowicz E, Lecollinet S, et al. Occurrence of West Nile virus antibodies in wild birds, horses, and humans in Poland. *Biomed Res Int* 2015;2015:234181.
18. Weitzel T, Jawień P, Rydzanicz K, et al. *Culex pipiens* s.l. and *Culex torrentium* (Culicidae) in Wrocław area (Poland): occurrence and breeding site preferences of mosquito vectors. *Parasitol Res* 2015;114(1):289-95.
19. Vazquez A, Jimenez-Clavero M, Franco L, et al. A. Usutu virus: potential risk of human disease in Europe. *Euro Surveill* 2011 Aug 4;16(31). pii: 19935.
20. Ashraf U, Ye J, Ruan X et al. Usutu virus: an emerging flavivirus in Europe. *Viruses* 2015;7(1):219-38.
21. Cavrini F, Gaibani P, Longo G, et al. Usutu virus infection in a patient who underwent orthotopic liver transplantation, Italy, August-September 2009. *Euro Surveill* 2009;14(50). pii: 19448
22. Pecorari M, Longo G, Gennari W, et al. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. *Euro Surveill* 2009;14(50). pii: 19446.
23. Cadar D, Becker N, Campos Rde M, et al. Usutu virus in bats, Germany, 2013. *Emerg Infect Dis* 2014;20(10):1771-3.
24. Allering L, Jöst H, Emmerich P, et al. Detection of Usutu virus infection in a healthy blood donor from south-west Germany, 2012. *Euro Surveill* 2012;17:e20341
25. Vilibic-Cavlek T, Kaic B, Barbic L, et al. First evidence of simultaneous occurrence of West Nile virus and Usutu virus neuroinvasive disease in humans in Croatia during the 2013 outbreak. *Infection*. 2014; 42(4):689-95.
26. Gaibani P, Cavrini F, Gould EA, et al. Comparative genomic and phylogenetic analysis of the first Usutu virus isolate from a human patient presenting with neurological symptoms. *PLoS One* 2013;31;8(5):e64761.
27. Hubálek Z, Wegner E, Halouzka J, et al. Serologic survey of potential vertebrate hosts for West Nile virus in Poland. *Viral Immunol* 2008;21(2):247-53.

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