Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON
Arboviruses: Update and Review

Dave Warshauer, PhD, D(ABMM)
Deputy Director, Communicable Diseases
Wisconsin State Laboratory of Hygiene
Objectives

- Describe the arboviruses that cause disease in the US and Wisconsin
- Describe the epidemiology of the arboviruses
- Describe arbovirus laboratory diagnosis
- Describe the arbovirus surveillance activities in Wisconsin
## Medically Important Arboviruses in the United States

<table>
<thead>
<tr>
<th>Family/Genus</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Togaviridae/Alphavirus</td>
<td>Eastern equine encephalitis</td>
</tr>
<tr>
<td>ss + RNA +; 70 nm particle</td>
<td>Western equine encephalitis</td>
</tr>
<tr>
<td></td>
<td>Venezuelan equine encephalitis</td>
</tr>
<tr>
<td>Flaviviridae/Flavivirus</td>
<td>St. Louis encephalitis</td>
</tr>
<tr>
<td>ss + RNA; 40-60 nm particle</td>
<td>Powassan</td>
</tr>
<tr>
<td></td>
<td>West Nile</td>
</tr>
<tr>
<td></td>
<td>Dengue</td>
</tr>
<tr>
<td>Bunyaviridae/Bunyavirus</td>
<td>California encephalitis</td>
</tr>
<tr>
<td>California serogroup</td>
<td>La Crosse encephalitis</td>
</tr>
<tr>
<td>ss -RNA; 3 segment genome</td>
<td>Jamestown Canyon</td>
</tr>
<tr>
<td></td>
<td>Snowshoe hare</td>
</tr>
<tr>
<td></td>
<td>Cache Valley (bunyamwera)</td>
</tr>
<tr>
<td>Reoviridae/Coltivirus</td>
<td>Colorado tick fever</td>
</tr>
<tr>
<td>ds RNA</td>
<td></td>
</tr>
</tbody>
</table>
Reported Arboviral Diseases, Wisconsin, 2007 - 2014 (N= 186)
Total Tickborne Cases in Wisconsin, 2009-2014 (n=3,550)

Anaplasma  E. chaffeensis  EML  Powassan  Babesia

Year of Illnes Onset

Number of Cases

<table>
<thead>
<tr>
<th>Year of Illnes Onset</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma</td>
<td>281</td>
<td>499</td>
<td>697</td>
<td>517</td>
<td>619</td>
<td>477</td>
</tr>
<tr>
<td>E. chaffeensis</td>
<td>34</td>
<td>17</td>
<td>31</td>
<td>10</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>EML</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Powassan</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Babesia</td>
<td>3</td>
<td>12</td>
<td>39</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>
Factors that Affect Arbovirus Incidence

- Weather
  - Temp and precipitation
- Zoonotic host and vector abundance
- Human behavior
  - Repellent use, outdoor activities
  - Use of air conditioning or screens
Clinical Manifestations

- 2-15 day incubation
- Usually mild and nonspecific
  - Headache,
  - Fever,
  - Fatigue
  - Muscle aches
  - Swollen lymph nodes
- Neuroinvasive disease
  - Flaccid paralysis
  - Encephalitis
  - Meningitis
Bush Sends Troops To West Nile

WASHINGTON, DC—Vowing to "exact justice for the taking of innocent American lives," a determined and defiant President Bush deployed more than 14,000 ground troops to the West Nile Monday.

"My fellow Americans, an enemy from overseas has attacked us in our own land, waging biological warfare against us on our home soil," Bush said in a nationally televised speech from the Oval Office. "We must send a strong message to our enemies in the West Nile region that this virulent aggression against America will not go unpunished; it will not stand."

Bush's decision to deploy troops came on the heels of three more West Nile virus deaths over the weekend—one in Louisiana and two in Illinois—bringing the national death toll to 51.

"These cowards want to bring down our very way of life," Bush said. "They have sought to rob us of our ability to leave the house without repellent. But what they did not count on is the tremendous spirit and resolve of the American people. No one, be they man or mosquito, will dictate what we put or don't put on our skin for protection."

Armed with anti-mosquito munitions, American Special Forces made landfall at Damietta near the mouth of the Nile early Tuesday, and by dawn had erected U.S. Army netting over the city. Bush promised that the netting, expected to extend all the way to Khartoum by the end of the week, will eventually stretch nearly 1,000 miles to the Nile's source and "as far to the west as necessary."

"The United States will not stand idly by while people or insects who despise everything we stand for develop weapons of mass infection," Bush said. "The only way to fight a pestilence such as this is to attack it right where it breeds—in this case, the lands to the west of the Nile River."

Though not made public until Monday, Operation
Clinical Manifestations

- WNV
  - 80% experience no symptoms
  - 20% relatively mild illness (WNV fever)
  - <1% (approx 1:150) seriously ill
    - Neuroinvasive disease
    - High fever
    - Neck stiffness
    - Extreme muscle weakness
    - Disorientation
    - Tremors, convulsions, disorientation
  - 10% mortality
Previous WNV Outbreaks/Isolations

- 1937  West Nile, Uganda
- 1951-54, 57  Israel
- 1962  France
- 1974  South Africa
- 1996  Romania
- 1999  Russia
- 1999-2000  USA, Israel
- 2002  Canada
Spread of WN Virus in the US

1999

2000

2001

2002

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Wisconsin Counties with West Nile Virus in 2001

Wisconsin Counties with West Nile Virus in 2002

LEGEND

- Counties with WNV positive birds or horses
- Counties with WNV positive people

DHFS, Division of Public Health
West Nile Virus – Transmission Cycle

Transmission Cycle

Mosquito vector

Reservoir hosts

Incidental infections
West Nile Virus-Human Infections

Novel Modes of Transmission

Transplantation

Transfusion

Breastfeeding

Transplacental transmission

Occupational exposure
West Nile Virus In Wisconsin

WNV Dead Bird Surveillance

- Sensitive indicator of viral activity in the environment
- Monitor the spread of the virus
- Crudely estimates intensity of epizootic
- Does not predict human risk
Surveillance
Crows Ideal Sentinels

- Widely distributed
- Found in multiple settings
- Highly susceptible
- Mortality > 90%
- Virus titers in tissues high enough to permit delayed testing
Avian Surveillance

Crows:
- Reported by residents
- Collected by LHD
- Tested at WVDL
West Nile Virus - Mosquitoes

175 mosquito species found in the U.S.

Over 50 species of mosquitoes in Wisconsin

Not all of them bite people

Only female mosquitoes seek blood meals

Very few mosquitoes are infected with virus

- Typically <1% mosquitoes of any species found with virus
## CDC Tests for WNV

<table>
<thead>
<tr>
<th>Specimen</th>
<th>1st Choice</th>
<th>Other</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum/CSF</td>
<td>IgM, IgG ELISA</td>
<td>NAAT</td>
<td>NAAT (57%) for acute CSF; &lt;10% serum</td>
</tr>
<tr>
<td></td>
<td>Plaque Reduction</td>
<td>Virus Isolation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neutralization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human tissue</td>
<td>NAAT</td>
<td>Virus Isolation</td>
<td>Fatal WN cases: NAAT positive ~ 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IHC</td>
<td></td>
</tr>
<tr>
<td>Non-Human</td>
<td>1st Choice</td>
<td>2nd Choice</td>
<td></td>
</tr>
<tr>
<td>Avian tissue</td>
<td>NAAT</td>
<td>VecTest Ag. Cap. ELISA</td>
<td>Ag.-based tests require 1000 pfu</td>
</tr>
<tr>
<td></td>
<td>Virus isolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosquito pool</td>
<td>NAAT</td>
<td>VecTestAg. Cap. ELISA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Virus isolation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DAYS POST ONSET

-5 | -4 | -3 | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10

#pfu/ml

IgM

IgG

WN viremia

illness

ELISA P/N

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IgM Capture ELISA

1. Coat With Goat anti-Human IgM
   ➢ 4° Overnight

2. Add Patient Serum @ 1:400
   ➢ 37° 1 Hour

3. Add West Nile Recombinant Antigen
   ➢ 4° Overnight

4. Add HRP anti-Flavivirus McAb
   ➢ 37° 1 Hour
Interpretation of Results

P/N > 3 = positive
P/N < 2 = negative
P/N 2-3 = equivocal

*ELISA Assay must be standardized in each lab*
### Flavivirus Cross-reactivities of IgM from WN Patient Serum*

<table>
<thead>
<tr>
<th>Serum</th>
<th>SLE</th>
<th>JE</th>
<th>WN</th>
<th>DEN2</th>
<th>YF</th>
<th>POW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.96</td>
<td>7.75</td>
<td>16.74</td>
<td>2.45</td>
<td>1.82</td>
<td>1.56</td>
</tr>
<tr>
<td>2</td>
<td>4.8</td>
<td>13.77</td>
<td>16.68</td>
<td>4.13</td>
<td>2.14</td>
<td>1.75</td>
</tr>
<tr>
<td>3</td>
<td>5.45</td>
<td>9.67</td>
<td>16.08</td>
<td>4.09</td>
<td>1.61</td>
<td>1.44</td>
</tr>
<tr>
<td>4</td>
<td>4.76</td>
<td>10.07</td>
<td>17.19</td>
<td>3.32</td>
<td>1.62</td>
<td>1.3</td>
</tr>
<tr>
<td>Positive Control</td>
<td>6.5</td>
<td>8.2</td>
<td>6.34</td>
<td>7.45</td>
<td>3.96</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* 1:400 screening dilution
Additional/Confirmatory Testing

- Plaque-reduction neutralization assay (PRNT)
- Microsphere immunoassay (MIA)
  - SLE/WNV
  - BioPlex instrument
**WN Serological Data**

*Typical Human WN Case*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days</th>
<th>IgM P/N</th>
<th>IgG P/N</th>
<th>PRNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>post-onset</td>
<td>WN</td>
<td>SLE</td>
<td>WN</td>
</tr>
<tr>
<td>Typical WN Case</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acute serum</td>
<td>8</td>
<td>12.75</td>
<td>4.00</td>
<td>1.37</td>
</tr>
<tr>
<td>conv. serum</td>
<td>31</td>
<td>11.35</td>
<td>4.21</td>
<td>6.38</td>
</tr>
</tbody>
</table>

In primary flavivirus infections;

- *Martin et al 2002*: IgM P/N to WN is 3-5X greater than SLE.
- *2002 data*: Use 2X criteria WN to SLE ratio: only 1 exception in 417 WN confirmed cases.
Longevity of Human WN Virus-Reactive IgM in Serum

<table>
<thead>
<tr>
<th>Days P.I.</th>
<th>N</th>
<th>Positive MAC-ELISA (%)</th>
<th>Equivocal (%)</th>
<th>Total (%)</th>
<th>Ave. P/N (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>22</td>
<td>13 (60)</td>
<td>4</td>
<td>17 (77)</td>
<td>6.0 (3.0-10.8)</td>
</tr>
<tr>
<td>300-400</td>
<td>21</td>
<td>9 (43)</td>
<td>2</td>
<td>11 (52)</td>
<td>4.0 (31.0-6.5)</td>
</tr>
<tr>
<td>500</td>
<td>12</td>
<td>5 (42)</td>
<td>2</td>
<td>6 (60)</td>
<td>5.0 (3.1-6.9)</td>
</tr>
</tbody>
</table>
CDC IgM ELISA Assay

Good Points

- Sensitive
- Relatively Specific (WN & SLE P/N ratio)
- Technology Transferable

Bad Points

- Cross-reactivity among flaviviruses
- Limited utility in secondary infections
- Two day test
- Technically complex
- IgM persistence
Serological Testing Algorithm for West Nile Virus

National Case Definition
Confirmed:
IgM pos csf
IgM pos serum + PRNT
>4-fold increase PRNT titer

IgM ELISA WN & SLE

POS

NEG

Plaque reduction
Neutralization test (PRNT) with:
SLE, WN, (other flaviviruses)

STOP
### WN Human Serological Data

*Lessons Learned 1999-2002*

- IgM Detectable in serum & csf by onset (99%)
  - 6 exceptions------ serum from 800 cases
  - 10 exceptions------ csf from 800
- IgG Positive by day 7 Post-Onset
- P/N 3-5X Higher to WN than SLE
- IgM Persistence > 1 Year
- Secondary Flavivirus Infections are Problematic
WNV-TO DO LIST

- Effective therapies
- Vaccine development
- Methods of vector control
- Basic research on the virus
- Development of commercial diagnostic tests that can be used in the clinical laboratory
  - Focus Laboratories FDA approved IgM IgG
  - Other commercial lab LDT assays
Powassan Virus

- Two types
  - Lineage 1 POW
    - Associated with *Ixodes cookei* or *I. marxi*
  - Lineage 2 (Deer Tick Virus) POW
    - Associated with *I. scapularis*
  - Both linked to human disease
Powassan Virus Transmission

- Maintained in a cycle between ticks and small-to-medium-sized rodents
  - I. cookei-----woodchucks
  - I. marxi-----squirrels
Ixodes scapularis (Blacklegged Tick)
Powassan Clinical Manifestations

- Incubation period 1-4 weeks
- Many people asymptomatic
- Fever, headache, vomiting, weakness, confusion, loss of coordination, speech difficulties, and seizures
- Encephalitis and meningitis
- 50% with permanent neurological symptoms
- 10% fatality rate
Powassan virus neuroinvasive disease cases reported 2004-2013
Powassan virus neuroinvasive disease cases reported by year, 2004–2013

Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention

Data Table: In the United States, the number of Powassan virus neuroinvasive disease cases reported each year varies. From 2004 through 2013, an average of 6 cases were reported annually (range 1–12).
Powassan Virus 2014

Pos Tests
Mass 4
NJ 1
NY 1
WI 2
Powassan Diagnosis

- Clinical features, activities, epidemiologic history of the location where infection likely occurred

- Laboratory Diagnosis—in fatal cases
  - Nucleic acid amplification
  - Histopathology w/ immunohistochemistry
  - Virus Culture

- Routine testing
  - IgM capture ELISA or MIA
  - IgG ELISA
Powassan Diagnosis

- CSF findings
  - Lymphocytic pleocytosis
    - Usually <500 WBCs/mm³
  - Granulocytes can predominate early in disease
  - Protein normal and mildly elevated
  - Glucose normal

- MRI brain scan
  - Changes consistent with microvascular ischemia or demyelinating disease in the parietal or temporal lobes
La Crosse 2014
EEE Virus 2014
Jamestown Canyon Virus

- California serogroup
- Wide distribution in North America
- Initially described in the early 1970s to cause mild human febrile disease
- Affects adults and more likely to cause meningitis
- Seroprevalence of up to 12% in NY and CT
- Retrospective studies shows JCV under-diagnosed
  - 1971-1981-----41/53 patients had antibody to JCV
- Reports are rare
- Became reportable in US in 2004
- Circulates primarily between deer and mosquitoes
Arbovirus Surveillance in Wisconsin

- Bird surveillance
  - Corvids---crow, blue jays, and ravens
    - Report all sick and dead corvids for WNV testing
      - Dead-bird hotline 800-433-1610

- Equine WNV surveillance
  - WVDL reports positive results to DPH
Arbovirus Surveillance in Wisconsin

- **Human Surveillance**
  - **Diagnostic testing at WSLH**
    - Panel—LAC, EEE, WNV, SLE,
      - POW, JC to CDC when requested by DPH
      - Also, consider Enterovirus
  - **Fee-exempt testing for patients who meet criteria**
    - Confirmatory testing of positive results from other labs
    - Patient >65 yr with CNS disease with no other Dx
    - Diagnosis of Guillain-Barre and no other lab Dx.
    - Request of LHD
  - **Fee-for-service available for patients who do not meet criteria**

- https://www.dhs.wisconsin.gov/arboviral/westnilevirus.htm
Thank you!