SEROLOGICAL INVESTIGATIONS OF WNV INFECTION IN HORSES FROM THE SOUTH-EAST OF ROMANIA

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Abstract. The WNV infection in human and animals is an actual subject in Romania. In the cadre of a research project we have done a survey of WNV antibodies in the horse population from 5 districts placed in the south-east of Romania (the inferior area of Danube).

A number of 167 samples of horse serum ingathered from 25 localities placed at the Danube river side or other adjacent rivers were examined using Elisa West Nile Test kit (made by Biogal Galed Laboratories and ID. VET Innovative Diagnostics). Overall serums tested, 56 samples were found positive, meaning a 33, 53% prevalence. The investigations are continuing.

INTRODUCTION

West Nile virus (WNV) is a mosquito-borne flavivirus, native to Africa. Infections are known for decades. The virus was first isolated in 1937, from a febrile woman blood, in the West Nile district in Uganda (19). After this, it was demonstrated that the WNV is one of the most widespread flaviviruses that infects humans, horses, birds and mosquito. Its geographic distribution is wide. The WNV was isolated from Europe (12, 13, 15), Africa, Middle East and USA (1, 10, 14, 15, 16, 17, 18). In many aspects, WNV is an outstanding example of a zoonotic pathogen.

In 1985 the most important epidemic episode of WN viral meningitis occurred in our country. At that moment 1.301 cases were registered. More recent, in July, October 1996 an epidemic episode of WN fever occurred in the south of Romania (Bucharest and the inferior area of Danube). There were detected 100 cases of neurological disease in human, from which 17 were fatal. The surveillance system during 1997-2000 detected 39 clinical human WN fever cases: 14 cases in 1997, 5 cases in 1998, 7 cases in 1999 and 13 cases in 2000. (9, 11).

Transmitted by mosquitoes, WNV infects a wide range of vertebrates. Naturally, the clinical symptomatology is rare excepting the horses. Birds are the main host and the natural cycle involving the infection of reservoir-competent birds and the spring migration is the principal reason for the amplification and spread of the virus in unaffected territories. However recent studies suggest that mammals, thought to be dead-end hosts are not only exposed to WNV, but at least some may also serve as competent WNV reservoirs(4, 7, 8, 9, 10).

The incubation period in the case of equine encephalitis preceding the transmission by mosquitoes is estimated at 3-15 days, a floating viremia with a low virus titre precedes the installation of clinical signs (2, 3, 6). The encephalitis is clinically manifested at a low percent of the infected horses and the symptomatology is characterized by ataxia and recumbency as a result of cranial nerves function damage (4, 5, 7, 8). Fever is not constant. The lethality rate in
symptomatic specimens is 30% and the mortality rate in infected equines vary between 28 and 45%. (1).

Differential diagnosis includes other arboviral encephalitis (eastern, western or Venezuelan equine encephalomyelitis, Japanese encephalitis), equine protozoal myelitis (*Sarcocystis neurona*), equine herpesvirus-1, Borna disease and rabies.

The identification of the agent use different methods: isolation in cell culture (Vero cell or mosquito cell), RT-PCR and immuno-histochemistry. Antibody can be identified in equine serum by IgM capture ELISA, haemagglutination inhibition (HI), IgG ELISA or plaque reduction neutralisation (PRN). The last two are more commonly for identifying WNV antibody in avian serum. (20).

**MATERIAL AND METHODS**

The period of the study was 2006-2007. The investigations were realized in the south east of Romania. From the 508 horses sampled in the investigation area, 167 serum specimens were tested.

The horses were selected from stud and private farms situated in Braila, Constanta, Galati, Ialomita and Tulcea districts. This area was chosen for study because here we found all the premises for the spread of West Nile encephalitis virus. In those districts we also have catalogued the number of birds and horses from the studied area, species directly implicated in WNV transmission.

The collection and stock of serum samples were made following a strict protocol and we have chosen 167 samples to be screened. The 167 horse equine sera were evaluated for the presence of anti-WNV antibodies by an Elisa assay „Kit for detection of West Nile anti prM-E antibodies in horse sera” made by ID.VET Innovative Diagnostics and WNV-ImunnoComb-Equine West Nile Virus Antibody Test Kit, made by Biogal Galed Laboratories.

**RESULTS AND DISCUSSIONS**

From the total of 508 samples stocked, 167 were selected for serological screening. The selection criteria was represented by the location of the localities following the Danube flow in the south east of Romania and the density of domestic and wild horse population from the studied areas in the Danube Delta.

After the screening using the two immunoenzymatic assays and referred to the number of samples studied we have obtained a significant number of positive results. Those results prove the existence of West Nile infection and the potential of its spreading is real. Knowing the implication of WNV infections as a zoonosis the presence of the infection is a tocsin for the veterinary and public health services.

As a validation and a comparison between the two tests, we have retested 5 samples screened first time by ImmunoComb assay (meaning two negative sample no.160 and 159, two medium positive sample no.82, 83 and one strong positive sample no.85) using the ID Vet Elisa test. The results were alike.
Table 1: Results of WNV serological investigations in the south east of Romania.

<table>
<thead>
<tr>
<th>Counties</th>
<th>Number of samples</th>
<th>Number of positive samples</th>
<th>Percent of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braila</td>
<td>20</td>
<td>10</td>
<td>50.00 %</td>
</tr>
<tr>
<td>Constanta</td>
<td>25</td>
<td>1</td>
<td>4.00 %</td>
</tr>
<tr>
<td>Galati</td>
<td>30</td>
<td>8</td>
<td>26.66 %</td>
</tr>
<tr>
<td>Ialomita</td>
<td>19</td>
<td>3</td>
<td>15.78 %</td>
</tr>
<tr>
<td>Tulcea</td>
<td>73</td>
<td>34</td>
<td>46.57 %</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>56</td>
<td>33.53 %</td>
</tr>
</tbody>
</table>

The biggest numbers of positive samples were found in Tulcea district: 34 samples from 73 analyzed the next one as seroprevalence is situated Braila 10 positive samples from 20 analyzed and after this Galati 8, Ialomita 3 and Constanta 1. Although the global seroprevalence is 33.53%, in Tulcea and Braila district the results are concludent and perfectly related to the presence of the mosquito and to the climate.

Fig. 1: Graphic representation of WNV serological investigations in the south east of Romania

Even if we have tested a relatively small number of samples, the positive results confirm the presence of WNV in the inferior area of Danube Delta and that this area represent a favorable environment for the development of WNV. The virus, due to its transmission cycle can be spread anytime by mosquito vector and birds.

**CONCLUSIONS**

Today is extremely difficult to predict the impact of WNV infections in veterinary medicine and in public health in Romania. The spread and the evolution of the disease depend of a lot of agents. The control of the migratory birds and the difficulties in the eradication of mosquito are one of the major inconvenient for the surveillance of WNV infections.
The development of WNV meningoencephalitis episode in 1996-1997 in Romania and other epidemic episodes in the whole world impose a stringent necessity for the knowledge of an accurate epidemiological situation and for the establish of efficient methods of prevention, control and diagnostic in order to be available in case of suspicion.

The serological survey of the horses in the south east of Romania in five districts with an important epidemiological role in WNV cycle, Brailia, Constanta, Galati, Ialomita and Tulcea, using two types of enzyme-linked immunosorbent assays, allowed us a first brief evaluation of the WNV presence in this area with a 33, 53% WNV seroprevalence in horse sera.

BIBLIOGRAPHY