

### Wisconsin State Laboratory of Hygiene UNIVERSITY OF WISCONSIN-MADISON



# Arboviruses: Update and Review

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- 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

Family/Genus	Pathogens
Togaviridae/Alphavirus	Eastern equine encephalitis
ss + RNA +; 70 nm particle	Western equine encephalitis
	Venezuelan equine encephalitis
Flaviviridae/ <mark>Flavivirus</mark>	St. Louis encephalitis
ss + RNA; 40-60 nm particle	Powassan
	West Nile
	Dengue
Bunyaviridae/ <mark>Bunyavirus</mark>	
California serogroup	California encephalitis
ss -RNA; 3 segment genome	La Crosse encephalitis
	Jamestown Canyon
	Snowshoe hare
	Cache Valley (bunyamwera)
Reoviridae/Coltivirus	Colorado tick fever
ds RNA	



### Reported Arboviral Diseases, Wisconsin, 2007 - 2014 (N=186)



### Total Tickborne Cases in Wisconsin, 2009-2014 (n=3,550)



# 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

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VOLUME 38 ISSUE 34



AMERICA'S FINEST NEWS SOURCE" 18 SEPTEMBER 2002

Front Page News

### NATION **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."



Above: Bush outlines the details of Operation Deep Desert Off! to reporters.

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

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## **Clinical Manifestations**

### • WNV

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

# Previous WNV Keres Conturbed Stress Content of Content

- 1937 <u>West Nile</u>, Uganda
- 1951-54, 57 Israel
- 1962 France
- 1974 South Africa
- 1996 Romania
- 1999 Russia
- 1999-2000 USA, Israel
- 2002 Canada



### **West Nile Virus In Wisconsin**

### Wisconsin Counties with West Nile Virus in 2001



Wisconsin Counties with West Nile Virus in 2002



DHFS, Division of Public Health

### WNV Neuroinvasive Disease, 2013





### West Nile Virus – Transmission Cycle

**Transmission Cycle** 



### 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**



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











# **IgM Capture ELISA**

- Coat With Goat anti-Human IgM
  → 4° Overnight
- Add Patient Serum @ 1:400
  ▶ 37° 1 Hour

Add West Nile Recombinant Antigen
 ▶ 4° Overnight

4. Add HRP anti-Flavivirus McAb
 ▶ 37° 1 Hour



### **Interpretation of Results**

P/N: O.D. patient serum/O.D. negative control serum.

- 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\*

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

\* 1:400 screening dilution

CEDEC Centers for Disease Control and Prevention

# **Additional/Confirmatory Testing**

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





### Typical Human WN Case

	Days	IgN	I P/N	IgG P/N		PRNT	
Sample	post-onset	WN	SLE	WN	SLE	WN	SLE
Typical WN Case							
acute serum	8	12.75	4.00	1.37	2.04	1:80	1:20
conv. serum	31	11.35	4.21	6.38	5.76	1:1280	1:80

### 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							
Days	Ν	<b>Positive</b> MA	AC-ELISA	Total	Ave. P/N		
<b>P.I.</b>				(%)	(Range)		
		Positive (%)	Equivocal				
200	22	13 (60)	4	17 (77)	<b>6.0</b> (3.0-10.8)		
<b>300-</b> <b>400</b>	21	9 (43)	2	11 (52)	<b>4.0</b> (316.5)		
500	12	5 (42)	2	6 (60)	<b>5.0</b> (3.1-6.9)		
					CDC		

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# **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



# 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 smallto-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













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

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# **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</p>
  - 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 mosquiotoes



### 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



