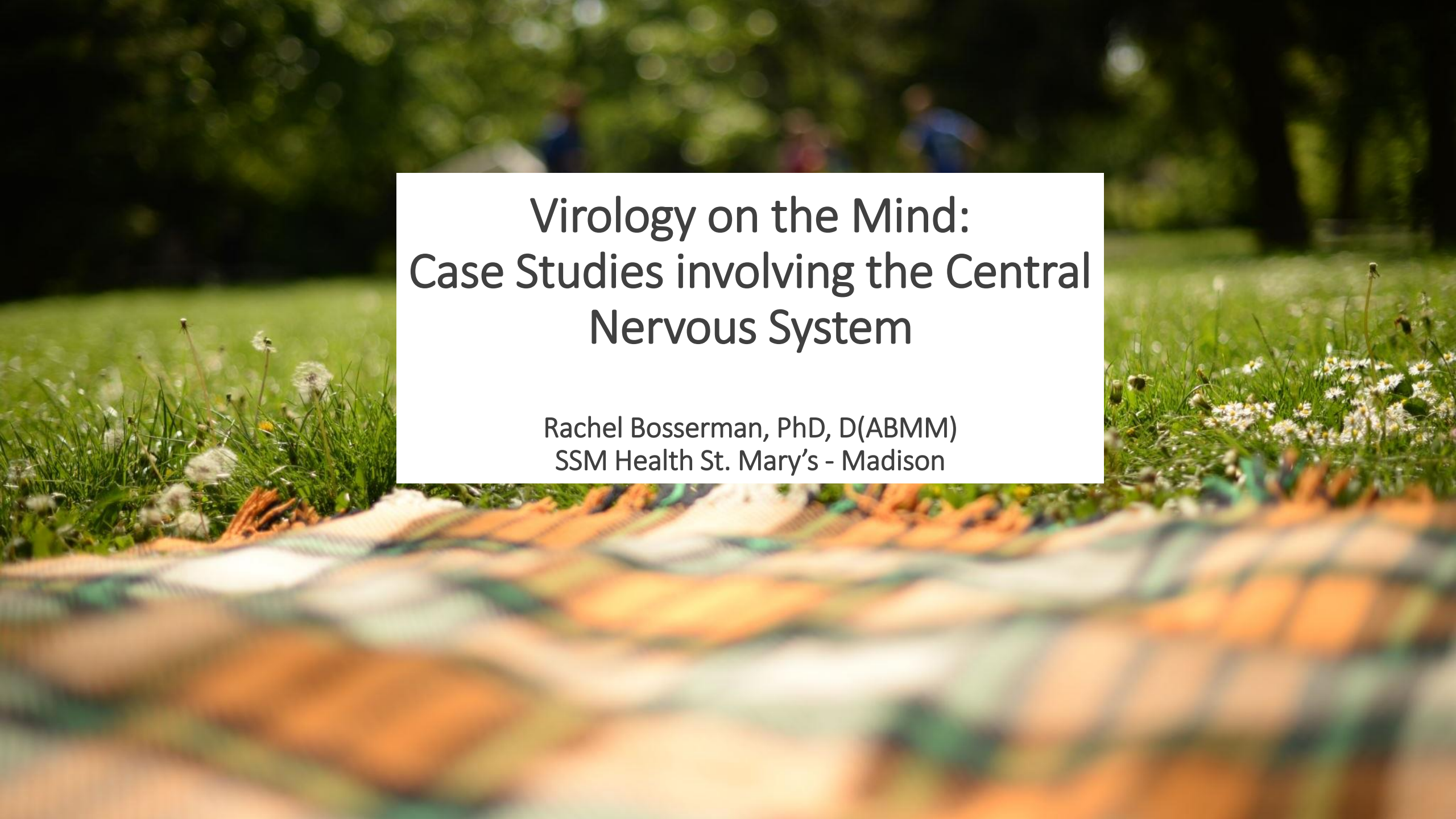


Virology on the Mind: Case Studies involving the Central Nervous System

Rachel Bosserman, PhD, D(ABMM)
SSM Health St. Mary's - Madison

Case #1 (Missouri)

- 52 y.o. male with past medical history of type 2 diabetes currently not taking any medications, presenting with multiple medical complaints in **mid August**.

- Developed nausea vomiting with “black diarrhea” about 1 week prior. Fevers began around this time as well

- For the past 3 days, he has been having **fevers**, generalized **malaise**, and epigastric **abdominal pain**.

- He states that he has had very dark stools, but no lightheadedness. He has also had intermittent **headaches and confusion**. Anorexia, weight loss, chills, night sweats.

Case #1, Continued

- He **denies international travel**, hiking, pet exposures. He gets his water from the city and has not tried new foods recently. He denies any tick bites.

- Denies periods of time being unhoused.

- Patient works in construction.

Core Lab Results

WBC 6.5K/cumm
Hgb 13.2 g/dL
Hct **38.1% (L)**
Plt 274 K/cumm

Sodium **134 (L)** mmol/L
Potassium, pl **5.6 (H)** mmol/L
Chloride 101 mmol/L
CO2 25 mmol/L
Anion gap 8 mmol/L
BUN 19 mg/dL
Creatinine **1.68 (H)** mg/dL
Glucose **206 (H)** mg/dL
Calcium 9.0 mg/dL

Imaging Summary

CT Chest, Abdomen, Pelvis Impression

No pulmonary embolus.

No acute abnormality within the chest, abdomen, or pelvis.

CT Head Impression

No acute intracranial process.

MRI Brain

Normal MRI of the brain

Microbiology Tests

- Blood culture: no growth
- Respiratory pathogen panel: all targets not detected
- RPR: nonreactive (checked for prozone)
- HepC Ab: nonreactive
- Rapid HIV, POC: negative
  - HIV ½ Ab + p24Ag: negative
- T-spot: negative
- Cryptosporidium/Giardia Ag: negative
- Stool culture: negative
- C. diff: negative

CSF Results

Opening pressure:
15cm

	CSF AND BODY FLUID
Tube 1	Tube Number, CSF
Colorless	Color, CSF
Clear	Clarity, CSF
Absent	Xanthochromia, CSF
95	Glucose, CSF
184 ▲	PROTEIN, TOTAL, CSF
4	Nucleated cells, CSF
4 ▲	RBC Count CSF
Test Compl...	Wright Stain

CSF Testing

- Bacterial culture and Gram stain- no growth, no organisms seen
- Cryptococcal Ag- negative
- Mycology culture – no growth

Clues from the CSF

	Normal	Bacterial
Opening pressure (cm CSF)	12-20	Raised
Appearance	Clear	Turbid/ Cloudy
CSF white cell count (cells/ uL)	<5	Raised (>100)
Predominant cell type	n/a	PMNs
CSF protein (g/L)	0.4 (40mg/dL)	Raised
CSF/ plasma glucose ratio	>2/3	Very low

	CSF AND BODY FLUID
Tube 1	Tube Number, CSF
Colorless	Color, CSF
Clear	Clarity, CSF
Absent	Xanthochromia, CSF
95	Glucose, CSF
184 ▲	PROTEIN, TOTAL, CSF
4	Nucleated cells, CSF
4 ▲	RBC Count CSF
Test Compl...	Wright Stain

Viruses?

Table modified from: Comprehensive Review of Infectious Diseases. 2020

Viral Causes of Encephalitis or Meningitis

Herpes Viruses	Arboviruses	Enteroviruses	Other
• HSV-1/2 • VZV • EBV • CMV • HHV-6	• West Nile • La Crosse • St. Louis encephalitis • EEEV, WEEV	• Poliovirus • Non-polio enteroviruses	• HIV • Measles • Mumps • Rabies

Encephalitis: inflammation of the brain parenchyma, associated with neurologic dysfunction (behavioral changes, altered consciousness, seizures, focal neurology)
Meningitis: inflammation of the meninges and subarachnoid space
Myelitis: inflammation of the spinal cord

Limited Amount of CSF Available: Pick One Test

Herpes Viruses	Arboviruses	Enteroviruses	Other
• HSV-1/2 • VZV • EBV • CMV • HHV-6	• West Nile • La Crosse • St. Louis encephalitis • EEEV, WEEV	• Poliovirus • Non-polio enteroviruses	• HIV • Measles • Mumps • Rabies

Limited Amount of CSF Available: Pick One Test

Per a study at BJH on >10,000 specimens: HSV, VZV, EV, and CMV testing on CSF is cancelled by a lab medicine resident if:

- Patient has <10 nucleated cells in the CSF AND patient is not immunocompromised

Herpes Viruses	Arboviruses	Enteroviruses	Other
• HSV • VZV • EBV • CMV • HHV-6	• West Nile • La Crosse • St. Louis encephalitis • EEEV, WEEV	• Poliovirus • Non-polio enteroviruses	• HIV • Measles • Mumps • Rabies

Wilen CB, et al. J Clin Microbiol. 2015 Mar;53(3):887-95. PMID: 25568435

Arbovirus Taxonomy

Flaviviridae/ Flavivirus	Togaviridae/ Alphavirus	Peribunyaviridae/ Orthobunyavirus
• West Nile • St. Louis Encephalitis • Powassan Virus • Dengue • Yellow Fever • Japanese Encephalitis	• Eastern Equine Encephalitis • Western Equine Encephaltiis • Venezuelan Equine Encephalitis • Chikungunya	• La Crosse virus • Jamestown Canyon Virus • Oropouche

Significant nucleic acid homology = significant serological cross-reactivity
Cross-reactive epitopes are often not cross-protective

Arboviruses

- Arthropod-borne viruses (aka Arboviruses)
- Transmitted by mosquitoes, ticks, sand flies, midges, and others
- **70+ different viruses are medically important**
- Most are enveloped RNA viruses-cannot survive outside of host for very long
- Capable of replication in both arthropod and mammalian cells

Manual of Clinical Microbiology, 13th edition. 2023

Transmission

Transmission via bite of infected arthropod

- Seasonal, depending on vector
- Can also have transmission from blood transfusion, organ donation, transplacental, sexual




Manual of Clinical Microbiology, 13th edition. 2023
Images: CDC

General Arboviral Symptoms

- Generally asymptomatic
- Incubation period ranges from a few days to 2 weeks
- Initial symptoms can include fever, gastrointestinal symptoms, and sometimes rash
- Can be neurotropic
  - Coma, meningitis, flaccid paralysis, encephalitis, microcephaly, Guillain Barre syndrome
  - Neuroinvasive disease is rare (<0.1%), can be higher in epidemic years
- Risk factors for severe infection: age, immunocompromised status

Manual of Clinical Microbiology, 13th edition. 2023

What arbovirus testing would you pick for this patient?

- Mayo Panel →
- West Nile Virus Testing

- Opted to prioritize the WNV testing

- Check ArboNet for information regarding potential outbreaks/ transmission

Test Id	Reporting Name
CAVPC	Calif(LaCrosse) Encep Ab Panel, CSF
EEPC	East Equine Enceph Ab Panel, CSF
STLPC	St. Louis Enceph Ab Panel, CSF
WEEPC	West Equine Enceph Ab Panel, CSF

Arboviruses in the US in 2022

Virus	#Cases	Median age (yrs)	Neuroinvasive	Mortality (neuroinv)	States
West Nile	**1132**	**63**	**73%**	**8%**	**42 states:** SD, CO, NE, CA, NY
La Crosse	22	9	86%	0	5 states: OH, WV, MN, TN, SC
Jamestown Canyon	12	60	92%	0	5 states: **WI**, RI, MI, MA, MN
Powassan	47 ↑	64	91%	15%	9 states: CT, ME, MA, VT, **WI**, MN, NJ, NY, PA
St. Louis Encephalitis	33 ↑	65	82%	9%	3 states: AZ, CA, TX

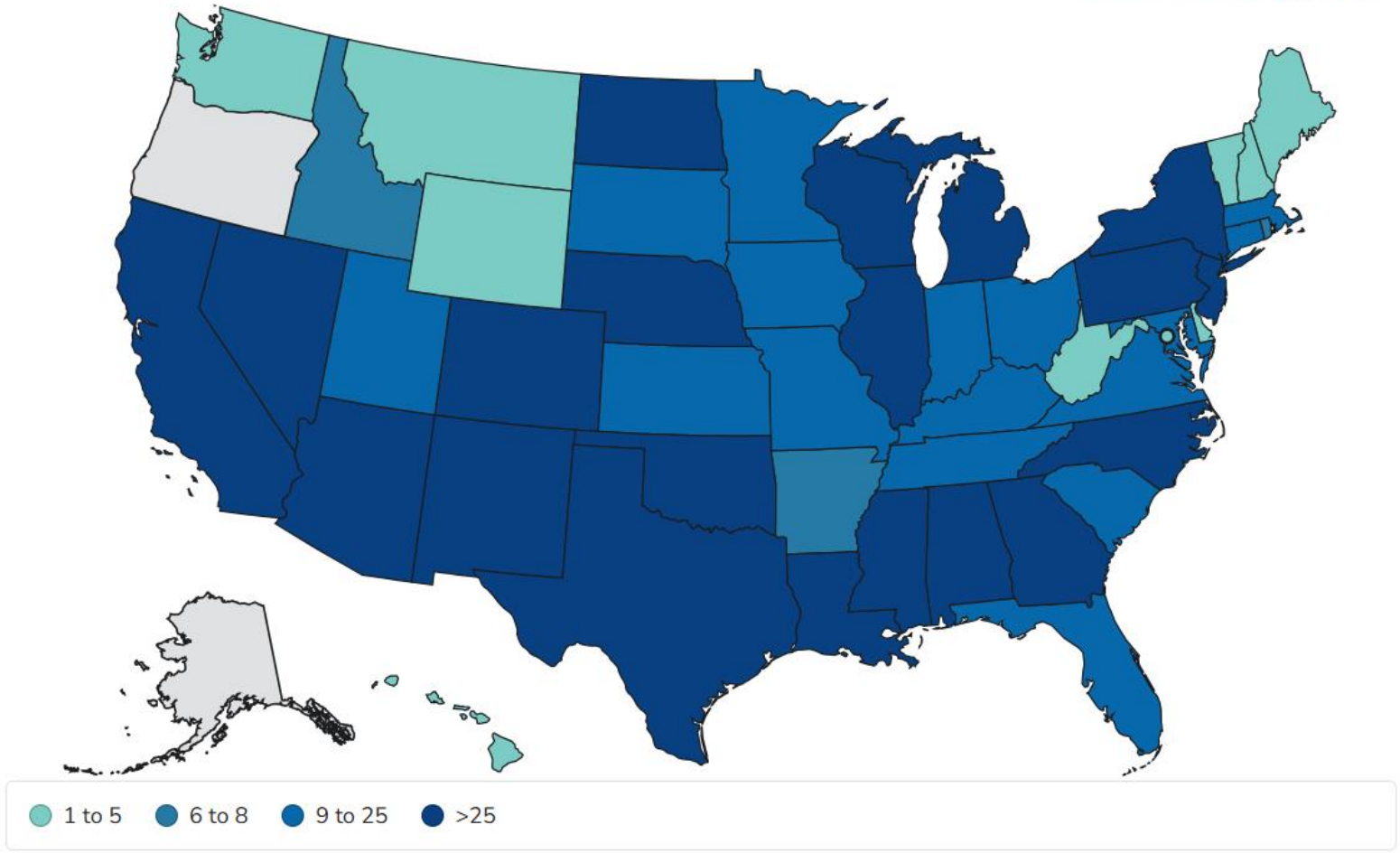
Numbers likely underestimate incidence and overestimate severity

Table modified from: Sutter RA, et al. MMWR Morb Mortal Wkly Rep. 2024 May 30;73(21):484-488. PMID: 38814815

WNV Cases in 2024

ArboNET
National Arbovirus Surveillance System

- 1466 Cases
  - 1063 Neuroinvasive
  - 49 States Reporting
  - WI: 34 Cases



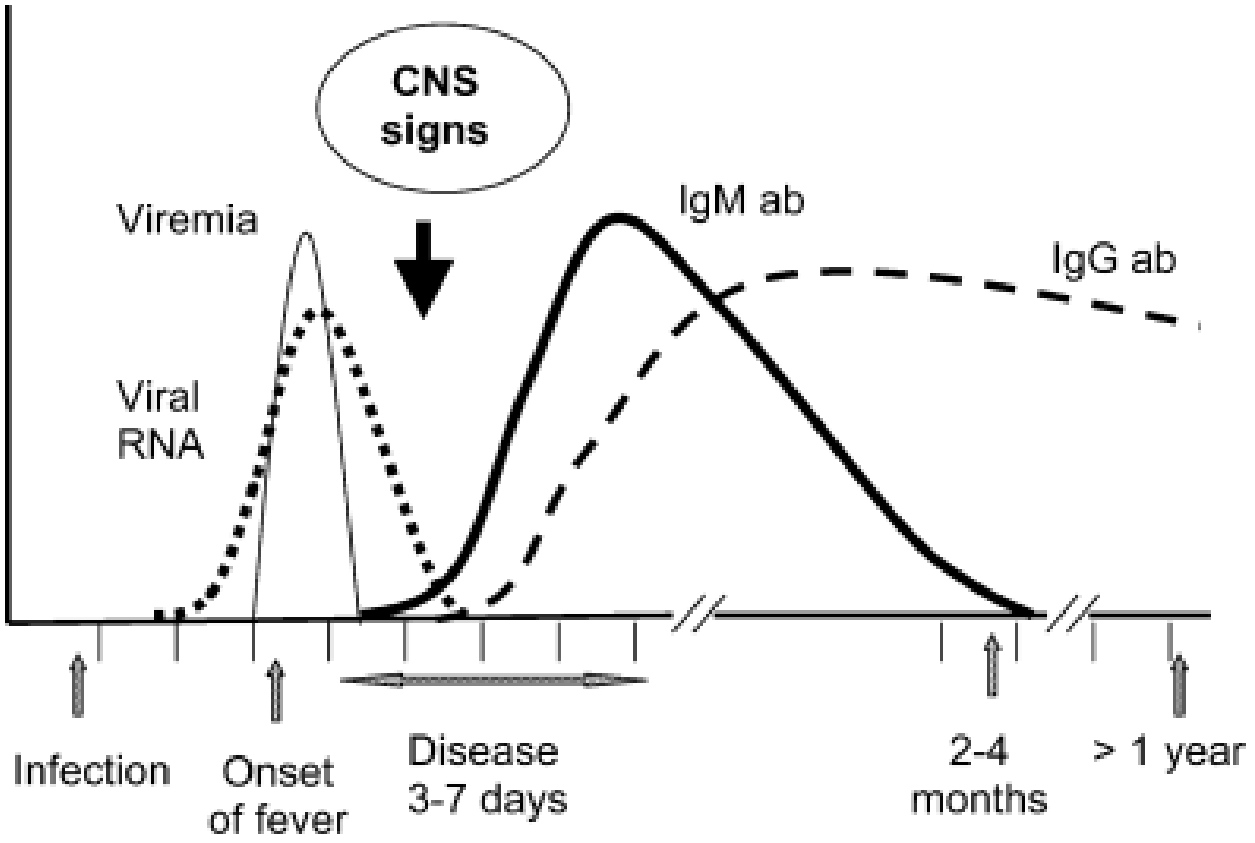
https://www.cdc.gov/west-nile-virus/data-maps/current-year-data.html

WNV
Testing Options

- PCR on CSF or Blood
- Serology IgM, IgG on Serum
- Serology IgM, IgG on CSF

Nucleic Acid Testing- specific, but short duration

- More specific
- Viremia particularly poor option for neurotropic arboviruses

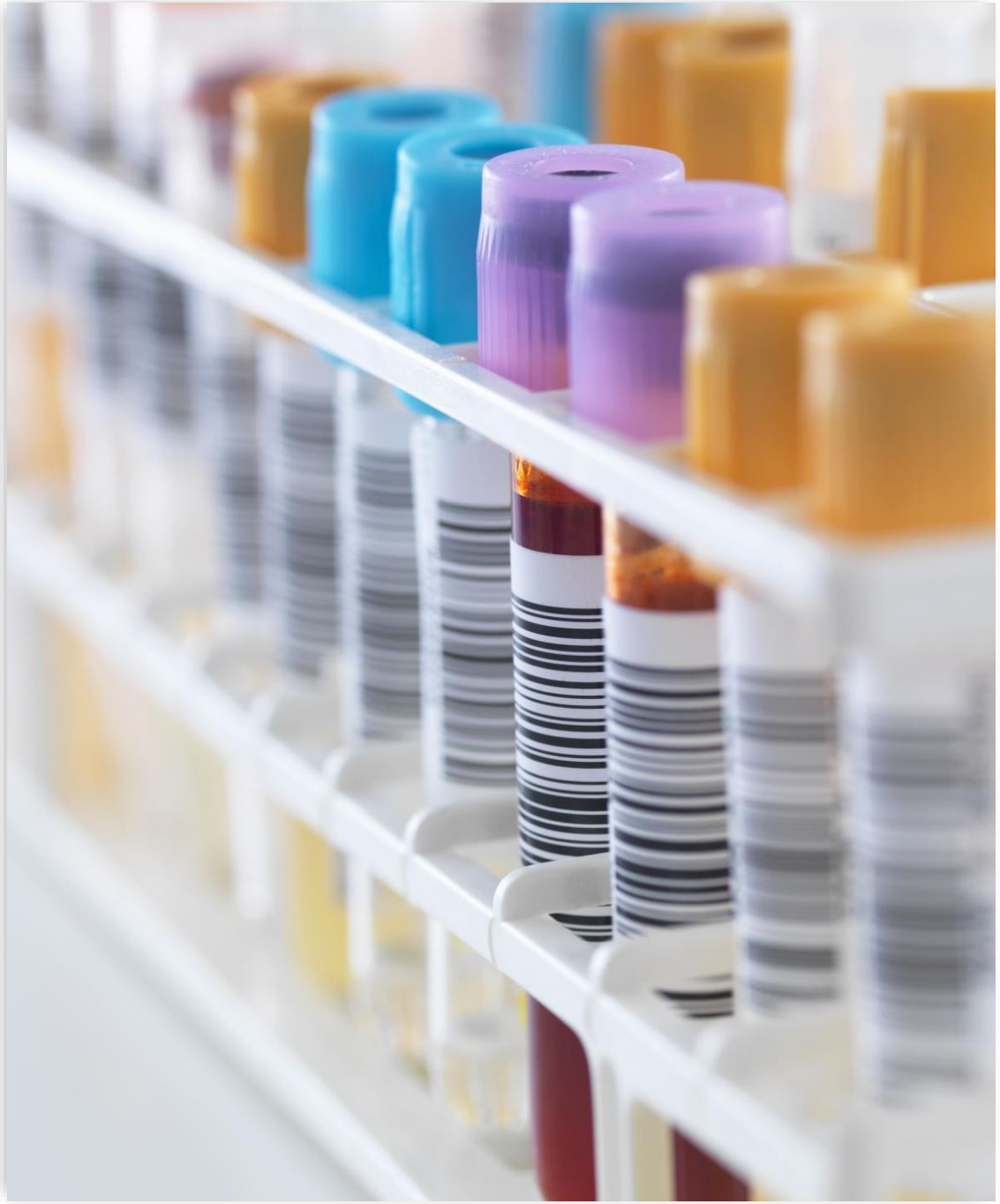


Zeller HG, Schuffenecker I.. Eur J Clin Microbiol Infect Dis. 2004 Mar;23(3):147-56. PMID: 14986160.

Serological Diagnosis

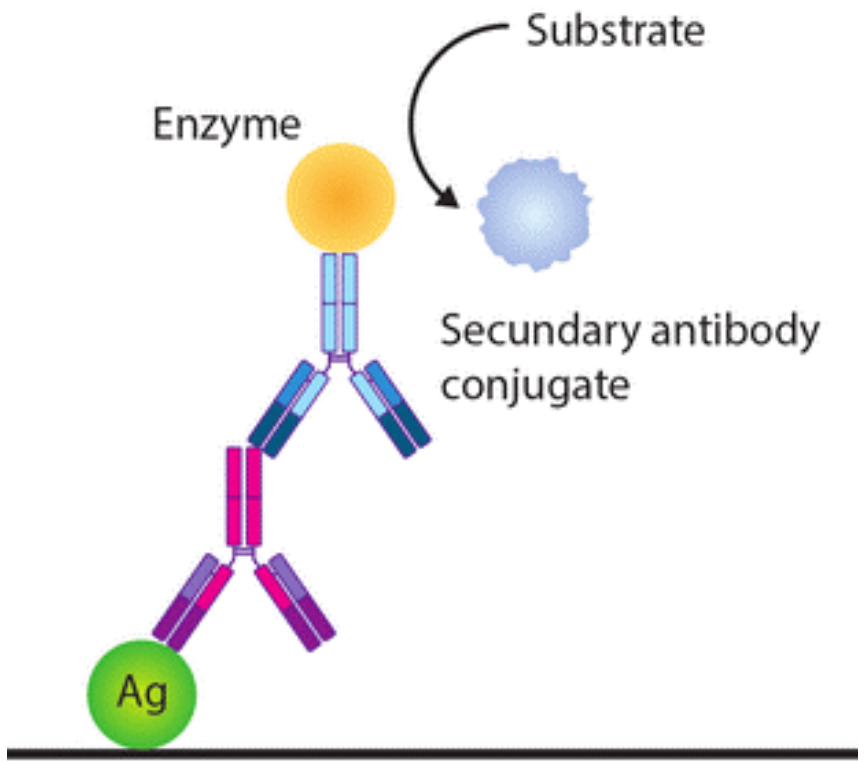
- WNV IgM antibodies appear a median of 4-10 days after the onset of symptoms, and typically last 1-3 months

- For neuroinvasive WNV, IgM may appear in the CSF earlier than serum

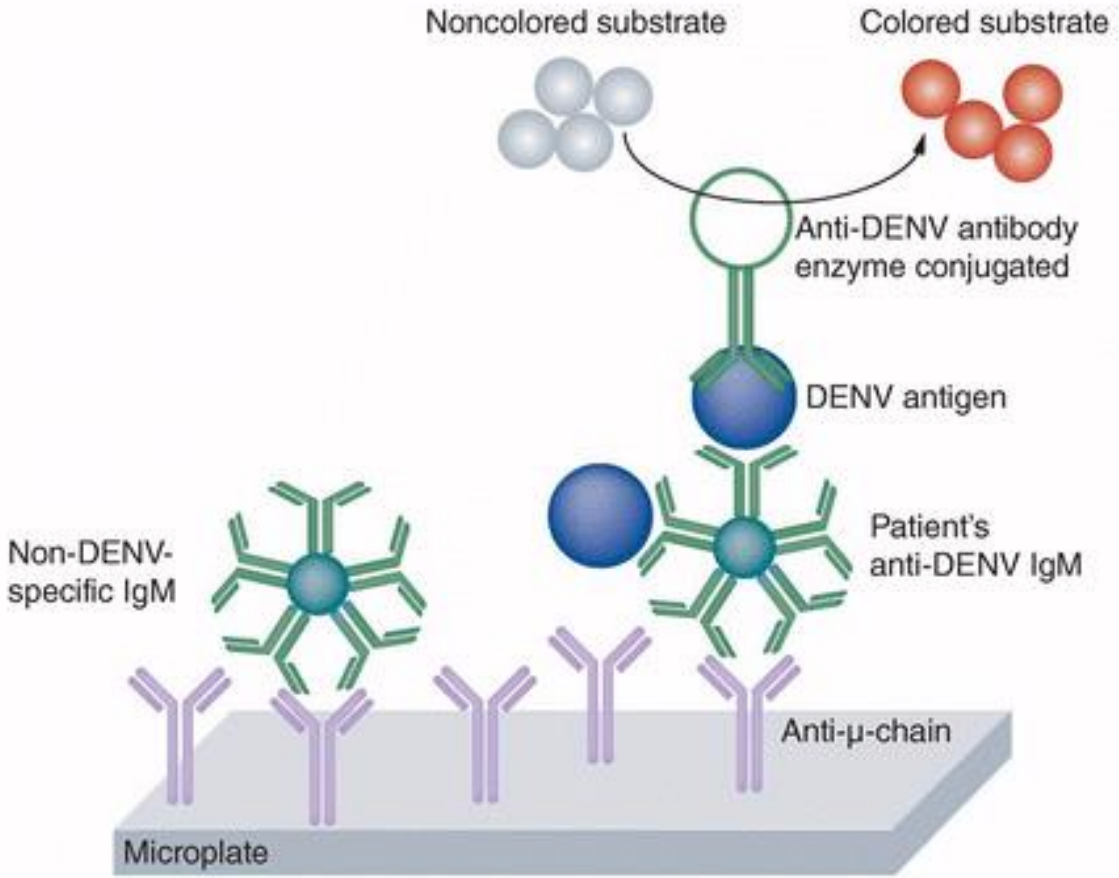


Piantadosi A, Kanjilal S. Diagnostic Approach for Arboviral Infections in the United States. J Clin Microbiol. 2020 Nov 18;58(12):e01926-19.

CSF or Serum Testing: IgM and IgG ELISAs



IgG Assay: Indirect ELISA



IgM Capture Assay (Example)

Images: https://novateinbio.com/

Which is preferred CSF or Serum?

WNV IgM Antibodies Persist Up to 81 Months

- Follow up of persons hospitalized with WNV in Colorado in 2003
- During acute illness all had WNV IgM antibodies in serum or CSF by CDC-developed ELISA
- Blood specimens were tested via ELISA (Focus Diagnostics) and by MIA (CDC)

TABLE 2
West Nile virus IgM antibodies results for participants by assay and time of sample collection postillness onset

		ELISA						MIA					
		Positive		Equivocal		Negative		Positive		Nonspecific		Negative	
Months after acute illness onset	Number of participants *N*	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
16–19	47	11	(23)	5	(11)	31	(66)	20	(43)	14	(30)	13	(28)
35–38	41	3	(7)	3	(7)	35	(85)	8	(20)	9	(22)	24	(59)
60–63	44	2	(5)	0	(0)	42	(95)	2	(5)	9	(20)	33	(75)
72–81	50	0	(0)	2	(4)	48	(96)	3	(6)	9	(18)	38	(76)

MIA = microsphere immunoassay.

Staples JE, et al. Am J Trop Med Hyg. 2022 Apr 11;106(6):1721–4.

West Nile virus, Ab, IgG and IgM, CSF

Collected [redacted] Status: Edited Result - FINAL Visible to patient: Yes (not seen)

Specimen Information: CSF

0 Result Notes

Component Ref Range & Units	
West Nile Virus Ab, IgG, CSF Negative	Negative
West Nile Virus Ab, IgM, CSF Negative	Positive ! VC
Comment: Telephone report made to: [redacted]	
West Nile virus Ab interp, CSF	See Footnote

West Nile virus Ab interp, CSF	See Footnote

Comment: IgM antibodies to WNV detected, suggesting current or recent infection.
False-positive results may occur in patients with other Flavivirus or enterovirus infections.
Clinical correlation with exposure history is required.
SEMI-URGENT RESULT
Some states may require follow up or confirmatory testing for patients testing positive for IgM antibodies to West Nile virus. Please consult your state health department.
Detection of organism-specific antibodies in the CSF may suggest central nervous system infection. WNV test results should be interpreted with other laboratory and clinical data prior to a diagnosis of central nervous system infection.

What other flavivirus(es) are we concerned about?

Arbovirus Taxonomy

Flaviviridae/ Flavivirus	Togaviridae/ Alphavirus	Peribunyaviridae/ Orthobunyavirus
• West Nile • St. Louis Encephalitis • Powassan Virus • Dengue • Yellow Fever • Japanese Encephalitis	• Eastern Equine Encephalitis • Western Equine Encephaltiis • Venezuelan Equine Encephalitis • Chikungunya	• La Crosse virus • Jamestown Canyon Virus • Oropuche virus

West Nile Virus IgM DxSelect Cross Reactivity

Focus Cross-reactivity without Background Subtract

Specimens characterized by Reference Assays	Site	Focus WNV IgM ELISA Results				
		Neg	Eqv	Pos	Total	% Positive
Dengue virus (secondary infections)	4	6	1	8	15	60.0% (9/15) 95%CI 32.3-83.7%
St. Louis encephalitis virus	1	6	0	7	13	53.8% (7/13) 95%CI 25.1-80.8%
Eastern equine encephalitis virus	4	2	0	0	2	0.0% (0/2) 95%CI 0.0-84.2%
Herpes simplex virus	4	18	1	1	20	10.0% (2/20) 95%CI: 1.2-31.7%
Epstein-Barr virus	4	19	0	0	19	0.0% (0/19) 95%CI 0.0-17.6%
Cytomegalovirus	4	13	0	1	14	7.1% (1/14) 95%CI 0.2-33.9%
Borrelia burgdorferi	4	7	0	1	8	12.5% (1/8) 95%CI 0.3-52.7%
Rheumatoid factor	4	15	1	4	20	25.0% (5/20) 95%CI 3.7-49.1%
Anti-nuclear antibodies	4	19	0	1	20	5.0% (1/20) 95%CI 0.1-24.9%
Polio virus	4	10	0	0	10	0.0% (0/10) 95%CI 0.0-30.8%

Focus Cross-reactivity with Background Subtract

Specimens characterized by Reference Assays	Site	Focus WNV IgM ELISA Results				
		Neg	Eqv	Pos	Total	% Positive
Dengue virus (secondary infections)	4	9	3	3	15	40.0% (6/15) 95%CI 16.3-67.7%
St. Louis encephalitis virus*	NA	NA	NA	NA	NA	Not tested.
Eastern equine encephalitis virus	4	2	0	0	2	0.0% (0/2) 95%CI 0.0-84.2%
Herpes simplex virus	4	20	0	0	20	0.0% (0/20) 95%CI: 0.0-16.8%
Epstein-Barr virus	4	19	0	0	19	0.0% (0/19) 95%CI 0.0-17.6%
Cytomegalovirus	4	14	0	0	14	0.0% (0/14) 95%CI 0.0-23.2%
Borrelia burgdorferi	4	8	0	0	8	0.0% (0/8) 95%CI: 0.0-36.9%
Rheumatoid factor	4	20	0	0	20	0.0% (0/20) 95%CI: 0.0-16.8%
Anti-nuclear antibodies	4	20	0	0	20	0.0% (0/20) 95%CI: 0.0-16.8%
Polio virus	4	10	0	0	10	0.0% (0/10) 95%CI 0.0-30.8%

* Positive SLE samples were not tested with the background subtract procedure.

Hogrefe WR, Moore R, Lape-Nixon M, Wagner M, Prince HE. J Clin Microbiol. 2004 Oct;42(10):4641-8.
West Nile Virus IgM DxSelect Package Insert (Focus Diagnostics)

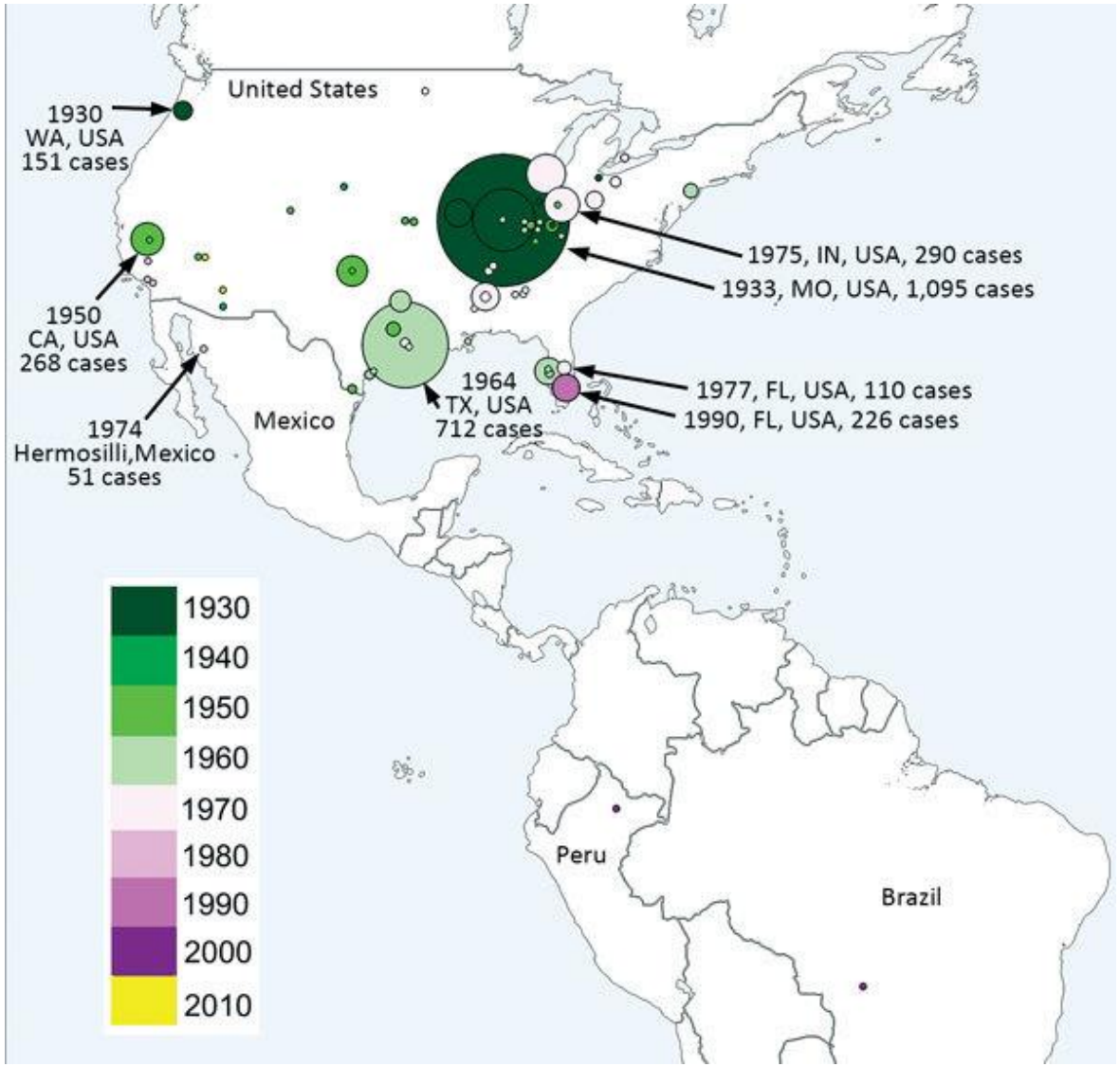
What other flavivirus(es) are in Missouri?

St. Louis Encephalitis Virus

- Prior to introduction of WNV in 1999, it was the most important epidemic mosquito-borne viral disease in the US
- In 1933 when over 1,000 cases of encephalitis reported in St. Louis/St. Louis County
- Another outbreak in St. Louis occurred in 1937

Other Outbreaks

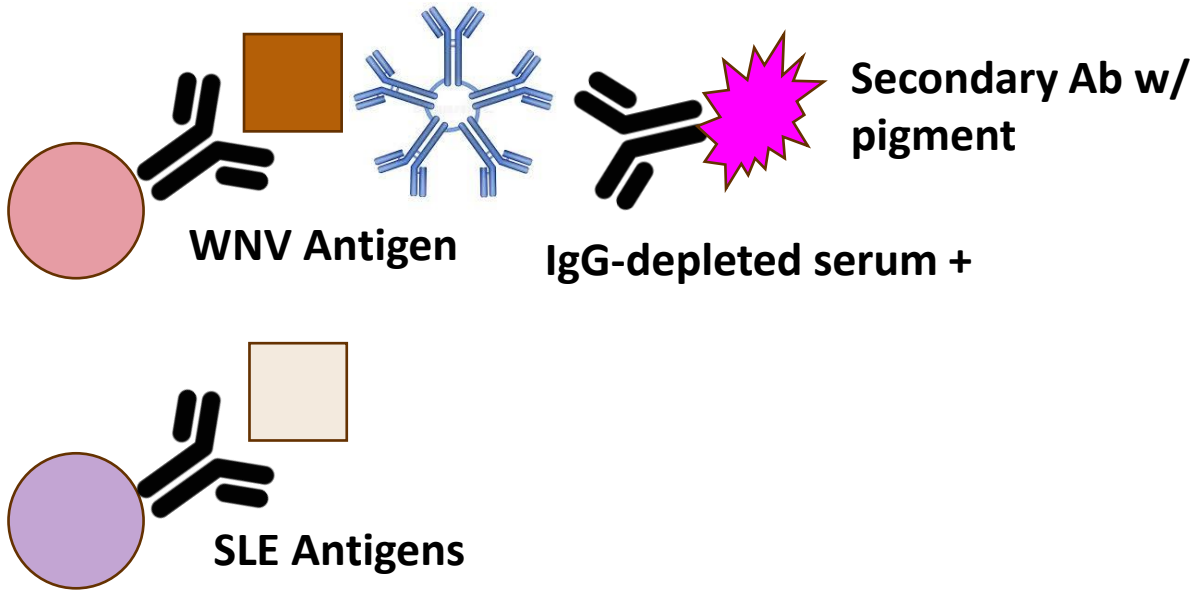
- 1975: Ohio River Valley (>2000 cases)
- 2001: Louisiana >50 cases were reported
- 2015: Arizona, 23 confirmed cases

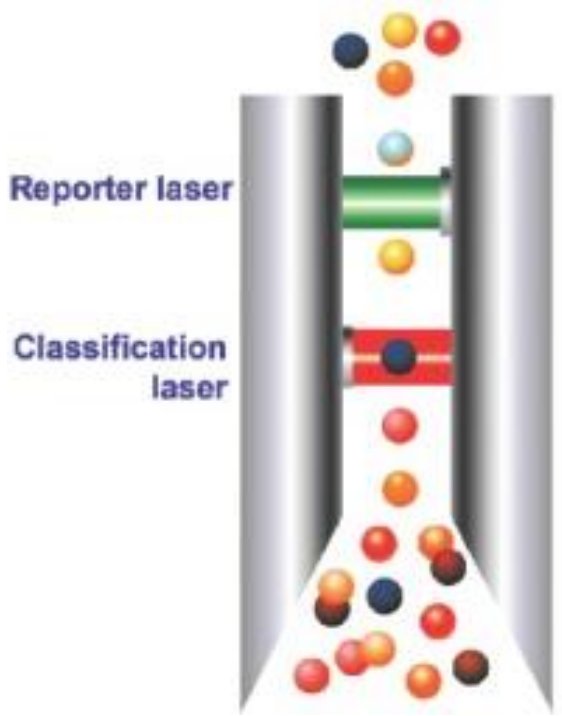


Diaz A, Coffey LL, Burkett-Cadena N, Day JF. Reemergence of St. Louis Encephalitis Virus in the Americas. Emerg Infect Dis. 2018 Dec;24(12):2150–7.

Sent to Missouri State Public Health Lab for Confirmatory Testing

- Microsphere-Based Immunoassay
  - Advantages: shorter turnaround time, multiplexed





West Nile Virus Antigen: E-prM protein
SLE antigens: Derived from SLE suckling mouse brain

Johnson AJ, et al. Clin Diagn Lab Immunol. 2005 May;12(5):566-74.
Johnson AJ, et al. Clin Vaccine Immunol. 2007 Sep;14(9):1084-93.

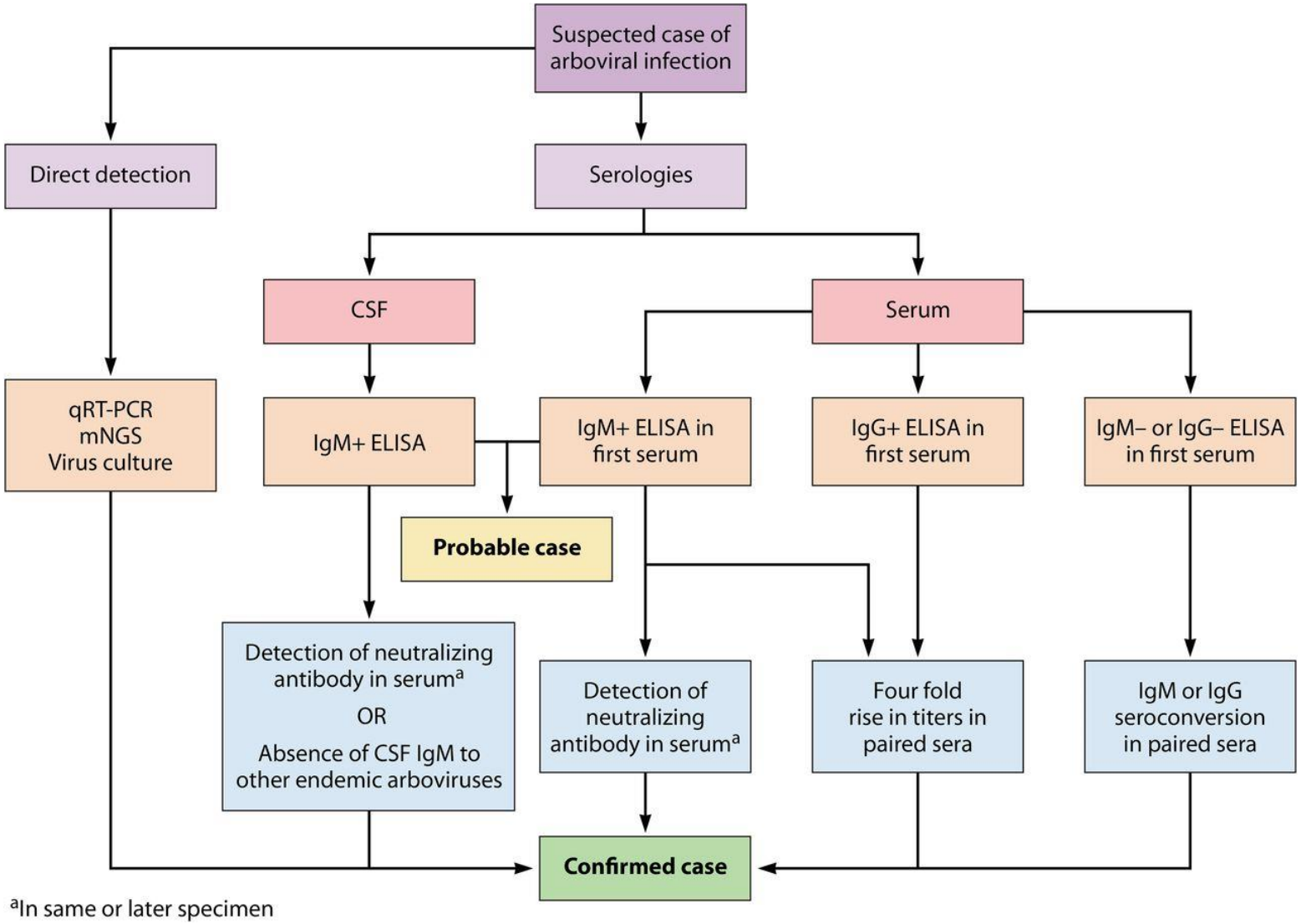
Our patient's results

RESULTS OF ANALYSIS - FINAL REPORT

TEST	RESULT
St Louis Encephalitis IgM, MIA	
St. Louis Encephalitis IgM AB	**Negative**
West Nile IgM, MIA	
West Nile Virus IgM Antibody	**Positive**

- **WNV IgM positive on CSF and Serum, no further testing needed**

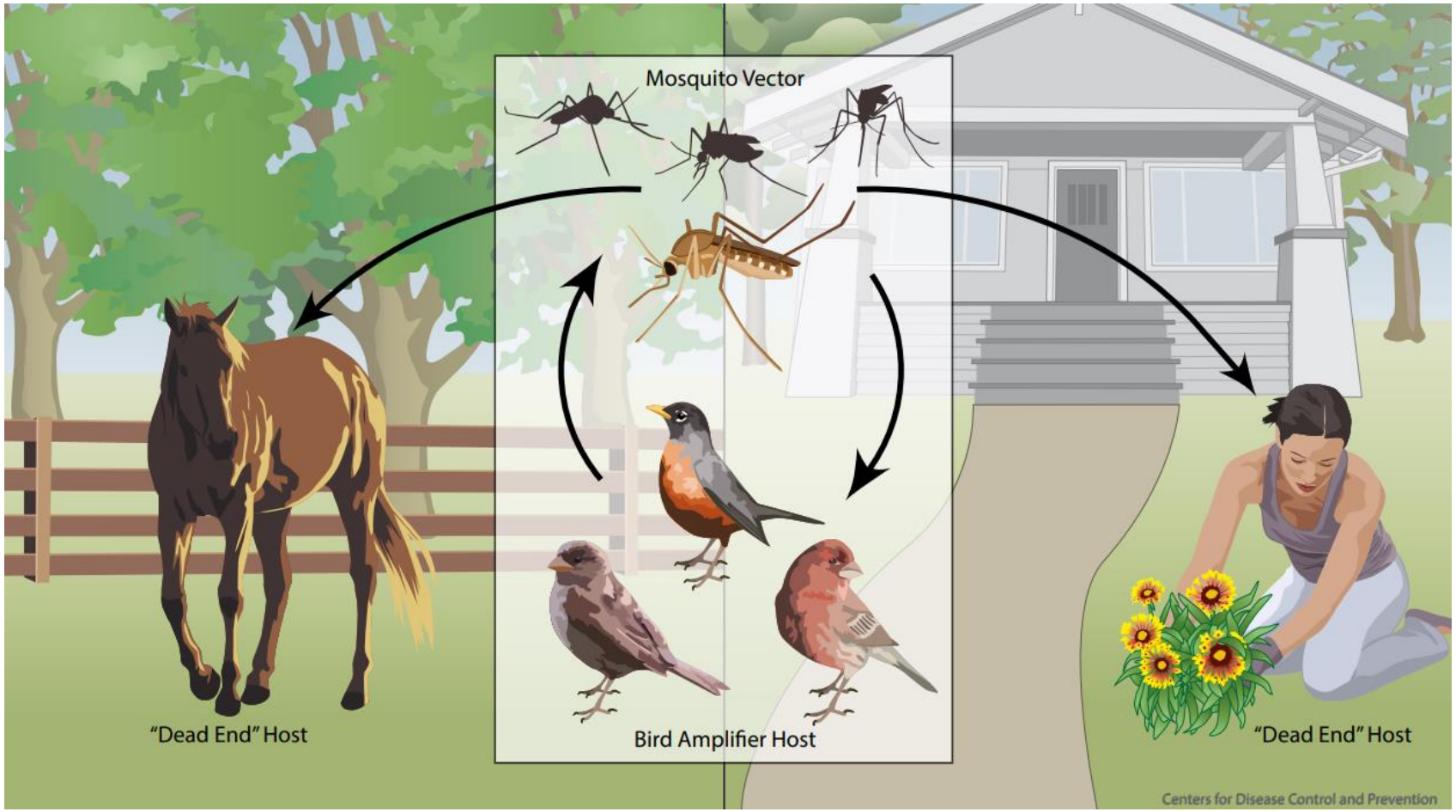
Diagnosis of Arboviral Infections



Piantadosi A, Kanjilal S. Diagnostic Approach for Arboviral Infections in the United States. J Clin Microbiol. 2020 Nov 18;58(12):e01926-19.

WNV Transmission

- Transmitted by *Culex* spp. from bird reservoirs



Do not develop high levels of viremia, do not pass on to mosquitoes

https://www.cdc.gov/westnile/transmission/index.html

West Nile Virus Symptoms

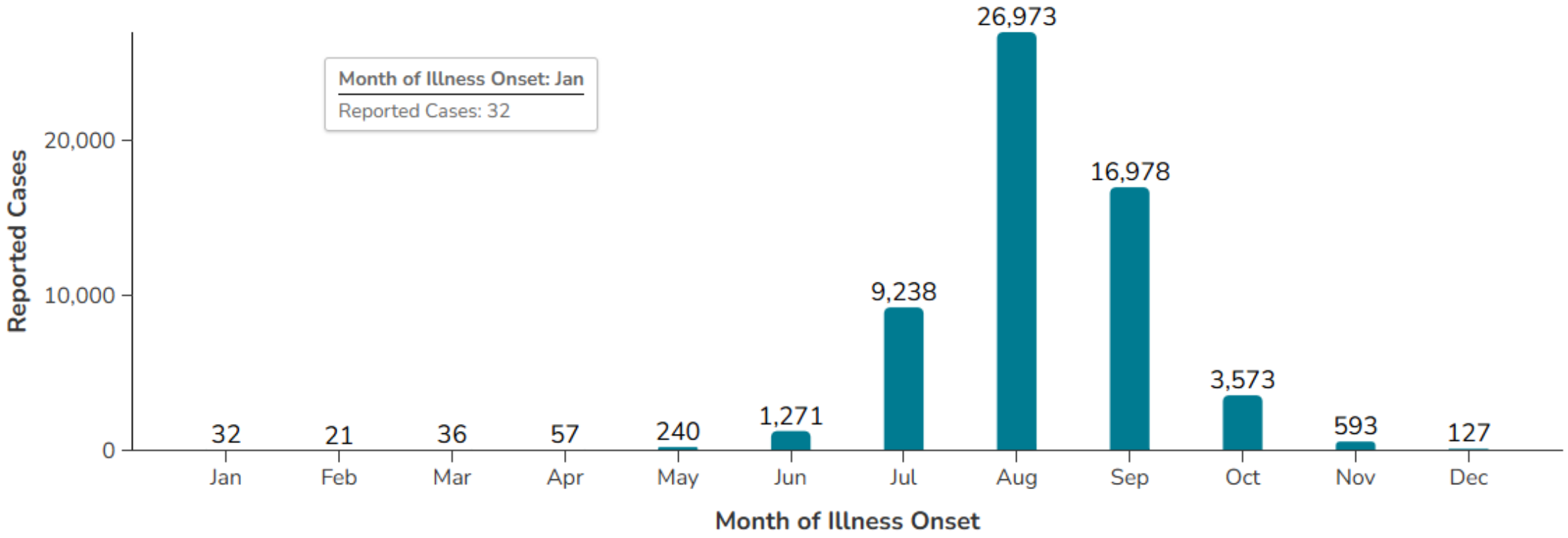
- 8 of 10 people infected with WNV are asymptomatic
- About 20% of people who are infected develop febrile illness
  - Fever with other symptoms such as headache, body aches, joint pains, vomiting, diarrhea, or rash.
  - Most recover completely, but fatigue/weakness can last weeks-months

- About 1 in 150 people develop encephalitis or meningitis
  - High fever, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, vision loss, numbness and paralysis
  - People > 60 yrs are at greater risk for severe illness if infected
  - People with cancer, diabetes, hypertension, kidney disease, and transplant patients also at greater risk

- Can cause poliomyelitis-like neurological symptoms, including flaccid paralysis
  - Similar to polio, neurons in the anterior horn of the spinal cord are vulnerable to WNV

https://www.cdc.gov/westnile/symptoms/

WNV Cases Peak in August



All Cases: 1999-2023

https://www.cdc.gov/

Treatment/ Prevention

- No antiviral or adjunctive therapies are approved or recommended for the treatment of West Nile virus (WNV) disease; clinical management is supportive

- CDC Review of Literature
  - **Polyclonal immune globulin intravenous (IVIG) – can interfere with other testing**
  - WNV Recombinant Monoclonal Antibody – trial terminated
  - Interferon
  - Ribavirin
  - Corticosteroids

- Vaccines in clinical trials (Phase I and II)

https://www.cdc.gov/west-nile-virus/hcp/treatment-prevention/

Gould et al. Combating West Nile Virus Disease—Time to Revisit Vaccination. N Engl J Med 2023;388:1633-1636

Case Wrap Up

- Patient was not treated for West Nile Virus infection
- Seen in clinic in January and reports significant improvement

Case #2 (Wisconsin)

- 74 year old woman presented to urgent care **Mid-May** with complaints of **tick bites and bulls-eye rash**
  - 3 tick bites the last few weeks. Rash appeared over her abdomen and chest.
  - Erythema noted around what appears to be a tick bite both on the upper shoulder and left flank
  - She does go in wooded areas to walk her dogs and spends time in her yard.
  - **Not treated- plan to wait for Lyme test results, follow up with PCP**

- She presented again to ED **13 days later** (end of May) **with fever and altered mental status**
  - Found confused and wandering in her yard by her neighbor day prior
  - Next day neighbor/police checked on her and she was found down in her bedroom
  - Unable to state how she arrived in the ED or elaborate on events leading up to ED
  - Temp elevated to 101.3F. **Left sided weakness on exam**.
  - COVID test was positive (CXR negative)

- Per daughter, patient had bouts of fevers and not feeling well off and on for a few weeks but was totally normal in between.

Core Lab Results

WBC 10.2 K/cumm
RBC **3.77 (L)**
Hgb 12.1 g/dL
Hct 34.8%
Plt 221 K/cumm

Sodium **128 (L)** mmol/L
Potassium 3.5 mmol/L
Chloride 98 mmol/L
CO2 **22 (L)** mmol/L
Anion gap 8 mmol/L
BUN 16 mg/dL
Creatinine 0.76 mg/dL
Glucose 135 mg/dL
Calcium **8.1 (L)** mg/dL

AST: 15 U/L
ALT: 29 U/L

Microbiology Workup

Bacteriology

- Bacteriology culture on CSF- Negative
- Blood cultures – Negative
- Q fever antibodies- Negative

Tickborne Illnesses

- Lyme Ab IgG / IgM
  - Negative on first visit
  - Negative on second visit
- Lyme CSF Screen - Negative
- Lyme PCR on Blood - Negative
- Borrelia PCRs on CSF – All Negative
  - *B. burgdorferi*, *B. garinii/afzelii*, *B. mayonii*
- Anaplasma/Ehrlichia PCRs on Blood - Negative

Viral/ Other

- ME Panel - All Negative
- Arboviral Panel CSF- All Negative
  - WNV, SLE, EEE, WEE, California Encephalitis
- Blood parasite exam - Negative
- **COVID – Positive (2nd visit)**

CSF results

	Normal	Bacterial	Viral	Fungal
Opening pressure (cm CSF)	12-20	Raised	Normal/ Mildly raised	Raised
Appearance	Clear	Turbid/ Cloudy	Clear	Clear or Cloudy
CSF white cell count (cells/ uL)	<5	Raised (>100)	Raised (5-100)	Raised (5-500)
Predominant cell type	n/a	PMNs	Lymphocytes	Lymphocytes
CSF protein (g/L)	0.4 (40mg/dL)	Raised	Mildly raised	Raised
CSF/ plasma glucose ratio	>2/3	Very low	Normal/ slightly low	Low

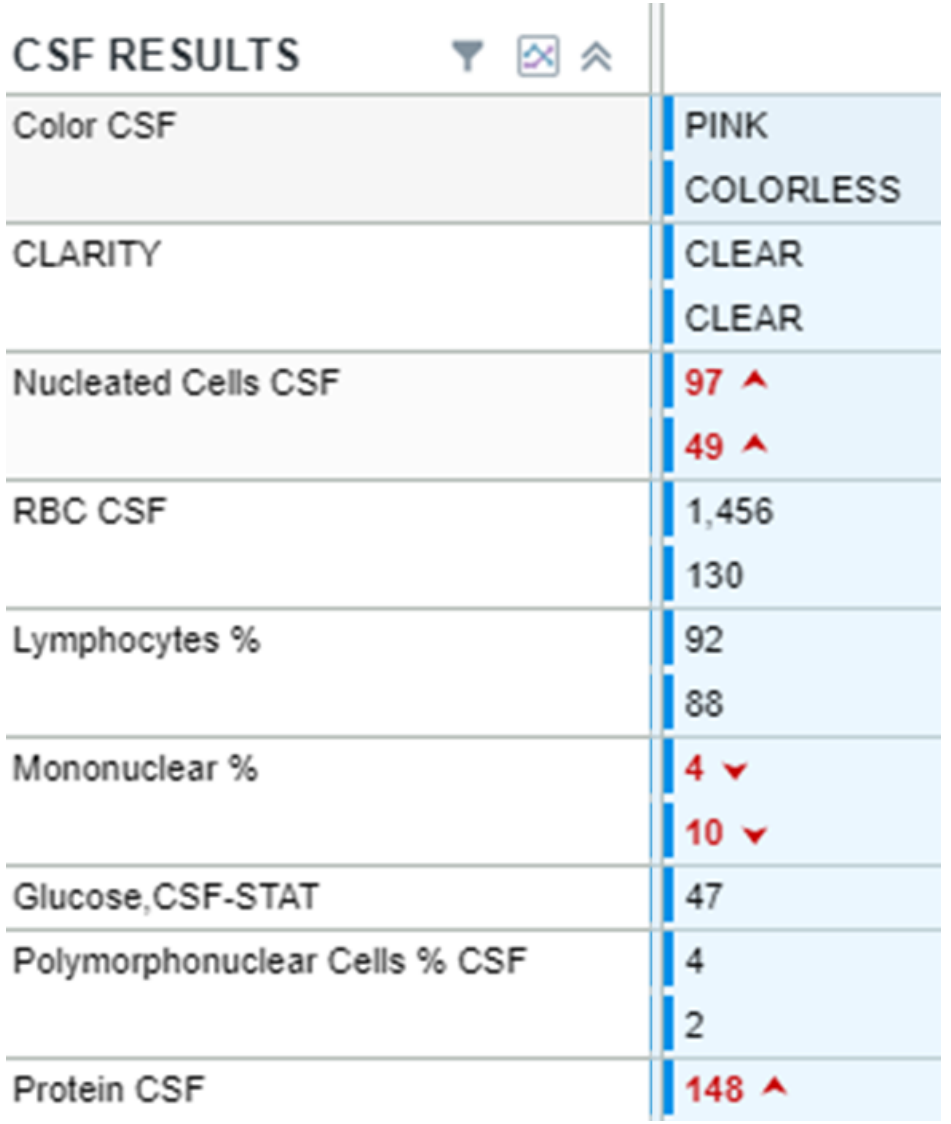


Table modified from: Comprehensive Review of Infectious Diseases. 2020

Case Wrap Up?

- Based on the CSF profile, **highest concern for a viral encephalitis**. After extensive lab testing, there was no positive culture or finding for any infectious meningitis
- She was found to be COVID 19 positive on admission, but without any respiratory symptoms.
  - COVID encephalopathy is possible diagnosis, however she did seem to improve significantly after initiation of antimicrobial therapy
- There was high suspicion for Lyme meningitis, but all testing has come back negative
  - IV antibiotics stopped, continue with just doxycycline per ID consultation.
- Discharged 7 days after her hospitalization.
- Her profound encephalopathy (and severe dysphagia, behavioral disturbance, etc) all improved dramatically, and she is 80% back to her baseline per family.

What was the virus?

- ID physician reached out to patient in July and suggested POWV testing as she recently had another positive patient with symptoms about the same time
- Serum specimen sent to WSLH

Powassan IgM Antibody (Final result)

ID: [redacted] Type/Src: Serum

	Result	Units
Powassan IgM Ab	13.948	
Powassan IgM Interpretation	**Presumptive Positive**	**(A)**

Comments:
Serologic evidence for recent infection. Specimen will be sent to CDC for confirmatory testing. In addition, it is recommended that a serum specimen be collected in 2-3 weeks for follow-up testing.

EIA result = optical density (OD) of patient serum (P) divided by the OD of the normal human sera control (N).

Result	Interpretations
> or = 3.0	Presumptive Positive
2.00 - 2.99	Equivocal
< 2.0	Negative

Arbovirus Taxonomy

Flaviviridae/ Flavivirus	Togaviridae/ Alphavirus	Peribunyaviridae/ Orthobunyavirus
• West Nile • St. Louis Encephalitis • Powassan Virus • Dengue • Yellow Fever • Japanese Encephalitis	• Eastern Equine Encephalitis • Western Equine Encephaltiis • Venezuelan Equine Encephalitis • Chikungunya	• La Crosse virus • Jamestown Canyon Virus • Oropuche virus

Powassan Virus/ Deer Tick Virus

- Transmitted by *Ixodes* ticks
  - Most cases occur in the Northeast and Great Lakes region from late spring – mid fall
- Potentially transmitted within 15 minutes (based on studies in mice)
- *Ixodes scapularis* transmits: MAPLE-B
  - Miyamotoi disease (*Borrelia miyamotoi*)
  - Anaplasmosis
  - Powassan Virus
  - Lyme Disease
  - Ehrlichiosis (*Ehrlichia muris eauclairensis*)
  - Babesia

Image: https://www.cdc.gov/powassan/about/index.html

Powassan Virus/ Deer Tick Virus Taxonomy

- Lineage I: Powassan Virus
  - *Ixodes marxi* (squirrel tick)
  - *Ixodes cookei* (groundhog tick)
- Lineage II: Deer Tick Virus
  - *Ixodes scapularis*

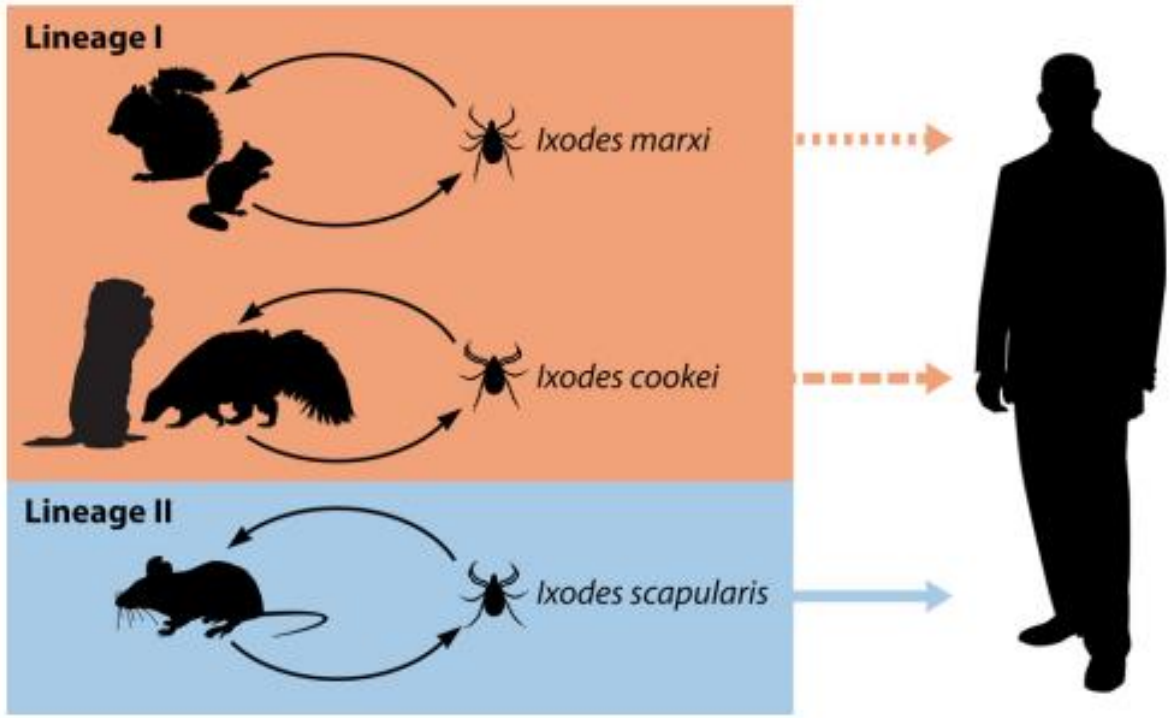


FIG 5 Enzootic cycles of lineage I and II POWVs linked to the main tick vector and some important vertebrate hosts. Humans are considered tangential hosts; arrows pointing to the silhouette on the right are indicative of the relative public health risk posed by different tick species (i.e., low risk for *Ixodes marxi* and *Ixodes cookei* and greater risk for *Ixodes scapularis*).

Kemenesi G, Bányai K. Tick-Borne Flaviviruses, with a Focus on Powassan Virus. Clin Microbiol Rev. 2018 Dec 12;32(1):e00106-17.

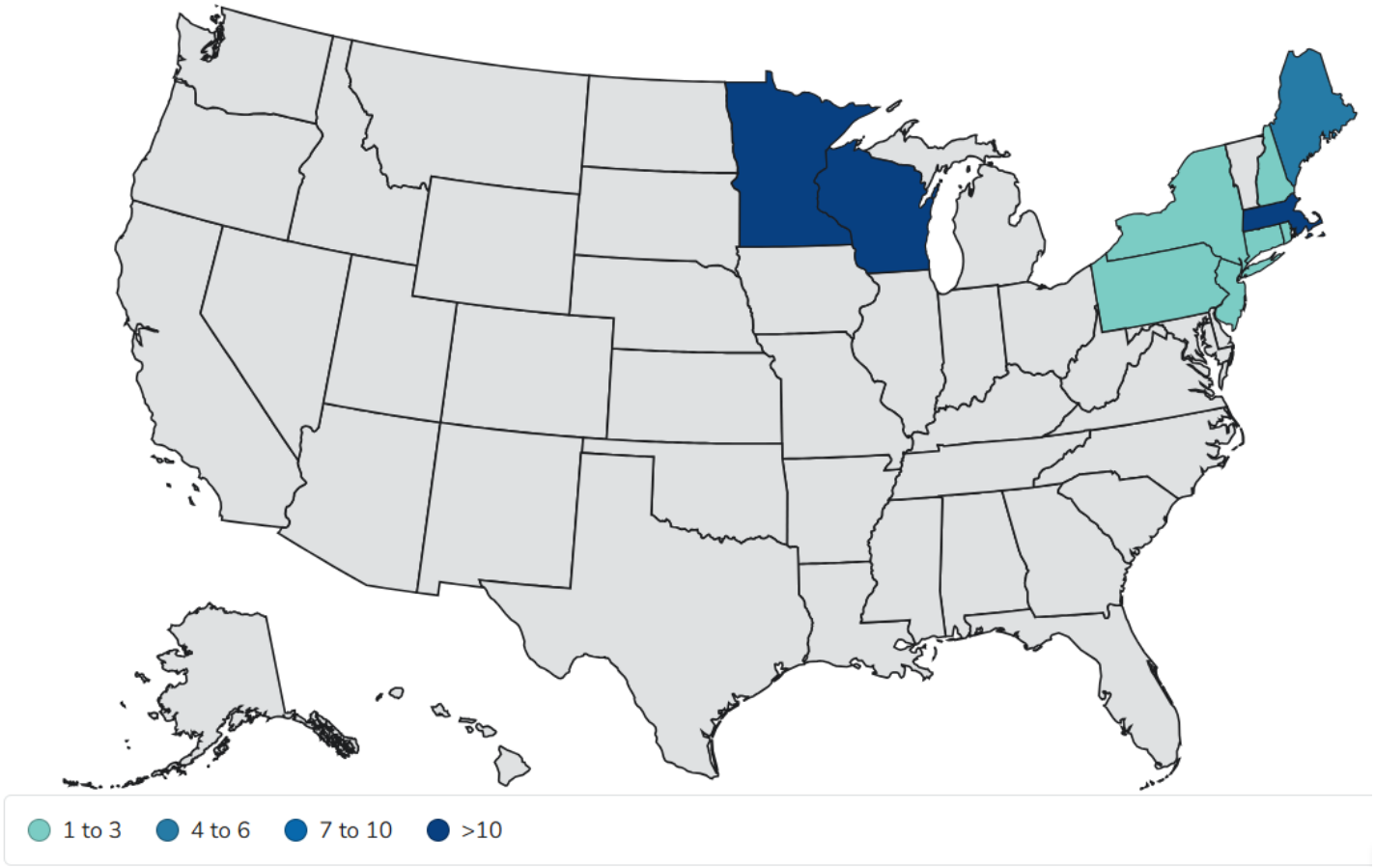
POWV Symptoms

- Many people infected with Powassan virus do not have symptoms. For people with symptoms, the time from tick bite to feeling sick ranges from **1 week to 1 month.**
- Initial symptoms can include fever, headache, vomiting, and weakness.
- Powassan virus can cause severe disease, including encephalitis and meningitis
- Symptoms of severe disease include confusion, loss of coordination, difficulty speaking, and seizures.
- Approximately 1 out of 10 people with severe disease die.
- Approximately half of the people who survive severe disease have long-term health problems. These can include recurring headaches, loss of muscle mass and strength, or memory problems.

https://www.cdc.gov/powassan/symptoms-diagnosis-treatment/index.html

POWV Cases in 2024

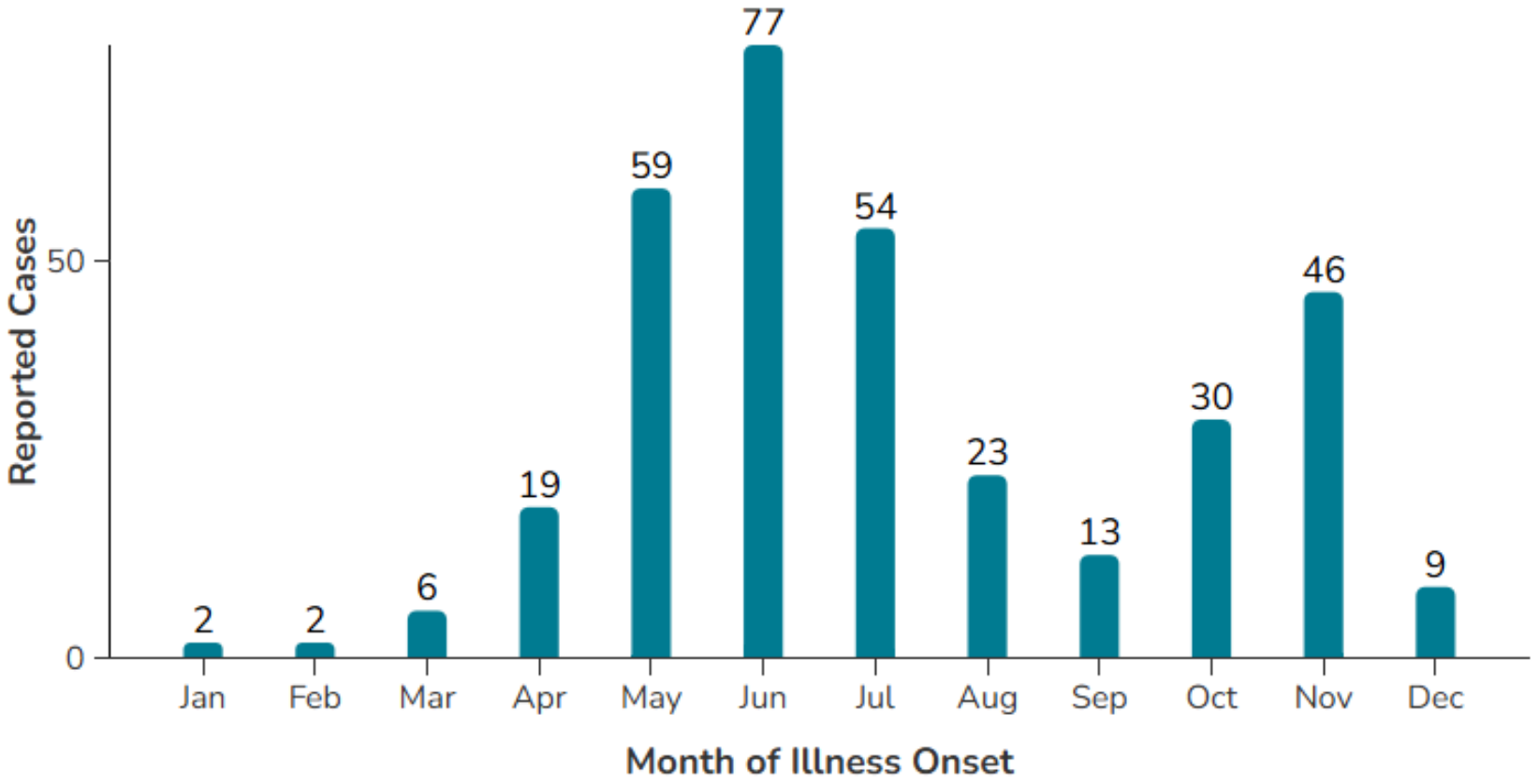
- 2024 Data
- 54 human cases
  - 54 neuroinvasive cases
  - 12 in Wisconsin
- Reported in 10 states



https://www.cdc.gov/powassan/data-maps/current-year-data.html

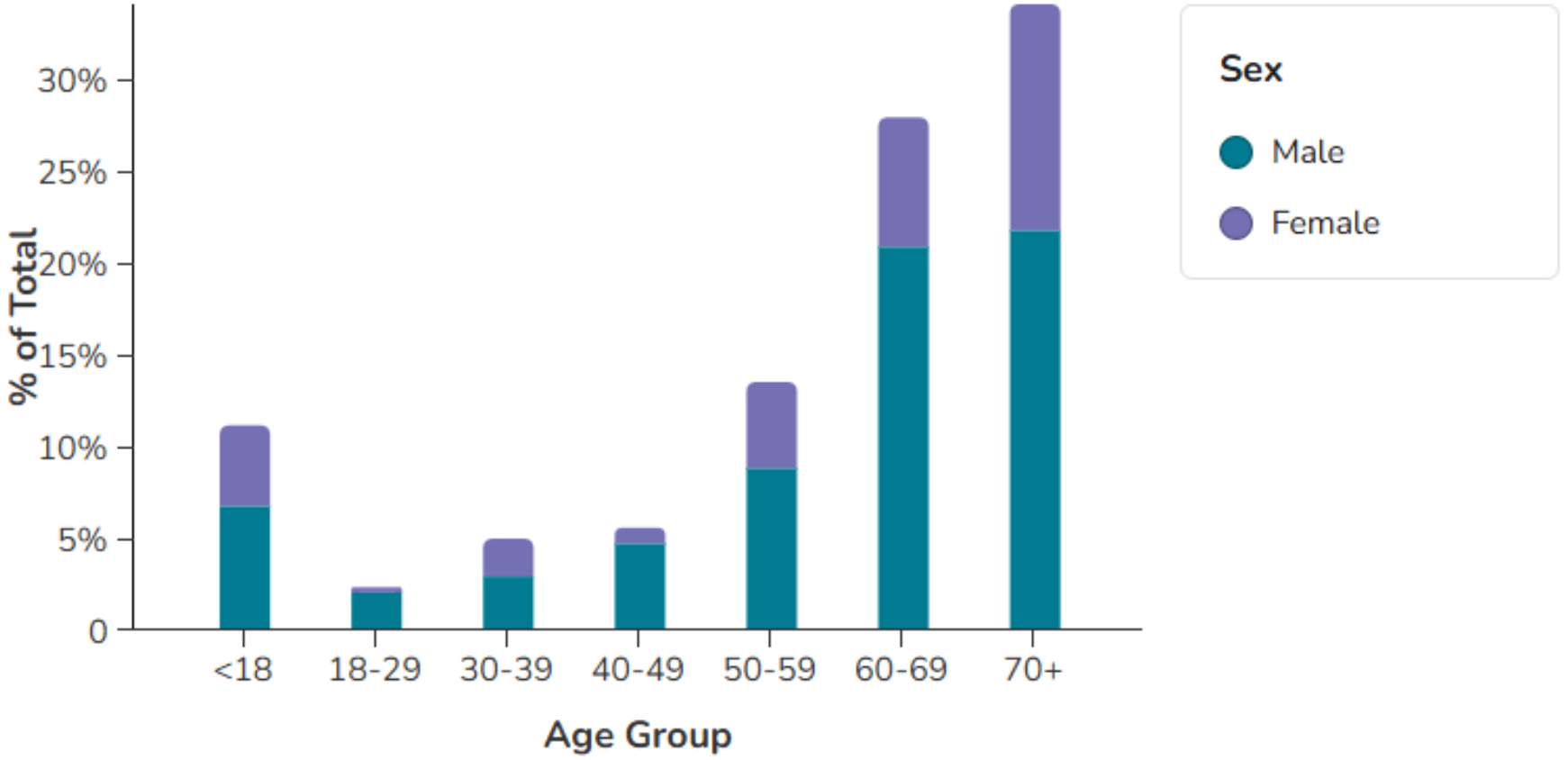
POWV Cases Peak in June

- CDC Data from 2004- 2023



Most POWV cases are in adults >60

- CDC Data from 2004- 2023



What about that rash?

- Not truly a bulls-eye rash/ erythema migrans?
- Example in the literature of possible coinfection (POWV + Lyme)
  - Patient with erythema migrans – treated for Lyme disease, 1 week later returns to ED with headache and encephalitis
  - Serology positive for Powassan virus; Lyme serologies were negative
  - Coinfection, due to prompt antibiotic treatment did not seroconvert

Cavanaugh CE, et al. Fatal Deer Tick Virus Infection in Maine. Clin Infect Dis. 2017 Sep 15;65(6):1043-1046. PMID: 28903511.

Case #3 (Wisconsin)

- Patient is a 2-week-old female born at 39 weeks via C-section
- Presented to ED in **November** for evaluation of fever, overall well-appearing
- No cough, runny nose, rash, vomiting, or diarrhea
- Brother (2.5 years, attends daycare)who was sick last week with a presumed viral illness (fever, headache, vomiting); mom also now has a fever and nausea
- Decreased feeding, decreased urination
- Blood cultures were collected, an LP performed, antibiotics (ceftazidime and ampicillin) started

Core Lab Results

WBC 9.6 K/cumm
RBC 4.39
Hgb 16.3 g/dL
Hct 45.5%
Plt 278 K/cumm

Sodium **134 (L)** mmol/L
Potassium 4.9 mmol/L
Chloride 104 mmol/L
CO2 20 mmol/L
Anion gap 10 mmol/L
BUN 7 mg/dL
Creatinine 0.4 mg/dL
Glucose **88 (H)** mg/dL
Calcium 10.1 mg/dL

CSF Results

CSF RESULTS	
Color CSF	COLORLESS
	COLORLESS
CLARITY	CLEAR
	CLEAR
Nucleated Cells CSF	43 ▲
	33 ▲
RBC CSF	51
	<1
Lymphocytes %	4
	2
Mononuclear %	2 ▼
	14 ▼
Glucose,CSF-STAT	48
Polymorphonuclear Cells % CSF	94 ▲
	84 ▲
Protein CSF	62

CSF Results

	Normal	Bacterial	Viral	Fungal
Opening pressure (cm CSF)	12-20	Raised	Normal/ Mildly raised	Raised
Appearance	Clear	Turbid/ Cloudy	Clear	Clear or Cloudy
CSF white cell count (cells/ uL)	<5	Raised (>100)	Raised (5-100)	Raised (5-500)
Predominant cell type	n/a	PMNs	Lymphocytes	Lymphocytes
CSF protein (g/L)	0.4 (40mg/dL)	Raised	Mildly raised	Raised
CSF/ plasma glucose ratio	>2/3	Very low	Normal/ slightly low	Low

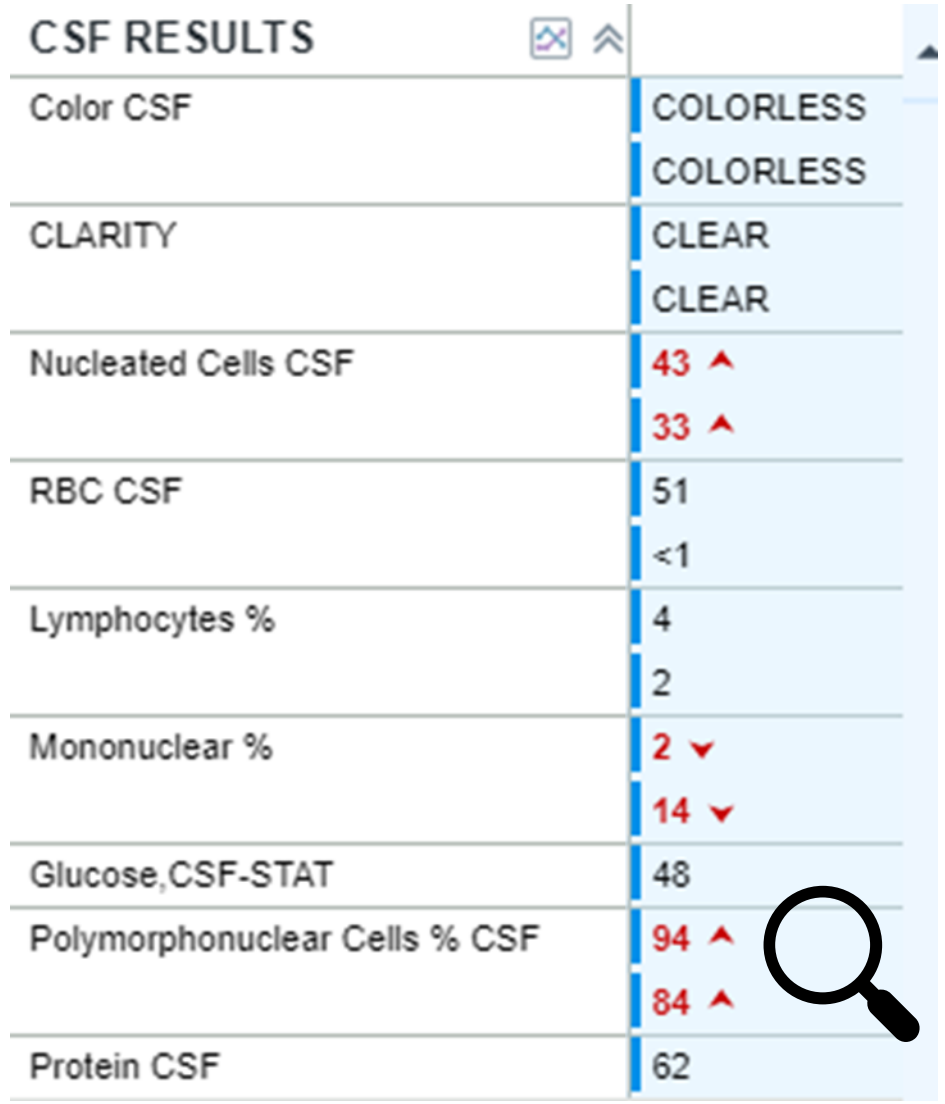


Table modified from: Comprehensive Review of Infectious Diseases. 2020

Viral Causes of Encephalitis or Meningitis

Herpes Viruses	Arboviruses	Enteroviruses	Other
• HSV-1/2 • VZV • EBV • CMV • HHV-6	• West Nile • La Crosse • St. Louis encephalitis • EEEV, WEEV	• Poliovirus • Non-polio enteroviruses	• HIV • Measles • Mumps • Rabies

Neutrophilic WBC predominance is most often found in Enterovirus infections

Retrospective study of patients with confirmed viral infections
Patients seen in New Orleans, LA or Houston, TX between 1999-2013

Etiology	No. (%) of cases Neutrophilic (n=45)	No. (%) of cases Lymphocytic (n=137)
Enterovirus	29 (64%)	45 (33%)
Herpes virus (HSV and VZV)	9 (20%)	63 (46%)
Arbovirus (WNV and SLE)	7 (16%)	29 (21%)

Table modified from: Jaijakul S, Salazar L, Wootton SH, Aguilera E, Hasbun R. Int J Infect Dis. 2017 Jun;59:77-81. PMID: 28435023.

Viral Causes of Encephalitis or Meningitis

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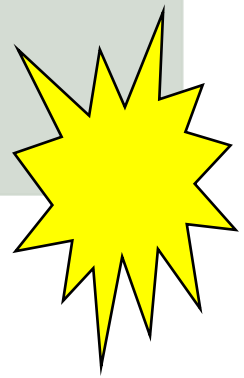


Testing for Enterovirus: PCR

FDA-approved tests

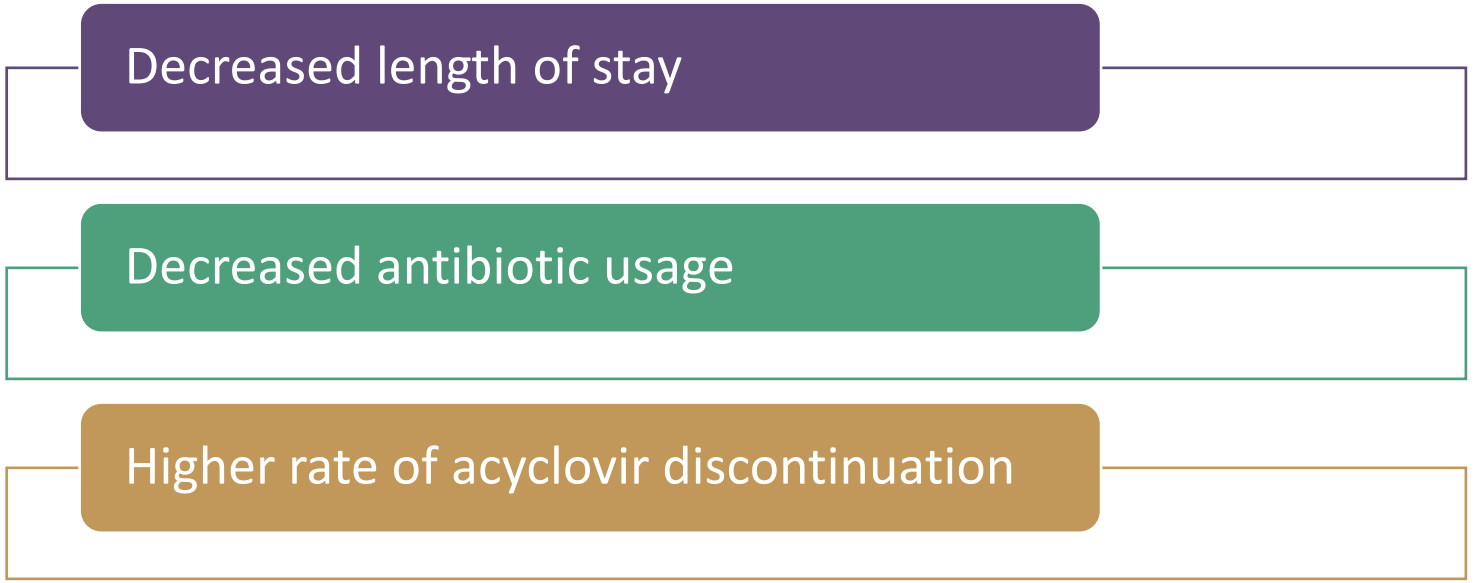
- Cepheid Xpert EV- Discontinued
- BioFire ME panel (14 targets)
- QIAstat-Dx Meningitis/Encephalitis Panel (9 targets) – FDA cleared Nov. 2024

Virus	Sensitivity/ PPA	Specificity/ NPA	Comparator	Reference
EV	BioFire ME: **93.8%**	BioFire ME: 99.3%	Virus-Specific PCR (3 studies, 6883 patients)	Meta-analysis Trujillo-Gomez et al. 2022
EV	BioFire ME: **89%** (226/252)	BioFire ME: 100%	Xpert EV	Schnuriger et al. 2022
EV	QIAstat-Dx ME: **77.8 %** (7/9)	QIAstat-Dx ME: 99.8% (569/570)	BioFire ME	Sundelin et al. 2023

Our patient’s results

- CSF ME panel performed- positive for **Enterovirus RNA**
- Patient continued to improve and was discharge the following afternoon.

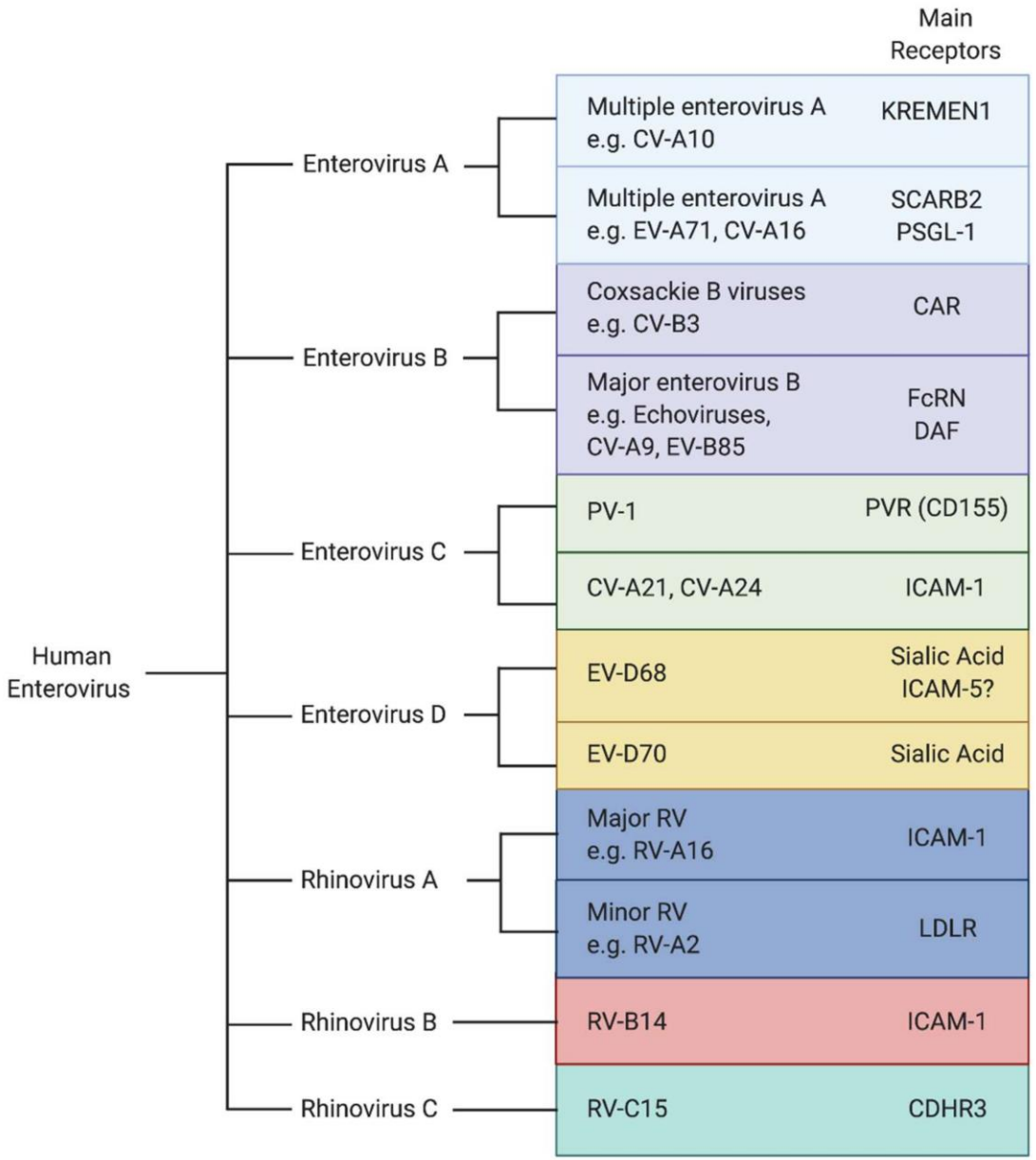
Advantages of Enterovirus Testing



Lee BR, Sasidharan A, Harrison CJ, Selvarangan R. J Clin Microbiol. 2020 Dec 17;59(1):e02106-20. PMID: 33055181

Enterovirus

- 7 species of Enterovirus
  - Includes: Enterovirus, Coxsackie, Poliovirus, Echovirus
- **RNA genome**, non-enveloped
- Stable in liquid environments and can survive for many weeks in water, body fluids, and sewage
- Temperate climates 70-80% of EV infections occur in **summer/ fall**
- Transmission: **fecal-oral, respiratory**, mother to infant prenatally, fomites
- Highest incidence in infants and children 5-10 years, children <1 year accounting for 1/3 of reported cases in US.



Manual of Clinical Microbiology, 13th edition. 2023

Image: Peters CE, Carette JE. Return of the Neurotropic Enteroviruses: Co-Opting Cellular Pathways for Infection. *Viruses*. 2021; 13(2):166.

Clinical Manifestations/ Symptoms

- Most common: acute, nonspecific febrile illness
- Other manifestations- respiratory, HFMD, neurologic (meningitis, encephalitis, AFM), gastrointestinal (vomiting, diarrhea, abdominal pain), eye (conjunctivitis), heart (myopericarditis), neonatal systemic disease

- Incubation time: 3-5 days
  - Exception: acute hemorrhagic conjunctivitis = 24-72 h

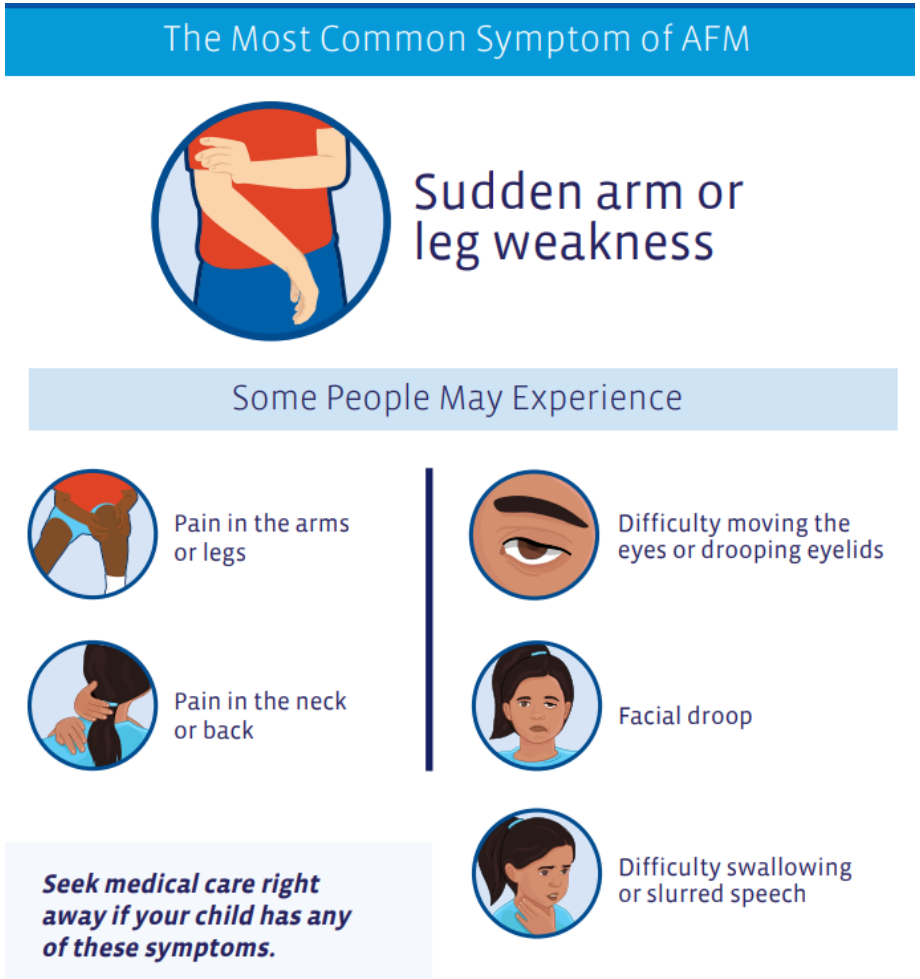
- EVs are **most common cause of meningitis** in the United states (80% of viral meningitis cases)

- Risk for severe disease: agammaglobulinemic patients, patients receiving anti-CD20 antibodies

Manual of Clinical Microbiology, 13th edition. 2023

Uncommon Complication: Acute Flaccid Myelitis

- **Uncommon neurologic condition**
- Sudden onset of acute flaccid limb weakness; most cases are in children
- Clinical presentation can mimic polio
- Viral causes include non-polio enteroviruses, WNV, herpesviruses, adenovirus



https://www.cdc.gov/acute-flaccid-myelitis/signs-symptoms/index.html

Enteroviruses linked to outbreaks of severe disease

- EV-A71
  - Common cause of HFMD, can cause neurologic disease
  - Outbreak in Colorado, 2018: 34 children with neurologic disease and specimens positive for EV-A71
    - Not all had positive PCR on CSF
  - Epidemics can occur in Asia/Pacific
- EV-D68
  - Can cause mild-severe respiratory illness
  - May be associated with **AFM**
  - Biennial cycle (2014, 2016, 2018…)
- FDA-approved tests do not provide Enterovirus typing

Manual of Clinical Microbiology, 13th edition. 2023

Messacar K, Burakoff A, Nix WA, et al. MMWR Morb Mortal Wkly Rep 2018;67:1017–1018.

Treatment of Enteroviruses

- Supportive
- Pocapavir: Capsid inhibitor with activity against polioviruses, has been used for compassionate use

Manual of Clinical Microbiology, 13th edition. 2023

Summer Virology Cases of the CNS: Summary

- Typical CSF profile for viral infection: normal-elevated WBCs, elevated protein
  - Typically, lymphocytic predominance, but can have neutrophilic predominance (EV)

- Arboviruses
  - West Nile Virus is the most common arbovirus in the United States
    - Typically affects older individuals; transmitted by mosquitoes
  - POWV cases have occurred in WI
    - Likely underreported due to lack of availability of testing at commercial ref. labs
    - Transmitted by *Ixodes*
  - PCR has limited utility due to short window of viremia; Serology is most useful

- Enterovirus
  - PCR is used for diagnosis
  - Complications of EV can include AFM

- No treatment available and no vaccines for WNV, POWV, and EV

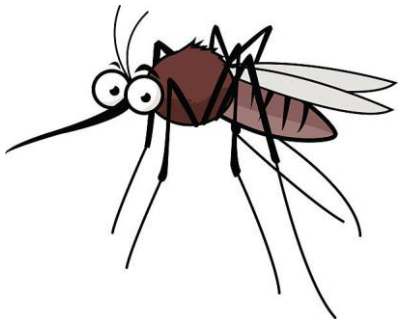
Summer Virology Cases of the CNS: Summary

	West Nile Virus	Powassan Virus	Enterovirus
Clinical	Neurotropic arbovirus	Neurotropic arbovirus	CNS, HFMD, respiratory, other
Virus	Enveloped RNA virus	Enveloped RNA virus	**Non-enveloped** RNA virus
Transmission	Mosquito	Tick	Fecal-oral, respiratory, fomites
Diagnosis	Serology (PCR)	Serology (PCR)	PCR
Population affected	Elderly	Elderly (Children)	Infants
Treatment	Supportive	Supportive	Supportive
Seasonality	Summer (peak Aug)	Spring-Fall (peak June)	Summer-Fall

Acknowledgements

- SSM Health St. Mary's Madison Microbiology/ Molecular Team
- WashU/BJH Microbiology/ Molecular Team
- ID physicians for sharing their interesting arbovirus cases

Questions?



Objectives

- Recognize CSF findings that are commonly associated with viral infections
- Review real-world cases to examine clinical symptoms– either typical or atypical – associated with each virus.
- Compare and contrast viral transmission, epidemiological trends, and diagnostic methods for each virus.

West Nile DxSelect IgM and IgG Performance

Patient Population		Reactivity	Focus WNV ELISA	
			IgM	IgG
Sensitivity	Meningioencephalitis (ME) Patients	Clinical Sensitivity (WNV PRNT positive and ME samples)	93%	97%
	WNV PRNT Positives	Agreement with WNV PRNT Positives	100%	36%*
Specificity	Meningioencephalitis (ME) Patients	Agreement with CDC WNV ELISA Negatives	100%	99%
	Samples submitted for non-WNV clinical testing	Agreement with CDC WNV ELISA Negatives	100%	97%

- Focus equivocal results were considered negative
- Specificity is used here to mean agreement with the CDC ELISA
- Focus WNV IgM results use the background subtract method

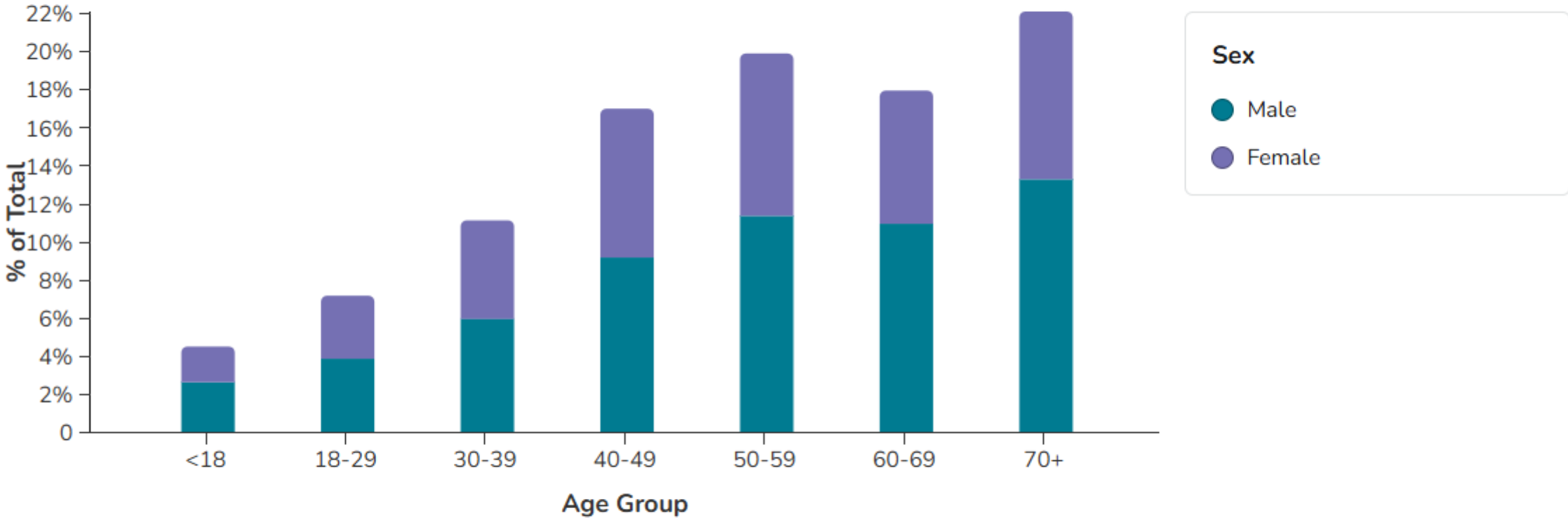
Enterovirus

TABLE 1 EVs, RVs, and PeVs affecting humans

Virus	Species	Serotypes, types, common name[a, b]
Enterovirus (EV)	EV A (21 types)	
	Coxsackievirus (CV)	CV-A2–A8, A10, A12, A14, A16
	Enterovirus (EV)	EV-A71, A76, A89–A92, A114, A119–A121
	EV B (62 types)	
	Coxsackievirus (CV)	CV-A9, B1–6
	Echovirus (E)	E1–7, 9, 11–21, 24–27, 29–33
	Enterovirus (EV)	EV-B69, B73–B75, B77–B88, B93, B97, B98, B100, B101, B106, B107, B110–B113
	EV C (23 types)	
	Coxsackievirus (CV)	CV-A1, A11, A13, A17, A19–A22, A24
	Poliovirus (PV)	PV1–3
	Enterovirus (EV)	EV-C95, C96, C99, C102, C104, C105, C109, C113, C116–118
	EV D (4 types)	
	Enterovirus (EV)	EV-D68, D70, D94, D111
Rhinovirus	RV A (80 types)	RV-A1, A2, A7, A9–A13, A15, A16, A18–A25, A28, A30–A34, A36, A38–A41, A43–A47, A49–A51, A53–A68, A71, A73–A78, A80–A82, A85, A88–A90, A94, A96, A98, A100–A109
	RV B (30 serotypes)	RV-B3–6, B14, B17, B26, B27, B35, B37, B42, B48, B52, B69–70, B72, B79, B83–84, B86, B91–93, B97, B99–104
	RV C (55 types)	RV-C1–55
Parechovirus (PeV)	PeV A (18 types)	HPeV A1–18

[a]From https://ictv.global/report/chapter/picornaviridae/picornaviridae/enterovirus.
[b]From https://ictv.global/report/chapter/picornaviridae/picornaviridae/parechovirus.

West Nile virus human disease cases by age and sex, 1999-2023



Data Table - West Nile virus human disease cases by age and sex, 1999-2023 +

Download Data (CSV)