

HRVATSKA AKADEMIJA
ZNANOSTI I UMJETNOSTI

Razred za medicinske znanosti

Odbor za animalnu i komparativnu patologiju



Simpozij

**EPIDEMIOLOŠKE I KLINIČKE OSOBITOSTI INFEKCIJE VIRUSOM
ZAPADNOG NILA U HRVATSKOJ I SUSJEDNIM ZEMLJAMA**

**Epidemiological and clinical aspects of West Nile virus infection in
Croatia and neighbouring countries**

SAŽETCI

Abstracts

palača Hrvatske akademije znanosti i umjetnosti, Zrinski trg 11, Zagreb

Zagreb, 25. listopada 2012.

Znanstveni odbor

Redoviti članovi

Razreda za medicinske znanosti

Hrvatske akademije znanosti i umjetnosti

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PROGRAM

10,00 - 10,10

Uvodna riječ

Marko Pećina, tajnik Razreda za medicinske znanosti HAZU

Chaired by Josip Madić and Ljubo Barbić

10,10 - 10,40

West Nile Virus infections in Europe – general features

Infekcija virusom Zapadnog Nila u Europi – osnovna obilježja

Norbert Nowotny, Tamás Bakonyi, Herbert Weissenböck, Bernhard Seidel, Jolanta Kolodziejek, Karin Sekulin, Helga Lussy (Viral Zoonoses, Emerging and Vector-Borne Infections Group, Institute of Virology, University of Veterinary Medicine, Vienna, Austria; Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman)

10,40 - 11,00

West Nile virus in Italy, five years of epidemic

Pet godina infekcije virusom Zapadnog Nila u Italiji

Giovanni Savini, Rossana Bruno (Department of Virology and Biotechnology, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise „G. Caporale”, Teramo, Italy)

11,00 - 11,20

Characterization of West Nile virus outbreaks in Hungary

Osobitosti infekcije virusom Zapadnog Nila u Mađarskoj

Tamas Bakonyi (Faculty of Veterinary Science, Department of Microbiology and Infectious Diseases, Budapest, Hungary)

11,20 - 11,40

West Nile virus in birds in Slovenia

Virus Zapadnog Nila u ptica u Sloveniji

Joško Račnik, Tomi Trilar, Mateja Jelovšek, Marko Zadavec, Brigita Slavec, Olga Zorman Rojs, Tatjana Avšič Županc (Institute for Poultry Health, Veterinary Faculty, University of Ljubljana, Slovenia)

11,40 - 12,00 Discussion - Rasprava

12,00 - 12,30 Coffe break - stanka za kavu

Chaired by Alemka Markotić and Tatjana Vilibić-Čavlek

12,30 - 12,50

Komarci vektori virusa Zapadnog Nila

Mosquitoes - vectors of West Nile virus

Enrih Merdić (Josip Juraj Strossmayer University of Osijek, Department of Biology, Croatia)

12,50 - 13,10

Serološki dokaz prisutnosti infekcije virusom Zapadnog Nila kod konja u Srbiji

Serological evidence of West Nile virus activity in horses in Serbia

Diana Lupulović, Sava Lazić, Ana-Belen Blázquez, Estela Escribano-Romero, Juan-Carlos Saiz, Tamas Petrović (Veterinary Institute, Novi Sad, Serbia; National Institute for Agronomic Research, Madrid, Spain)

13,10 - 13,30

Proširenost infekcije virusom Zapadnog Nila u konja u Hrvatskoj

West Nile virus serosurveillance in horses in Croatia

Ljubo Barbić, Vladimir Stevanović, Josip Madić (Faculty of Veterinary Medicine, Department of Microbiology and Infectious Diseases, University of Zagreb, Croatia)

13,30 - 13,50

Prvi slučajevi neuroinvazivne bolesti uzrokovane virusom Zapadnog Nila u ljudi u Hrvatskoj

First outbreak of West Nile virus neuroinvasive infection in humans in Croatia

Iva Pem-Novosel, Tatjana Vilibić-Čavlek (Croatian National Institute of Public Health, Zagreb, Croatia)

13,50 - 14,10

Kliničke osobitosti infekcije virusom Zapadnog Nila u ljudi u Hrvatskoj

Clinical aspects of West Nile virus infection in humans in Croatia

Ljiljana Perić, **Danijel Šimašek** (Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia)

14,10 - 14,40 Rasprava - Discussion

WEST NILE VIRUS INFECTIONS IN EUROPE – GENERAL FEATURES

Norbert Nowotny^{1,2}, Tamás Bakonyi^{3,1}, Herbert Weissenböck⁴, Bernhard Seidel⁵, Jolanta Kolodziejek¹, Karin Sekulin¹, and Helga Lussy¹

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In the past West Nile virus (WNV) infections occurred only sporadically in Europe, limited in time and geographic distribution. This changed dramatically since the emergence of an exotic lineage 2 WNV strain in Hungary in 2004 [Bakonyi et al., *Emerg. Infect. Dis.* 12, 618-623 (2006)]. Following a few years of adaptation, this virus strain dispersed widely in 2008, and was identified all over Hungary and in the eastern part of Austria [Wodak et al., *Vet. Microbiol.* 149, 358-366 (2011)]. Most likely during that time this WNV lineage also spread to Croatia, Slovenia and Serbia. In 2010, a clinically severe outbreak occurred in the Thessaloniki area of Greece with more than 200 human neuroinvasive cases and more than 30 deaths. Genetic analysis demonstrated the above-mentioned lineage 2 WNV as the causative agent, however with an amino acid exchange, which might have been responsible for the increased neuroinvasiveness [Papa et al., *Emerg. Infect. Dis.* 17, 920-922 (2011)]. In 2012, overt West Nile disease in humans and animals was also reported from several Balkan states.

Since 2008 widespread outbreaks of lineage 1 WNV have been reported in northern Italy as well as outbreaks of a different type of a lineage 2 WNV (Volgograd strain) in Romania.

We demonstrated that the virus is overwintering in mosquitoes in outbreak areas, indicating that central, southern and eastern Europe must be aware of further WNV outbreaks in the future.

In my talk I will present general features of WNV infections and an overview of WNV epidemiology in Europe.

Infekcija virusom Zapadnog Nila u Europi – osnovna obilježja

Norbert Nowotny, Tamás Bakonyi, Herbert Weissenböck, Bernhard Seidel,
Jolanta Kolodziejek, Karin Sekulin, Helga Lussy

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U prošlosti se infekcija virusom Zapadnog Nila (ZN) u Europi javljala sporadično te vremenski i geografski ograničeno. To se stubokom promijenilo otkad se 2004. godine u Mađarskoj pojavila egzotična linija 2 virusa ZN [Bakonyi et al., *Emerg. Infect. Dis.* 12, 618-623 (2006)]. Nakon pet godina prilagodbe, soj te linije nadaleko se proširio te je 2008. dokazan na cijelom području Mađarske i istočnom dijelu Austrije [Wodak et al., *Vet. Microbiol.* 149, 358-366 (2011)]. Ta se linija vjerojatno u tom razdoblju proširila i na Hrvatsku, Sloveniju i Srbiju. Klinički teški oblik infekcije virusom ZN pojavio se 2010. u Grčkoj s više od 200 neuroinvazivnih slučajeva i više od 30 umrlih. Genetska analiza je pokazala da je uzročnik te epidemije bila spomenuta linija 2 virusa ZN s izmijenjenim aminokiselinskim sastavom, što je vjerojatno bio uzrok njezine pojačane neuroinvazivnosti [Papa et al., *Emerg. Infect. Dis.* 17, 920-922 (2011)]. Kliničko očitovanje infekcije virusom ZN zabilježeno je 2012. u ljudi i životinja u nekoliko balkanskih država. Od 2008. zabilježeno je nekoliko pojava infekcije linijom 1 virusa ZN u sjevernoj Italiji kao i pojava različitih tipova linije 2 virusa ZN (soj Volgograd) u Rumunjskoj. Dokazali smo da virus prezimljuje u komaraca na područjima gdje se trajno javlja, što upućuje na zaključak da se u budućnosti mogu očekivati nove pojave infekcije virusom ZN u središnjim, južnim i istočnim dijelovima Europe.

U izlaganju će biti riječi o općenitim značajkama infekcije virusom ZN i općem pregledu epidemiologije te infekcije u Europi.

WEST NILE VIRUS IN ITALY, FIVE YEARS OF EPIDEMIC

Giovanni Savini and Rossana Bruno

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West Nile virus (WNV) first appeared in Italy in 1998. Although the infection caused severe disease and deaths in horses, no cases were reported in humans at that time. Since 2008, the Italian epidemiological scenario of WNV has been dramatically changing with neurological diseases being increasingly observed in both, humans and horses. In order to monitor and control the WNV circulation, the Italian government implemented both a national serological, entomological and virological surveillance program and a national plan for West Nile Neuroinvasive Disease. Within the framework of WNV surveillance activities, it was possible to detect and monitor the progression and evolution of different strains of WNV from their entry in the North Eastern part to their spread into the South and main Islands of Italy. In some areas co-circulation of WNV strains belonging to lineage 1 and 2 was also evidenced. The presentation will describe the main features observed during these last five years of outbreak.

Pet godina epidemije uzrokovane virusom zapadnog Nila u Italiji

Giovanni Savini, Rossana Bruno

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Virus Zapadnog Nila (ZN) prvi put je u Italiji bio dokazan 1998. godine. Premda je tada uzrokovao tešku bolest i uginuća u konja, bolest nije bila zabilježena u ljudi. Epidemiologija infekcije virusom Zapadnog Nila u Italiji znatno se promijenila nakon 2008. godine. Od tada se sve češće javlja s nervnim poremećajima u ljudi i konja. S ciljem promatranja kruženja i kontrole virusa ZN, u Italiji je na državnoj razini donesen nacionalni plan za provođenje programa seroloških, entomoloških i viroloških istraživanja neuroinvasive bolesti uzrokovane virusom Zapadnog Nila. U sklopu promatranja proširenosti infekcije virusom ZN proučavana je evolucija različitih sojeva virusa od njegove pojave u sjevernim i istočnim područjima Italije do širenja na jug i glavne talijanske otoke. U nekim područjima dokazana je istodobna aktivnost sojeva linije 1 i linije 2 virusa ZN. U izlaganju će biti govora o osnovnim epidemiološkim obilježjima infekcije posljednjih pet godina.

CHARACTERIZATION OF WEST NILE VIRUS OUTBREAKS IN HUNGARY

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The occurrence the West Nile virus (WNV) in Hungary was first reported on the basis of serological studies on geese in 1969. However, clinical manifestation of the virus infection has never been observed in the country until 2003. In that year an outbreak of encephalitis occurred in a goose flock at the southern region of Hungary. A lineage 1 WNV strain was detected in the brain of the affected birds. One year later an exotic, lineage 2 strain of WNV emerged in south-eastern Hungary. This virus strain differed from the strains distributed in Europe, Asia, America and Australia; its closest relatives were isolated in the sub-Saharan region of Africa. It is likely that migratory birds introduced the strain to Hungary from their African wintering places. The first neurological and lethal cases were observed in goshawks (*Accipiter gentilis*) in 2004, and the virus was subsequently detected in 2005 in diseased or dead goshawks, sparrowhawks (*Accipiter nisus*) and in a sheep. The cases were observed in a close geographic proximity (approx. 30 km distance from each other). In 2007 we detected WNV infections in three goshawk and three red-footed falcon (*Falco vespertinus*) samples collected in the territories of the Körös-Maros National Park and the Hortobágy National Park. A moderate geographic spread of the virus was observed (approximately 80 km distance from the location of the year 2004-2005 cases). An unexpected and explosive geographic spread of the virus strain was detected in 2008. We found 25 WNV positive wild bird cases. Goshawks were the most seriously involved (17 diagnosed cases) but other species, mainly birds of prey were also found WNV infected. The virus strain emerged in the central and western regions of Hungary and in the eastern part of Austria. In 2009 we detected WNV in 16 wild bird specimens. The highest number of cases was again diagnosed in goshawks, but small songbirds were also found positive. Cases were distributed over the whole territory of Hungary and in the eastern regions of Austria. In 2010, a lower intensity of WNV activity was observed in Hungary, compared to the previous two years. Three wild bird samples were found positive for WNV. In 2011 further four WNV infections were diagnosed in wild birds. Genetic comparisons of the viruses detected between 2004 and 2011 revealed a high level of genetic relatedness of the strains, which indicate that the previously exotic WNV strain, which was introduced to Hungary around 2004, established itself in the country and became a resident pathogen.

Besides wild birds, WNV-associated central nervous illnesses were diagnosed in horses (2007: 1 case, 2008: 12 cases, 2009: 5 cases, 2010: 5 cases, 2011: 4 cases, and 2012: 3 cases. Many of these horses died due to encephalomyelitis), and in humans (2008: 19 cases, 2009: 7 cases, 2010: 19 cases, 2011: 6 cases, 2012: 7 cases. One case in 2010 was fatal). We have also detected the virus in mosquito vectors. Serological investigations were made on serum samples of wild birds and horses for the detection of anti-WNV antibodies, in order to estimate the frequencies of subclinical infections. Antibodies were detected in 10-40% of the serum samples, which indicate a relatively high infection rate. The frequent and widespread subclinical infection is a possible explanation for the reduced number of clinical cases in Hungary in 2010, 2011, and 2012, compared to 2008 and 2009. Nevertheless, the WNV strain emerged in 2010 in the north-eastern part of Greece and caused a serious outbreak in people with several encephalitic cases and a high fatality rate. The same virus strain was also detected in a human case in Italy in 2011, as well as in Serbia in 2011 (in mosquitoes) and in 2012 (in horses and in humans). Our genetic investigations demonstrated that the strain is practically identical with the one that emerged in Hungary and which has been circulating in the country since.

Osobitosti infekcije virusom Zapadnog Nila u Mađarskoj

Tamas Bakonyi

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Pojava virusa Zapadnog Nila (ZN) u Mađarskoj prvi put je bila zabilježena na osnovi seroloških istraživanja u gusaka 1969. godine. Međutim kliničko očitovanje zaraze nije bilo ustanovljeno sve do 2003. Te se godine pojavio encefalitis u jatu gusaka u južnom dijelu Mađarske. U tkivu mozga zaraženih gusaka ustanovljena je linija 1 virusa Zapadnog Nila. Sljedeće godine pojavila se egzotična linija 2 virusa ZN u jugo-istočnom dijelu Mađarske. Taj soj bio je različit od sojeva virusa koji su kružili u Europi, Aziji, Americi i Australiji. Njegovi najbliži srodnici bili su izdvojeni u subsaharskom području Afrike. Soj je u Mađarsku vjerojatno bio prenesen pticama selicama iz afričkih područja gdje one prezimljuju. Prvi neurološki slučajevi i uginuća zapaženi su u jastreba (*Accipiter gentilis*) 2004., a virus je bio dokazan i 2005. u oboljelih ili uginulih jastrebava, kobaca (*Accipiter nisus*) i u ovaca. Svi slučajevi bili su ustanovljeni na uskom geografskom području (u razmaku oko 30 km jedan od drugoga). Godine 2007. infekcija virusom ZN bila je dokazana u tri jastreba i tri sokola (*Falco vespertinus*) na području Nacionalnog parka Körös-Maros i Nacionalnog parka Hortobágy. Tada je primijećena značajna geografska proširenost virusa

(na udaljenosti oko 80 km od mjesta gdje su bili utvrđeni slučajevi 2004. i 2005.). Neočekivano i eksplozivno širenje virusa ustanovljeno je 2008. Virus je tada bio dokazan u 25 divljih ptica. Najčešće se bolest javljala u jastrebova (17 dijagnosticiranih slučajeva), ali su bile zahvaćene i druge vrste, pretežito grabljivice. Virus se pojavio u središnjim i zapadnim područjima Mađarske te istočnom dijelu Austrije. Godine 2009. virus ZN bio je dokazan u 16 uzoraka divljih ptica. Najviše slučajeva ponovo je bilo dokazano u jastrebova, ali i u malih ptica pjevica. Zaraza se proširila na cijelo područje Mađarske i na istočni dio Austrije. Godine 2010. ustanovljena je smanjena aktivnost virusa ZN u odnosu na prijašnje godine. Svega tri uzorka divljih ptica bila su pozitivna na virus. Godine 2011. virus je bio dokazan u daljnja četiri slučaja. Usporedba genetskih svojstava sojeva dokazanih od 2004. do 2011. pokazala je njihovu veliku genetsku srodnost, što upućuje na zaključak da se prethodno egzotični virusni soj, koji je bio unesen u Mađarsku oko 2004., tu udomaćio i postao prirodan mađarskom području.

Osim u divljih ptica virus ZN bio je u svezi i s dijagnosticiranim poremećajima središnjeg živčanog sustava u konja (2007: 1 oboljeli, 2008: 12 oboljelih, 2009: 5 oboljelih, 2010: 5 oboljelih, 2011: 4 oboljela i 2012: 3 oboljela). Mnogi od tih konja uginuli su pod znakovima encefalomijelitisa. Infekcija je bila dijagnosticirana i u ljudi (2008: 19 oboljelih, 2009: 7 oboljelih, 2010: 19 oboljelih, 2011: 6 oboljelih, 2012: 7 oboljelih. Godine 2010. jedan je pacijent umro).

U Mađarskoj je virus bio dokazan i u komaraca, njegovih prenositelja. Poduzeta su bila i serološka istraživanja u divljih ptica i konja na prisutnost specifičnih protutijela, s ciljem procjene učestalosti supkliničkih infekcija. Protutijela su bila dokazana u 10-40% pretraženih uzoraka seruma, što govori o relativno visokoj stopi infekcije. Sve većom učestalošću i proširenošću supkliničke infekcije mogao bi se objasniti smanjeni broj kliničkih slučajeva u Mađarskoj 2010., 2011. i 2012. godine u odnosu na 2008. i 2009. Soj virusa ZN koji se 2010. pojavio u sjeverno-istočnom dijelu Grčke uzrokovao je teški oblik encefalitisa s visokom smrtnošću u ljudi. Isti virus bio je također dokazan u ljudi u Italiji 2011., zatim u Srbiji 2011. (u komaraca) i 2012. u konja i ljudi. Naša genetska istraživanja pokazala su da je taj soj zapravo identičan soju koji je bio dokazan u Mađarskoj, a sada kruži cijelom zemljom.

WEST NILE VIRUS ANTIBODIES IN PASSERINE BIRDS IN SLOVENIA

Joško Račnik¹, Tomi Trilar², Mateja Jelovšek³, Marko Zadavec¹, Brigita Slavec¹, Olga Zorman Rojs¹ and Tatjana Avšič Županc³

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Wild living passerine birds can be infected by a number of pathogenic microorganisms (avian influenza virus - AIV, West Nile virus - WNV ...). They carry emerging pathogens as a reservoir hosts. Many of them are transmissible to humans and to other domestic or free living animals. Backyard poultry flocks, cattle or other domestic animals reared outside can attract migrating wild passerine birds to stop on their territory where transmission of infectious agent could happen (1,2,3). The West Nile virus transmission is related to the mosquito carrier, songbirds, however, are an important natural host for WNV (3). In our research the presence and epidemiology of WNV from 2003 until 2009 among wild passerine birds was investigated. Passerine birds were captured into mist nets. The captured birds were clinically examined; the ornithologist fixed the ring, determined the species, sex and age, and measured the body weight and the length of the wings. In molting birds the way of molting was described. Songbirds' blood was taken with a puncture of the jugular vein. The blood sample was centrifuged; the serum was poured off and stored at -20°C until serologic testing (2,4,5,6). For the detection of the West Nile virus the indirect immunofluorescence (IIF) was used. As an antigen the West Nile virus (WN (E101)01.71) was used. The commercial anti-wild bird fluorescein isothiocyanate (FITC) conjugated antibodies were used in the test (Benthy Laboratories, Inc., Montgomery) (2,5,6,7). Because of small sample volume (< 0,03 ml of sera) no additional testing with other serological methods was performed. During our research in the years 2004, 2005 and 2006, 2008 and 2009 altogether 976 sera of songbirds from 34 different species, with the IIF were tested. Specific antibodies against WNV were found in the serum of 46 songbirds (4,7 %). Positive species were blackcap, house sparrow, goldfinch, common whitethroat, lesser whitethroat, greater whitethroat, reed warbler, robin, sedge warbler, icterine warbler, blackbird and garden warbler (2, 5, 6). The songbirds that were captured during spring migration as well as the ones during autumn migration were positive. Most of the positive birds on autumn

migration were young, first year birds that were hatched some were in Europe. The proof of antibodies in young birds could indicate the circulation of WNV in some areas of Europe. WNV positive birds were detected also among songbirds that were hatched in Slovenia and song birds (house sparrow) that constantly live here. This could indicate a local circulation of WNV in our country (2). In the light of presented data and reports from neighboring countries of increased WNV activity, further research is needed to investigate the epidemiology of WNV in Slovenia.

Literature: (1) Reed KD, Meerce J, Henkel JS, Shukla SK. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, Influenza A and enteropathogenes. Clin Med Res 2003; 1: 5-12; (2) Račnik J. Epidemiological study of avian influenza and West Nile virus in birds in Slovenia, (PhD thesis). Ljubljana: University of Ljubljana; 2008; (3) Hubalek Z. An annotated checklist of pathogenic microorganisms associated with migratory birds. J Wildl Dis 2004; 40: 639-59; (4) Račnik J, Slavec B, Trilar T, Zadavec M, Dovč A, Krapež U, Barlič Magajna D, Zorman - Rojs O. Evidence of avian influenza virus and paramyxovirus subtype 2 in wild living passerine birds in Slovenia. Eur J Wildl Res. 2008; 54: 529-532; (5) Račnik J, Trilar T, Slavec B, Marhold C, Jelovšek A, Vergles Rataj A, Avšič Županc T, Zorman Rojs O. Study of some pathogens in living wild passerine birds captured during autumn migration in Slovenia. Slov Vet Res 2011; 48; Suppl 13: 146 -9; (6) Račnik J, Zorman Rojs O, Trilar T, Jelovšek M, Duh D, Dovč A, Avšič Županc T. Demonstration of West Nile virus antibodies in wild birds in Slovenia. In: Proceedings of 8th symposium Poultry days 2009 with international participation, Poreč, Croatia; 69 – 71; (7) Ebel GD, Dupuis AP, Nicholas D, Young D, Maffei J, Kramer LD. Detection by enzyme linked immunosorbent assay of West Nile virus in birds. Emerg Infect Dis 2002; 8: 979-82.

Protutijela za virus Zapadnog Nila u ptica u Sloveniji

Joško Račnik, Tomi Trilar, Mateja Jelovšek, Marko Zadavec, Brigita Slavec,
Olga Zorman Rojs, Tatjana Avšič Županc

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Ptice vrapčarke mogu se zaraziti različitim patogenim mikrobima, primjerice virusom ptičje gripe, virusom Zapadnog Nila i dr. One su rezervoar prijetećih uzročnika zaraza. Mnogi od njih prenose se na čovjeka te druge domaće i životinje koje slobodne žive u prirodi. Domaća perad, goveda i druge domaće životinje držane ekstenzivno mogu na svoj teritorij privući vrapčarke pri čemu se mogu prenositi uzročnici zaraznih bolesti (1,2,3). Virus Zapadnog Nila prenose komarci dok su ptice pjevice njegov prirodni domaćin (3). U našem radu istraživana je prisutnost i epidemiologija virusa Zapadnog Nila u vrapčarki i to od 2003. do 2009. godine. Ptice su bile hvatane s posebnim mrežama. Uхваćene ptice bile su klinički pretražene, prstenovane, bila im je određena vrsta, spol i dob te izmjerena tjelesna težina i dužina krila. Kod onih u fazi

mitarenja, opisan je i način mitarenja. Krv im je bila uzeta iz jugularne vene. Svaki uzorak krvi bio je centrifugiran, a serum odvojen i pohranjen pri -20 °C do pretraživanja (2,4,5,6). Za dokazivanje specifičnih protutijela rabljena je neizravna imunofluorescencija. Kao antigen rabljen je virus Zapadnog Nila (E101)01.71. Rabljena su komercijalna protutijela obilježena fluorescein izotiocijanatom (FITC) (Benthy Laboratories, Inc., Montgomery) (2,5,6,7). Zbog male količine (<0,03 ml), uzeti se uzorci nisu mogli dodatno pretražiti drugim serološkim metodama. Od 2004. do 2006., zatim od 2008. do 2009. pretražili smo ukupno 976 uzoraka seruma podrijetlom od 34 vrste različitih ptica pjevica. Specifična protutijela bila su dokazana u njih 46 (4,7%). Pozitivne su bile sljedeće vrste: crnokapa grmuša, vrabac, češljugar, grmuša pjenica, grmuša čevrljinka, siva grmuša, trstenjak cvrkutić, crvendać, trstenjak rogožar, žuti voljić, kos i siva grmuša (2,5,6). Pozitivne su bile jednako pjevice uhvaćene za vrijeme proljetne kao i one za vrijeme jesenske selidbe. Za vrijeme jesenske selidbe većinom su bile pozitivne mlade ptice izležene negdje u Europi. Dokaz protutijela u mladim ptica upućuje na zaključak o cirkulaciji virusa Zapadnog Nila na području Europe. Pozitivne ptice na virus Zapadnog Nila dokazane su i među pjevicama koje su se izlegle u Sloveniji te onima (vrabac) koje trajno žive u Sloveniji. To upućuje na lokalno kruženje virusa Zapadnog Nila u Sloveniji (2). U svjetlu predstavljenih rezultata i izvješća o pojačanoj aktivnosti virusa u susjednim zemljama, potrebno je poduzeti daljnja istraživanja epidemiologije virusa Zapadnog Nila u Sloveniji.

KOMARCI VEKTORI VIRUSA ZAPADNOG NILA U HRVATSKOJ

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Hrvatsku faunu komaraca čini 50 vrsta. Od njih medicinsko značenje ima 15 vrsta, a samo četiri vrste sposobne su prenijeti virus Zapadnog Nila. Uloga komaraca u širenju svakog arbovirusa pa tako i virus Zapadnog Nila je vrlo značajna. Komarci su najznačajniji vektori, ali ne i jedini prenositelji virusa Zapadnog Nila. Da bi komarac bio vektor moraju se poklopiti neki faktori. Prije svega virus u komarca ulazi ishranom i to u krvnom obroku. Virus se replicira u želudcu, jajniku, živcima, masnom tkivu, prelazi u hemolimfu i potom dolazi u žlijezde slinovnice. Novim hranjenjem ubrizgava se sadržaj slinovnica u domaćina i prenosi virus. Različit je vektorski kapacitet komarca u Hrvatskoj. Najbolji prenositelji virusa Zapadnog Nila su vrste *Culex pipiens* i *Aedes albopictus*. S obzirom na njihovu rasprostranjenost u Hrvatskoj i njihovu brojnost, može se reći da je rizično područje za prijenos virusa Zapadnog Nila cijela Hrvatska. U istočnoj Hrvatskoj, *Culex pipiens* je prisutan u fauni komaraca s 5-10% i predstavlja dominantnu vrstu, a u jadranskom priobalju i *Culex pipiens* i *Aedes albopictus* su danas eudominantne vrste (više od 10 %). Druge dvije vrste *Aedes vexans* i *Aedes cinereus* pojavljuju se u poplavnim prostorima sjeverne Hrvatske u većem broju, ali je njihov vektorski kapacitet znatno manji. Tijekom 2012. godine ekološki uvjeti za razvoj komaraca nisu bili najbolji tako da broj komaraca diljem Slavonije nije bio velik, osim u porječju Drave gdje je visok vodostaj u srpnju donio jednu generaciju poplavnih komaraca. Legla komaraca vrste *Culex pipiens* su raznolika: od manjih vodenih tijela u blizini čovjeka (burad, kante slivnici, septičke jame) do prirodnih, srednje velikih legala (kanali, depresije). Tijekom 2012., koja je bila sušna godina, nije bilo mnogo legala, pa je i brojnost jedinki ove vrste bila mala. U vrijeme pojave bolesti uzorkovani su komarci CDC (skraćenica od: Center for Disease Control) klopama na 46 postaja u Osječko-baranjskoj i Vukovarsko-srijemskoj županiji. Od ukupno 2254 uzorkovane jedinke 109 ih je bilo vrste *Culex pipiens* koji su adekvatno pohranjeni i poslani na analizu prisutnosti virusa. Odmah nakon informacije o pojavi bolesnika zaraženih virusom Zapadnog Nila organizirana je protuepidemijska dezinfekcija u svim mjestima gdje su boravili bolesnici. Dezinfekcija je obuhvatila hladno zamagljivanje na ukupnoj površini 1540 ha i suzbijanje ličinki u malim leglima u naseljima od vrata do vrata na oko 3000 objekata.

Mosquitoes - vectors of West Nile virus in Croatia

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Croatian fauna comprises 50 mosquitoes species, and 15 of them have medical importance. Only 4 species are capable to transmit West Nile virus (WNV). Mosquitoes play important role in spreading of arboviruses. Mosquitoes are the most important vectors of WNV but not the only way of spreading. To transmit virus, mosquito must allow replication in body. Virus enters in mosquito through blood meal, replicates in gut, ovarium, nerves, fat tissue and moves into hemolymph and finally infects salivary glands. With new feeding mosquito transmit virus in another host. Vector capacity is different in different mosquito species. In Croatia the most capable vectors are *Culex pipiens* i *Aedes albopictus*. According to their distribution and abundance all Croatia is risk area for WNV transmission. *Culex pipiens* is dominant species (share of 5-10%) in east Croatia, but both *Culex pipiens* and *Aedes albopictus* are now eudominant species in Adriatic area. Other two species *Aedes vexans* and *Aedes cinereus* appear in flooded areas in northeast Croatia in larger populations, but their vector capacity is lower. Ecological conditions for mosquito development during 2012 were not favourable and there were not too many mosquitoes in Slavonia, except area along the Drava River where we noted one flood pulse and one generation of flooded mosquitoes. *Culex pipiens* breeding sites are various small human made water bodies and natural small size water bodies. The year 2012 was dry and abundance of this species was small. During outbreak dry ice CDC traps were used in 46 localities for sampling mosquitoes in Osječko-baranjska and Vukovarsko-srijemska counties. In total 2254 mosquitoes were caught and 109 were determined as *Culex pipiens*. These mosquitoes were adequately stored and send for further virus analysis. Immediately epidemic control measures were done. ULV method is applied in places of disease appearance on 1540 ha and larvaeciding door to door in 3000 small water bodies around houses.

SEROLOŠKI DOKAZ PRISUTNOSTI INFEKCIJE VIRUSOM ZAPADNOG NILA KOD KONJA U SRBIJI

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West Nile virus (WNV) prvi put je bio izdvojen u Ugandi 1937. Danas je najrasprostranjeniji flavivirus širom svijeta. Ciklus prijenosa virusa podrazumijeva komarce i ptice, a ljudi i konji su slučajni krajnji domaćini. Infekcija kod ljudi i konja često je asimptomatska, a klinički znaci bolesti uključuju groznicu i meningitis i/ili encefalitis. Virus je bio odgovoran za povremene epidemije u Europi, Africi, Bliskom Istoku i nekim dijelovima Azije prije mnogo godina. Od 1990. porastao je broj slučajeva kod ljudi, kao i učestalost i težina infekcije. U posljednjih nekoliko godina prijavljen je sve veći broj teških epidemija kod konja u Francuskoj, Italiji, Rumunjskoj i Španjolskoj.

Pošto je rizik od širenja infekcije izazvane WNV prisutan i postao je prijetnja za zdravlje ljudi i životinja, proveli smo serološko ispitivanje na prisutnost specifičnih antitijela protiv WNV kod zdravih konja. Ukupno je bilo prikupljeno 349 uzoraka seruma konja tokom 2010. godine u Beogradu, općini Šabac i 26 općina u Vojvodini i svi uzorci su testirani na prisutnost anti-WNV IgG. Testiranje je izvršeno validiranom "in house" ELISA metodom zasnovanom na korištenju djelomično pročišćenog rekombinantnog glikoproteina E virusa iz ekstrakta inficiranih larvi insekata, dobivenog metodom ekspresije u bakulovirusu kao i neutralizacijskim testom plak redukcije (PRNT). Rezultati su pokazali da je 42/349 (12%) testiranih konjskih uzoraka seruma bilo pozitivno i da posjeduje anti-WNV IgG neutralizacijska antitijela.

Rezultati našeg istraživanja ukazuju da virus Zapadnog Nila cirkulira u populaciji konja u Srbiji. Nedavno, tokom ljeta 2012., zabilježeni su i prvi slučajevi infekcije ljudi WNV sa smrtnim ishodom u Srbiji. Iako do sada nije bilo prijavljenih slučajeva kliničke bolesti kod konja, epidemiološka situacija u susjednim zemljama u proteklih nekoliko godina nas upozorava da je neophodno da se provedu opsežna istraživanja u našoj zemlji, kao i mogućnost provođenja posebnog programa nadzora i kontrole bolesti.

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Serological evidence of West Nile Virus activity in horses in Serbia

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West Nile virus (WNV) was isolated in Uganda in 1937. Today, it is the most widely spread Flavivirus around the world. The transmission cycle of virus involves mosquitoes and birds, but humans and horses are accidental dead-end hosts. The infection in humans and horses is often asymptomatic, but the clinical signs of the disease include fever and meningitis and/or encephalitis. The virus has been responsible for occasional outbreaks in Europe, Africa, the Middle East and some parts of Asia for many years, but since 1990's the number, frequency, and severity of cases in humans has increased. In the past few years an increasing number of severe outbreaks in horses have been reported in France, Italy, Romania and Spain.

Since the risk of spreading of WNV infection is present and became threat for animal and human health, we have conducted a serological survey for the presence of anti-WNV antibodies in healthy horses. In total, 349 horse's sera were randomly collected during 2010 in Belgrade district area, the municipality of Sabac, and 26 municipalities in Vojvodina province and were tested for the presence of anti-WNV IgG by a validated *in house* ELISA, based on the use of a baculovirus expressed recombinant E viral glycoprotein partially purified from infected insect larvae extracts and by plaque reduction neutralization test (PRNT). The results showed that 42/349 (12%) tested horse sera were seropositive and presented anti-WNV IgG neutralizing antibodies.

The results of our serosurvey indicate that West Nile virus is circulating in the horse population in Serbia. Recently, during the summer 2012, the first cases of human WNV infection with fatal outcome in Serbia have been reported. Although so far there were no reported cases of clinical disease in horses, the epidemiological situation in the neighboring countries over the past few years, warns us that it is necessary to conduct extensive tests in our country, as well as the possibility of implementing a special program of surveillance and disease control.

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PROŠIRENOST INFEKCIJE VIRUSOM ZAPADNOG NILA U KONJA U HRVATSKOJ

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Virus Zapadnog Nila uzročnik je bolesti u ljudi i različitih vrsta životinja. Uz primarnog domaćina (ptice) virus sporadično uzrokuje bolest s mogućim smrtnim ishodom u ljudi i konja. U konja se bolest najčešće javlja supklinički, ali se u određenog broja životinja javljaju i različiti klinički oblici. Klinički oblik bolesti u konja očituje se sa simptomima sličnim influenci, a životinje se obično oporave za 7-10 dana. Međutim, u oko 30% oboljelih konja zaraza se očituje encefalomijelitisom, koji u vrlo visokom postotku završava uginućem ili medicinski opravdanom eutanazijom. Konji koji prebole neurološki oblik bolesti uglavnom se u potpunosti oporave za 1-6 mjeseci, dok u 10% životinja ostaju trajne posljedice.

Podaci dobiveni istraživanjem infekcije virusom ZN u konja mogu biti od važnosti za javno zdravstvo. Naime, program kontrole virusa Zapadnog Nila na određenom području provodi se pretraživanjem uginulih ptica, kontrolom vektora, pretraživanjem klinički oboljelih konja i pretraživanjem "sentinel" životinja, a u tu svrhu se najčešće koriste konji i/ili domaća perad. U dosadašnjim istraživanjima dokazano je da se na područjima s visokim postotkom serološki pozitivnih konja (IgG), može očekivati pojava kliničkih oblika u konja i u ljudi. Stoga praćenje aktivnosti virusa Zapadnog Nila u konja kao "sentinel" odnosno „prokazivačkih“ životinja omogućava predviđanje rizičnih područja za pojavu kliničkih oboljenja u ljudi. Prateći pojavu zaraze u ljudi i aktivnost virusa, spoznalo se da njegova aktivnost u ptica i komaraca i akutne infekcije u konja prethode kliničkim slučajevima u ljudi. Iz navedenoga se razabire da program kontrole virusa Zapadnog Nila u životinja daje vrijedne podatke za javno zdravstvo.

Iako je u Hrvatskoj prva prijava slučajeva akutno inficiranih konja i oboljenja ljudi zabilježena ljeti 2012., istraživanja prisutnosti i proširenosti virusa provedena su mnogo ranije. Infekcija virusom Zapadnog Nila u konja u Hrvatskoj prvi put je dokazana pretragom uzoraka seruma iz 2001. i 2002. godine u Istočnoj Slavoniji. Opsežnije istraživanje provedeno je tijekom 2010. godine kada je aktivnost virusa Zapadnog Nila dokazana u 8 županija Hrvatske s najvišom seroprevalencijom u Istočnoj Hrvatskoj, području koje graniči s Mađarskom, Srbijom i Bosnom i Hercegovinom, te u Istri graničnom području prema Italiji. Seroprevalencija po županijama iznosila je od 1,4% do 9,1%, a na razini države 3,5%. Na osnovi navedenih rezultata, 2011. godine

uveden je program kontrole proširenosti virusa Zapadnog Nila na području RH. Obuhvaćeno je osam županija, a aktivnost virusa dokazana je u sedam. Ponovo je najviša seroprevalencija dokazana na području Istočne Slavonije, a ukupna seroprevalencija u pretraživanim županijama iznosila je 3,7%. Program kontrole proširenosti virusa Zapadnog Nila u konja 2012. godine nastavljen je dokazivanjem specifičnih IgM protutijela. Akutne infekcije konja dokazane su u najistočnijem dijelu države u uzorcima uzorkovanim od 20. 7. 2012. do 1. 8. 2012. Oko mjesec dana nakon toga, dokazani su i slučajevi oboljenja ljudi na istom području. Naknadnim pretraživanjem uzoraka na prisutnost IgG protutijela dokazano je da je seroprevalencija u konja u županijama s kliničkim očitovanjem u ljudi bila viša od 7,5% dok je u županijama bez zabilježenih slučajeva bolesti u ljudi bila manja od 3%. Istraživanja provedena tijekom 2012. godine pokazuju da su rezultati kontrole aktivnosti virusa Zapadnog Nila u konja bili indikativni za mjesto i vrijeme pojave kliničkih oboljenja ljudi.

Zaključno, višegodišnji dokaz aktivnosti virusa Zapadnog Nila u konja u najmanje 9 županija RH ukazuje na moguće enzootsko pojavljivanje bolesti. Nadalje, RH je vjerojatno dio značajno većeg enzootskog područja i bolest se najvjerojatnije širi iz dvaju pravaca, s jedne strane iz Srbije i Mađarske te s druge strane iz Italije. Stoga je pojava budućih epidemija bolesti u sljedećim sezonama vrlo izvjesna. Prikazani rezultati istraživanja u konja i klinički slučajevi u ljudi tijekom 2012. godine govore o značenju konja kao "sentinel" životinja. Rezultati istraživanja omogućuju predviđanje mjesta i vremena pojave kliničkih slučajeva u ljudi. Navedeno obvezuje na daljnje provođenje programa kontrole virusa Zapadnog Nila u još većem opsegu.

West Nile virus serosurveillance in horses in Croatia

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West Nile virus (WNV) is well known cause of infection in the different animal species and also humans. Birds are primary hosts but clinical illness with fatal end can also be seen in humans and horses. Most of the infections in the horses are subclinical, but in some portion of infected animals different clinical symptoms can occur. Most common manifestation of WNV infection in horses is influenza like syndrome that is self limiting and full recovery is expected in 7 to 10 days. On the other hand in 30% of clinically diseased horses symptoms of encephalomyelitis predominate and in many cases animals die or they have

to be euthanized. Horses that survive neurological form of the disease can fully recover but in 10% of animals permanent disorders persist.

Data obtained in horse population are of outmost importance for public health. Surveillance program in some area is based on examination of the dead or dying birds, the vector control, examination of diseased horses and using of the sentinel animals, usually poultry or horses. Some studies showed that the areas with high prevalence of the IgG positive horses are the areas in which clinical manifestation of WNV infection in the humans and in the horses is expected. Even more, usually evidence of WNV circulation, in terms of increased viral activity in birds, WNV positive mosquito pools and acute infections in horses precede clinical cases in humans. All these data make possible predicting the occurrence of illness in human population. Accordingly, data of surveillance program of WNV circulation in animals gives invaluable data for public health.

Despite that the first cases of acute infected horses and clinical cases in humans were reported in the summer 2012, monitoring of the presence and prevalence of the WNV has been done for some years before. First serological evidences of the WNV infection in horses date from 2001 and 2002 and it was made in sera samples of the horses in Eastern Slavonia. More extensive study was made in the year 2010 when the WNV activity was confirmed in the 8 Croatian counties with the highest seroprevalence in eastern Croatia in the area that lies next to the state borders with Hungary, Serbia and Bosnia and Herzegovina as well as in Istria, county next to Italian border. The seroprevalences, on the county level, were from 1.4% to 9.1% and on the state level it was 3.5%. In the 2011, based on the results from previous study, official surveillance system on a state level has been established. In 2011 in 8 of Croatian counties surveillance was made and in the 7 of them the WNV activity was confirmed. Again, like in previous year, the highest seroprevalence was in Eastern Slavonia with average value of 3.7% in all counties included. Surveillance program has been continued in 2012 with focus on testing horse sera samples for the WNV specific IgM antibodies as a proof of acute infections. Confirmation of acute WNV infections in horses was made in the easternmost part of country, in sera sampled between 20th of July and 1st of August. In a month, from first confirmation of acute infection in horses, first clinical cases in humans were confirmed in same area as well. Testing of the sera samples for the presence of IgG specific antibodies that had been done afterwards, seroprevalences in counties with human cases were uniformly higher than 7.5% and in the rest of counties had been less than 3%. Results of surveillance program in 2012 showed that it had been possible to make the good prediction of the time and the place of occurrence of human cases.

In the conclusion, evidence of the WNV activity for many years in at least 9

counties, indicates possibility of WNV endemisation in Croatia. There is high possibility that Republic of Croatia is the part of the larger endemic area and the spreading of WNV is probably coming from two directions, Serbia and Hungary on the one side and Italy on the other. Possible endemisation make occurrence of new epidemics in future years almost inevitable. All the showed results of the studies in horses and clinical cases in humans in 2012 highlight the importance of the horses as sentinel animals that could give us possibility to predict place and the timing of occurrence of human cases. All together should result in the extending of the surveillance system in the coming years because of the importance for veterinary medicine and even more because the obtaining invaluable information for public health.

PRVI SLUČAJEVI NEUROINVAZIVNE BOLESTI UZROKOVANE WEST NILE VIRUSOM KOD LJUDI U HRVATSKOJ

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U radu prikazujemo prve slučajeve neuroinvazivne bolesti uzrokovane West Nile virusom (WNV) kod ljudi u Hrvatskoj.

WNV je prvi puta opisan 1937. god. na području Zapadnog Nila u Ugandi. Tijekom dva posljednja desetljeća, u svijetu je zabilježeno više epidemija WNV. Uz SAD, velike su epidemije opisane u Europi i na području Mediterana. U zadnjih nekoliko godina WNV neuroinvazivne infekcije su bilježene u Italiji (2008-2010. god.), Rumunjskoj (2010-2012. god.) i Grčkoj (2010-2012. god.). U srpnju 2012. god. prvi put je registrirana epidemija WNV infekcije u Srbiji s 44 laboratorijski potvrđena slučaja. Životna dob oboljelih je 17-82 god. Do sada je od WNV infekcije u Srbiji umrlo šestoro ljudi, a u Makedoniji i Kosovu po jedna osoba.

Od 1970. do 1988. god. u Hrvatskoj je provedeno nekoliko seroepidemioloških studija o prisutnosti WNV koje su pokazale prevalenciju od 0,28% do 4,9%. U pilot istraživanju provedenom 2011. god. u skupini od 360 slučajno odabranih zdravih ispitanika u dobi od 30-60 god., WNV IgG protutijela su dokazana u samo jednog ispitanika s područja istočne Hrvatske (0,3%).

U rujnu 2012. god. zabilježeni su prvi slučajeви neuroinvazivne WNV bolesti u ljudi u Hrvatskoj koja je do sada dokazana u šest bolesnika. U prvog su se bolesnika simptomi pojavili 04. kolovoza, a u posljednjeg 07. rujna. Četiri su bolesnika imala kliničku sliku meningoencefalitisa te dva bolesnika sliku encefalitisa. Medijan dobi iznosio je 62 god. (raspon 48-77 god.). U prvom se slučaju radilo o importiranoj WNV infekciji iz Srbije (oboljeli je boravio u selu Ratkovo u Srbiji od 24.-31. srpnja, tijekom razdoblja inkubacije), dok su ostali slučajeви autohtono stečeni. Petero bolesnika se potpuno oporavilo.

Serološko testiranje je učinjeno u Nacionalnom referentnom laboratoriju za arbovire i riketije pri Hrvatskom zavodu za javno zdravstvo. WNV IgM i IgG protutijela dokazana su pomoću komercijalnog ELISA testa (Euroimmun, Lübeck, Germany). Serumi su također testirani na druge flavivire (virus krpeljnog encefalitisa i virus dengue) zbog mogućih križnih reakcija. Četiri su uzorka potvrđena u OIE Referentnom laboratoriju za WNV, Istituto G. Caporale, Teramo, Italija neutralizacijskim testom redukcije plakova (PRNT) i mikroneutralizacijskim testom (NT). Real-time RT-PCR (WNV linija 1 i 2) je bio

negativan u svim testiranim uzorcima. Rezultati seroloških pretraga prikazani su u tablici.

Bolesnici s dokazanom neuroinvazivnom WNV bolesti su s područja tri županije istočne Hrvatske koje se nalaze uz granicu sa Srbijom (Brodsko-posavska, Osječko-baranjska i Vukovarsko-srijemska županija).

U svrhu suzbijanja komaraca, odmah su provedeni adulticidni i larvicidni tretmani kao učinkovite protuepidemijske mjere na područjima gdje su otkrivene oboljele osobe.

Tablica. Serološki rezultati šest bolesnika s neuroinvazivnom WNV infekcijom u Hrvatskoj, 2012.

Slučaj	Dob	Mjesto boravka	Klinička dijagnoza	WNV ELISA IgM / IgG	PRNT*	NT*
1	65	Nova Gradiška	Meningoencefalitis	Poz / Poz	20	20
2	77	Vukovar	Meningoencefalitis	Poz / Poz	10	10
3	48	Budimci	Encefalitis	Poz / Poz	10	20
4	76	Belišće	Meningoencefalitis	Poz / Neg	5	10
5	60	Slavonski Brod	Meningoencefalitis	Poz / Poz	-	-
6	48	Osijek	Encefalitis	Poz / Poz	-	-

*Titar protutijela

First outbreak of West Nile virus neuroinvasive infection in humans in Croatia

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We are reporting the first outbreak of West Nile virus (WNV) neuroinvasive infection in humans in Croatia.

WNV was originally identified in 1937 in West Nile District of Uganda. During the last two decades, several WNV outbreaks have occurred throughout the world. In addition to USA, large outbreaks of WNV have been reported in Europe and in the Mediterranean basin. In the past few years, WNV neuroinvasive disease was notified in Italy (2008-2012), Romania (2010-2012) and Greece (2010-2012). In July 2012 first outbreak of WNV disease has occurred in Serbia with 44 laboratory diagnosed cases aged 17-82 years and six deaths up to now. In Macedonia as well as in Kosovo one death of West Nile virus was reported.

In Croatia, several seroepidemiological studies on WNV in humans have been conducted during 1970-1998 which have shown the prevalences ranging from 0.28% to 4.9%. In 2011, a pilot seroprevalence study has been performed in a group of 306 randomly selected healthy adult people aged 30-60 years. Only one participant (0.3%) from eastern Croatia showed positive WNV IgG antibodies.

In September 2012, first outbreak of WNV neuroinvasive infection in humans in Croatia was reported. Six patients with WNV disease were detected up to now. The first WNV case had symptoms onset on 04 August and the last on 07 September. Four patients presented with clinical symptoms of meningoencephalitis and two patients with encephalitis. The median patient age was 62 years (range 48-77 years). The first case was imported from Serbia (patient resided in Ratkovo village in Serbia from 24-31 July, during the incubation period) while the others represent first autochthonous WNV cases. All but one patient were recovered.

WNV IgM and IgG testing was performed at the National Reference Laboratory for Arboviruses at Croatian National Institute of Public Health using commercial ELISA (Euroimmun, Lübeck, Germany). Sera were also tested for the presence of other flaviviruses (tick-borne encephalitis virus and dengue virus) to rule out cross-reactions. Four samples were confirmed at the OIE Reference Laboratory for West Nile Disease, Istituto G. Caporale, Teramo, Italy by plaque-reduction neutralization assay (PRNT) and micro-neutralization assay (NT). Real-time RT-PCR (lineage 1 and 2) was negative in all tested samples. Laboratory test results of six patients with WNV neuroinvasive infection are presented in the Table.

Patients with WNV disease are residents of three counties (Brod-Posavina County, Osijek-Baranja County and Vukovar-Srijem County) located in eastern Croatia, near the Serbian state border. Adulticide and larvicidal treatment as effective epidemic control measures were implemented immediately in patient's places of residence in Osijek-Baranja County and Vukovar-Srijem County.

Table. Serological results of six patients with neuroinvasive WNV infection in Croatia, 2012

Case	Age	Place of clinical residence	Clinical Diagnosis	WNV ELISA IgM / IgG	PRNT*	NT*
1	65	Nova Gradiška	Meningoencefalitis	Poz / Poz	20	20
2	77	Vukovar	Meningoencefalitis	Poz / Poz	10	10
3	48	Budimci	Encefalitis	Poz / Poz	10	20
4	76	Belišće	Meningoencefalitis	Poz / Neg	5	10
5	60	Slavonski Brod	Meningoencefalitis	Poz / Poz	-	-
6	48	Osijek	Encefalitis	Poz / Poz	-	-

*Antibody titre

KLINIČKA OBILJEŽJA INFEKCIJE VIRUSOM ZAPADNOG NILA KOD LJUDI U HRVATSKOJ

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Tijekom kolovoza i rujna 2012. godine, u Klinici za infektologiju KBC Osijek, liječeno je 5 bolesnika s meningoencefalitisom, u kojih je serološki (ELISA IgG i IgM pozitivni) dokazan virus Zapadnog Nila.

To su prvi dokazani slučajevi meningoencefalitisa uzrokovano virusom Zapadnog Nila u istočnoj Slavoniji (Budimci, Vukovar, Belišće, Kuševac, Petrijevci). Bolesnici su u dobi od 48 do 77 godina. Tri su bolesnika prije godinu dana bila liječena na neurologiji: dva zbog cerebrovaskularnog infarkta, a treći zbog vertiginoznog sindroma. Hospitalizirani su od 4. do 6. dana prisutnih općih infektivnih simptoma, a prvog dana prisutnog poremećaja svijesti. Smetenost, dezorijentiranost i halucinacije imala su dva bolesnika. Somnolenciju i sopor također dva bolesnika, a u dva bolesnika bio je prisutan i tremor.

Povišenu temperaturu svi su bolesnici imali prva dva dana liječenja, od 37,2 °C do 38,3 °C. U svih je bolesnika nalaz likvora govorio u prilog seroznog meningitisa. Broj leukocita (pretežno limfocita) varirao je od 10 do 517 x10⁶/L. Prisutna je bila umjerena proteinorahija i granično povišene vrijednosti laktata. Glukoza i kloridi su bili u granicama normale.

U svih bolesnika je EEG bio disritmički promijenjen, difuzno ili postranično. CT mozga je bio uredan u dva bolesnika, u dva bolesnika prisutna je bila difuzna atrofija mozga, a u jednog kronična vaskularna lezija. Nalaz MR mozga u dva bolesnika je bio uredan.

Uz antiedematoznu terapiju (infuzije 10% Manitola i 10% Dekstroze) i drugu simptomatsku terapiju, unutar 5 dana liječenja uslijedilo je poboljšanje. Bolničko liječenje je trajalo 2-3 tjedna. Svi su otpušteni bez neuroloških posljedica.

Clinical aspects of West Nile virus infections in humans in Croatia

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During August and September of the year 2012th there were 5 patients with meningoencephalitis treated at Clinic for Infective Diseases, University Hospital Centre Osijek. In all of them West Nile virus was proved to be cause, serologically (ELISA IgG and IgM positive).

These are the first confirmed cases of West Nile encephalitis in Eastern Slavonia (Budimci, Vukovar, Belišće, Kuševac, Petrijevci). Patients ranged in age from 48 to 77 years. One year ago, three of these patients were treated in Neurology; two of them due to the stroke, and third because the vertigo. Patients were admitted to the hospital on the 4th to 6th day from the beginning of general infectious symptoms, and the first day of consciousness disorders. Two patients had confusion, disorientation and hallucinations, two patients had somnolence and sopor, and two patients had tremor.

During first two days of treatment, all patients had raised body temperature in range from 37.2 °C to 38.3 °C. Cerebrospinal fluid analysis in all patients spoke in favor of serous meningitis. The number of leukocytes (mainly lymphocytes) ranged from 10 to 517 x10⁶/L. There was a moderate protein elevation and marginal lactate elevation. Glucose and chlorides were within normal range.

All patients had EEG changes dysrhythmic, diffuse or laterally. CT scan of the brain was normal in two patients, two patients had diffuse brain atrophy, and one had chronic vascular lesions. MRI scan of the brain in two patients was normal.

Patients were treated with anti-edematous therapy with infusion 10% Mannitol and 10% Dextrose and other symptomatic therapy. Clinical signs improved within 5 days of treatment. Hospitalization lasted 2-3 weeks. All patients were discharged without neurological sequelae.

