West Nile Virus Infections from a clinician’s point of view

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West Nile virus can cause a fatal neurological disease in humans.
- the virus can cause severe disease and death in horses

- However, approximately 80% of people who are infected will not show any symptoms

- West Nile virus is mainly transmitted through the bites of infected mosquitoes. Birds are the natural hosts of West Nile virus.

- Vaccines are available for use in horses but not yet available for people.
WNV INVASION

- 1937 Uganda
- 1960-90 Eastern Europe and Mediterranean region

- 1999: New York
  - next 7 years: North America
  - 24,213 eq
  - 23,975 hu (962 †)
  - 2012: Texas (286 †)

- 2003 Hungary
- 2010 Greece
  - 262 hu (35 †)
**WNV GENETIC LINEAGES**

**Genetic lineage 1**
- worldwide
- neuroinvasive

**Genetic lineage 2**
- Africa, Madagascar
- Europe
- 2008<neuroinvasive

Kutasi et al., 2011, JVIM
In Hungary: both lineages 1&2 are circulating, clinical disease only by lineage 2 strains
migratory routes of white storks coincides with lineage 2 WNV spread

routes of migratory birds between Africa and Europe
In Europe many flaviviruses are endemic
- West Nile
- Usutu
- Tick-borne encephalitis

or occasionally imported
- Dengue
- Yellow fever viruses
WNV, USUV AND TBE IN EUROPE

Mancini et al., Veterinaria Italiana 53(2):97-110
DOI: 10.12834/VetIt.114.933.4764.2

Beck et al., Int. J. Environ. Res. Public Health 2013, 10(11), 6049-6083; https://doi.org/10.3390/ijerph10116049
The amino acid sequence identity in the E-protein ranges from 40–44% for unrelated flaviviruses and 60–70% within closely related flaviviruses.

The extent and duration of cross-neutralization and even cross-protection is strongly dependent on the degree of amino acid similarity in the E-proteins (Beck et al. 2013).

Neutralizing antibodies have a critical role in the long-term protection from disease and their present measurement provides the best correlate of flavivirus immunity. The flavivirus envelope (E) protein, which is involved in host cell attachment and membrane fusion, is the major target of virus neutralizing antibodies (Ahlers and Goodman, 2018).
**WHY ARE SIMULTANEOUSLY CIRCULATING FLAVIVIRUSES PROBLEMATIC FOR A CLINICIAN?**

- **Disease:**
  - similar clinical symptoms: encephalomyelitis
  - West Nile Virus Neuroinvasive Disease (WNND)

- **Diagnostics:**
  - cross-reaction

- **Prevention:**
  - cross-protection: after vaccination or natural infection by different flaviviruses
  - antibody-dependent enhancement of infection (ADE)
    - presence of non-neutralizing antibodies or neutralizing antibodies at suboptimal concentrations can facilitate virus entry through Fc-receptor binding leading to increased infection instead of protection
INDEX CASE: 2007

- colic signs
- muscle twiching, weakness
- rapid deterioration: recumbency, coma

2018: lineage 2 WNND outbreak
16 horses
(Kutasi et al, 2011, JVIM)
HUNGARY: SEROSURVEY

Seropositivity IgG ELISA:

- **2011**: 25.71% (27 out of 105 samples positive)
- **2014**: 15.85% (13 out of 82 samples positive)
- **2018**: 66.52% (236 out of 357 samples positive)
- **2019**: 72.32% (81 out of 112 samples positive)

Number of samples and number of positive horses.
Distribution of West Nile virus infections among humans and outbreaks among equids in the EU
Transmission season 2018; latest data update 15 Nov 2018

<table>
<thead>
<tr>
<th>year</th>
<th>equine</th>
<th>human cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 HU</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>2016 HU</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>2017 HU</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>2018 HU</td>
<td>91</td>
<td>215</td>
</tr>
<tr>
<td>2018 EU</td>
<td>285</td>
<td>1503</td>
</tr>
</tbody>
</table>
Distribution of West Nile virus infections among humans and outbreaks among equids in the EU
Transmission season 2019; latest data update 5 Sep 2019

- Human cases, with or without outbreaks among equids
- Outbreaks among equids
- No reported cases
- Not included

Germany endemic
HUNGARY: HUMAN CASES

Incidence of human cases in Hungary (unpublished data); Department of Communicable Diseases Epidemiology and Infection Control; National Reference Laboratory for Viral Zoonoses; National Public Health Center, Budapest, Hungary
Incidence of equine cases in Hungary (unpublished data); arrows: first case and peak of incidence; WNND: west nile virus neuroinvasive disease
source: National Food Chain Safety Office Budapest, Hungary and University of Veterinary Medicine Budapest, Hungary


**SEASONALITY**

- Between June and November?

- Clinician’s point of view: seasonality defines vaccination schedules and helps in diagnostics

<table>
<thead>
<tr>
<th>year</th>
<th>first case day</th>
<th>week</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>26th August</td>
<td>35.</td>
</tr>
<tr>
<td>2016</td>
<td>2nd August</td>
<td>31.</td>
</tr>
</tbody>
</table>

Forrás: European Center for Disease Prevention and Controll (ECDC)
www.ecdc.europa.eu
SEASONALITY

- mosquito population and viral load
  - warm weather – increase in viral load
  - warm and wet – increase in mosquito number

- August:
  - change in the behavior of birds
  - migratory birds leave

Climate change!!!
Lineage 1 (France and Spain) and Lineage 2 (Hungary)

Seasonality is less strict

Cases: cumulative ratio, %

1st June to second half of August and before end of October
OUTBREAKS FROM TIME TO TIME

- overwintering or reintroduction
- new strains
- new vectors, vector number and activity
- wheather
- seroprevalence, vaccination, rate of immunity in the population

Outbreaks: non predictable, „disappears” for a while

propensity for vaccination decreases, awareness lost
TRANSMISSION CYCLE

The Carrier (Culex p.)

The Virus

amplifier
reservoir
distributor

Incidental hosts

Image: Purdue U.
humans
- 80% subclinical
- 10-20% west nile fever
- 1% WNND

horses
- more sensitive
- west nile fever?????
- 10% WNND

Clinical manifestation:
- strain: neuroinvasive
- individual factors: age, genetics, concurrent disorders
- presence of antibodies against other flaviviruses
**Pathogenesis**

- Peripheral inoculation
- Replication in skin dendritic cells, cells migrate to lymph nodes
- Primary viraemia, infection of peripheral tissues
- Cleared or CNS (hematogenous, Trojan horse, retrograde axonal)
- Incubation period:
  - 2-14 days
# Clinical Signs

<table>
<thead>
<tr>
<th>Primary Symptoms</th>
<th>Percentage of Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethargy</td>
<td>65.2 %</td>
</tr>
<tr>
<td>Fever</td>
<td>47.8 %</td>
</tr>
<tr>
<td>Loss of Appetite</td>
<td>39.1 %</td>
</tr>
<tr>
<td>Colic Symptoms</td>
<td>15.2 %</td>
</tr>
<tr>
<td>Lameness</td>
<td>13.5 %</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td>Percentage of occurrence</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Ataxia</td>
<td>80.4%</td>
</tr>
<tr>
<td>Hind limb ataxia</td>
<td>56.8%</td>
</tr>
<tr>
<td>Forelimb ataxia</td>
<td>10.8%</td>
</tr>
<tr>
<td>All 4 limb ataxia</td>
<td>32.4%</td>
</tr>
<tr>
<td>Weakness</td>
<td>50.0%</td>
</tr>
<tr>
<td>Muscle fasciculation</td>
<td>47.8%</td>
</tr>
<tr>
<td>Recumbency</td>
<td>41.3%</td>
</tr>
<tr>
<td>Hyperaesthesia</td>
<td>37.2%</td>
</tr>
<tr>
<td>Muscle rigidity</td>
<td>26.1%</td>
</tr>
<tr>
<td>Abnormal behavior (aggressivity, obtunded, comatose)</td>
<td>26.1%</td>
</tr>
<tr>
<td>N. Facialis paralysis</td>
<td>15.2%</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>8.7%</td>
</tr>
</tbody>
</table>
OUTCOME AND SURVIVAL RATE OF EQUINE WNND

- 2008: 70% survival rate
  - 30% residual neurologic signs
- 2016: 77% survival rate
- 2018: 80% survival rate
LABORATORY PARAMETERS

- **Haematology**: +/- increased white blood cell count & neutrophilia
- **Biochemical parameters**: non diagnostic
CSF SAMPLING

- high protein +/- pleocytosis with lymphocytes or neutrophils
- inflammatory protein, *glucose*, lactate, ALP
- timing of sampling, age of patient, presence of meningitis, genetics-immunology, severity of inflammation (tissue damage)

Never do this!
Differential Diagnosis

Colic → Nervous system → Lamness

- Flaviviruses: Usutu, TBE
- Wobbler-syndrome (CVM)
- Trauma
- Equine herpesvirus encephalomyelitis
- Rabies
- Borna
- Verminous meningoencephalomyelitis, parasitic migration
- Bacterial meningitis
- EDM, NAD
- Botulism
- ..................
DIAGNOSTICS

- viraemia
- viral RNA
- CNS signs
- IgM ab
- IgG ab
- infection
- onset of fever
- disease 3-7 days
- 2-4 months
- > 1 year
DIAGNOSING WNV (OR USUTU OR TBE)

season + horses showing neurological symptoms + serology

- serology
  - IgM ELISA
    - acute infection
    - less cross-reaction
  - IgG ELISA
    - strong cross-reaction!
    - serosurvey
- VNTs
  - gold standard: paired samples
  - less sensitive, more specific, BSL3, time consuming
  - comparative VNTs that include every flavivirus suspected to circulate within a given area
  - length of protection after natural infection
POSITIVE IgM ELISA IS NOT A DIAGNOSIS

- IgM means recent infection only (e.g., a horse with traumatised spinal cord can be bitten by a WNV transmitting mosquito)

- IgM sometimes can be detected after vaccination, vaccination history should be clear (Joó et al, 2017)
SEASON + NEUROLOGIC SIGNS + NEGATIVE IG M?

- in season, non vaccinated, typical clinical signs and clinical progression (other tests negative)

- negative on IgM Elisa
  - repeat **IgM Elisa**: some days later positive – slow seroconversion
  - **IgG Elisa**: positive - previous infection by WNV or another Flavivirus
  - **VN (WNV + USUTU + TBE)**: positive (pairs of sera) - comparison of titres
  - If negative then ???????????????????????????????
DEFINITIVE DIAGNOSIS OF WNV

- **Antemortem**: PCR, virus isolation (PBLC, CSF, urine) – NO!
- **Postmortem**: histopathology + immunohistochemistry, PCR, virus isolation

The routine diagnostic techniques in most of the European public health and veterinary laboratories are designed to detect lineage 1 WNV strains. In a recent PCR external quality assurance multicenter test, <40% of the involved laboratories could detect lineage 2 WNV strains. (Bakonyi et al, 2006)
HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

neuron death
HE 400*

lympho-histiocytic perivascular infiltration
gliosis, HE400*

WNV Ag conglomerates
JH400*
REAL-TIME PCR

- spinal cord (1)
- med. oblongata (2)
- pons (3)
- cerebellum (4)
- cortex (5)
- control
- control

DNA marker
TREATMENTS

NO SPECIFIC TREATMENT, SUPPORTIVE CARE AS EARLY AS POSSIBLE

- Antiinflammatories
  - Flunixin 1.1 mg/ttkg 12 q12 h iv.
  - dexamethasone ??? 0.05–0.1 mg/ttkg q24 h IV

- Sedation
  - acepromazine
  - seizures: phenobarbital

- Vitamin E 6,000 NE PO sid and vitamine C 10-15 gramm/day

- B vitamine (B1) complex in recovery phase

- DMSO 10% solution (5% dextrose, or Salsol), 1 mg/ttkg 3 days

- Antiviral agents and else: interferon, ribavirin, angotensin II….

- Hyperimmune plasma (2-4l)

- Antibiotics

- Sling, art. feeding, infusion therapy, eye protection, etc.
PREVENTION

- Management strategies to reduce exposure to mosquitos
- Vaccination
PREVENTION: VACCINE

- Equip WNV, Zoetis
  - inactivated
- Equilis West Nile, MSD
  - inactivated chimeric flavivirus
- Proteq West Nile, Boehringer Ingelheim
  - recombinant of canarypox virus

...but there is vaccine against WNV

There is no vaccine against stupidity.

—Albert Einstein—
VACCINE

- When to vaccinate?......in endemic areas what we suggest: double primovaccination, then 6 months booster, then yearly booster
- Should we vaccinate during an outbreak?.....better than nothing
- Vaccinate after natural infection?....no need (???)
- Length of immunity?.......after natural infection and after vaccination?
- Lineage 1 vaccines....cross protection
REDUCE EXPOSURE TO MOSQUITOS

BEST WAY TO PREVENT MOSQUITO BITES UNDER NORMAL CIRCUMSTANCES.

BEST WAY TO PREVENT MOSQUITO BITES WHEN WEST NILE VIRUS IS PRESENT.

One bite. One life changed forever. Protect yourself.

West Nile Virus can strike anyone of any age. And one out of five who are infected will suffer a debilitating illness that can last a lifetime. Worse yet, some will actually lose their life. Take action and protect yourself all summer.

Remember the four Ds:
• Use DEET-enhanced insect repellent. (2% soy-based, organic products also available.)
• DRESS in long sleeves and pants.
• Avoid the outdoors from DUSK to DAWN.
• DRAIN standing water outside your home.

For more information call the West Nile Virus Hotline at 303.441.1460. Or visit www.bouldercountymosquito.net.
IS WNV A SIGNIFICANT HUMAN HEALTH PROBLEM IN THE EU?

  - „…underrecognition or underreporting of TT-WNV cases contribute to the present situation”

- To prevent transfusion-transmitted WNV infections, EU/EEA countries should implement 28-day blood donor deferral or individual donation nucleic acid testing (ID-NAT) of prospective donors who have visited or live in an affected area.
THANK YOU FOR YOUR ATTENTION!

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