West Nile Virus Infection in Southeastern Romania

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General considerations

• “WNV, usually regarded as a cause of endemic dengue-like illness, erupted in a unique urban encephalitis epidemic in Romania…as a public health threat in eastern Europe”
  – Theodore Tsai, Division of Vectorborne Infectiuous Diseases, CDC. 1997
General considerations

- The appearance was confined to Bucuresti (especially rural area) and 14 districts neighboring Danube River, a well-known site for migratory birds from Africa.
- While considered as having neurological manifestations as the most important, the estimated clinical-to-subclinical infection ratio was in Bucuresti one to 140-320.
First lessons learned

• Cluster of idiopathic aseptic meningitis, ME and encephalitis observed by clinicians in Bucuresti after mid-July 1996

• Reported by mid-August, when already hundreds of cases existed

• MOH established an active surveillance system hospital-based and a clinical case definition was introduced
Case finding
The 1996 clinical case definition

- An acute encephalitis or meningoencephalitis with a suspected viral etiology
- With fever, chills, agitation, somnolence, coma, cranial nerve paralysis, rash
- And abnormal CSF with 30-500 white blood cells mm³ (predominantly polymorphonuclear cells)
The mysterious Agent

- August 1996
- Clinicians excluded bacterial etiology, as well as looping-ill or TB encephalitis
- Epidemiologists showed unusual age-specific incidence and geographical distribution
- Cantacuzino Institute infirmed enteroviral etiology
- Institute of Virology suggested the introduction of a “new” arbovirus
The mysterious Agent

- September 1996
- Lymphocytic choriomeningitis virus confirmed by Pasteur Institute
- Army Research Laboratory showed serological evidence for WN
- Pasteur Institute confirmed the evidence of anti-WN virus IgM antibodies in sera of 44 patients
- CDC and MOH conducted serosurvey and case-control studies
Case confirmation

- Patients meeting clinical criteria were considered as laboratory-confirmed if:
  - anti-WN virus IgM antibodies in serum or CSF,
  - anti-WN virus IgG antibodies in CSF,
  - conversion from negative to positive anti-WN virus IgG antibodies,
  - was demonstrated.
- Reagents were provided by USAMRIID
Summary of suspected West Nile Encephalitis
Bucuresti, 1996

Total
Reported
Suspected Cases
835

Meet
case definition
767

Did
not meet 
casedef
68

Serotested
441

Not
tested
326

Serotested
68

Not
tested
0

Positives
352

Negatives
89

Unknown
326

Positives
41

Negatives
27

Unknown
0
West Nile encephalitis by week of onset and residence
Romania, 1996

No. cases

Week

Districts
Bucuresti
West Nile Encephalitis incidence by district
Romania, 1996
Age specific incidence of West Nile Encephalitis Romania, 1996

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No. cases/100000</th>
</tr>
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<tbody>
<tr>
<td>0-9</td>
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<tr>
<td>10-19</td>
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<td>50-59</td>
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<tr>
<td>60-69</td>
<td>60</td>
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<tr>
<td>&gt;= 70</td>
<td>70</td>
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Male

Female

Total
Clinical description

- Among the 352 confirmed cases 40% had meningitis, 44% meningoencephalitis and 16% encephalitis
- Onset with fever (91%) and headache (77%), also neck stiffness, vomiting, chills, confusion
- Coma was registered in 13% of cases
- Fatality-case ratio was 4.3%, all 17 deaths being in patients older than 50 years
Clinical status of serologically confirmed West Nile cases by week of onset
Romania, 1996

<table>
<thead>
<tr>
<th>Week of Onset</th>
<th>No. Cases</th>
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<tr>
<td>8 Jul</td>
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<tr>
<td>15 Jul</td>
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<td>22 Jul</td>
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<td>2 Sep</td>
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<td>9 Sep</td>
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<td>16 Sep</td>
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<td>23 Sep</td>
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<td>30 Sep</td>
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<tr>
<td>7 Oct</td>
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<tr>
<td>14 Oct</td>
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Legend:
- Other
- Neurological
Serosurvey

• To measure the magnitude of the epidemic
• Age-representative sample of patients being bled for other medical indications
• IgG antibodies to WN virus were detected in 39 residents of Bucuresti (4.1%) and 18 of them also had detectable IgM antibodies (1.9%) a month after the peak of epidemic
• In other 7 districts seroprevalence was significantly lower (0.9%)
<table>
<thead>
<tr>
<th>Agegroup</th>
<th>IgG (%)</th>
<th>IgM (%)</th>
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<tbody>
<tr>
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<tr>
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West Nile agegroup specific seroprevalence survey study in general population Bucuresti, October 1996

IgG seroprevalence 4.1%
IgM seroprevalence 1.9%
• Proportions of outpatients with IgG and IgM antibodies were similar in both sexes and in all ages, suggesting a uniform risk of infection among the city residents.

• Also it suggests that the virus had not been previously present or, if infections had occurred before, that they were rare.

• By extrapolation, between 43000 and 96000 residents of Bucuresti were infected.

• The estimated clinical-to subclinical infection ratio was 1 to 140-320.
Case - control study

Internal risk Factors

External risk Factors

Sero+

Sero-
Risk factors for WN infection

• The risk for infection was associated with mosquitoes in the home and for those living in apartment buildings, the flooded basement

• The risk for ME was associated with spending more time daily outdoor rather than previous medical conditions

• The above risk factors may be correlated with the number of mosquito bites / day
Mosquito survey

- Heavy infestation with mosquitoes found due to numerous breeding sites
- Cx.pipiens - 94% of the mosquitoes
- A virus was identified antigenically as WN virus, recovered from a pool of Cx.pipiens collected in central Bucuresti
- The estimated infection rate was 0.3/1000
- A greater than usual population of Aedes vexans in the city outskirts was observed in June
Bird survey

- Neutralising antibodies to WN virus were detected in 41% of domestic fowl (30/73)
- Proportion of seropositive birds was 37% in chicken, 42% in duck, 67% in turkey
- Neutralising antibodies to WN virus were detected in 8% of wild birds (1/12 - robin)
Other Data

- **Illness in Equines**
  - no unusual occurrence of neurological illness in horses in the Bucuresti area in 1996

- **Meteorological Data**
  - no significant differences were found in rainfall and temperature during the period May-September 1996 compared to the same months in the preceding ten year interval

- **Historical Data**
  - no clinical cases confirmed or WN virus isolated prior to the epidemic in Romania
  - serologic studies to prove enzootic viral transmission in the Danube plain
  - serological data were conflicting (lack of specificity of tests and cross-reactivity with TBE virus)
Genesis of the epidemic

• Migratory routes follow a flyway in which Danube delta is an important avian refuge
• Establishment of a local enzootic transmission in the Danube basin is possible
• Cx.modestus, enzootic vector, is frequent in the Danube basin and could mediate a sylvatic cycle
• The virus could be transferred in an urban cycle or could infect direct humans
• All above are considerations and could not be proved
The postepidemic period 1997-2002

• A regional seasonal hospital-based surveillance system is in place from 1997

• A suspect case is still neurological oriented (viral aseptic meningitis or encephalitis), a probable case is a suspect case with IgM antibodies to WN virus, and a confirmed case is a probable case with the WN virus isolated or IgM antibodies were detected in CSF.

• The surveillance system proved that sporadic cases occurred each year within the districts neighbouring the Danube.
Frequency of WN serologically confirmed encephalitis
Bucuresti, 1997 - 2002

<table>
<thead>
<tr>
<th>Year</th>
<th>No. cases</th>
<th>% confirmed</th>
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<tbody>
<tr>
<td>1997</td>
<td>4</td>
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<td>2001</td>
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<tr>
<td>2002</td>
<td>0</td>
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Proportion of West Nile Cases by Month of Onset
Bucuresti, 1996 - 2002

1996
- July 3%
- August 61%
- September 35%
- October 1%

n = 292

1997 - 2002
- July 17%
- August 67%
- September 17%

n = 12
The postepidemic period

- A national reference laboratory within Cantacuzino Institute is responsible for all confirmations in humans, and also for mosquito and bird studies.
- Community-based control programs using insecticides (including larvicide) was established since, at least in Bucuresti.
- The general public is yearly warned about the specific preventive measures against mosquitoes.
Essential elements for future epidemics

• The vector mosquitoes
  – chances that infected female mosquitoes survive over winter

• Susceptible vertebrate amplifying hosts (birds)
  – a high prevalence in resident birds could limit the transmission

• The virus itself

• A susceptible human population
The future, issues and responses

• Is the southeastern part of Romania neighboring the Danube River (and similar other countries) subject to become an endemic area of WNF? Is the Delta or other sites for migratory birds along the Danube river a place for enzootic foci?

• Serosurveys should be routinely conducted (like the ones from 1996) and sentinel places, at least, should be established (floods all around Europe and climate abnormalities can determine at any time good conditions for the development of mosquitoes populations in unexpected areas).

• Control measures against mosquitoes should be more carefully designed, implemented and evaluated, while being statutory regulated.