

# Mosquito-Borne Viruses of the Midwestern United States

(As if you needed another reason to dislike mosquitoes)

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WCLN Webinar Series

Eric T. Beck, PhD, D(ABMM), MB(ASCP)<sup>CM</sup>

Technical Director-Microbiology

ACL Laboratories/Advocate Aurora Health



## Outline

- Brief overview on mosquito-borne diseases
- Mosquito-borne viruses of the Midwestern United States
  - Viral Characteristics
    - Mosquito vectors
    - Primary hosts
  - Symptoms
  - Diagnosis
- Clinically relevant travel-related mosquito-borne viruses
- Overwintering Mechanism
- Summary
- Questions



## Mosquito-Borne Diseases

- Worldwide, vector-borne diseases have been estimated to account for >17% of infectious disease burden
- Over 1 billion vector-borne infections each year with the majority being mosquito-borne<sup>1-3</sup>
  - Malaria – 228 million cases in 2018; 405,000 deaths
  - Dengue – 100 – 400 million cases per year; severe disease in less than 1%
- The US CDC considers the mosquito the deadliest animal in the world



## Vector-Borne Disease in WI: 2016 – 2018<sup>4</sup>

- From 2016 – 2018 WI Averages
  - 110 cases of mosquito-borne infection each year
  - 3200 cases of tick-born infection each year
- Of mosquito-borne infections, viruses account for approximately 80%
  - Remainder are almost exclusively imported malaria cases
  - Most common locally acquired viruses are
    - West Nile Virus
    - California Encephalitis Virus
  - Most common imported viruses are
    - Dengue
    - Zika

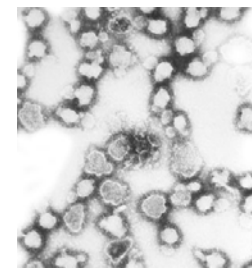
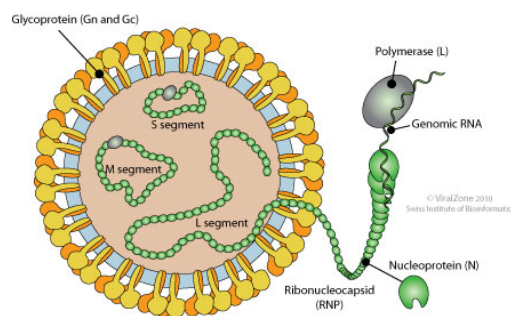


# California Serogroup Viruses



## California Serogroup Viruses

- Family *Peribunyaviridae*
- Genus *Orthobunyavirus*
- Includes:
  - California Encephalitis Virus
  - La Crosse Virus
  - Jamestown Canyon Virus
- Enveloped virion
- Genome
  - Three segments
  - Negative-sense
  - Single stranded RNA



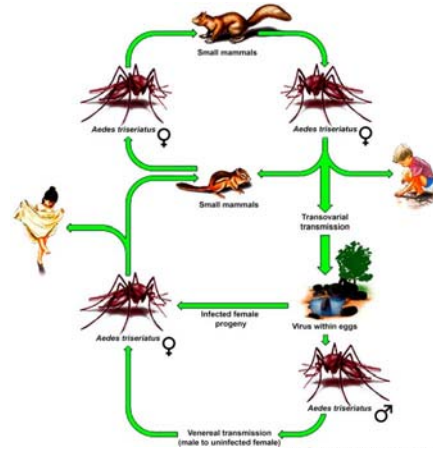
<https://www.cdc.gov/lac/tech/virus.html>

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Seis Institute of Biomedicine



## California Serogroup Viruses

- Traditional life cycle between small mammals and mosquitoes
- Humans are dead end hosts
- Transmitted by a variety of mosquitoes including: *Aedes*, *Ochlerotatus*, *Culiseta*
- May be transmitted from female to her eggs (transovarial transmission)
- Venereal transmission from males to females
- Extrinsic incubation period 7-14 days



## La Crosse Encephalitis Virus<sup>5-6</sup>

- Named for La Crosse, WI
  - Virus identified in 1964 (case from 1960)
- Primary vector: *Ochlerotatus triseriatus*
- Distribution: Midwestern and Southeastern US
- Incubation Period: 5 – 15 Days
- Disease Manifestation:
  - Often asymptomatic
  - Mild disease includes fever, malaise, headache, nausea, vomiting
  - Severe disease includes encephalitis and seizures

*Ochlerotatus triseriatus* – primary vector of LACV in WI



# La Crosse Encephalitis Virus

- Epidemiology:
  - Can infect all age groups
  - Most common around wooded areas
  - Almost all serious infection occurs in children < 16 years of age
- Mortality rate: < 1 % in patients with encephalitis
- Annual cases:
  - Mild infections likely very underreported
  - Neuroinvasive cases approximately 70 per year in US
- Prevention: Mosquito repellent
- Treatment: Supportive care

   
La Crosse virus neuroinvasive disease cases reported by state of residence, 2009-2018



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



# La Crosse Encephalitis Virus

- Immunity: Infection is thought to confer life-long immunity
- Long-Term Issues:
  - Neurologic sequelae include:
    - Recurrent seizures
    - Cognitive abnormalities
    - Hemiparesis (weakness on one side of body)
  - Medical costs associated with sequelae can range from \$50K – \$3M<sup>7</sup>



## Jamestown Canyon Virus<sup>8</sup>

- Discovered in *Culiseta* mosquitoes in Jamestown, CO in 1961
- Vectors include: *Aedes*, *Ochlerotatus*, *Culex*, and *Culiseta* sp.
- Primarily found in MN and WI
- Incubation Period: 5 – 15 Days
- Disease Manifestation:
  - Often asymptomatic
  - Mild disease includes fever, malaise, headache, mild respiratory symptoms
  - Severe disease includes encephalitis and meningitis

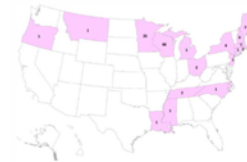
*Culiseta inornata* - a competent JCV vector in WI



## Jamestown Canyon Virus

- Epidemiology:
  - Can infect all age groups
  - Most common around wooded areas
  - No clear age group at risk
- Mortality rate: < 1 % in patients with encephalitis
- Annual cases:
  - Mild infections likely very underreported
  - Neuroinvasive cases approximately <50 per year in US

Jamestown Canyon virus neuroinvasive disease cases reported to CDC by state of residence, 2009 –2018



# Jamestown Canyon Virus

- Prevention: Mosquito repellent
- Treatment: Supportive care
- Long-Term Issues: Thought to be very rare
- Immunity: Infection is thought to confer life-long immunity

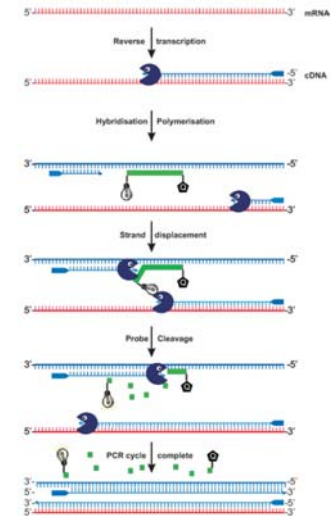


# Diagnosis of California Serogroup Viruses



## Real-Time RT-PCR

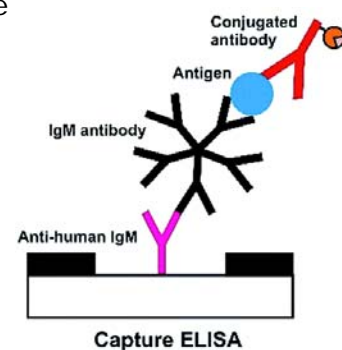
- Reverse Transcriptase Polymerase Chain Reaction
- Detects the presence of virus
- High specificity
- Virus often only present at detectable levels early during course of infection
- La Crosse and Jamestown Canyon Assays available
  - Performed at CDC
  - Best specimen is usually CSF



From Bustin. 2005. Real-time reverse transcription PCR and its potential use in clinical diagnosis. *Clin Sci*. 109: 365-379

## IgM Capture ELISA (MAC-ELISA)

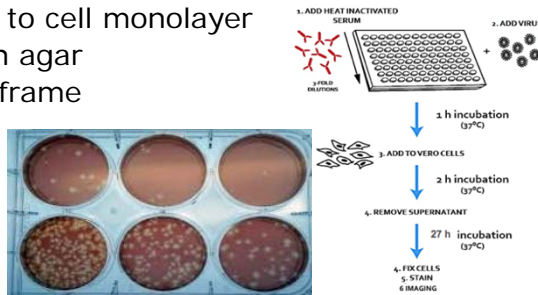
- Detects early immune response to virus
- IgM testing via MAC-ELISA (at WSLH)
  - Anti-human IgM antibodies conjugated to plate
  - Patient specimen added to capture IgM molecules
  - Specific virus antigen added
  - Conjugated virus specific IgG antibody used for detection
- Positive result generally indicative of recent infection
- Many arboviral IgM antibodies are cross reactive





# Plaque Reduction Neutralization Test (PRNT)

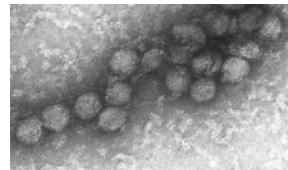
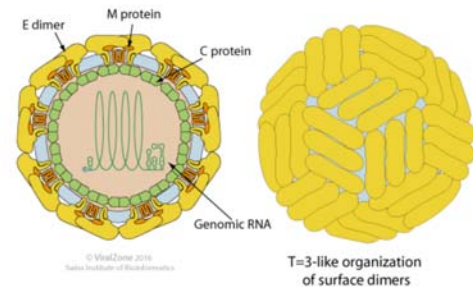
- Plaque Reduction Neutralization Test (PRNT) performed at CDC
  - More specific than MAC-ELISA
  - Prepare serial dilutions of serum
  - Combine dilutions with standard viral inoculum
  - Add serum/virus combination to cell monolayer
  - Remove and overlay cells with agar
  - Incubate for appropriate timeframe
  - Identify serum titer that reduces viral plaques by 50%



## Mosquito-Borne *Flaviviruses* of the US

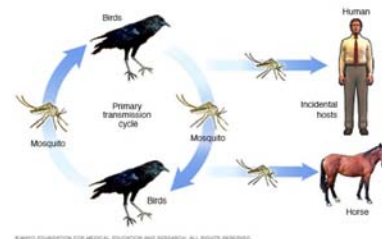
## Mosquito-Borne *Flaviviruses*

- Family *Flaviviridae*
- Genus *Flavivirus*
- Viruses circulating in the United States include:
  - West Nile virus
  - St. Louis Encephalitis
- Other common mosquito-borne *Flaviviruses*:
  - Japanese encephalitis virus
  - Dengue virus
  - Yellow fever virus
- Viral properties:
  - Enveloped
  - Non-segmented, positive sense, single stranded RNA genome



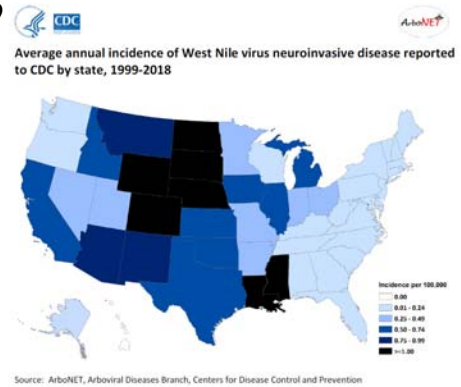
## Mosquito-Borne *Flaviviruses*

- Endemic in the United States:
  - West Nile Virus
  - St. Louis Encephalitis virus
- Traditional life cycle between birds and mosquitoes
- Primary vectors: *Culex tarsalis* and *Culex pipiens* mosquitoes
- Humans and horses are dead end hosts
- Generally cause asymptomatic or mild febrile illness
- Neuroinvasive infections are relatively rare



## West Nile Virus<sup>9-11</sup>

- First identified in West Nile District of Uganda in 1937
- First US cases identified in New York in 1999
  - Believed to have originated from Israel
  - Unknown if virus entered US in infected birds or mosquitoes
- Cases in 3 states in 2000
- Cases in 45 states by 2003
- Leading mosquito-borne disease in the United States including WI
- Found in all 50 states



## West Nile Fever

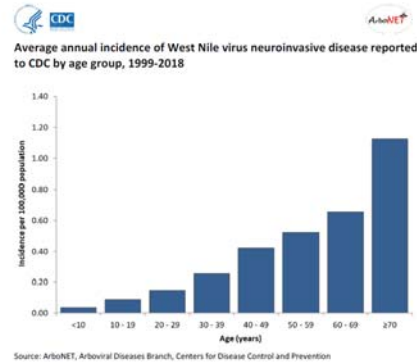
- Occurs in about 25% of cases
- Affects all ages
- Common symptoms:
  - headache, fever, myalgia, rash (generally at time of defervescence)
- Less common symptoms:
  - nausea, joint pain, eye pain
- Can last days to weeks, with prolonged fatigue

*Culex* sp. mosquitoes – primary vector of WNV in WI



## Neuroinvasive West Nile

- Occurs in < 1% of cases
- Disease Manifestation:
  - Meningitis
  - Encephalitis
  - Acute flaccid paralysis
- Lasts weeks to months
- Long term functional and cognitive difficulties are common
- Mortality rate ~10% in neuroinvasive disease



## West Nile Virus

- Prevention: Mosquito repellent
- Treatment: Supportive care
- Vaccine available for horses
- Average 2000-3000 reported cases annually
- Can be transmitted via blood transfusion
- Minimal transmission possible during pregnancy or breastfeeding



## West Nile Virus Diagnosis

- IgM Capture ELISA (MAC-ELISA) or Microsphere Immunoassay (MIA)
  - Generally present by day 8 of disease
  - Disappears within a few months post infection
  - May be detectable in CSF earlier than blood
  - MIA performed at WSLH
- IgG draw at day 7 and 21 to compare titers
- RT-PCR
  - CSF or Blood
    - Only useful very early in infection prior to Ab production
    - Sensitivity is very low ~50% in CSF; 10% in blood



## St. Louis Encephalitis Virus<sup>12-13</sup>

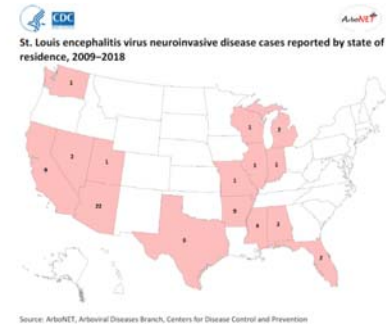
- First identified in St. Louis, MO in 1933
- Transmitted primarily by *Culex* mosquitoes
- Primarily found in Central and Western states
- Largest US epidemic was in 1975
  - Almost 2000 cases of SLEV neuroinvasive disease
  - Ohio/Mississippi River basin
- Incubations period is 5-15 days
- Less than 1% of cases are clinically apparent disease

*Culex* sp. mosquitoes – primary vector of SLEV in WI



# Neuroinvasive St. Louis Encephalitis Virus

- Abrupt onset:
  - Fever, headache, dizziness, nausea
- Disease may end or progress to include:
  - Stiff neck, confusion, dizziness, tremors or other signs of CNS disease
- Risk of severe disease increases with age
- Up to 90% of elderly develop encephalitis
- Case fatality rate of about 10%
- Symptoms can persist for up to three years



# St. Louis Encephalitis Virus

- Diagnosis consists of:
- MAC-ELISA or MIA
  - Performed on serum or CSF
    - IgM present one week after onset; declines rapidly (~ 3 months)
    - IgM positive indicates acute infection
  - Performed at WSLH
- IgG 4-fold rise in titer between acute and convalescent serum

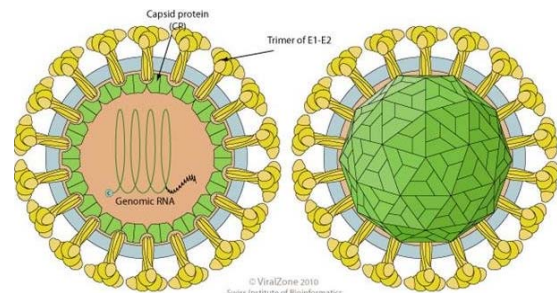


# Mosquito-Borne *Alphaviruses* of the US



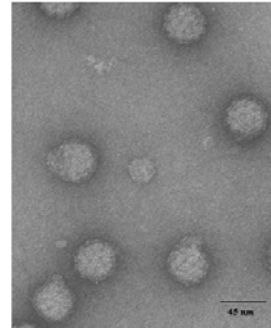
## Mosquito-Borne *Alphaviruses*

- Family *Togaviridae*
- Genus *Alphavirus*
- Viruses circulating in the United States include:
  - Eastern Equine Encephalitis Virus
  - Western Equine Encephalitis Virus
- Other common mosquito-borne *Alphaviruses*:
  - Chikungunya
  - Venezuelan Equine Encephalitis Virus
- Viral properties:
  - Enveloped
  - Non-segmented, positive sense, single stranded RNA genome



## Mosquito-Borne *Alphaviruses*

- Endemic in the United States:
  - Eastern and Western Equine encephalitis viruses
- Traditional life cycle between birds and mosquitoes
- Primary vectors: *Culex tarsalis* and *Culex pipiens* mosquitoes
- Humans and horses are dead end hosts
- Generally cause asymptomatic or mild febrile illness
- Neuroinvasive infections are relatively rare



## Eastern Equine Encephalitis Virus<sup>14-15</sup>

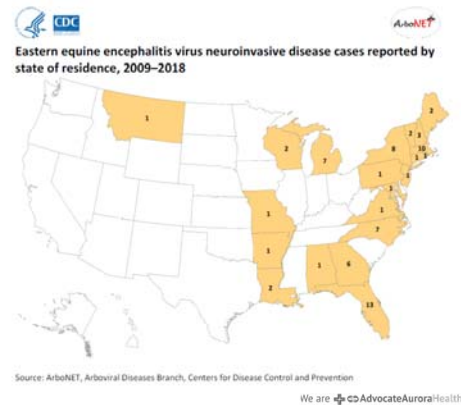
- First human cases in 1938 in Massachusetts
- Maintained in nature between *Cs. Melanura* and birds
- *Aedes*, *Culex*, and *Coquilletidia* mosquitoes transmit from birds to humans
- Greater risk for patients:
  - Under 15 years of age
  - Over 50 years of age
- Systemic febrile illness
  - Fever, chills, arthralgia, myalgia
  - Last 1-2 weeks
  - Generally complete recovery occurs





# Eastern Equine Encephalitis Virus Infections

- Neurologic illness
  - Fever, headache, vomiting, diarrhea, seizures, behavioral changes
- Average 7 cases reported annually
  - Almost all reported cases are neuroinvasive; systemic cases rarely reported
- No treatment
- Vaccine available for horses
- Most cases since 1930s occurred in 2019
  - 34 cases; 35% fatality rate



## EEEV in the News

### Deadly mosquito-borne disease EEE reportedly on the rise

By Lisa Eichenbaum  
June 9, 2020 | 10:00am | Updated



**This rare mosquito-borne virus may be a threat this summer — what you need to know**

**WASH DC: MOSQUITOES**  
A report about growing outbreaks of EEE virus, or Eastern equine encephalitis, has gone viral. Vaccine made of mosquito spit could help stop next epidemic.



### A tiny mosquito bite took away this Michigan teen's ability to talk, walk. Now, she fights.

Kristen Jordan Shamus, Detroit Free Press  
Published 1:07 p.m. ET Feb. 2, 2020 | Updated 4:40 a.m. ET Feb. 4, 2020



Savannah DeHart, 15, works through her rehabilitation at the Mary Free Bed Rehabilitation Hospital in Grand Rapids after contracting the EEE virus Detroit Free Press

### Mass. Senate OKs bill to fight mosquito-borne virus EEE

Last year, Massachusetts saw a resurgence of EEE, with more than 200 communities designated as moderate to critical risk by the state Department of Public Health.



In this Aug. 16, 2019, file photo, Salt Lake City Mosquito Abatement District biologist Taylor Baleson examines a mosquito in Salt Lake City. State and federal health officials are reporting a higher than usual number of deaths and illnesses from a rare, mosquito-borne virus this year. (AP Photo/Mark Duncan, File)

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AP  
June 14, 2020 | 10:00am

**BOSTON (AP)** — A bill intended to help protect the public from Eastern Equine Encephalitis has been approved by the Massachusetts Senate.

# Western Equine Encephalitis Virus<sup>16</sup>

- First isolated from horses in San Joaquin Valley, CA in 1930
- Maintained in nature between *Culex* mosquitoes and birds
- No reported cases in US since 1994
- Milder than EEEV
- Case fatality rate is < 10%
- Greater risk for infants and elderly
- No vaccine or treatment available



# Common Travel-Related Mosquito-Borne Viruses



# Zika Virus<sup>17</sup>

- Family *Flaviviridae*; Genus *Flavivirus*
- Maintained in nature between *A. aegypti* and humans
  - Possible transmission between mosquitoes and non-human primates
- Low level circulation prior to first reported outbreak in 2007 in Micronesia
- Transmitted via
  - Mosquito bite
  - Blood transfusion
  - Congenital transmission
  - Sexual transmission
- Symptoms generally mild; last < 7 days



# Zika Virus

- Congenital infection associated w/ microcephaly
  - Brain and head smaller than expected
  - 6671 cases in Brazil 10/22/15 – 3/19/16
  - Of 2378 cases investigated 907 linked w/ congenital Zika infection
  - 163 cases on average from 2001 – 2014
- Association with Guillain-Barré syndrome
  - Auto-immune disease body attacks peripheral nervous system
- 1 travel related Zika case in US in 2020
- 8 locally acquired cases in US territories in 2020



## Zika Virus Diagnosis

- Cases have dramatically decreased and testing only recommended on symptomatic pregnant women with recent travel history
- RT-PCR testing
  - Serum or urine (virus shed in urine longer)
- Serology
  - IgM can persist for months to years
  - Antibody tests are cross reactive with DENV
    - Must be confirmed by PRNT



## Japanese Encephalitis Virus<sup>18-19</sup>

- Family *Flaviviridae*; Genus *Flavivirus*
- Leading cause of vaccine preventable encephalitis in Asia
- Maintained in nature between *Culex tritaeniorhynchus* and pigs and birds
- Humans are dead-end hosts
- <1% of those infected develop clinical disease
- Incubation time 5-15 days

Geographic Distribution of Japanese Encephalitis Virus



# Japanese Encephalitis Virus

- Encephalitis Cases:
  - Most common in children <14 years
  - 50K – 175K reported cases/year
  - 30% fatality rate
  - 30% report neurological sequelae
- Symptoms include:
  - Parkinsonian syndrome
  - Acute flaccid paralysis similar to polio has been reported



# Japanese Encephalitis Virus

- One of few mosquito-borne viruses with vaccine available
  - Highly efficacious
  - 2 dose vaccine series
  - Recommended for US citizens only if long term travel to endemic countries
- Diagnosis
  - IgM Serology from Serum or CSF
    - IgM detectable for 30 – 90 days post infection
    - Plaque reduction neutralization testing used to confirm positive serological tests
  - RT-PCR testing may be used in fatal cases



## Dengue Virus<sup>20-22</sup>

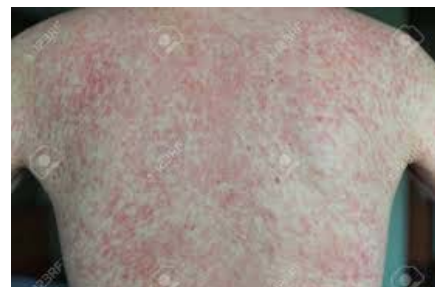
- Family *Flaviviridae*; Genus *Flavivirus*
- Dengue outbreaks described dating back to the 1600s
- Virus first isolated in 1943-44
- Maintained in nature between *A. aegypti* and humans
- Nearly half of the world lives in endemic regions;  
endemic in over 100 countries
- Primarily travel-related infections in US
- Occasional local transmission seen in Southern US
- 4 Serotypes (DENV-1, -2, -3, and -4)
- 400 million infections annually

*Aedes aegypti* – primary vector of DENV worldwide



## Dengue Fever

- Symptoms are sudden onset
  - Fever, eye pain, muscle pain, joint pain, and rash
  - Originally referred to a “break-bone fever”
- Recovery occurs after a few days
- 114 cases reported in the US already in 2020



## Dengue Hemorrhagic Fever

- More common following secondary infection with different serotype
- Progression from Dengue Fever usually evident around time of defervescence
- Transitional Symptoms include:
  - Severe abdominal pain, vomiting, hypothermia
- Mild hemorrhagic symptoms:
  - Petechiae, nose bleeds, gum bleeding
- Severe hemorrhagic symptoms:
  - Vaginal bleeding, vomiting blood, bloody stool, intracranial bleeding
- >20,000 deaths annually worldwide



## Dengue Virus Diagnosis

- Molecular diagnosis days 1-7 post infection
- FDA approved NS1 antigen detection tests
  - Detect NS1 protein of DENV
  - Similar timeframe as Molecular tests
- Serologic diagnosis
  - IgM:
    - typically present about 5 days post infection
    - Persist for approximately 3 months
  - IgG:
    - Required paired acute and convalescent serum
- Immunity is lifelong



## Yellow Fever Virus<sup>23</sup>

- Family *Flaviviridae*; genus *Flavivirus* (type virus)
- Transmitted by *A. aegypti*
- Most infections are asymptomatic
- Mild illness includes:
  - Sudden onset of fever, chills, headache, nausea, vomiting, fatigue
  - Symptoms improve in about a week
- Severe illness includes:
  - Initial symptoms may appear to resolve briefly
  - High fever, jaundice, bleeding from eyes, nose, mouth,
  - Stomach, vomiting blood, shock, organ failure
  - 30-60% mortality



## Yellow Fever Virus

- Primarily circulating in South America and Africa
- Cases in US are travel related
  - In 1793, over 5% of Philadelphia's population died of yellow fever
  - Began with refugees fleeing yellow fever epidemic in Caribbean
- Treatment is supportive
- Yellow fever virus has a highly efficacious live, attenuated vaccine
- IgM serology
  - Can persist for years
  - Plaque reduction neutralization testing used to confirm positive serological tests
- RT-PCR testing may be used in fatal cases





## Chikungunya Virus<sup>24-25</sup>

- First recognized as human pathogen after isolation from outbreak of arthritic disease in Tanzania in 1952
- In Kimakonde language translates to “that which bends up”
- Very little asymptomatic infection
- Chikungunya fever
  - Rapid onset fever
  - Polyarthralgia and arthritis
  - Rash
  - Myalgia and headache
- Rarely fatal



## Chikungunya Virus

- Recurring musculoskeletal disease affecting peripheral joints
  - Can last months to years
- Transmitted by *A. aegypti*
- United States currently has 8 cases in 2020 all travel related
- Last few years in US, typically a few hundred cases annually
- Viral RNA present during first few days of infection, RT-PCR testing available
- Acute and convalescent IgM should be collected (acute may be sufficient if positive)

Countries and territories where chikungunya cases have been reported\*  
(as of September 17, 2019)



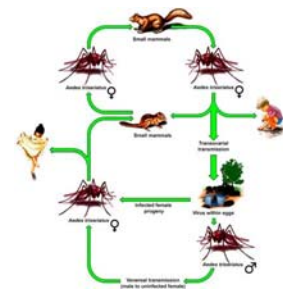
\*Does not include countries or territories where only imported cases have been documented.

# Viral Survival in Temperate Climates



## *Orthobunyaviruses*

- Includes La Crosse and Jamestown Canyon Viruses
- Generally transmitted by *Aedes* and *Ochlerotatus* mosquitoes
  - Lay eggs in dry environment likely to flood or just above water line
  - When flooded, the eggs hatch
- In fall eggs remain dormant through winter
- Many *Orthobunyaviruses* found in temperate climates can be transmitted from female mosquito to her eggs
- In spring, emergence of infected females from eggs allow viruses to resumes their life cycles



## *Flaviviruses*

- Zika, Yellow Fever, Dengue viruses
  - Viruses generally not transmitted transovarially
  - Predominantly transmitted by *Aedes aegypti* mosquitoes
  - *Aedes* mosquitoes survive winter as eggs
  - Winter generally would break any local transmission cycle
- West Nile and St. Louis Encephalitis viruses
  - Viruses generally not transmitted transovarially
  - Predominantly transmitted by *Culex* mosquitoes
  - *Culex* mosquitoes survive winter as adults
    - Avoid freezing remaining dormant in sewers, subways, etc.
  - In spring, emergence of infected adults allow viruses to resume their life cycles



## Questions



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