West Nile virus infection

Neil M. Ampel, M.D.
Professor Emeritus of Medicine and Immunobiology
University of Arizona
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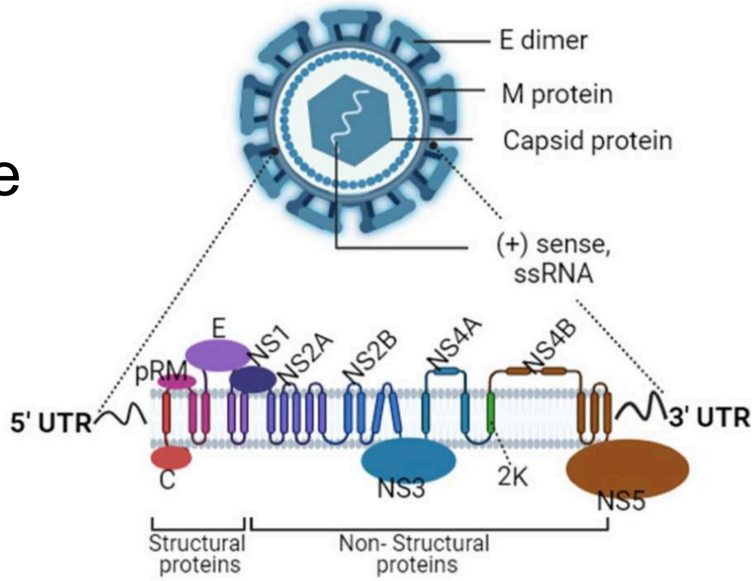
Case presentation

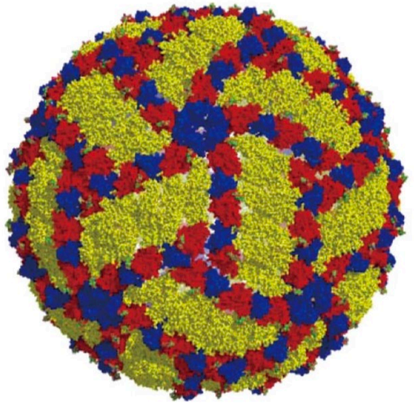
- A 78 year-old man from Peoria, AZ presented with a history of fever, headache, with concentration and memory issues while vacationing in Ohio in late August 2021
  - in Ohio, lumbar CSF analysis revealed 110 wbc/µl (90% lymphocytes); glucose 75 mg/dL; protein 110 mg/dL; no other tests were performed
- He was generally healthy
  - hypertension and hyperlipidemia
  - followed for pulmonary coccidioidomycosis (Valley fever)
- When evaluated in Arizona in September, the fever and headache had resolved but he still had concentration problems and some issues with balance
- Serum IgM for West Nile virus was positive
- He was given a diagnosis of West Nile neuroinvasive disease and referred to Neurology for further care

The virus

- **West Nile virus** (WNV) is a **neurotropic arbovirus** first identified in the blood of a febrile woman in the West Nile district of Uganda in 1937
  Am J Trop Med Hyg 1940; s1-20:471
- Single-stranded positive-sense RNA virus in the genus ***Flavivirus***
  - single open reading frame (11kb) encoding structural and non-structural proteins; translated into a single polyprotein
    - outer protein icosahedral nucleocapsid covered with lipid bilayer envelope
  - belongs to the Japanese encephalitis virus (JEV) antigenic complex that includes the St. Louis encephalitis virus
  - related to dengue, Zika, and yellow fever viruses



Emerg Microbe Infect 2025; 14:2437244

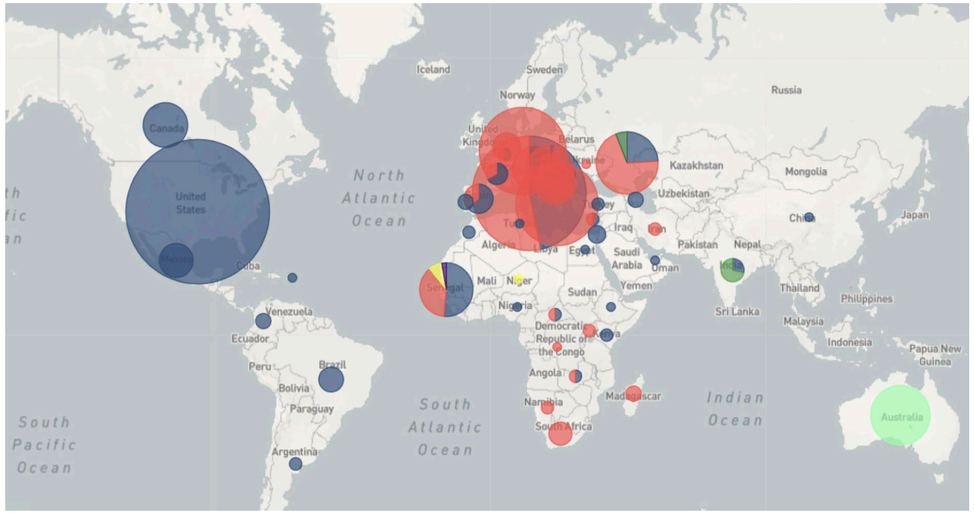


World J Virol 2012; 1:51

Viral genomics and hosts

- Globally dispersed with at least 9 lineages
  - **lineages 1a** and **2** associated with human disease
  - emerged in Africa in 16th or 17th century
  - introduced into North America as a single lineage (1a) from an infected bird or mosquito from the Middle East in 1998
- Life cycle involves arthropod vectors, avian reservoirs and incidental hosts (humans, horses, reptiles)

  Pathogens 2020; 9:589
  - primary arthropod carriers are *Culex* mosquitoes

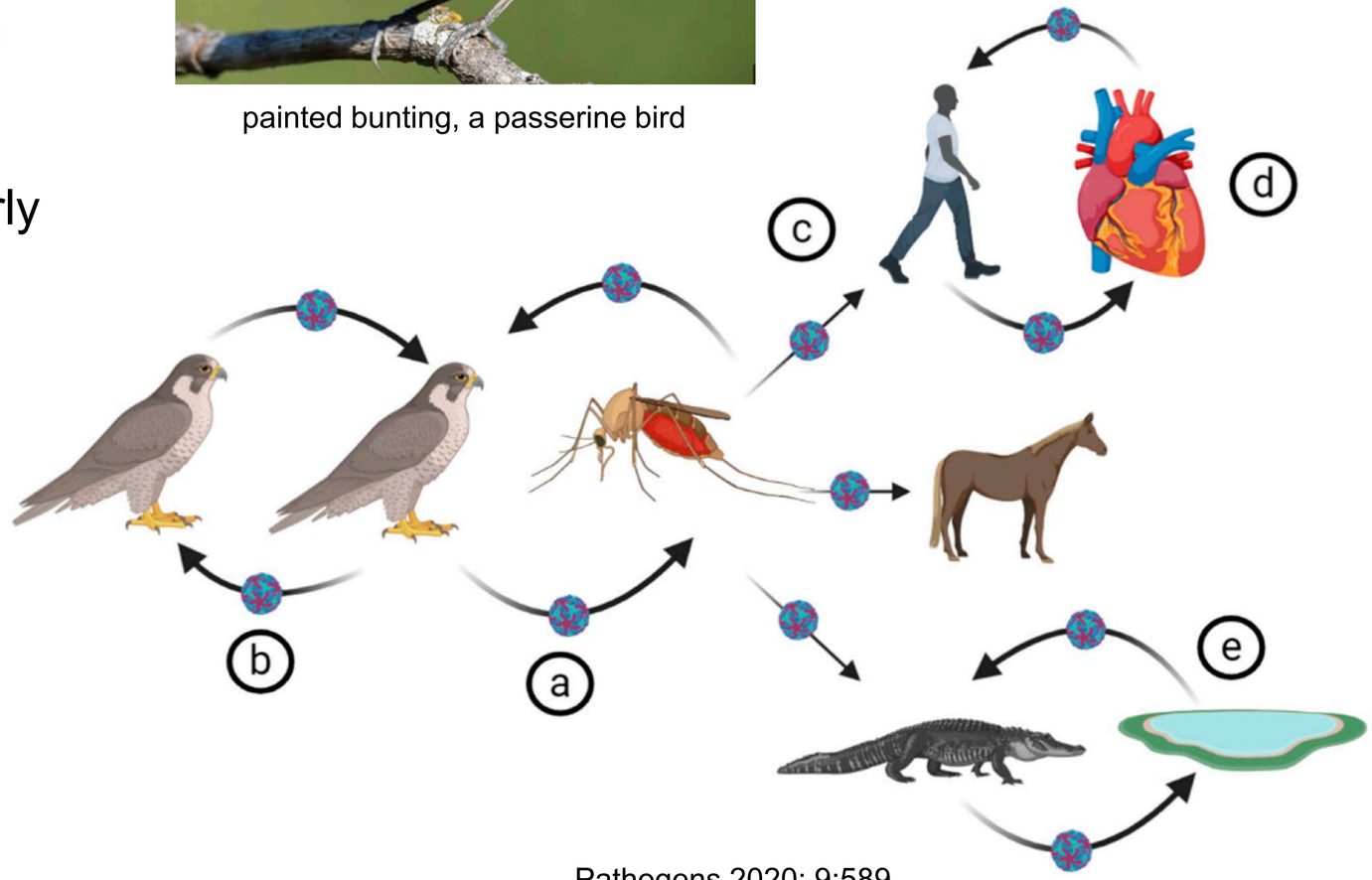


One Health 2024; 18:100664

Life cycle

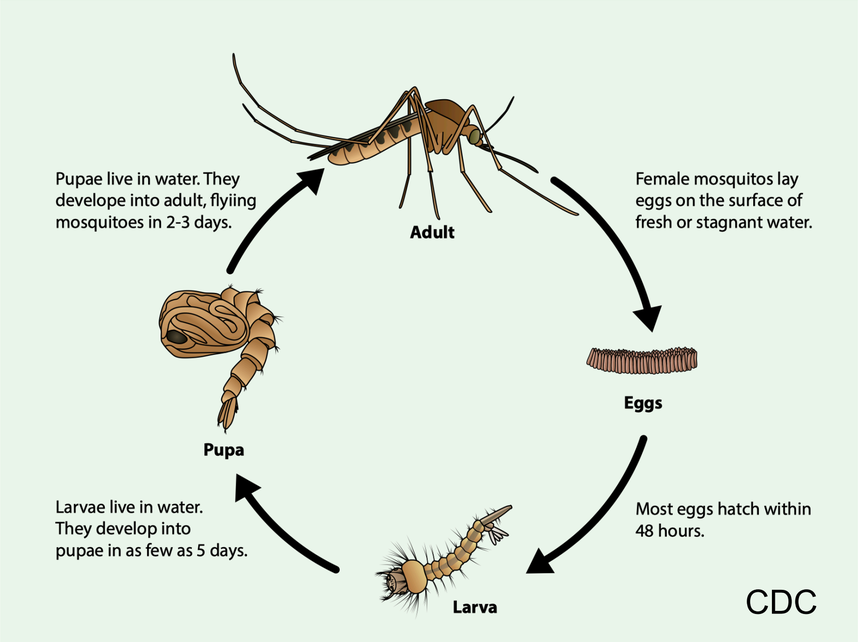
- Persistent reservoir between birds and mosquitoes
  - passerine (perching) birds [four toes, three directed forward and one backward] have high levels of viremia without illness (**amplifying hosts**)
  - high avian mortality in other species, particularly corvids (ravens, crows, and jays) and exotic birds
- Incidental transmission to end-hosts (mammals, reptiles)
  - but crocodiles are amplifying hosts
- Human-to-human transmission via blood transfusion and organ donation

painted bunting, a passerine bird



Pathogens 2020; 9:589

The mosquito

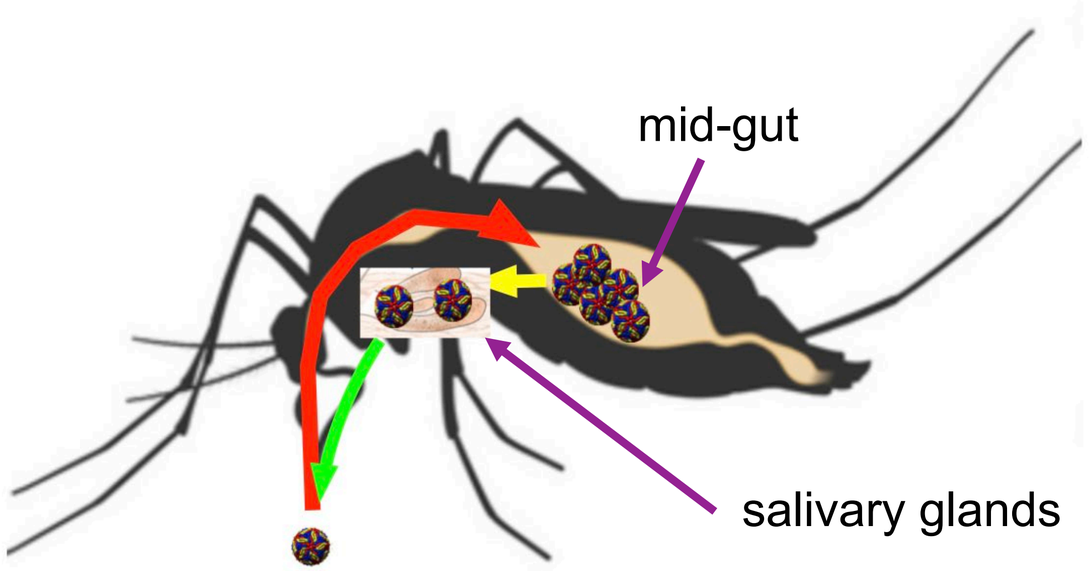


- ***Culex*** mosquitoes are worldwide in distribution
  "common house mosquito"
- **Life cycle** (7-10 days)
  - Females lay <u>eggs</u> in rafts on stagnant water →
    - including artificial sources such as rain barrels, stagnant swimming pools
  - In water, <u>larvae</u> breathe & feed → non-feeding pupae → adult emerges from pupa and flies away (<500m)
  - Development increases with increasing temperature
  - ***Culex tarsalis***, *Culex quinquefasciatus*, and *Culex pipiens* are found in Arizona
- **Females bite** around **dusk and dawn** (crepuscular) at low-light
  - prefer birds (**ornithophilic**) but will feed on mammals and reptiles
  - need blood to lay eggs
  - can enter homes

- **Transmit**
  - WNV, St. Louis encephalitis (SLE) virus, Japanese encephalitis (JE) virus, western and eastern equine encephalitis (WEE & EEE) virus, Rift Valley fever (RVF) virus
  - *Dirofilaria immitis*: cause of heartworm disease in dogs
  - Avian malaria

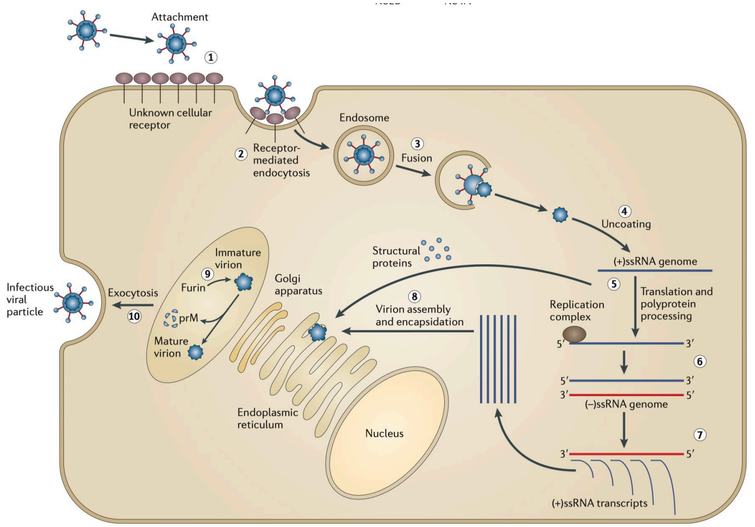
WNV life cycle in the *Culex* mosquito

- Female *Culex* mosquitoes acquire virus during blood meal of infected vertebrate host
- Virus reaches mosquito mid-gut and replicates (amplifying stage)
- Virus spreads to salivary glands as well as to other tissues
  - does not appear to cause disease in the mosquito
- Transmission to next vertebrate host occurs upon feeding with injection of virus from salivary glands

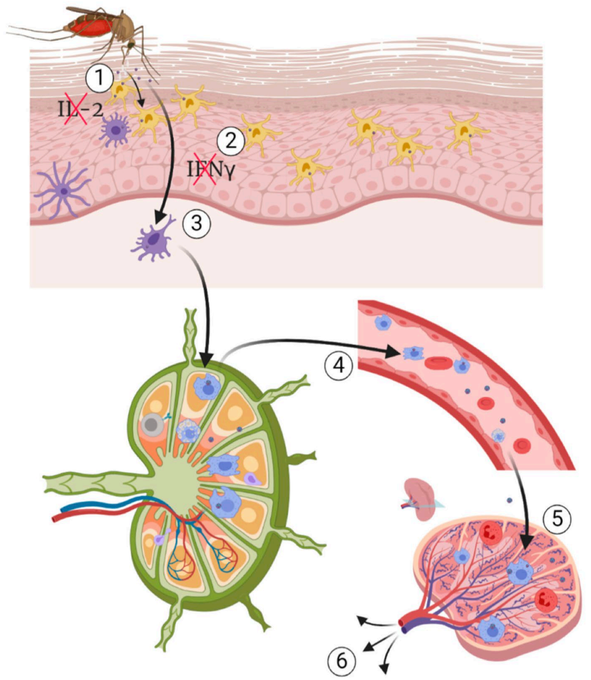


Pathogenesis

- Virus infected saliva is injected intradermally with the mosquito bite
- Virus infects via cell-receptor mediated endocytosis following cell-virus fusion
- Keratinocytes are infected and virus replicates in dendritic cells
- These then migrate to draining lymph nodes and cause an initial viremia
- Virus spreads throughout reticuloendothelial system leading to secondary viremia and organ spread
  - occurs ~1-2 days after bite and persists for ~1 week that ends with the development of IgM antibodies
  - primary infection is presumed to confer life-long immunity and associated with circulating neutralizing antibodies



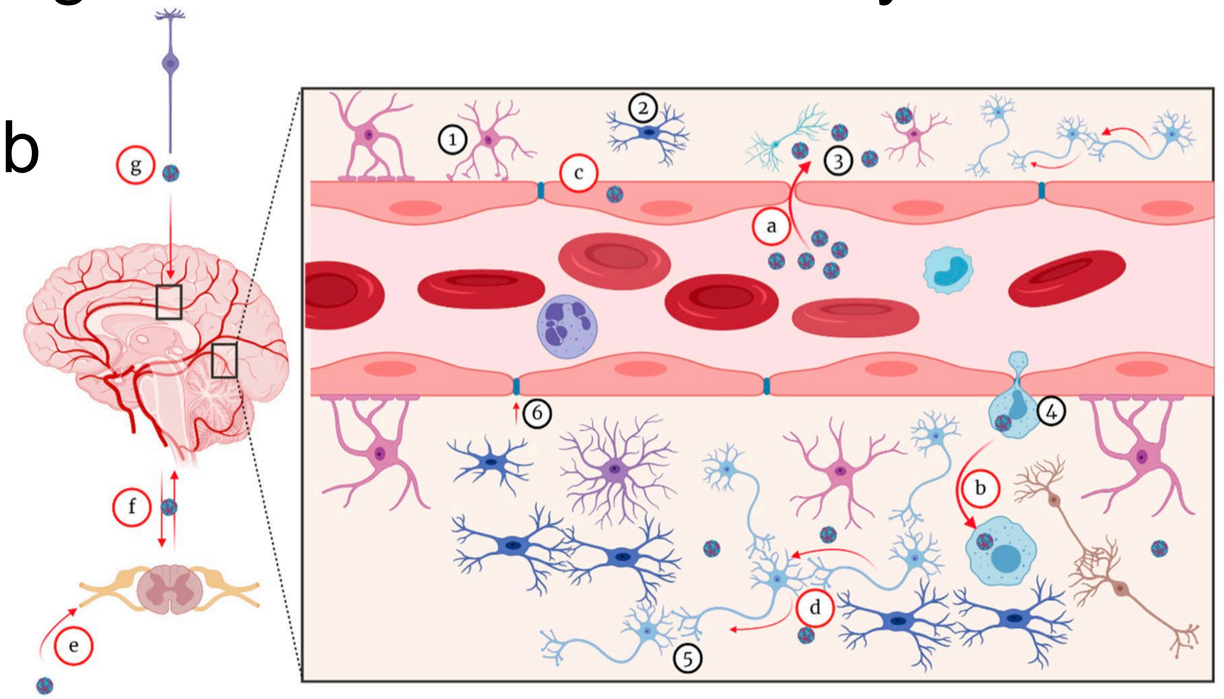
Nature 2013; 11:115



Pathogens 2020; 9:589

Neuroinvasion

- Several possible mechanisms:
  - direct viral infection of endothelial cells of the the blood-brain barrier (BBB)
  - passive migration of free virus through increased permeability of the BBB
  - direct invasion by infected macrophages into central nervous system (“Trojan horse”)
  - neuroinvasion through olfactory bulb
- Retrograde axonal transport
- Migration to spinal cord and brain



Pathogens 2020; 9:589

Epidemiology in the United States

- First noted in NYC as a cluster cases of meningoencephalitis occurring between August and September, 1999

  Science 1999; 286:2333 ; NEJM 2001; 344:1807; One Health 2024; 18:100664

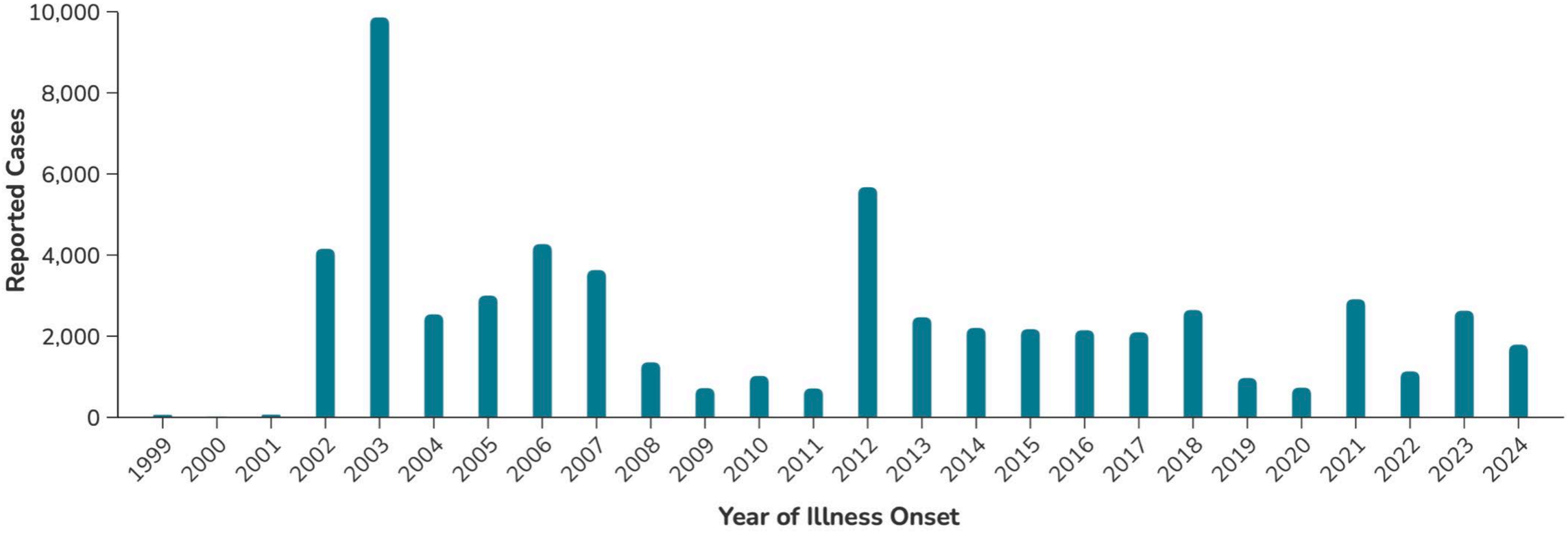
  - 59 hospitalized cases; average 71 years; 7 deaths; attack rate 6.5/1,000,000
  - The outbreak was coincident with extensive mortality in crows and exotic birds

- ArboNET created in 1999
  - passive reporting to state health departments and CDC
  - tracks infections in humans, blood donors, animals (including mosquitoes, dead birds, and sentinel animals), and veterinary cases

- So far in 2025
  - 24 cases WNV disease among 14 states
    - 10 neuroinvasive

Epidemiology in the United States

WNV human disease cases by year of illness onset, 1999-2024



CDC

Epidemiology in the United States

WNV human neuroinvasive disease average incidence/100,000 by county of residence, 1999-2024

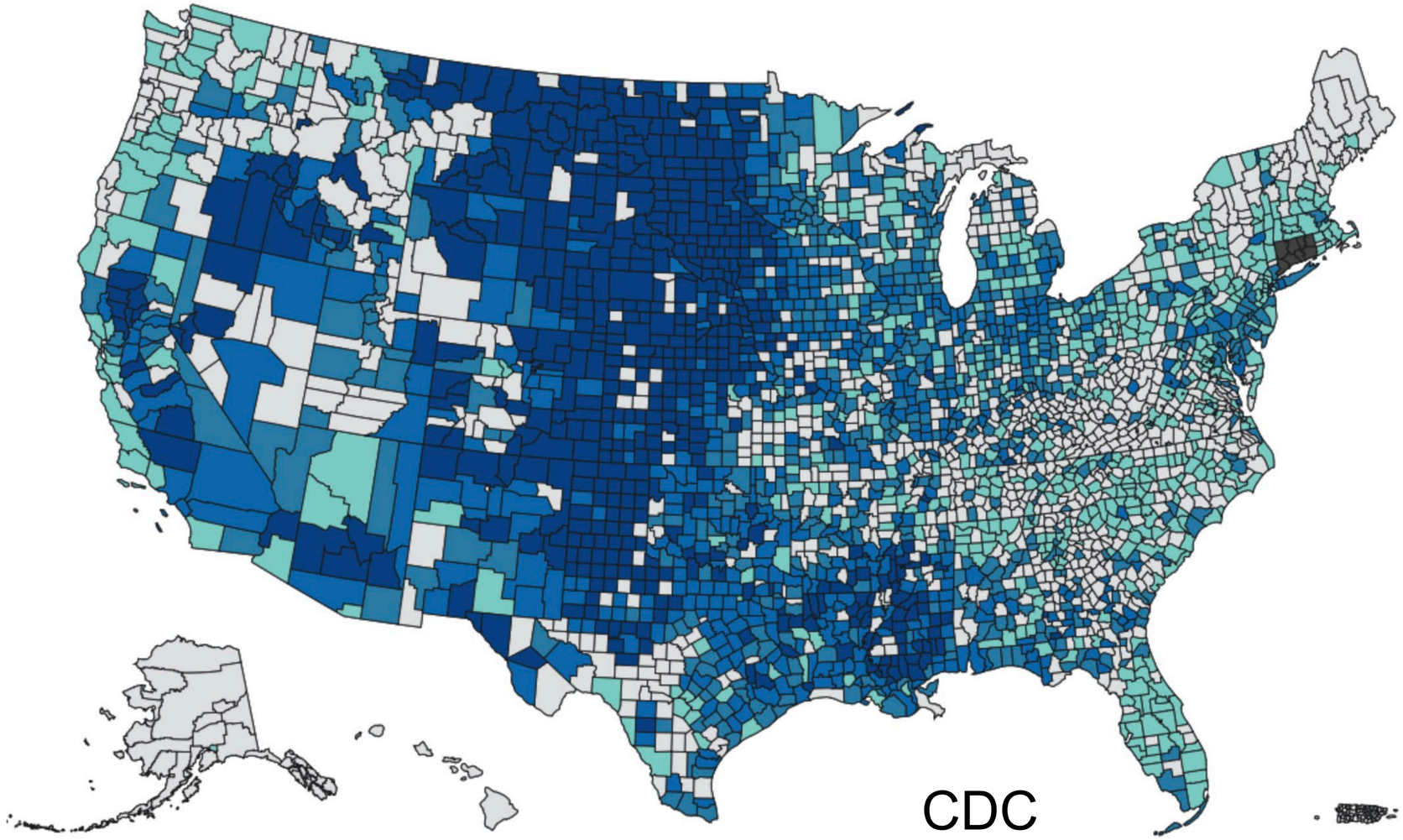
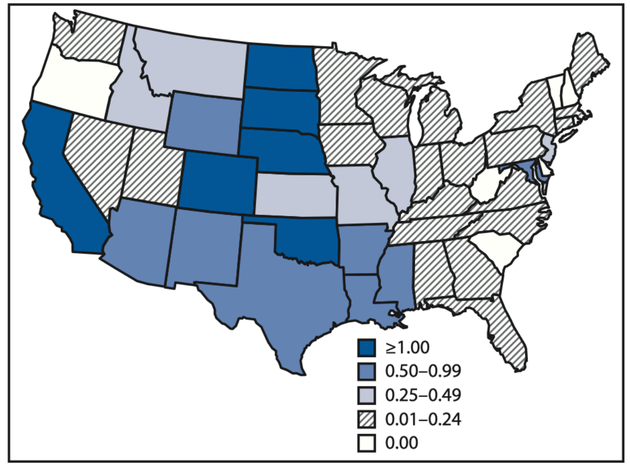


FIGURE. Rate* of reported cases of West Nile virus neuroinvasive disease — United States, 2015

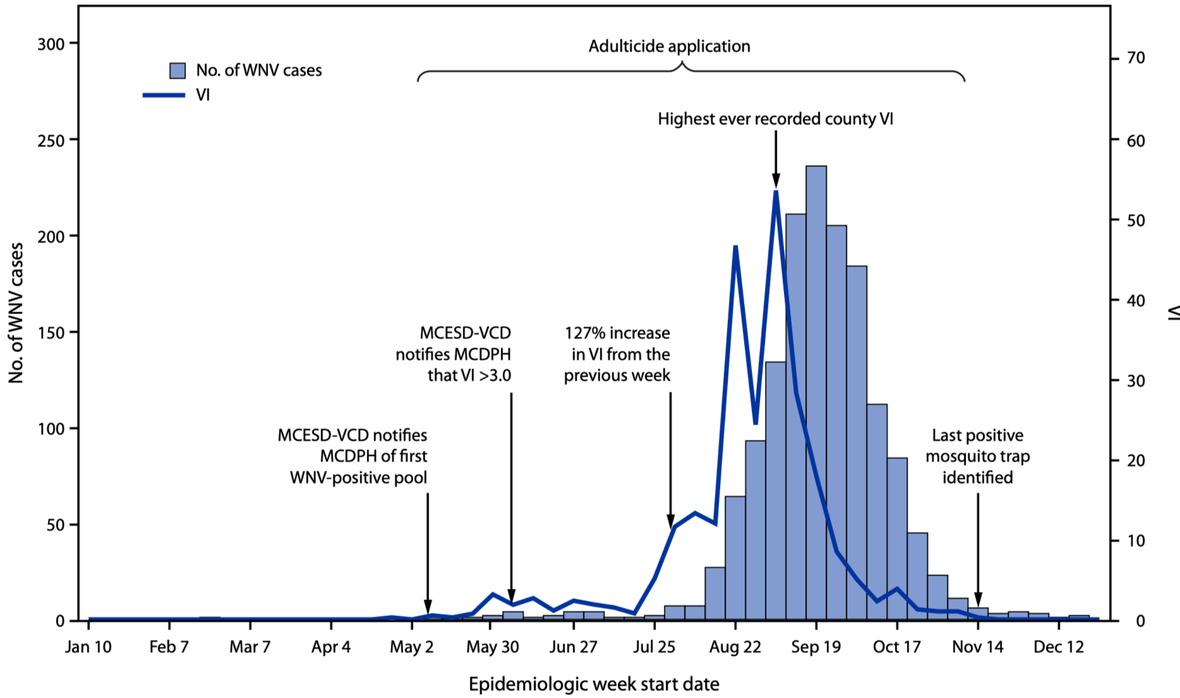


* Per 100,000 population.

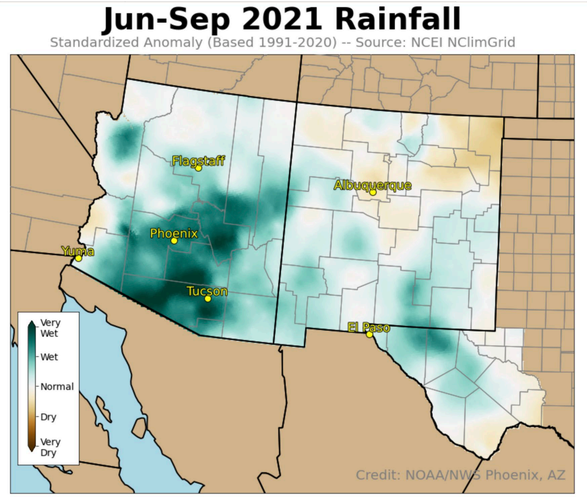
Krow-Lucal, et al. MMWR 2017; 66:51

Epidemiology in Arizona

- The largest reported county outbreak of WNV disease occurred between May-December 2021 in Maricopa County
  - 1487 cases
    - 956 (63%) were neuroinvasive
    - median age 66 years
    - 1,014 hospitalizations
    - 101 (7%) died, median age 79 years
    - 78 (5%) were found among blood donors.
    - 99 cases in Pima;115 cases in Pinal counties
- Associated with a spike in virus-infected mosquitoes (VI)
- Occurred during a 23-day period with 6.6" rain from June-September
  - ↑moisture presumed to maintain mosquito breeding sites

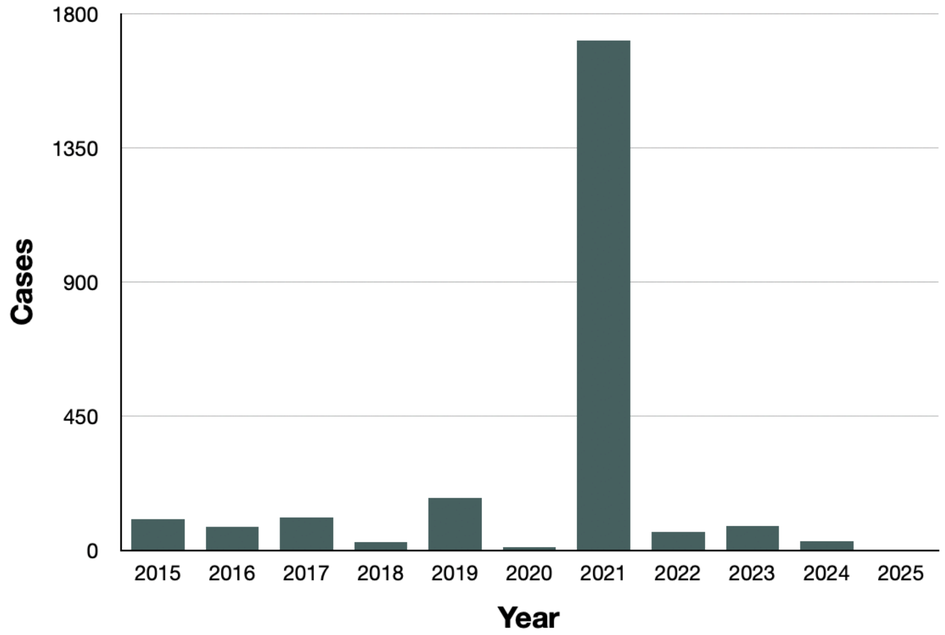


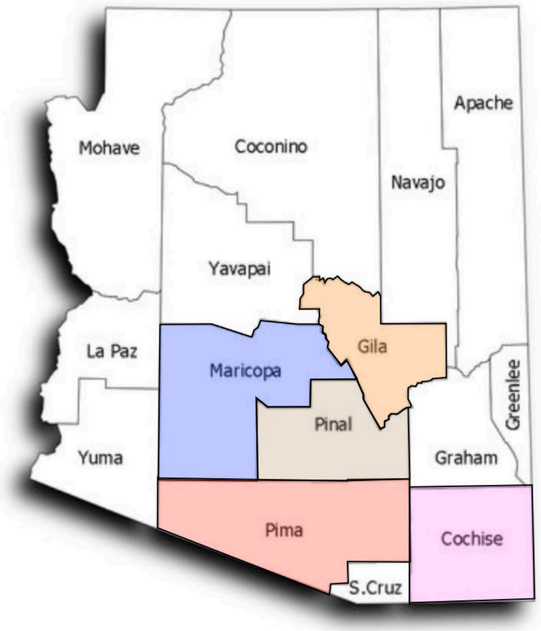
Kretschmer M, et al. MMWR 2023; 72:452



Current Arizona epidemiology

- The number of cases has abated since 2021
  - <100 cases/year
- In 2024
  - 31 cases
    - Maricopa: 20
    - Pinal: 4
    - Pima: 3
    - Gila: 3
    - Cochise: 1
- 17 WNV infections this year (22 July, 2025)





July 22, 2025; Arizona Daily Star

1st West Nile-virus death of season

MICHELLE CRUZ
Arizona Republic

Maricopa County health officials announced the first West Nile virus-related death of the season, in "an older adult with underlying health conditions."

A total of 17 human cases of West Nile virus were confirmed in Maricopa County so far this year, the Maricopa County Department of Public Health said in a news release Monday.

36 cases reported to date: 34 in Maricopa; 1 each in Pinal and Pima; *Culex* mosquitoes infected with WNV and SLEV have been found.

Other modes of transmission

- **Blood transfusion**
  - during 2002, WNV infection was found to be associated with transfusion blood components (PRC, FFP, platelets) in 23 patients
    NEJM 2003; 349:1239
  - Starting in 2003, all blood components screened for WNV through RNA nucleic acid amplication testing (NAT)
  - Fatal case in immunocompromised patient occurred in 2012 after platelet transfusion with NAT-negative component
    MMWR 2013; 31:622

- **Organ Transplantation**
  - First observed in 2002
    - 4 organ recipients from one donor
    NEJM 2003; 22:2196
  - Screening currently not uniformly performed
  - 10 reported cases since then
    • 8 not screened
  - Consider screening organs, at least during months when WNV active
    Emerg Infect Dis 2022; 28:403

Clinical disease

- Symptoms occur in 20-40% of all those infected from 2-14 days after infection
  - **West Nile fever**
    - abrupt onset of fever, headache, fatigue, myalgias, anorexia
      - maculopapular rash in 25-50%
    - persists 3-10 days
  - **Neuroinvasive disease**
    - occurs in ~1% of infections
    - more common in immunocompromised
      - solid organ transplants
      - B-cell depleting agents (e.g. rituximab)
  - **Chorioretinitis**

Clinical experience of WNV infection in Arizona in 2021

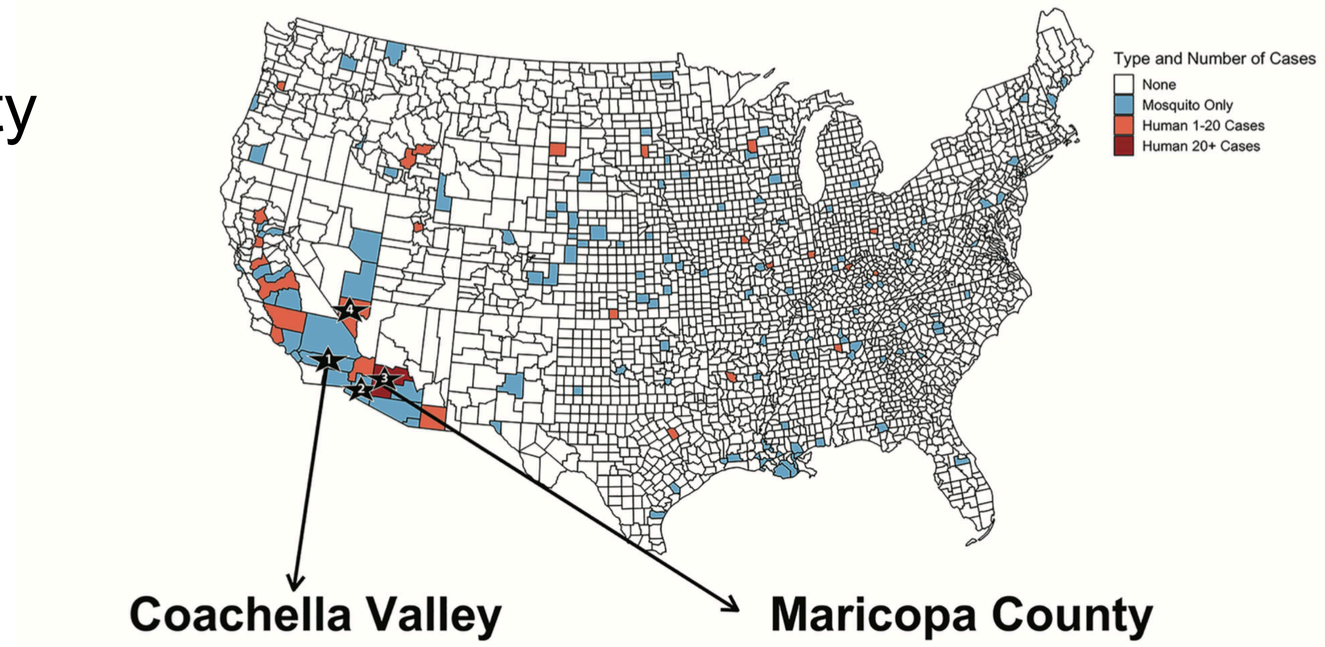
- Among 190 patients tested at MCA with probable or proven WNV infection
  - median age 65 years
  - 143 (75%) required hospitalization
    - 20 (14%) ICU admission
  - 127 (67%) neuroinvasive
  - 43 (23%) immunocompromised
  - 7 (5%) had severe neurological sequelae
  - 7 (5%) died
- Median duration of symptoms before admission: 5 (0-30) days
- Median duration of hospitalization: 6 (0-64) days

Kasule et al. Open Forum Infect Dis 2024. DOI:10.1093/ofid/ofae188

St. Louis encephalitis virus (SLEV)

- Presents similarly to WNV infection
- Outbreak occurred in Maricopa County in 2015
  - 62 WNV cases
  - 20 SLEV cases

- Genetically analyzed SLEV from mosquito traps in SW United States
  - introduced between 2011 & 2014
  - multiple clades
  - multiple subsequent introductions



Sites of *Culex* mosquitoes + for SLEV 2015-2019

Frontiers in Genetics 2029; 10.3389/fgene.2021.667895

Neuroinvasive WNV disease

- Roberts, et al. reviewed the 47 studies of neuroinvasive WNV infection
- Presenting symptoms in addition to West Nile fever:
  - altered mental status
  - focal muscle weakness
  - tremors
  - nuchal rigidity
  - other manifestations: acute flaccid paralysis; Parkinson's syndrome; seizures
- Hospitalization occurred 3-9 days after symptom onset; hospitalization lasted 4-30 days
- Overall mortality was 9%
- Neurocognitive impairment, memory, concentration deficits, and depression are common post-hospitalization

Ann Neurol 2025; 98:94

Neuroinvasive disease: laboratory profile

- Peripheral blood studies are generally normal

- **Lumbar puncture** with CSF profile should be done if neurological abnormalities are present
  - typical “viral” pattern:
    - RBC: few
    - WBC: ~50-100 cells/μL (usually <500/μL) with lymphocytic pleocytosis
    - Glucose: not depressed (>50 mg/dL)
    - Protein: ~100 mg/dL (<150 mg/dL)
    - Anti-WNV **IgM+** in CSF is diagnostic

- **Neuroimaging**
  - MRI demonstrate abnormalities in about 50% of those with neuroinvasive disease

  AJNR Am J Neuroradiol 2005; 8:1986

- **Serum anti-WNV EIA IgM (MAC-ELISA) positivity**
  - occurs 3-8 days after symptoms
  - persists 30-90 days

Diagnosis

- **Serum EIA IgM** (IgM antibody capture enzyme-linked immunosorbent assay; **MAC-ELISA**) useful ≥5 days after symptom onset
  - less sensitive in immunocompromised patients
  - some cross-reactivity (St. Louis encephalitis virus, SLEV)
  - **CSF EIA IgM** should be ordered on all samples obtained

- **Plaque reduction neutralization test (PRNT)**
  - useful if cross-reactivity with SLEV suspected

- **Whole-blood reverse-transcribed polymerase chain reaction** (**WB RT-PCR**)
  - may be helpful in immunocompromised patients
  - 190 patients at MCA with possible WNV infection retrospectively reviewed
    - WB RT-PCR was ordered in 56 (29.5%)
      - 45 (80%) positive
        - includes 7 cases where EIA IgM negative
  - RT-PCR of urine may be more sensitive

Kasule et al. Open Forum Infect Dis 2024. DOI:10.1093/ofid/ofae188
Cvjetkovic et al. Diag Microbiol Infect Dis 2023. DOI: 10.1016/j.diagmicrobio.2023.115920

Treatment

- There are no specific antiviral therapies for WNV or SLEV
  - Drug development against structural and non-structural proteins on-going

  Front Cell Infect Microbiol 2025; 10.3389/fcimb.2025.1568031

- Agents of possible benefit
  - **Corticosteroids**
    - case reports suggest rapid improvement in neuroinvasive disease with solumedrol 1000 mg daily x 5 days

    Cureus 2022; doi: 10.7759/cureus.31971

    Front Med (Lausanne) 2019; 10.3389/fmed.2019.00081

  - **Immunoglobulin (IVIG)**
    - no efficacy noted from high-titer preparation

    Emerg Infect Dis 2019; 11:2064

Vaccines

- Four horse vaccines are currently licensed by USDA
  - Two whole inactivated virus with adjuvant
  - Non-replicating recombinant canary pox with WNV expressed antigens
  - Inactivated flavivirus chimera vaccine: WNV antigens expressed in yellow fever virus vector clone with adjuvant

- No human vaccines available but Phase II trials on-going
  - **ChimeriVax-WN02** (Sanofi Pasteur)
    - insertion of the genes encoding the pre-membrane (prM) and envelope (E) proteins of WNV (strain NY99 [7]) into the yellow fever 17D vaccine clone (YFV-17D)
    - live, attenuated - contraindicated in immunocompromised
    - seroconversion >90% after one dose

Vector management and control

- **Vector monitoring**
  - Provided by Arizona counties
    - Maricopa County Environmental Services:
      - sets 800 mosquito traps; counts, speciate, and tests
        - *Culex*: WNV, SLEV
        - *Aedes*: Zika, chikungunya, and dengue
      - abatement provided when needed
        - larviciding and adulticiding
        - provides *Gambusia* fish at request for contained areas
      - reports results to AZDHS (Vector-Borne & Zoonotics Program)

- **Personal control**
  - Limit outside activity, particularly at dawn and dusk
  - Wear long-sleeved shirts and pants when outside
  - Use insect repellent (DEET)

- **Reducing mosquito transmission competence and/or numbers**
  - **Reduce breeding grounds**
    - remove standing water around homes and other structures
    - clean gutters and drainage pipes
    - maintain pools and other water features
    - keep screen and other doors closed

  - Most **other control studies** have been done in *Aedes*
    - Release of sterile males
    - *Wolbachia*
      - A stable colony of *Aedes* mosquitoes infected with the bacterium *Wolbachia* has reduced ability to transmit viral pathogens; males and females can persist in wild populations when released
      - Not shown to reduce transmission of WNV with *Culex*

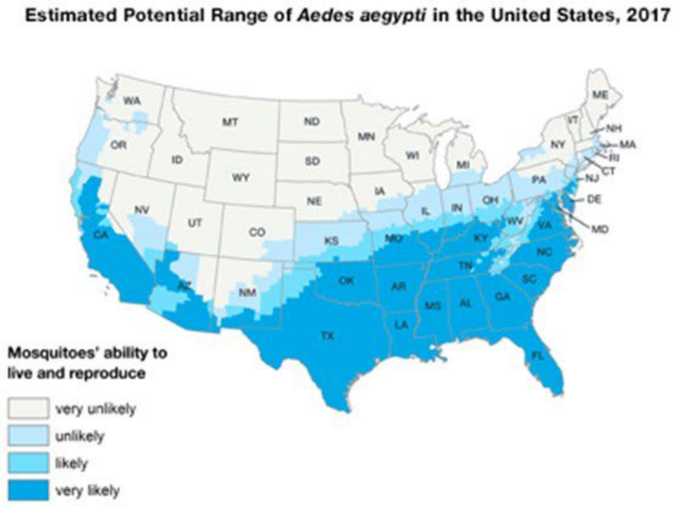
    Nature 2018; 16:508

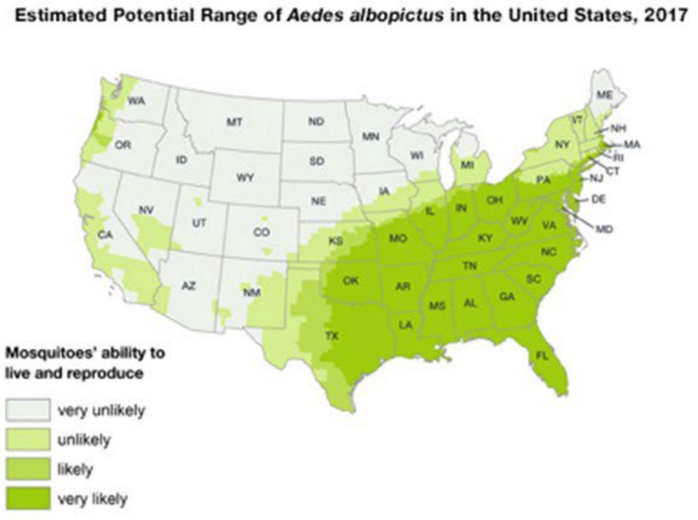
Zika, chikungunya, and dengue

- **Transmitted by *Aedes* mosquitoes**
  - Three species
    - ***Aedes aegypti*** (Yellow fever mosquito)
      - lives near humans, small water containers
    - ***Aedes albopictus*** (Asian tiger mosquito)
      - adapted to cooler climates
    - ***Aedes solicitans*** (Eastern saltmarsh mosquito)
      - not a significant vector

Aedes aegypti

Aedes albopictus





Aedes vs *Culex*

Feature	*Aedes*	*Culex*
Biting time	Daytime (early morning and late afternoon)	Nightime (dusk to dawn)
Appearance	Small, black, with white silver stripes on body and legs	Medium sized, brownish, generally without distinct markings
Breeding sites	Small, artificial containers with clean water (i.e. flower pots, tires)	Large bodies of stagnant water with organic material (i.e. ponds, gutters)
Egg laying	Eggs laid singly above waterline; resistant to drying; can survive months	Eggs laid in “rafts” on water surface; not resistant to drying
Main diseases transmitted	Dengue, Zika, chikungunya, yellow fever	WNV, SLEV, WEEV, EEEV, dog heartworm
Feeding Preferences	Strong preference for humans	Often feed on birds, mammals, and sometimes humans

Zika, chikungunya, and dengue in Arizona

- *Aedes aegypti* mosquitoes are present in central and southern Arizona

BUT

- **Zika**
  - no locally transmitted cases in Arizona

- **Chikungunya**
  - only travel-related cases; local cases reported in 2019

- **Dengue**
  - only travel-related cases in Arizona; Puerto Rico and U.S. Virgin Islands endemic
  - dengue is endemic to Mexico and 14 imported cases have been reported in Arizona this year

Oropouche virus

- A newly described arbovirus (Orthobunyavirus)
- Found principally in the Amazon basin
  - outbreaks have occurred in Brazil, Peru, Bolivia, Colombia, Ecuador, Panama, French Guiana, Trinidad and Tobago, Cuba, and the Dominican Republic
- Transmitted mostly by biting midges (*Culicoides paraensis*) but also some mosquitoes (*Culex quinquefasciatus*, *Aedes serratus*, *Coquillettidia venezuelensis*)
- Reservoir likely non-human primates, sloths, and possibly birds
  - no human-to-human transmission
- Abrupt onset of fever, severe headache, chills, myalgias, and arthralgias
  - acute illness lasts 2-7 days
  - may relapse weeks later
  - 5% experience aseptic meningitis, encephalitis, or bleeding

Questions or comments?