5. SEROLOGIC INVESTIGATION OF ACTIVITY OF WEST NILE VIRUS IN HUMANS IN THREE DISTRICTS OF VOJVODINA

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Abstract

West Nile virus is RNA virus from the family Flaviviridae, genus Flavivirus. It is one of the most widely distributed arboviruses being found across much of Africa, southern Europe, the Middle East, Asia, Australia (Kunjin subtype), and America. Virus is transmitted by Culex mosquitoes. At least 300 different bird species are reservoir of West Nile virus. At least 30 vertebrate species can be infected. Most human infections are asymptomatic. WN virus may cause fever and sometimes encephalitis in large mammals, especially in horses and also in humans. Sporadic cases and outbreaks of febrile disease (WN fever) were reported in humans in the Middle East and Africa. Since 1996 WN virus has been recognized as a major public health concern in Europe. That year the first outbreak of WN virus infection in Europe was registered in Romania where 393 laboratory confirmed neuroinvasive cases were reported. During the 2010 Greece, Romania, Turkey and Russian Federation experienced WN virus infections outbreaks in humans with a large percentage of neuroinvasive cases. Sporadic human cases during 2010 were registered in Hungary, Spain, Portugal, Italy and France.

The aim of this study was to investigate the presence of West Nile virus specific antibodies in the serum samples from three districts of Vojvodina using IgG ELISA assay. During the period 2007 – 2010, 426 serum samples were collected from residents of South Backa District. During September and October 2011, 91 serum samples were collected from residents of Central Banat and North Backa District. All samples were tested for West Nile Virus IgG by commercial enzyme-linked immunosorbent assay (ELISA) test, produced by Euroimmun, Luebeck, Germany in Institute of Public Health of Vojvodina. Testing, calculation and interpretation of results were performed strictly following the manufacturer’s instructions on the automatic device Euroimmun Analyzer I-2P. X2 test was used for statistic calculations. In the serosurveys, conducted during period 2007-2010 in South Backa District, IgG antibody against WN virus was found in 16 (3.75%) out of 426 serum samples. In the study conducted in 2011 in North Backa District IgG antibody to West Nile virus was found in 1 out of 43 serum samples (2.32%). In samples from Central Banat seroprevalence of WN IgG antibody was 12.5% (6/48) that was significantly higher than seroprevalence for two other district (X² = 13.07, p<0.01). According to the results of this study the virus was present in all investigated districts and circulates among humans.

Key words: West Nile virus, IgG antibody, Vojvodina

Introduction

West Nile virus is RNA virus from the family Flaviviridae, genus Flavivirus. Virus was first isolated in 1937 in West Nile district of Uganda. It is one of the most widely distributed arboviruses being found across much of Africa, southern Europe, the Middle East, Asia, Australia (Kunjin subtype), the Americas. Virus is transmitted by Culex mosquitoes. At least 300 different bird species are reservoir of West Nile virus; At least 30 vertebrate species can be infected. Humans and horses are accidental hosts, unsuitable for infection of mosquitoes (because of low virus titer and short duration of viremia). Most human infections are asymptomatic. WN virus may cause fever and sometimes encephalitis in large mammals, especially in horses and also in humans. Only a few human cases (1%) develop a neuroinvasive disease. Sporadic cases and outbreaks of febrile disease (WN fever) were reported in humans in the Middle East and Africa. Since the 1990s the epidemiology of WN virus has changed. Mild cases of fever, predominant in previously described outbreaks in Africa and Israel have been replaced by outbreaks of cases with severe neurological manifestations and even death. The first outbreak of WN virus infection in Europe was in Romania in 1996 where 393 laboratory confirmed neuroinvasive cases were reported. Since 1996 WN virus has been recognised as a major public health concern in Europe. During 2010 Greece, Romania, Turkey and Russian Federation experienced WN virus infection outbreaks in humans with a large percentage of neuroinvasive cases. Sporadic human cases during 2010 were registered in Hungary, Spain, Portugal, Italy and France (1, 2).

Seroepidemiological surveys carried out in many European countries have identified antibodies against this virus which confirmed the activity of virus outside its endemic range. Studies conducted in almost all countries in our region have shown that population has been in contact with the mentioned virus. Seroprevalence of West Nile virus by hemagglutination inhibition test was 1-3% in Croatia, 1% in Bosnia, 3% in Bulgaria, 2-12% in Romania and 1% in Central Macedonia in Grace (3, 4). First serological investigation in humans in Vojvodina was conducted in 1972 and antibody against West Nile virus was found in 2.6% - 4.7% in different rural parts of province using hemagglutination inhibition test (5).

The aim of this study was to investigate the presence of West Nile virus specific antibodies in the serum samples from Vojvodina at the beginning of the 21st century using IgG ELISA assay.

Materials and methods

During the period 2007 – 2010 426 serum samples were collected from residents of Backa South District. Seventy two children aged from 1 to 18 and 354 adults aged from 19 - 84 were tested. Serum samples were collected from 180 females and 246 males. During September and October 2011, 91 serum samples were collected from residents of North Backa and Central Banat District. Respondents were aged 0 - 83. 59 respondents were males and 32 females. All samples were tested for West Nile virus specific antibodies in the serum samples from Vojvodina using IgG ELISA assay.
Virus IgG by commercial enzyme-linked immunosorbent assay (ELISA) test, produced by Euroimmun, Luebeck, Germany in the Institute of Public Health of Vojvodina.

Testing, calculation and interpretation of results were performed strictly following the instructions of manufacturer on the automatic device Euroimmun Analyzer 1-2P. Results were evaluated semi quantitatively by calculating a ratio of the extinction value of patient sample over the extinction value of the calibrator 2 which was included into the test. Results were considered as positive if ratio was equal to or greater than 1.1, intermediate if ratio was between 0.8 and 1.1 and negative if ratio was less than 0.8. X² test was used for statistic calculations.

Results

In the serosurveys conducted during the period 2007-2010 in South Backa District IgG antibody against WN virus was found in 16 (3.75%) out of 426 serum samples. In the study conducted in 2011 in North Backa District IgG antibody to West Nile virus was found in 1 out of 43 serum samples (2.3%). In samples from Banat Central District seroprevalence of WNV IgG antibody was 12.50% (6/48) that was significantly higher than seroprevalence for two other district (X² = 13.07, p<0.01 ). Results of serological test for WN antiviral antibody were given in table 1.

Table 1. Serologic results for anti West Nile Virus IgG ELISA in three districts of Vojvodina

<table>
<thead>
<tr>
<th>DISTRICT</th>
<th>IgG POSITIVE</th>
<th>IgG BORDERLINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Central Banat District</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>North Backa District</td>
<td>43</td>
<td>1</td>
</tr>
<tr>
<td>South Backa District</td>
<td>426</td>
<td>16</td>
</tr>
<tr>
<td>TOTAL</td>
<td>517</td>
<td>23</td>
</tr>
</tbody>
</table>

Persons aged above 45 were significantly more frequent among the seropositive subjects (7/23; 56.52%)(X²=20.72, p<0.01) Out of total of 6 seropositive subjects from the Central Banat District 5 aged above 45. Only one seropositive subject from the North Backa District and 7 seropositive from the South Backa District also belonged to this age group (Figure 1).

Discussion

Results of the study conducted in the period 2007-2011 in three districts of Vojvodina indicated that West Nile virus was not a virus of far exotic countries. In this investigation 4.45% of inhabitants had IgG antibodies to the West Nile virus. Serological findings show that the West Nile virus is active in the territory of three districts of Vojvodina. Results are consistent with virological research conducted in other European countries as well as with studies conducted in our country.

In the sample of patients from Vojvodina who were treated from viral encephalitis or meningoencephalitis of uncertain etiology at Clinic for infectious diseases in Novi Sad during five year period the frequency of IgG antibodies to West Nile virus by ELISA test was 7.4% (6). In the sample of 105 patients from Serbia, occupationally exposed to mosquito bites, in 4.76% IgG antibodies were confirmed by indirect immunofluorescence assay (7). West Nile seroprevalence has been proven in many European countries. In different parts of Italy the incidence of hemagglutinin inhibition antibodies to West Nile virus ranges from 2 to 23%, in Greece from 1 to 27%, in Bulgaria 3%, in Slovakia from 1 to 4% and in Austria from 1 to 6% (3). In more recent studies conducted in Hungary and Germany seroprevalence was less than in this study. In Hungary 5312 persons were examined and 30 (0.56%) had antibodies against
West Nile virus (8). In the study in Germany 14 437 blood donors were tested by ELISA IgG test for West Nile virus and 5.9% were initially anti-WNV reactive (9).

As expected in the tropics the incidence of antibodies was higher than that reported for Europe. Thus, in Ghana seroprevalence of IgG antibodies to West Nile virus ranges from 4.8% in children to 27.9% in adults. The same study found that 2.4% of children had IgM antibodies against this virus (10). In the similar study IgG antibodies to West Nile virus ranged from 18.4% in children aged under 14 to 51.7% in patients aged over 44 (11).

Climate change is affecting the appearance of vector in geographic areas outside the earlier traditional territory, which facilitates the spread of the virus into new areas. Activity of West Nile virus and human and animal cases of infection were registered in geographically close countries. In north-eastern Italy and Hungary human West Nile infections with involvement of the CNS have been diagnosed since 2008. There is the tendency of increased number of patients in these countries (12, 13). Human infections were registered in France (14), Germany, Denmark and Scandinavia (15, 16). Recently conducted investigation has confirmed presence of antibodies to WNV in horses in Serbia (17).

Conclusion
The results of this preliminary research conducted at the beginning of the twenty-first century, using contemporary serological tests confirmed the results of other authors and indicated the presence of virus in humans in the investigated parts of Vojvodina.

Recommendation
In Vojvodina the routine diagnosis of West Nile virus infection has not been carried out. The incidence of clinically manifested human cases is not known. For many doctors it is the just one in a long line of ARBO viruses transmitted by mosquitoes in remote tropical regions. According to the results of this and previous studies the virus is present in Vojvodina. The introduction of diagnosis of this viral infection would contribute to resolving the etiology and better treatment of encephalitis and other neurological disorders.

Reference
SEROLOGICAL INVESTIGATIONS OF WEST NILE FEVER IN BULGARIA DURING 2010

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Abstract:
In the past decade numerous arthropod-borne diseases re-emerged causing large health and economical damages. Annually multiple West Nile fever outbreaks are documented around the world. The disease is endemic in many regions in Africa, the Mediterranean basin and the Middle East from where the infection could be introduced into new areas by migratory birds.

In this study two types of animal species (equines and domestic birds) permanently stabled in four risk zones (Tutrakan, Staro Oriahovo, Durankulak and Shabla) were surveyed for the presence of neutralizing antibodies against West Nile fever. Overall high percentage of samples tested positive: Tutrakan (28.6%), Staro Oriahovo (63.6%), Durankulak (43.5%), Shabla (39.7%). In addition IgM antibodies were detected in serum samples from equines.

The results indicate that the virus circulate in the study areas involving different animal species (birds and equines) as well as variety of mosquito species.

Key words: West Nile Fever, arthropod-borne diseases, sentinel animals, seroprevalence

Introduction
West Nile fever (WNF) is a mosquito - borne disease belonging to the Flaviviridae family, genus Flavivirus. Due to the close antigenic relationship it is a part of the Japanese Encephalitis serum-complex which includes also Murray Valley virus, Saint Louis virus, Usutu virus, Cacipacore, Koutango, Yaounde (Murphy et al., 1995; OE, 2008).

West Nile Virus (WNV) isolates have been grouped in two lineages. Strains from lineage I were isolated in Europe, North America, Africa and Australia. Lineage 2 contains strains isolated in Madagascar and South Africa. Recently additional two lineages were proposed: lineage 3 including the Rabensburg virus, isolated in the Czech Republic and lineage 4 consisting from strains isolated in Caucasus region. Within different lineages WNF isolates are segregated into clusters. Strains from European Mediterranean/Kenyan cluster (lineage 1) are characterized by moderate pathogenicity for humans and equidae and limited or no pathogenicity for birds. Conversely the representatives of the Israeli/American cluster (lineage 1) are characterized by high rates of illness and deaths in humans, equines and birds (Calistri et al., 2010).