UDK 616-022.1(497.5-11)
Original scientific paper
Received: 6 June 2013
Accepted: 18 September 2013

HUMAN WEST NILE VIRUS INFECTION IN EASTERN CROATIA, AUGUST-SEPTEMBER, 2012

Tatjana Vilibić-Čavlek[1], Iva Pem-Novosel[2], Ira Gjenero-Margan[2], Nenad Pandak[3], Ljiljana Perić[4], Ljubo Barbić[5], Karlo Kožul[6], Eddy Listeš[7], Pavle Jeličić[8], Vladimir Stevanović[5], Giovanni Savini[9]

[1]Department of Virology, Croatian National Institute of Public Health; School of Medicine University of Zagreb;
[2]Epidemiology Service, Croatian National Institute of Public Health, Zagreb;
[3]Department of Infectious Diseases, General Hospital "Dr Josip Benčević", Slavonski Brod;
[4]Department of Infectious Diseases, Clinical Hospital Centre Osijek;
[5]Department of Microbiology and Infectious Diseases with Clinic, Faculty of Veterinary Medicine University of Zagreb;
[6]Osijek-Baranja County Institute of Public Health, Osijek;
[7]Croatian Veterinary Institute, Regional Veterinary Institute Split;
[8]Ecology Service, Croatian National Institute of Public Health, Zagreb;
[9]National Reference Center for West Nile Disease, Istituto G. Caporale Teramo, Teramo, Italy

Summary

In the period from 6 to 21 September, Croatia reported the first clinical cases of human West Nile virus (WNV) neuroinvasive infection in three counties (Brod-Posavina, Osijek-Baranja and Vukovar-Srijem County). The first case was most probably imported from Serbia where the patient resided during the incubation period, while the others represent autochthonous cases. Five patients presented with meningoencephalitis and two with meningoencephalitis followed by acute flaccid paralysis. In all samples WNV IgG and/or IgM antibodies were detected using enzyme-linked immunosorbent assay (ELISA) and confirmed by plaque-reduction neutralization (PRNT) and micro-neutralization test (MNT). Following the outbreak, a total of 178 serum samples were collected from healthy inhabitants of Osijek-Baranja County where the incidence of cases were highest. WNV IgG antibodies were detected by ELISA in 4 (2.2%) participants indicating the increased WNV circulation in this area in comparison with season 2011 resulting in the first reported clinical cases.

Keywords: West Nile virus; outbreak; seroprevalence; Croatia.

INTRODUCTION

West Nile virus (WNV) is a small, enveloped virus that belongs to the genus *Flavivirus* of the family *Flaviviridae* [1]. The virus is maintained in an enzootic cycle between *Culex* mosquitoes and birds, whereas humans and horses are considered dead-end hosts due to low-level and transient viremia [2]. However, humans may occasionally transmit or acquire virus in utero, through breast milk, via blood transfusion or organ transplantation, or through occupational exposure [3]. Most human infections (~80%) are asymptomatic. Approximately 20% of infected persons develop a non-specific febrile illness ("WNV fever"). A small number of symptomatic cases (<1%) progresses to the neuroinvasive form of infection characterized by meningitis, encephalitis and acute flaccid paralysis [4]. The epidemiology of WNV is continuously changing and currently the virus has a wide geographic distribution. After the first isolation of WNV in Uganda in 1937, few outbreaks in humans or horses were recorded until the beginning of 1990s [5]. A series of outbreaks in the 1990s (Algeria 1994, Morocco 1996, Romania 1996, Tunisia 1997, Italy 1998, Israel 1998, France 2000, and Russia 1999) were associated with severe complications, including neuroinvasive disease [6-11]. In 1999, WNV was detected for the first time in the Western Hemisphere during the outbreak in New York [12]. In the past few years, in European countries, WNV neuroinvasive disease was notified every year: Italy (2008-2012), Hungary (2008-2012), Romania (2010-2012) and Greece (2010-2012). In July 2012, the first outbreak of WNV disease has occurred in Serbia with 44 laboratory diagnosed cases and six deaths [13].

We are presenting the first human cases of WNV infection in Croatia, August-September, 2012 and the results of seroepidemiological study performed after the transmission season 2012 in Osijek-Baranja County, a region with the highest incidence of WNV neuroinvasive infection during 2012.

MATERIALS AND METHODS

During September-November 2012, a total of 54 serum samples and 15 cerebrospinal fluid (CSF) samples from patients with clinical symptoms of WNV infection (neuroinvasive disease and WNV fever) were tested at the National Reference Laboratory for Arboviruses, Croatian National Institute of Public Health (CNIPH) for the presence of WNV IgM and IgG antibodies using commercial ELISA (Euroimmun, Lübeck, Germany). Samples were obtained from hospitalized and non-hospitalized patients with clinical diagnosis of meningoencephalitis (27/50.0%), encephalitis (12/22.2%), non-specific febrile disease (13/24.1%) and febrile disease with rash (2/3.7%). IgM/IgG reactive samples were further tested for IgG avidity (Euroimmun, Lübeck, Germany) to confirm acute/recent WNV infection. The interpretation of

AI results was determined as follows: AI <40% = low avidity antibodies indicating acute/recent WNV infection; AI 40-60% = moderate avidity; AI >60% = high avidity antibodies indicating past WNV infection. Any IgM or IgG positive samples were evaluated at the OIE Reference Laboratory for West Nile Disease, Istituto G. Caporale, Teramo, Italy by plaque-reduction neutralization (PRNT) and micro-neutralization test (MNT) for result confirmation. Nine (5 CSF and 4 serum) samples were tested for WNV RNA using real-time pan-flavivirus RT-PCR and qRT-PCR assays for detection of WNV lineage 1 and 2 [14,15].

Following the outbreak of WNV infection, from September 2012 to February 2013, a total of 178 serum samples were collected from healthy inhabitants of Osijek-Baranja County aged 7-86 years where the incidence of neuroinvasive cases was highest to evaluate the seroprevalence of WNV.

RESULTS

Human cases of WNV neuroinvasive infection

Between 6 and 21 September 2012, 7 human cases of WNV neuroinvasive infection have been diagnosed in three eastern Croatian counties (Brod-Posavina, Osijek-Baranja and Vukovar-Srijem County), four of which were reported in Osijek-Baranja County (*Figure 1*). Patient's demographic and clinical characteristics are presented in the *Table 1*. The first case resided in northern Serbian province of Vojvodina during the incubation period, while the other represents autochthonous WNV cases.

Laboratory results

WNV IgM antibodies were detected in all patients. In six patients, IgG antibodies of low avidity were found indicating acute WNV infection. Using PRNT and MNT, WNV neutralizing antibodies were confirmed in all samples (*Table 1*). For two patients, paired serum samples were available, which showed a four-fold increase of WNV-specific antibody titre. None of the tested CSF samples were WNV antibody positive. Real-time RT-PCR was negative in all tested samples.

WNV IgG seroprevalence results

Of 178 tested subjects in Osijek-Baranja County, four (2.2%) were reactive for WNV IgG antibodies by ELISA (28-200 RU/ml) while one participant (0.6%) showed equivocal result (20 RU/ml). Two seropositive subjects were residents of urban settings (Osijek region) and two lived in rural area (Semeljci, Strizivojna) (*Figure 1*).

Table 1. Demographic, clinical and laboratory data of 7 patients with West Nile virus neuroinvasive disease in Croatia, August-September, 2012.

Characteristic	Results
Age	
Median	62 years
Range	48 - 77 years
Sex	
Male	3 (57.1%)
Female	4 (42.9%)
Area of residence	
Rural	5 (71.4%)
Urban	2 (28.6%)
Clinical symptoms	
Meningoencephalitis	5 (71.4%)
Meningoencephalitis, AFPa	2 (28.6%)
History of hypertension	4 (57.1%)
Outcome	
Recovered	5 (71.4%)
Persistent neurologic sequelae	2 (28.6%)
Laboratory results	
WNV ELISA - IgM (Ratio)b	1.7 - 4.1
WNV ELISA - IgG (RU/ml)c	40 - 170
WNV IgG avidity	11-38%
WNV PRNTd (Titre)	5 - 40
WNV MNTe (Titre)	5 - 80

aAFP = acute flaccid paralysis; bRatio <0.8 neg, 0.8-1.1 equivocal, >1.1 pos; c<16 neg, 16-22 equivocal, >22 pos; dPRNT = plaque-reduction neutralization test; eMNT = micro-neutralization test



Figure 1. Map of Croatia representing human WNV neuroinvasive infection notified in 2012 and IgG positive participants.

DISCUSSION

In Croatia, clinical cases of human WNV infection were not reported so far although cases occurred in many European countries (Italy and Hungary 2008-2012, Spain 2009, Romania and Greece, 2010-2012) [13]. However, serologic evidence for the presence of WNV in Croatia dates back to 1970s. These studies were usually based on the detection of hemagglutination inhibiting (HI) antibodies that are group-specific. In two studies conducted among residents of the Island Brač in 1970 and 1974, the prevalence of HI antibodies was 4.9% and 0.28%, respectively [16]. In another survey (1980), positive HI results were found in 1.7% Croatian inhabitants: 1.2% from North-East Croatia, 3.4% from Middle Dalmatia and 0.8% from Southern Dalmatia [17]. In a study conducted in 2007 among voluntary blood donors in north-east Croatia, antibodies against WNV were detected in 0.3% participants by neu-

tralization [18]. A pilot study performed in a group of 306 randomly selected adult people aged 30-60 years in 2011 showed similar results. Only one participant (0.3%) from eastern Croatia (Osijek-Baranja County) showed positive neutralizing WNV antibodies (data from the CNIPH). In addition to humans, antibodies to WNV were detected in brown bears in 1993 [19] and horses in 2001-2002 [20]. WNV infection in horses was documented in a large study during 2011-2012, the results of which indicate a possible endemic appearance of WNV in Croatia (WNV activity was documented in at least 9 counties) [21].

The first clinical cases of WNV neuroinvasive disease in Croatia were laboratory confirmed in September 2012. A serological survey in sentinel horses demonstrated asymptomatic acute WNV infection (detection of IgM antibodies) in 12 animals in the same counties where human cases were reported (data from the Faculty of Veterinary Medicine University of Zagreb). The exception is Osijek-Baranja County where human clinical cases, but not infections in horses were reported. These results could be explained by searching the horses for WNV before the increase in vector activity. In addition, acutely infected horses were documented in Virovitica-Podravina County in which there were no human cases reported. Since most infected people do not show any symptoms or have non-specific febrile disease, it is possible that many WNV infections remained unrecognized.

In areas where cases of WNV infection occurred and in neighboring municipalities, mosquito control measures (adulticidal and larvicidal treatment) were immediately implemented.

Results of seroepidemiological study performed after the transmission season 2012 in Osijek-Baranja County showed that the seroprevalence rate in healthy inhabitants increased compared to 2011. The increase in WNV seropositivity in these two consecutive years indicates increased viral activity during season 2012. These results are in accordance with the first reported clinical cases.

References

[1] *Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB*. Phylogeny of the genus Flavivirus. J Virol. 1998;72:73-83.

[2] *Rossi SL*. West Nile virus. Clin Lab Med. 2010;30:47-65.

[3] *Gould LH, Fikrig L*. West Nile virus: a growing concern? J Clin Invest. 2004;113:1002-7.

[4] *Sejvar JJ, Haddad MB, Tierney BC, et al*. Neurologic manifestations and outcome of West Nile virus infection. J Am Med Assoc. 2003;290:511-5.

[5] *Calistri P, Giovannini A, Hubalek Z, et al*. Epidemiology of West Nile in Europe and in the Mediterranean Basin. Open Virol J. 2010;4:29-37.

[6] *Murgue B, Murri S, Zientara S, Durand B, Durand JP, Zeller H.* West Nile outbreak in horses in southern France, 2000: the return after 35 years. Emerg Infect Dis. 2001;7:692-6.

[7] *Tber AA.* West Nile fever in horses in Morocco. Bull OIE. 1996;108:867-9.

[8] *Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI.* West Nile encephalitis epidemic in southeastern Romania. Lancet. 1998;352:767-71.

[9] *Triki H, Murri S, Le Guenno B, et al.* West Nile viral meningoencephalitis in Tunisia. Med Trop (Mars). 2001;61:487-90.

[10] *Cantile C, Di Guardo G, Eleni C, Arispici M.* Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. Equine Vet J. 2000;32:31-5.

[11] *Platonov AE, Shipulin GA, Shipulina OY, et al.* Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. Emerg Infect Dis. 2001;7:128-32.

[12] *Lanciotti RS, Roehrig JT, Deubel V, et al.* Origin of the West Nile virus responsible for an outbreak of encephalitis in northeastern United States. Science. 1999;286:2333-7.

[13] www. http://ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever maps/Pages/index.aspx

[14] *Johnson N, Wakeley PR, Mansfield KL, et al*. Assessment of a novel real-time pan-flavivirus RT-polymerase chain reaction. Vector Borne Zoonotic Dis. 2010;10:665-71.

[15] *Eiden M, Vina-Rodriguez A, Hoffmann B, Ziegler U, Groschup MH.* Two new real-time quantitative reverse transcription polymerase chain reaction assays with unique target sites for the specific and sensitive detection of lineages 1 and 2 West Nile virus strains. J Vet Diagn Invest. 2010;22:748-53.

[16] *Vesenjak-Hirjan J, Galinovic-Weisglass, M Brudnjak Z*, et al. Island of Brač - Focus of Arbovirus Infections. In: Vesenjak-Hirjan J (ed). Arboviruses in the Mediterranean Countries. ZbL. Bakt. Suppl 9, Gustav Fischer Verlag: Stuttgart-New York, 1980; pp 311-7.

[17] *Vesenjak-Hirjan J*. Arboviruses in Yugoslavia. In: Vesenjak-Hirjan J (ed). Arboviruses in the Mediterranean Countries. ZbL. Bakt. Suppl 9, Gustav Fischer Verlag: Stuttgart-New York, 1980; pp 165-77.

[18] *Golubić D, Dobler G*. Flavivirusi u sjeverozapadnoj Hrvatskoj. Infektol Glasn. 2012;32:153-7.

[19] *Madić J, Huber D, Lugović B.* Serological survey for selected viral and rickettsial agents of brown bears (*Ursus arctos*) in Croatia. J Wildl Dis. 1993;29:572-6.

[20] *Madić J, Savini G, Di Gennaro A, et al*. Serological evidence for West Nile Virus infection in horses in Croatia. Vet Rec. 2007;160:772-3.

[21] *Barbić Lj, Listeš E, Katić S, et al.* Spreading of West Nile virus infection in Croatia. Vet Microbiol. 2012;159:504-8.

Sažetak

Infekcija ljudi virusom Zapadnog Nila u istočnoj Hrvatskoj, kolovoz – rujan 2012.

U razdoblju od 6. do 21. rujna 2012. zabilježeni su prvi humani klinički slučajevi neuroinvazivne infekcije virusom Zapadnog Nila (VZN) u tri hrvatske županije (Brodsko-posavska, Osječko-baranjska i Vukovarsko-srijemska). U prvom se slučaju vjerojatno radilo o infekciji podrijetlom iz Srbije, gdje je bolesnik boravio tijekom inkubacijskog perioda, a u ostalih bolesnika o autohtonoj infekciji VZN-om. Pet je bolesnika imalo kliničku sliku meningoencefalitisa, dok je u dva bolesnika nakon početnih simptoma meningoencefalitisa nastupila akutna mlohava kljenut. U svih su dokazana VZN IgG i/ili IgM protutijela pomoću imunoenzimnog testa (ELISA) te su potvrđena neutralizacijskim testom redukcije plakova (PRNT) i mikroneutralizacijskim testom (MNT). Po završetku sezone sakupljeno je ukupno 178 uzoraka seruma zdravih osoba s područja Osječko-baranjske županije, gdje je tijekom epidemije zabilježen najveći broj slučajeva. Pomoću ELISA testa VZN IgG protutijela dokazana su u 4 (2,2%) ispitanika. Ti rezultati ukazuju na povećanu cirkulaciju VZN-a na tom području u usporedbi sa sezonom 2011. godine, što je rezultiralo prvim humanim kliničkim slučajevima.

Ključne riječi: virus Zapadnog Nila; pojava; seroprevalencija; Hrvatska**.**

Corresponding author:
Tatjana Vilibić-Čavlek
E-mail: tatjana.vilibic-cavlek@hzjz.hr