

**Epidemics of the central nervous system infections caused by West Nile virus in the  
territory of the South Bačka District, Vojvodina, Serbia**

Epidemija West Nile virus infekcija centralnog nervnog sistema na teritoriji Južnobačkog  
okruža, Vojvodina, Srbija

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Abbreviated title: WEST NILE VIRUS INFECTIONS

## **Apstrakt**

**Uvod/Cilj:** West Nile Virus je neurotropna RNA virusna partikula, pripada porodici *Flaviviridae*, rod *Flavivirus*. Održava se u artropodama u okviru transmisivnog ciklusa između komaraca i ptica. Infekcije WNV kod čoveka su u 80% slučajeva asimptomatske. Manje od 1% obolelih osoba razvije neuroinvazivni oblik bolesti - meningitis, encefalitis ili akutnu flakcidnu paralizu. Utvrditi najčešće subjektivne tegobe, laboratorijske i kliničke manifestacije kod pacijenata obolelih od West Nile Virus infekcije. Istaći prisustvo komorbiditeta, trajanje i ishod lečenja, sekvele.

**Materijal i metode:** Retrospektivna studija sprovedena je u periodu od 01.01.2012. do 31.12.2013. godine. Praćena su 32 bolesnika, kojima je na osnovu kliničkog nalaza, laboratorijskog i serološkog testiranja dijagnostikovana WNV infekcija. Podaci su obrađeni standardnim statističkim metodama. U svrhu ocene statističke značajnosti korišćeni su  $\chi^2$  i t-test.

**Rezultati:** Najviše obolelih osoba je muškog pola, uzrasta od 31 do 65 godina. Na prijemu bilo je 50% febrilnih, 84.4% sa pozitivnim meningealnim znacima, 53,2% sa neurološkim znacima i 31,3% sa poremećajem svesti. Kod svih obolelih WNV infekcije je potvrđena ELISA metodom, RT-PCR test je bio pozitivan u 30 % nalaza. Od komorbiditeta dominirali su kardiovaskularni 21,9%. Potpuni oporavak postignut je u 87,5% slučajeva.

**Zaključak:** Odsustvo meningealnih znakova i febrilnosti sedmog dana hospitalnog lečenja pokazatelj su dobrog toka i prognoze neuroinvazivnih oblika WNV infekcije. Prisustvo komorbiditeta ne povećava rizik od oboljevanja. ELISA test predstavlja suveren metod u postavljanju dijagnoze. U najvećem broju slučajeva nakon sprovedene simptomatske terapije postiže se potpuni oporavak obolelih

**Ključne reči:** West Nile virus, epidemiologija, transmisija, dijagnoza, prognoza

## **Abstract**

**Background/Aim :** *West Nile virus (WNV)* is a neurotropic RNA virus particle of the *Flaviviridae* family, genus *Flavivirus*. It is sustained in arthropods within the transmission cycle between the mosquitoes and birds. Most commonly (in 80% of cases), WNV infections are asymptomatic among people. Less than 1% of patients develop neuroinvasive form of the disease - meningitis, encephalitis, or acute flaccid paralysis. To determine most common subjective symptoms, laboratory, and clinical manifestations among patients with West Nile virus infection. To emphasise the presence of comorbidities, duration, and outcome of treatment, sequelae.

**Material and Methods:** A retrospective study was conducted in the period from January 1<sup>st</sup>, 2012 to December 31<sup>st</sup>, 2013. We evaluated 32 patients who were diagnosed WNV infection based on clinical findings, laboratory, and serological tests. Data were analyzed using standard statistical methods .To assess statistical significance we used  $\chi^2$ , and t – test.

**Results:** Most affected individuals were male, aged from 31 to 65 years. On admission, there were 50% of febrile individuals, 84.4% with positive meningeal signs, 53.2% with neurological signs, and 31.3% with consciousness disorders. WNV infection was confirmed by ELISA method in all patients, while RT-PCR test was positive in 30% of findings. Cardiovascular comorbidities dominated in 21.9% of cases. Full recovery was accomplished in 87.5 % of cases.

**Conclusion:** The absence of meningeal signs and fever on the seventh day of hospital treatment are indicators of good course and prognosis of neuroinvasive forms of WNV infection. Comorbidities do not increase the risk of disease. ELISA test is a sovereign diagnostic method. In most cases, after the administered symptomatic therapy, the complete recovery of patients was achieved.

**Keywords:** West Nile Virus, epidemiology, transmission, diagnosis, prognosis

## Introduction

West Nile Virus – WNV is a small neurotropic RNA virus particle that belongs to the *Flaviviridae* family, genus *Flavivirus*. It is sustained in arthropods within the transmission cycle between the mosquitoes and birds. WNV is the most widely spread arbovirus type in the world <sup>1</sup>.

Virus particles that are transmitted via arthropods (arboviruses) represent a public health problem and threat to the health of the entire human population, cattle fund, and wild animals around the world. The transmission of arbovirus is directed and determined by ecological interaction of vectors, the host, and pathogen in the environment. Most arboviruses circulate primarily between the arthropod vectors that are fed on blood and wild vertebrates. Consequently, they can spread onto accidental hosts – humans and/or cattle <sup>1</sup>

Birds are natural reservoirs and hosts of WNV infection, while human population and other mammals could be occasionally infected after being bitten by infected mosquitoes <sup>2</sup>. West Nile virus was originally identified in 1937 in the endemic region of Uganda in a female patient with moderate febrile condition. It is the cause of sporadic infection cases in Europe and epidemics in endemic regions of Africa, southern Asia, and northern Australia. Starting from 1996, the WNV infection has been getting in significance in western countries causing massive epidemics or smaller cluster epidemics of WNV encephalitis in Europe <sup>2,3,4</sup>.

In the United States of America (USA), the WNV was recognised for the first time in 1999 when it became the key public health problem spreading across several countries. Today, this flavivirus represents the leading cause of neuroinvasive arboviral disease in the USA and it is responsible for the occurrence of focal seasonal epidemics. Until 2010 almost 1.8 million people in the USA were infected with the West Nile virus, and 12,851 cases of meningoencephalitis and 1,308 lethal outcomes of infection were reported <sup>2,4,5</sup>.

In 80% of cases, the WNV infection is asymptomatic. The symptoms occur in insignificant number of patients (around 20%), most frequently in the form of acute system febrile condition – West Nile fever. It is believed that in clinical manifested cases the incubation period lasts from 3 to 14 days. Monitoring of the described epidemics indicates the presence of febrile condition (body temperature above 39 °C accompanied by fatigue, anorexia,

nausea, vomiting, myalgias, arthralgia, headache, maculopapulose rash and swollen lymph glands. The disease lasted for 3 to 6 days. In the epidemics occurring before 1999, there were also reported myocarditis, pancreatitis, and fulminant hepatitis cases. Less than 1% of patients with WNV infection develop a neuroinvasive form of disease that is manifested as meningitis, encephalitis, or acute flaccid paralysis and accompanied by higher morbidity and mortality<sup>4,5,6</sup>

Transitional viremia occurs in patients infected with WNV directly prior to the occurrence of initial symptoms, i.e. in the early disease stage (5 -14 days after inoculation) and it lasts for 4 to 9 days. The production of neutralising anti-bodies leads to termination of viremia and transiting of the disease in the symptomatic phase. Prolonged viremia is found in immunocompromised patients who cannot develop an adequate immune response in the presence of virus particles. Specific immunoglobulins of IgM class are detected in the patient's serum 2 to 8 days after the occurrence of initial disease symptoms and this is accompanied with the increase of WNV-specific IgG antibodies<sup>5</sup>.

WNV genome is a single-stranded positive-polarity ribonucleic acid (RNA) molecule with approximately 11,000 nucleotides built up by 3 structural and 7 non-structural proteins. Out of five WNV lineages, lineages 1 and 2 are most widely spread in the world. Until 2004, only the WNV of lineage 1 circulated across Europe. That same year the WNV particle of lineage 2 was isolated for the first time in Hungary in the sample taken from the infected goshawk<sup>7</sup>.

The establishing of etiological WNV infection diagnosis is based on a routine use of serological tests: ELISA (*enzyme-linked immunosorbent assay*), IFA (*immunofluorescence assay*), NT (*neutralization test*), and hemagglutination-*inhibition test*. The neutralization tests is considered most specific, it is difficult to perform, time consuming and it can be performed only in *Biosecurity Level 3* ( BSL-3 ) laboratories. Serological ELISA test is currently most widely used since it is fast, easy to perform, cheap and it enables detecting of immunoglobulin, i.e. IgM and IgG WNV antibodies [8]. The reverse transcription polymerase chain reaction – RT-PCR is used to detect viral RNA during the acute disease phase. Sequencing of RT-PCR products can confirm the presence of WNV particle<sup>9</sup>.

WNV is transferred onto people primarily via infected mosquitos' bites (*genus Culex*). The risk factors for infection among humans include: length of stay in natural environment, irregular use of repellents, dwelling in premises with flooded basements and presence of mosquitoes in the space used for living. First cases of transmission of WNV infection from person to person via blood transfusion or solid organ transplantation were registered in the USA in 2002. The post-transplantation WNV infection can be found in rare individual cases and it leads to the development of neuroinvasive forms of the disease with lethal outcome, or retaining of long-term neurological sequelae. Starting from 2003 the screening of blood derivations for presence of WNV RNA has become the routine practice in the USA <sup>5,9,10</sup>.

Prevention of WNV transmission via organ transplantation has motivated a large number of donors' organisations in Canada and USA to introduce the routine donor's serum screening for WNV. In Italy, the study protocols set forth that WNV screening should be carried out within the initial 72 hours after transplantation for all organ donors living in endemic regions <sup>5,10</sup>.

The WNV infection epidemics in human population in the territory of the Republic of Serbia were reported for the first time in the period from August to October 2012 in northern parts of the country, including the AP Vojvodina <sup>3,7,11</sup>.

The detection and understanding of a complex group of factors that condition the creating of the focus of WNV infection and consequential spreading of this pathogen in the environmental is of the key significance for forecasting and mitigating of future epidemics <sup>1</sup>.

## **Objectives**

1. Determine the most frequent subjective health problems and clinical manifestations in patients with neuroinvasive forms of WNV infection.
2. Determine the presence of comorbidity in patients.
3. Significance of laboratory and serological methods compared to other procedures in diagnostics of neuroinvasive forms of WNV infection.
4. Determine the length of treatment, treatment outcomes, and sequelae in patients with neuroinvasive forms of WNV infection.

## **Material and methods**

The study of WNV infection was conducted as retrospective study by analysing medical documentation of patients who were treated at the Clinic for Infectious Diseases of the Clinical Centre of Vojvodina in the period from January 1<sup>st</sup>, 2012 to December 31<sup>st</sup>, 2013. The study included 32 patients who were diagnosed neuroinvasive form of WNV infection based on clinical findings, laboratory analyses, and serological tests.

In all the patients we analysed clinical (febrile condition), laboratory indicators of inflammation process (sedimentation value, C – reactive protein (CRP) and leukocytes, liquor findings) depending on age and sex, on the day of admission and on the seventh day of hospital treatment.

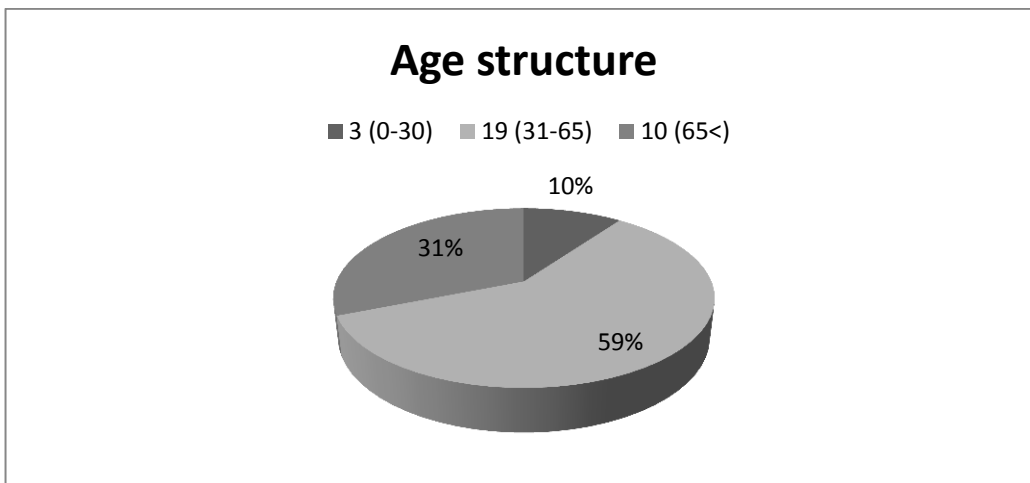
We monitored clinical and demographic indicators (patients' age, presence of health problems on admission of patients to hospital treatment, duration of hospitalisation, place of residence).

Etiological diagnostics was carried out for all patients in the studied sample by means of isolation and identification of the virus and virus antigens from the patients' material using ELISA method and/or by means of RT-PCR methods that were carried out at the Institute of Public Health of Vojvodina, Virology Department.

The results of the study are presented in Tables and graphs and they were processed using standard statistical methods. The  $\chi^2$  and t-test were used for the purpose of statistical significance evaluation.

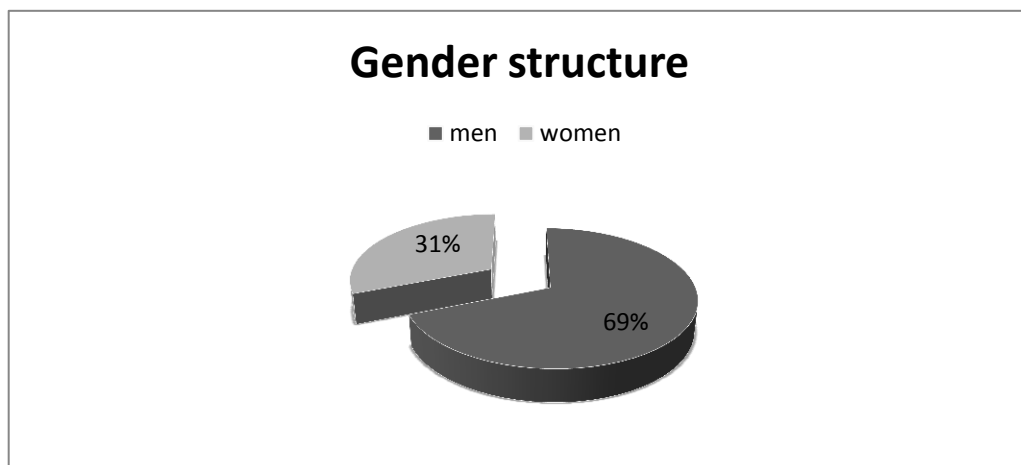
## Results

Retrospective analysis showed that 32 patients with the diagnosed neuroinvasive form of WNV infection were hospitalised at the Clinic for Infectious Disease of the Clinical Centre of Vojvodina in Novi Sad within a two-year period from the beginning of January 2012 to the end of December 2013. The distribution of patients by age and gender has been presented in Fig. 1 and 2. The existence of statistically significant difference in distribution of patients by gender has not been determined and neither it was determined among the studied age groups ( $p>0.001$ ).



$p>0.001$

**Fig. 1 - Demographic distribution of patients by age**

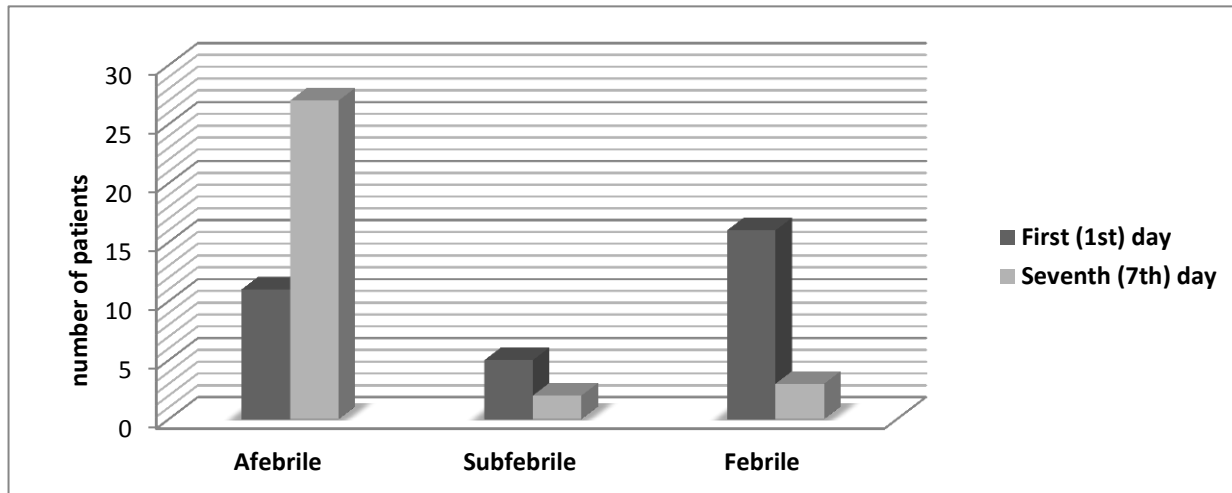


$p>0.001$

**Fig. 2 - Demographic distribution of patients by gender**



We analysed the presence of febrile condition among patients at admission and on the seventh day of hospitalisation – afebrile (body temperature up to 37 °C, sub-febrile (body temperature from 37 to 38 °C febrile (body temperature above 38 °C. We presented the obtained results in Fig. 3.



**p<0.001**

**Fig. 3 - Presence of febrile condition at admission and on the seventh day of hospital treatment**

When it comes to indicators of inflammatory response, we analysed the CRP (C-reactive protein) values, as well as lymphocytes and total leukocytes count. Leukocytosis is the value of the total leukocytes count above  $10 \times 10^9/L$ ; lymphocytosis is the share of lymphocytes in the percentage exceeding 40%. CRP is elevated for the values higher than 5. There is no statistically significant difference between the analysed parameters ( $p>0.001$ ). The distribution of the above-mentioned parameters at admission and on the seventh day of hospital treatment is presented in Table 1.

**Table 1.****Values of inflammatory parameters on the first and seventh day of treatment**

Laboratory findings		
Type of findings	First day	Seventh day
Leukocytosis	0	0
Lymphocytosis	2 (6.25%)	2 (6.25%)
CRP	14 (43.75%)	12(37.5%)

p>0.001

Etiological diagnostics was carried out at the Clinic for Infectious Diseases of the Clinical Centre of Vojvodina by applying serological testing of patients. ELISA test was carried out for all hospitalised patients and all the findings were positive (100%). Out of 32 patients in total, PCR was carried out for 31.25% (10/32) of them. Positive PCR findings for presence of WNV genome were found in 30% (3/10) of patients out of the total number of tests that were carried out.

**Table 2.****Diagnostic procedures carried out in patients**

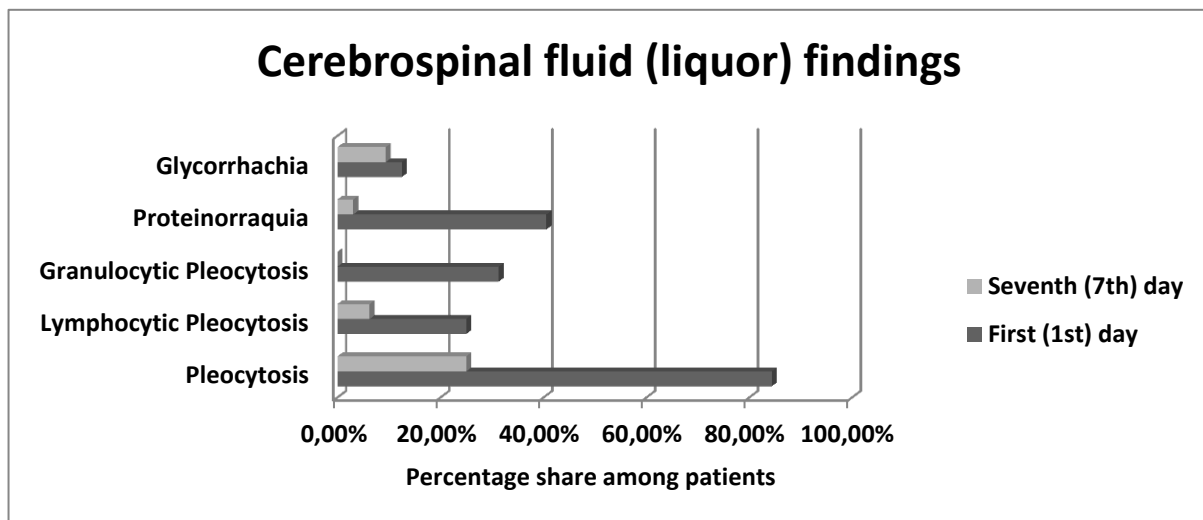
Laboratory diagnostics			
Type of diagnostics	Number of the obtained findings	Number of positive findings	Success percentage
ELISA	32	32	100%
PCR	10	3	30%

We also analysed the clinical findings of patients at admission and on the seventh day of hospitalisation. Among subjective problems and objective signs of disease at the moment of

patients' admission, we can single out positive meningeal signs with the total share of 84.4% (27/32). The frequency of individual symptoms within the meningeal syndrome at admission was registered as – headache (78.1%), vomiting (34.4%), neck rigidity (43.8%), and photophobia (6.25%).

Neurological signs were determined in 53.2% (17/32) of cases and consciousness disturbances 31.3% (10/32) cases. On the seventh day of hospital treatment, we registered the absence of consciousness disturbances and each individual symptom within the meningeal syndrome. The presence of neurological signs on the seventh day of hospitalisation was confirmed in 12.5% (4/32) of cases. We found the statistically significant difference between the share of the analysed clinical parameters (meningeal signs, neurological signs, consciousness disturbances) among individuals at admission and on the seventh days after the admission to hospital treatment ( $p < 0.001$ ).

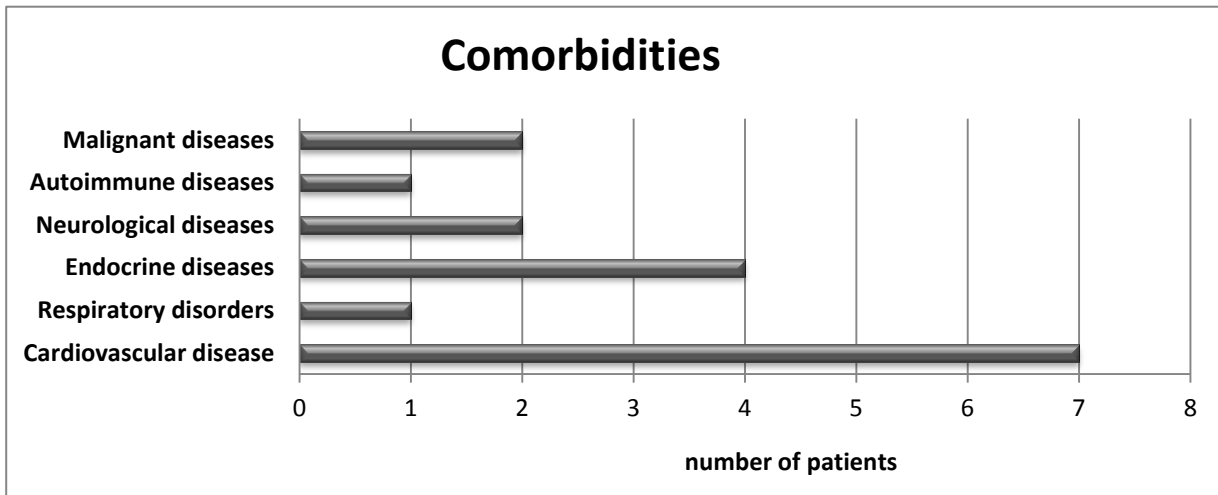
Lumbar puncture was performed at admission and on the seventh day of hospital treatment in all the patients within the analysed sample (Fig. 4).



**Fig 4. Registered values of cerebrospinal fluid (liquor) findings parameter**

We determined the presence of comorbidity and predisposing factors of the disease occurrence among patients. Out of the total number of patients, the presence of accompanying diseases was registered in 37.5% (12/32) cases. The highest frequency among patients with comorbidity is exhibited by cardiovascular diseases (21.9%). We presented the distribution of the

analysed groups of diseases in a graphic form (Fig. 5) and we found that there is no statistically significant difference in the share of the analysed groups of diseases ( $p>0.001$ ). The largest number of patients were released from the hospital treatment fully recovered 87.5% (28/32). Among the remaining 12.5% (4/32) of patients – 6.25% (2/32) of patients developed sequelae. Lethal outcome was registered in 3.13% (1/32) of cases.



$p>0.001$

**Fig. 5- The share of registered groups of comorbidity among patients**

### Discussion

Arboviruses, RNA viruses with huge inherent evolution potential, have been evolving very slowly until now. The life cycle and survival of the viruses of this group relies on vertical transmission that reduces their pathogen potential for the vertebrate host<sup>12</sup>.

Ciota et al. confirmed that transmissive potential of *Culex pipiens* mosquito population decreased after WNV of the MP02 lineage infection despite high sustainability of those chains in the host's organism<sup>12</sup>.

The development of WNV infection in endemic regions is linked with multiplication of population of *Culex* genus mosquitoes (*Culex pipiens*, *Culex restuans*, and *Culex tarsalis*) that are primary vectors. The above-mentioned mosquito genus pullulates and reproduces itself in stale, dirty, and putrid collections of ground water areas and flood prone regions, landfills and septic pits that are located within the residential infrastructure<sup>13</sup>.

Within the Project titled “Detekcija virusa Zapadnog Nila kod populacije komaraca na teritoriji Republike Srbije“ (“Detection of the West Nile virus among the mosquito population in the territory of the Republic of Serbia“), the Institute for Biocides and Medical Ecology from Belgrade performed the tests on mosquitoes in the territories of municipalities of Novi Sad and Temerin in September, 2013. The traps were set at potential sites and live mosquitoes of *Culex* genus were isolated under the professional supervision of doctors of veterinary medicine. They were transported in adequate conditions and subsequently tested by PCR method. Out of 10 sites in the territory of Novi Sad, the presence of the viral genome was proved at 5 sites (50%). In the municipality of Temerin, the result of the PCR method was positive to the presence of the viral genome at one site out of 5 tested (20%)<sup>14</sup>.

In the sample of 32 patients who were treated at the Clinic for Infectious Diseases of the Clinical Centre of Vojvodina during 2012 and 2013 and who were included in our study, there were 59.4% (15/32) of patients with the residence in the territory of the municipality of Novi Sad and 9.4 (3/32) in the territory of municipality of Temerin. Our results are in compliance with the data obtained in the analysis of mosquitoes from the same territory.

Thanks to the data of the Institute for Public Health from Novi Sad and Institute for Biocides and Medical Ecology from Belgrade, we produced the map of the analysed sites in the territory of municipality of Novi Sad and residence of citizens with positive laboratory findings.



**Fig. 6- Addresses of citizens with positive laboratory findings on West Nile virus and locations of mosquito traps with positive results of PCR analysis in Novi Sad, in 2013**  
 + – Positive laboratory findings to West Nile virus  
 W – Locations of traps with infected mosquitoes

The European and American researchers agreed that more than 90% of infections caused by arboviruses show seasonal character and occur in the period from July to September<sup>15,16</sup>. Both epidemic waves analysed in our study were recorded in the period from the beginning of August to the end of September 2012 and 2013.

In a long-term period from 1999 to 2012, more than 36,000 cases of WNV infection in human population were reported to the Centre for Disease Control and Prevention – CDC. Epidemiological department of this institution assess that starting from 1999 there have been 2 to 4 million infected people in the territory of the USA, while some 400,000 to 1 million of

infected people had some sort of symptomatology <sup>14</sup>. In the territory of the Republic of Serbia, there are no records on the number of infected people and number of people with the West Nile virus.

In the Climatological Analysis of the Republic Hydrometeorological Service of Serbia that was conducted by Smailagić et al., it was confirmed that the summer of 2012 was one of the hottest within the last few years <sup>17</sup>. The obtained climatological data correlate with multiplication of the *Culex* genus mosquito population, and the occurrence of first registered cases of infection with the West Nile virus in the territory of the Republic of Serbia.

The US Centre for Disease Control and Prevention registered 5,245 cases of WNV infection until the end of November, 2012, including 256 lethal outcomes for the current year. Nasci states in his report that 51% of the total number of infected individuals had neuroinvasive form of the disease (meningitis/meningoencephalitis). This was the highest annual incidence of WNV infection diseases in the USA since 2003 <sup>18</sup>.

Immediately after the WNV RNA lineages were isolated from the serum of goshawk in the territory of Hungary and after it was proven they circulated in the blood of the infection reservoir (birds, horses) in the territory of Hungary, Austria, and Italy, a sharp wave of epidemics among humans occurred in the territory of Greece in 2010. During two seasons, there were 273 registered cases of neuroinvasive forms of West Nile virus infection in Greece <sup>19</sup>.

In our region, all registered cases of WNV infection had neuroinvasive forms of the disease. The data from our study indicate the presence of neuroinvasive form of WNV infection (meningitis/meningoencephalitis) in all the patients within the analysed sample. It is assumed that the number of persons infected with WNV was much higher in both epidemic waves, during 2012 and 2013. Only those with the most severe forms of clinical picture were hospitalised and serologically tested.

Thanks to co-operation between the Clinic for Tropical and Infectious Diseases of the Clinical Centre of Serbia and Institute for Virology Torlak, the report was compiled and the epidemic of WNV infection in the territory of Belgrade and its surroundings was evaluated in 2012. Within this study, Popović et al. emphasise the predominance of neuroinvasive forms of

the disease <sup>3</sup>. The results of our study are in compliance with the data from the study published by this group of authors from Belgrade.

The group of authors from Belgrade believe that the number of infected persons within the WNV epidemics in 2012 was higher than the registered one and that a significant number of patients with febrile condition resembling to influenza remained undiagnosed. It is assumed that these patients with non-specific inflammatory symptomatology did not ask for medical assistance or they reported to the institutions of primary health care where they were treated symptomatically <sup>3</sup>.

The results of national studies are in compliance with the conclusions of wider professional public. Kwan, Park et al. determined in the study that encompassed a six-year period (2004-2010) that the number of reported cases of West Nile fever and neuroinvasive forms of WNV infection in the territory of the state of California was not a representational sample compared to the entire population. The above-mentioned authors indicate the reduced number of performed serological tests in persons with febrile condition, which reduces the total number of reported disease cases <sup>20</sup>.

According to the estimates of the US Centre for Disease Control and Prevention, 30 to 70 cases of moderate neuroinvasive forms of disease could be added to each reported case of neuroinvasive form of WNV infection <sup>15</sup>.

Epidemiological analysis of WNV infections in Texas was carried out in January 2013 for a multi-annual period from 2002 to 2011 and it was determined male Caucasian individuals aged 54 on the average dominate among the reported cases (59%) <sup>21</sup>. The data of the US Centre for Disease Control and Prevention show insignificant differences in age and gender distribution in the territory of the USA – the share of male individuals among the ill makes 56% with the average age of 56 <sup>15</sup>. Our results in the analysed sample indicate the predominance of male individuals (68.75%) with a high frequency of diseases among the working population aged 30 to 65 (59.38%), which is in compliance with the conclusions of the American authors.

Nolan et al. point to the mortality rate of 6.3% among patients with neuroinvasive WNV infection in the territory of Texas. The report from June 2013 describes that mortality or



people with similar diseases in the entire territory of the USA reaches 5%, with the average age of 77<sup>15,21</sup>. Contrary to American studies, our study indicates a lower mortality rate among the patients – lethal outcome was found in 3.13% (1/32) of cases in the analysed sample.

PCR can be useful diagnostic procedure during an early stage of WNV infection. After seroconversion, it is much more likely that etiological diagnosis will be established via serological tests<sup>22</sup>. PCR testing was performed in 31.25% of patients in our study. Positive result was obtained in 30% of the analysed samples.

South African researchers Zaayman and Venter from the University in Pretoria described a lower incidence of positive PCR results stating 2 factors that conditioned a small number of positive PCR WNV tests: 1. arbovirus viremia lasts for a short time and cerebrospinal fluid retains a small number of viruses, and 2. certain percentage of falsely negative results will occur if the samples are not stored properly<sup>22</sup>. Turkish experts pointed out that they did not obtain a single positive result of RT-PCR to presence of WNV RNA in the analysed sample<sup>23</sup>.

Serological testing using ELISA method was performed in all the patients who were included in our study, which enabled etiological diagnosing of the disease. The obtained results are in compliance with the data of the Clinic for Tropical and Infectious Diseases of the Clinical Centre of Serbia described by Popović et al. in their study<sup>3</sup>. A larger number of European authors emphasise the significance and advantages of use of ELISA method for final diagnosing and determining of causes of infection<sup>19,22,23</sup>.

Bocanegra et al. believe that supervision over the domestic horses can be useful for monitoring of WNV genome circulation in endemic region, which is important from the aspect of public health. These attitudes are based on the established seroprevalence in horses who have not got ill and determined elevated frequency of neurological symptoms among horses compared to birds and humans<sup>24,25</sup>.

Birds are the main natural WNV reservoirs. Local mobility of domestic birds and huge distances that migratory birds travel could contribute to spreading of the WNV infection<sup>26,27,28,29</sup>. Veterinary monitoring related to WNV infections was performed in the territory of the Republic of Serbia within the recent years. Petrović et al. from the Scientific Institute for

Veterinary Medicine in Novi Sad performed epidemiological monitoring and testing over domestic birds and migratory birds from the territory of the AP Vojvodina in the period from January to September 2012. The WNV presence and circulation among birds in the territory of the Republic of Serbia was confirmed serologically for the first time in 8% (7/91) of the analysed samples<sup>7</sup>.

Preventive procedures aimed at prevention of WNV infection occurrence imply public-health measures that will preclude mosquito bites in human population. The fact is that vaccines against domestic forms of arbovirus are not available. Forms of prevention of arbovirus infections rely on the activities of social community and personal efforts to reduce the vector population. Education of population on significance of prevention and methods of implementation of personal protection measures (via posters, leaflets, media campaigns) and implementation of system measures directed to the vector-mosquito population (destruction of habitats, larvae, and adult mosquitoes, use of insecticides) would enable combating new epidemic waves of WNV infection. The relevant bibliography states that the use of repellents correlates with the reduction of the risk of development of WNV infection<sup>3,5,17</sup>. During the analysed epidemic wave in 2013, only the insignificant number of patients (3.13%) carried out personal protection measures and used repellents.

## **Conclusion**

1. The absence of meningeal signs and fever on the seventh day of hospital treatment are indicators of good course and prognosis of neuroinvasive forms of WNV infection.
2. The presence of comorbidities does not increase the risk of development of neuroinvasive forms of WNV infection.
3. ELISA test is a sovereign diagnostic method RT-PCR test can serve as an indicator of acute infection.
4. In most cases, after the administered symptomatic therapy, the complete recovery of patients is achieved.

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