


Arboviruses: Update and Review

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Objectives

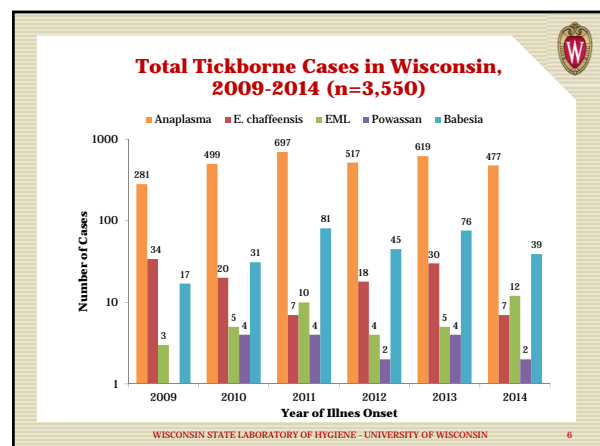
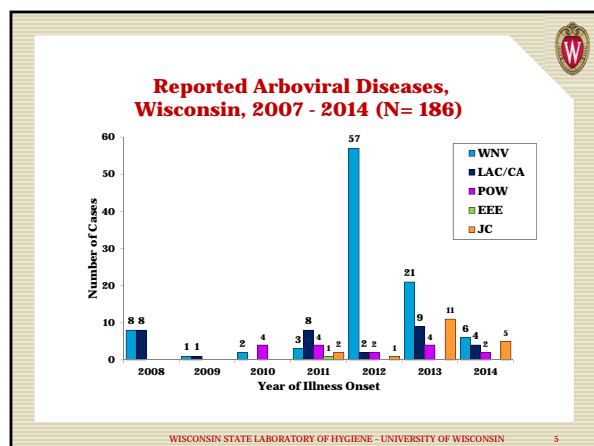
- Describe the arboviruses that cause disease in the US and Wisconsin
- Describe the epidemiology of the arboviruses
- Describe arbovirus laboratory diagnosis
- Describe the arbovirus surveillance activities in Wisconsin

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Medically Important Arboviruses in the United States

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

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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



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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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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

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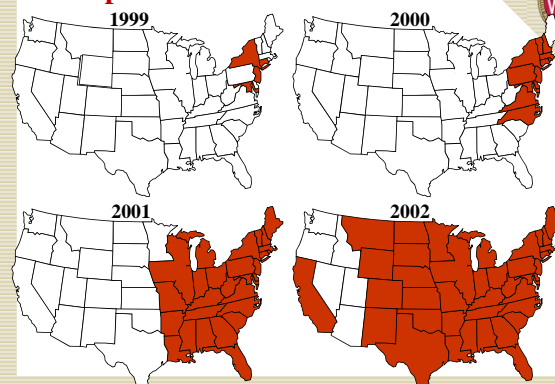
Previous WNV Outbreaks/Isolations

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

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Spread of WN Virus in the US

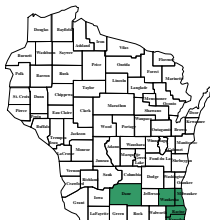


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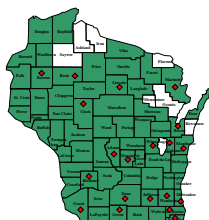
West Nile Virus In Wisconsin

Wisconsin Counties with West Nile Virus in 2001



LEGEND
■ Counties with WNV

Wisconsin Counties with West Nile Virus in 2002



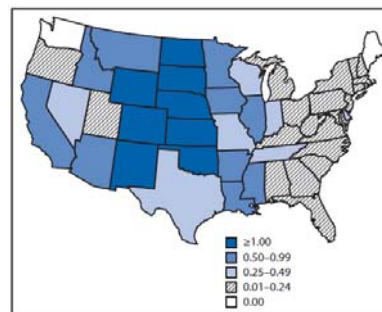
LEGEND
■ Counties with WNV positive
♦ Counties with WNV positive people

CDC, Division of Public Health

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WNV Neuroinvasive Disease, 2013

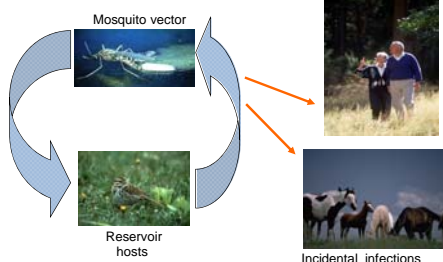


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West Nile Virus – Transmission Cycle

Transmission Cycle



CDC

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West Nile Virus-Human Infections Novel Modes of Transmission

Transplantation

Transfusion

Breastfeeding

Transplacental transmission

Occupational exposure

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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

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Surveillance Crows Ideal Sentinels

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



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Avian Surveillance

Crows:

- Reported by residents
- Collected by LHD
- Tested at WVDL



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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

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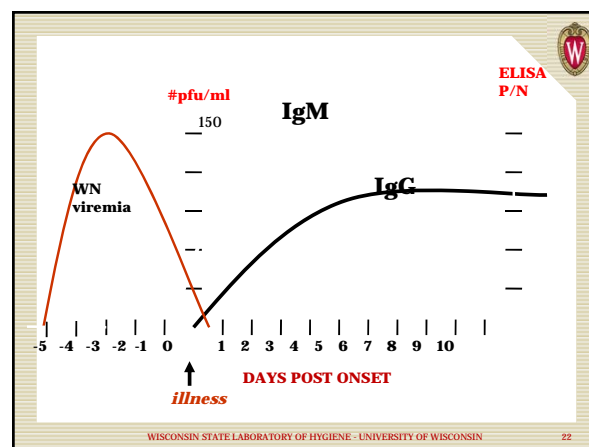
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CDC Tests for WNV

Specimen	1 st 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 st Choice	2 nd Choice	
Avian tissue	NAAT Virus isolation	VecTest Ag. Cap. ELISA	Ag.-based tests require 1000 pfu
Mosquito pool	NAAT Virus isolation	VecTest Ag. Cap. ELISA	

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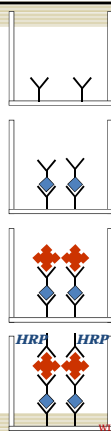
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IgM Capture ELISA



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

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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

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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	4.8	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

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Additional/Confirmatory Testing

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

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WN Serological Data

Typical Human WN Case

Sample	Days post-onset	IgM P/N		IgG P/N		PRNT	
		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.

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Longevity of Human WN Virus-Reactive IgM in Serum

Days P.I.	N	Positive MAC-ELISA		Total (%)	Ave. P/N (Range)
		Positive (%)	Equivocal		
200	22	13 (60)	4	17 (77)	6.0 (3.0-10.8)
300-400	21	9 (43)	2	11 (52)	4.0 (3.1-6.5)
500	12	5 (42)	2	6 (60)	5.0 (3.1-6.9)

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CDC IgM ELISA Assay

Good Points

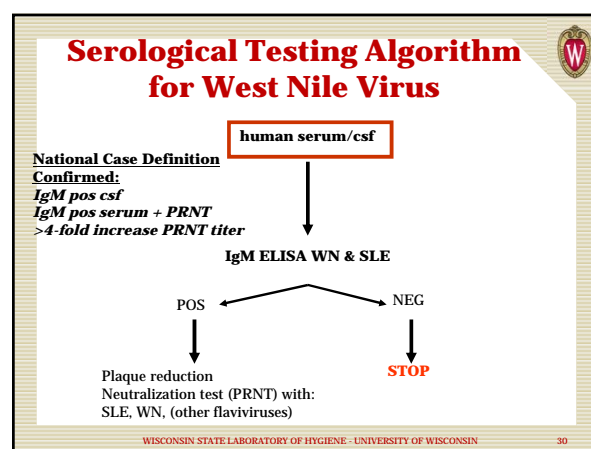
- Sensitive
- Relatively Specific (WN & SLE P/N ratio)
- Technology Transferable

Bad Points

- Cross-reactivity among flaviviruses
- Limited utility in secondary infections
- Two day test
- Technically complex
- IgM persistence

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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

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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

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POWASSAN



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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



I. cookei

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Powassan Virus Transmission

- Maintained in a cycle between ticks and small-to-medium-sized rodents
 - *I. cookei*-----woodchucks
 - *I. marxi*-----squirrels



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Ixodes scapularis (Blacklegged Tick)



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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

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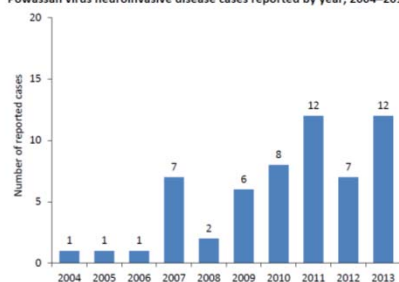
Powassan virus neuroinvasive disease cases reported 2004-2013



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Powassan virus neuroinvasive disease cases reported by year, 2004–2013



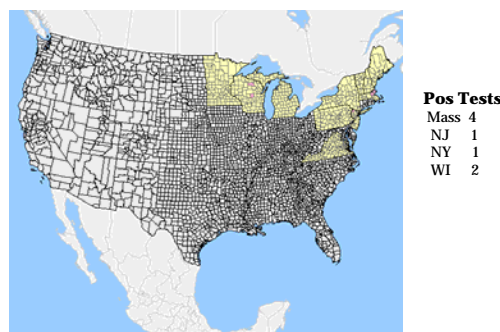
Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention

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).

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Powassan Virus 2014



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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

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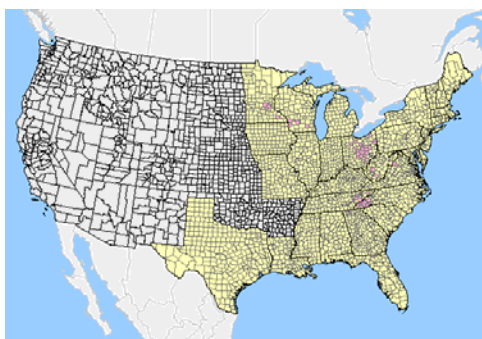
Powassan Diagnosis

- 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

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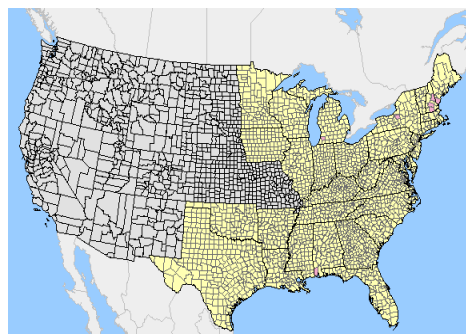
La Crosse 2014



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EEE Virus 2014



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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

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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



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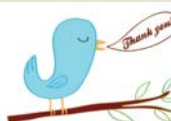
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Arbovirus Surveillance in Wisconsin

- 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>

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