Diagnostic Strategies for Arboviral Diseases in Wisconsin

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Vectorborne Disease Epidemiologist
May 28, 2025

FIGHT the BITE

WISCONSIN DEPARTMENT of HEALTH SERVICES

Agenda

- **Arboviral Disease Epidemiology**
- **Arboviral Disease Diagnostics**
  - Domestic Arboviruses
  - Imported Arboviruses
  - Case Studies
- **Resources for Providers and Laboratorians**

Arboviral Disease Epidemiology

Arboviral Diseases Can be Spread **Locally** or During **Travel** to Other States or Countries.

West Nile virus

Jamestown Canyon virus

Powassan virus

La Crosse virus

Eastern equine encephalitis virus

Dengue virus

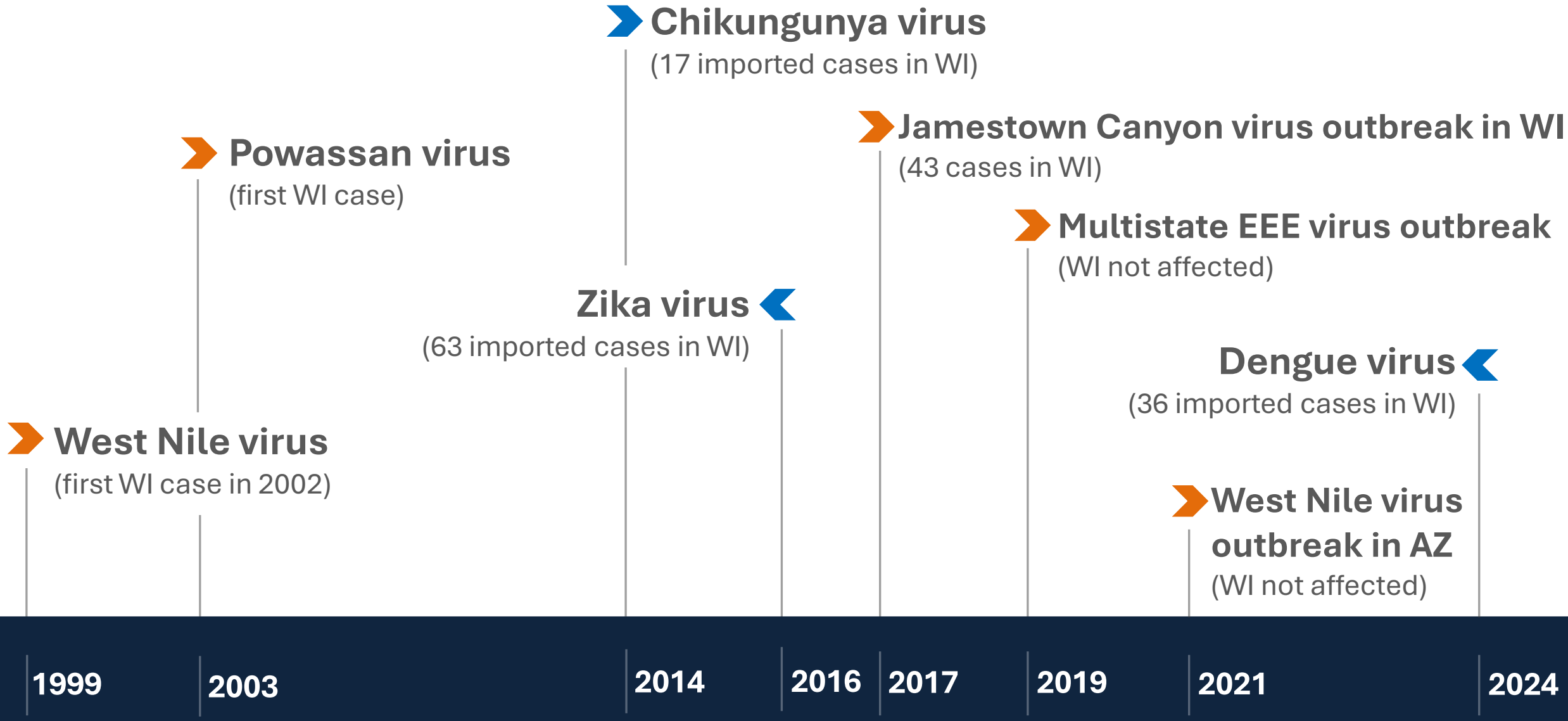
Oropouche virus

Zika virus

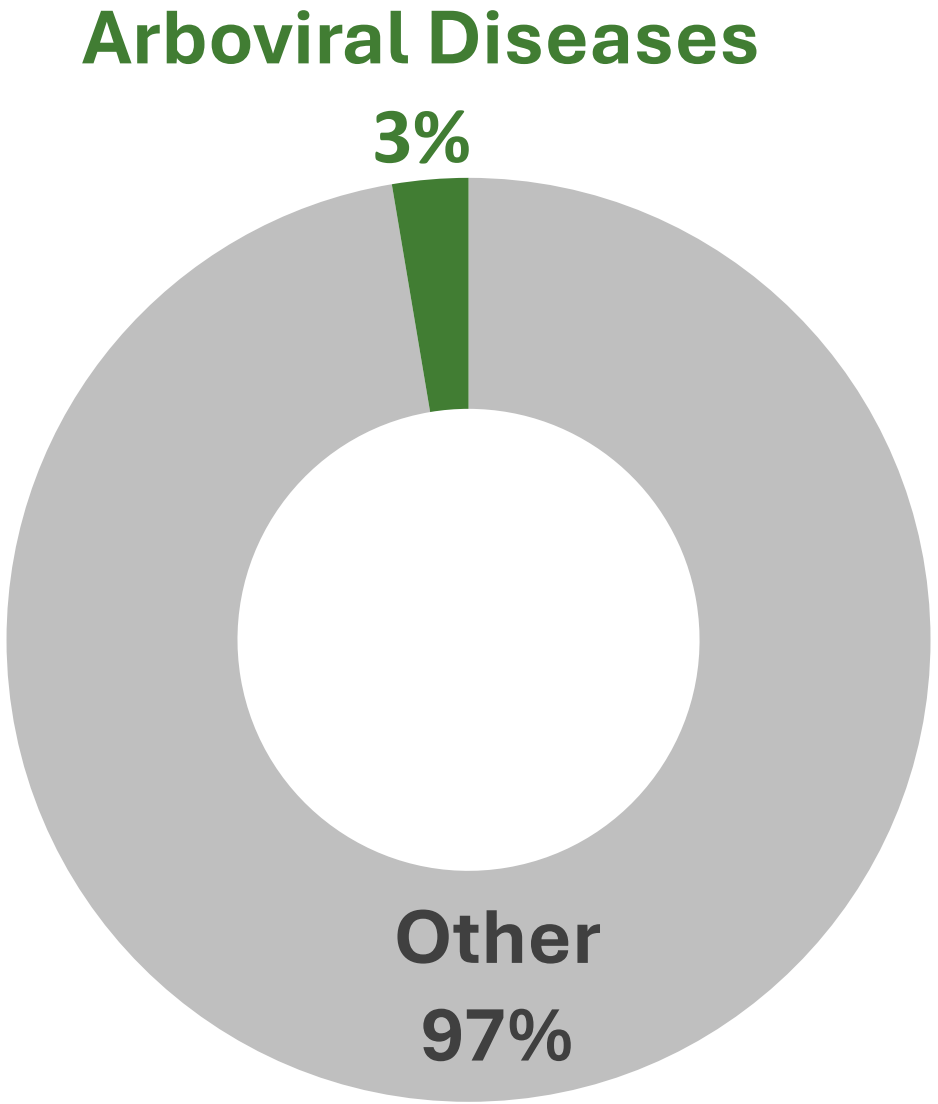
Chikungunya virus

Yellow fever virus

Arboviral Disease Emergence in the U.S.



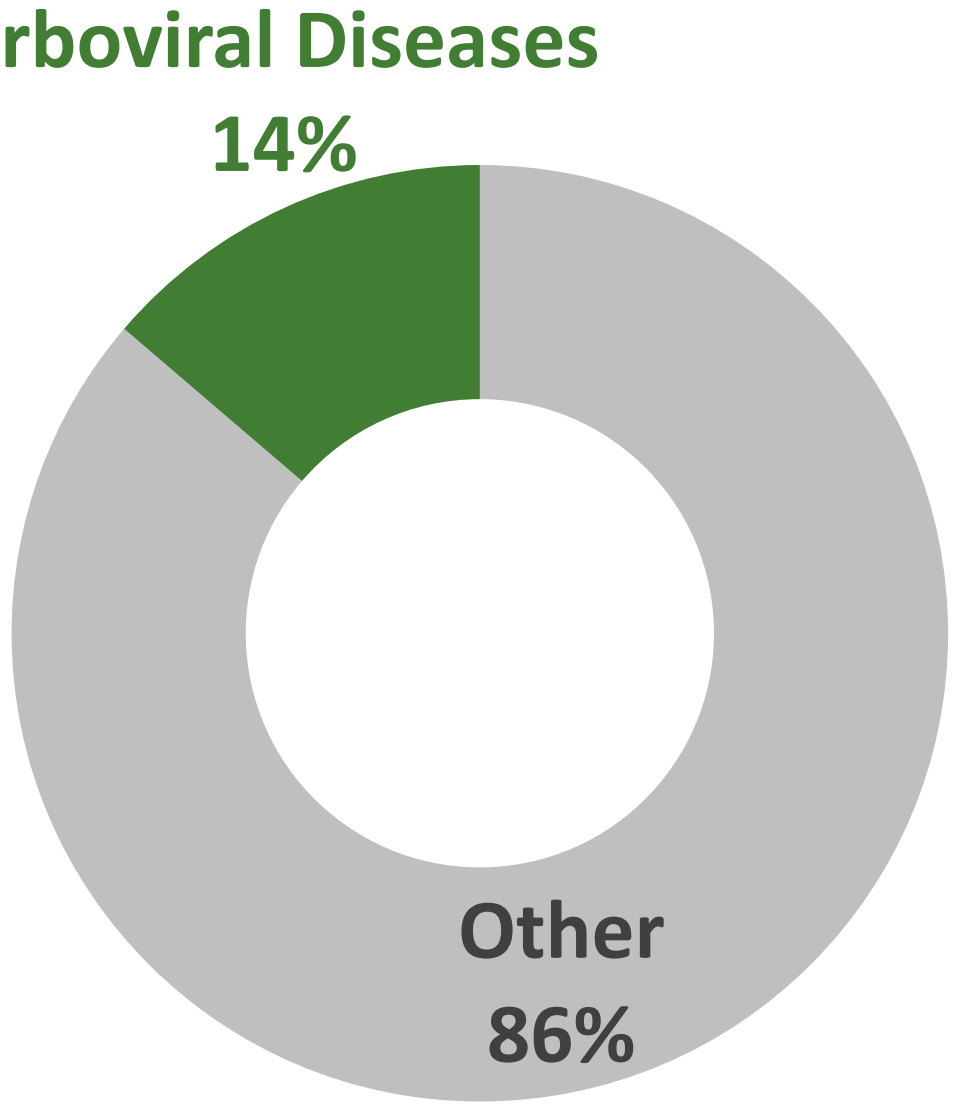
Vector-borne Disease Burden 2014–2023



3%

of all reported vector-borne diseases in Wisconsin are from **arboviruses**.

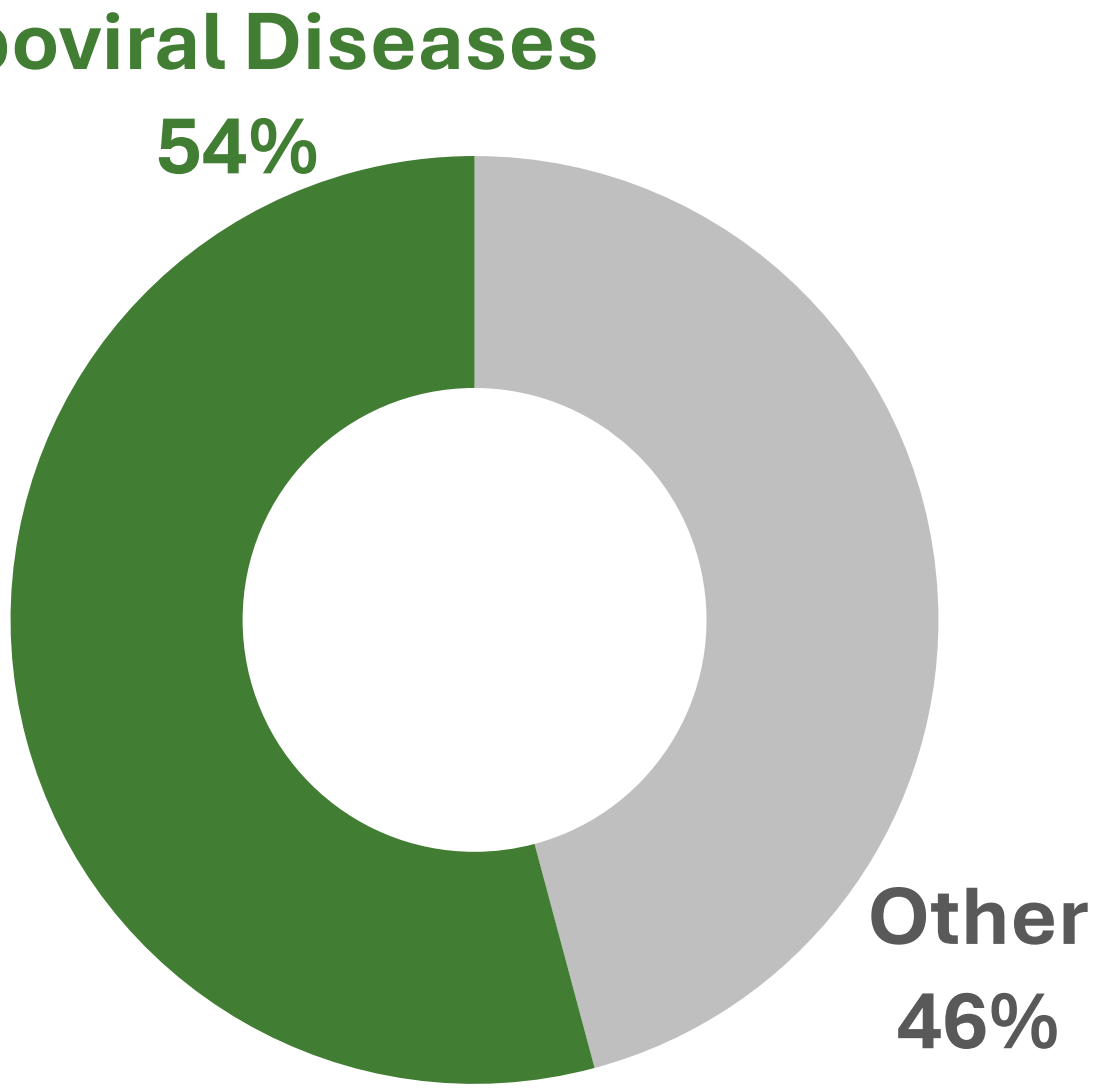
Hospitalizations (2014–2023)



14%

of all vector-borne disease **hospitalizations** in Wisconsin are from **arboviruses**.

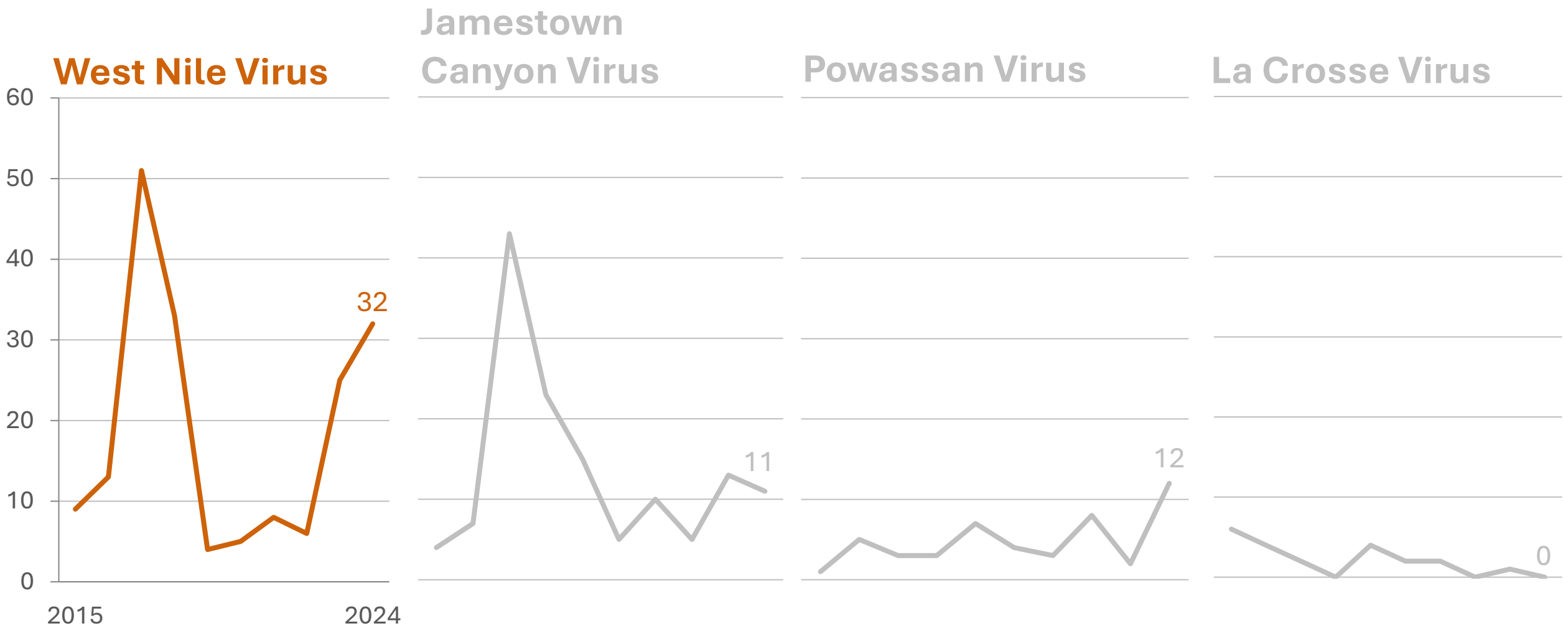
Deaths (2014–2023)



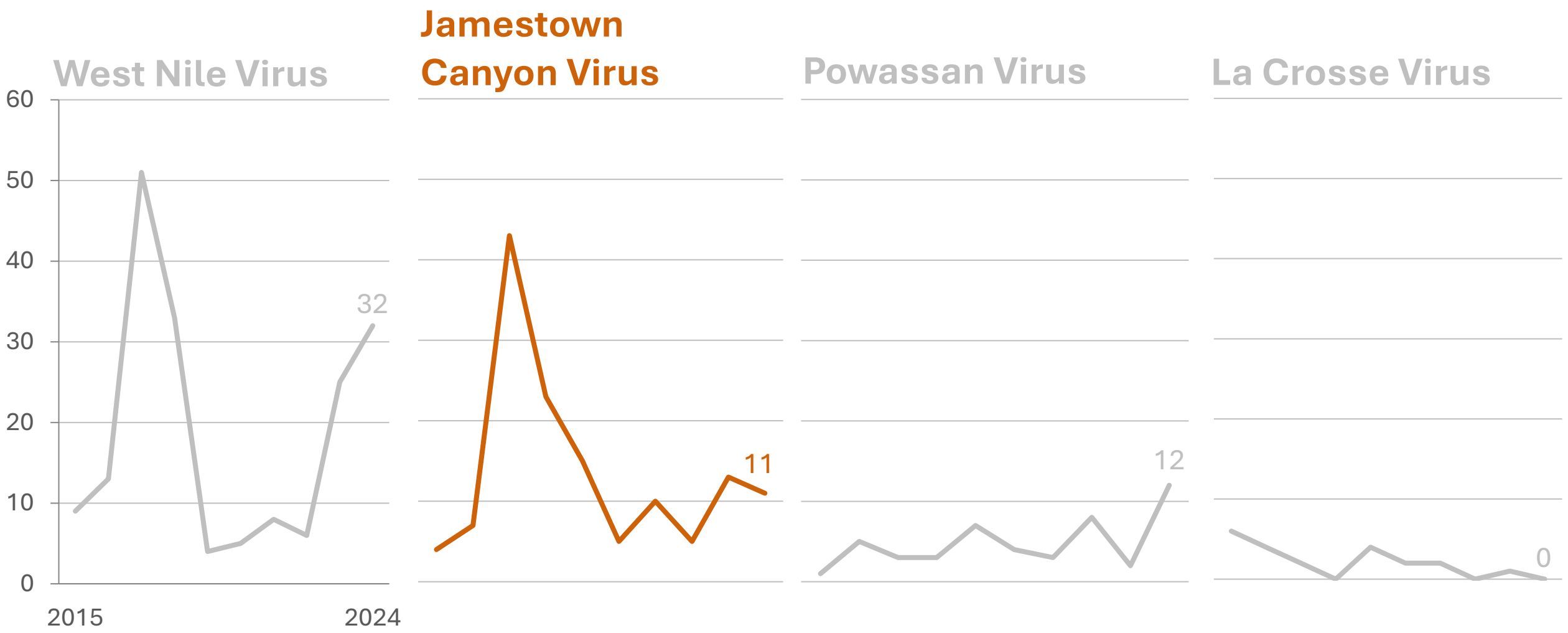
54%

of all vector-borne disease **deaths** in Wisconsin are from **arboviruses**.

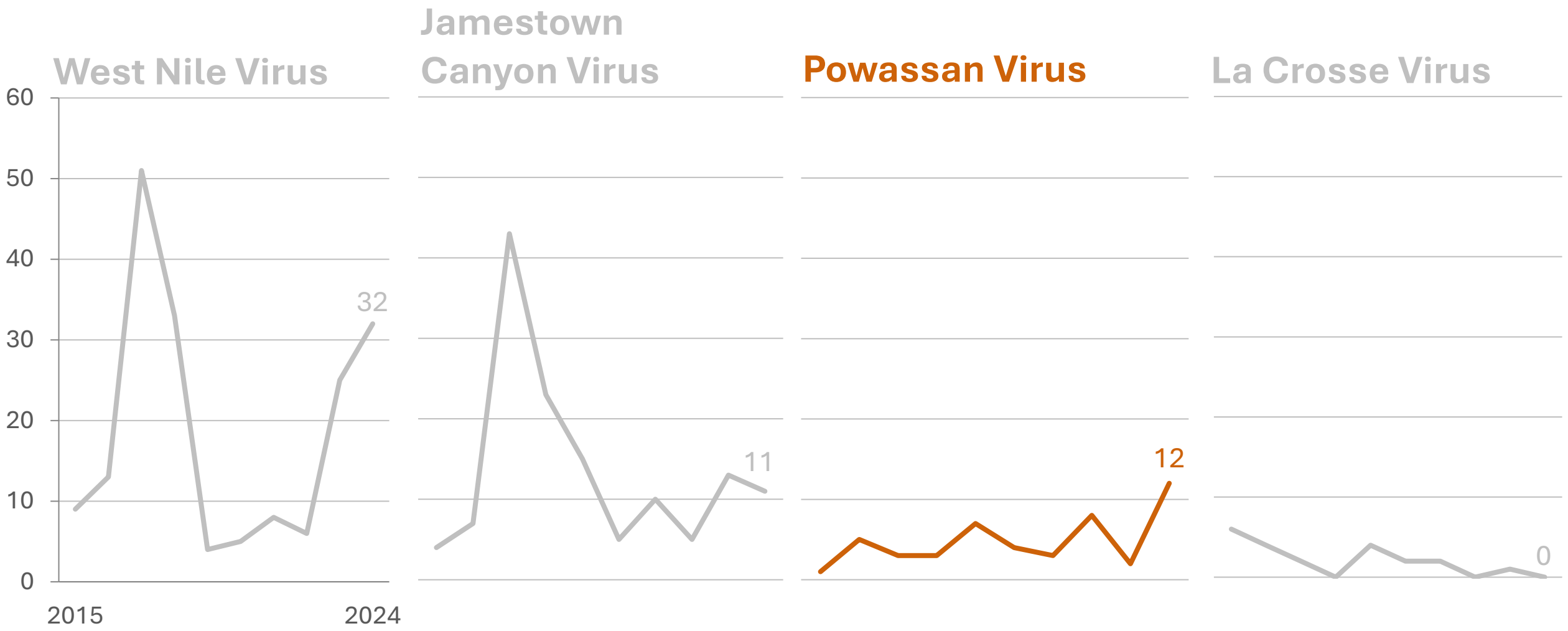
Arboviral Diseases in Wisconsin (2015–2024)



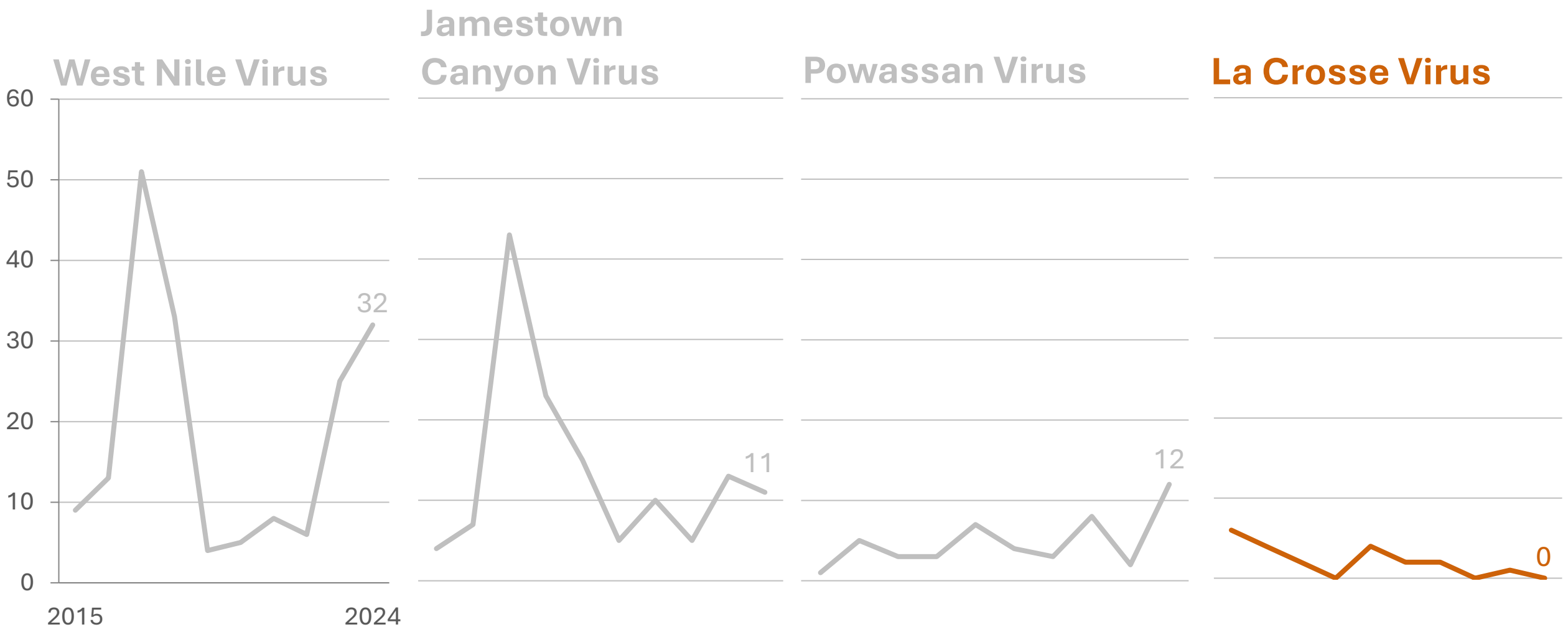
Arboviral Diseases in Wisconsin (2015–2024)



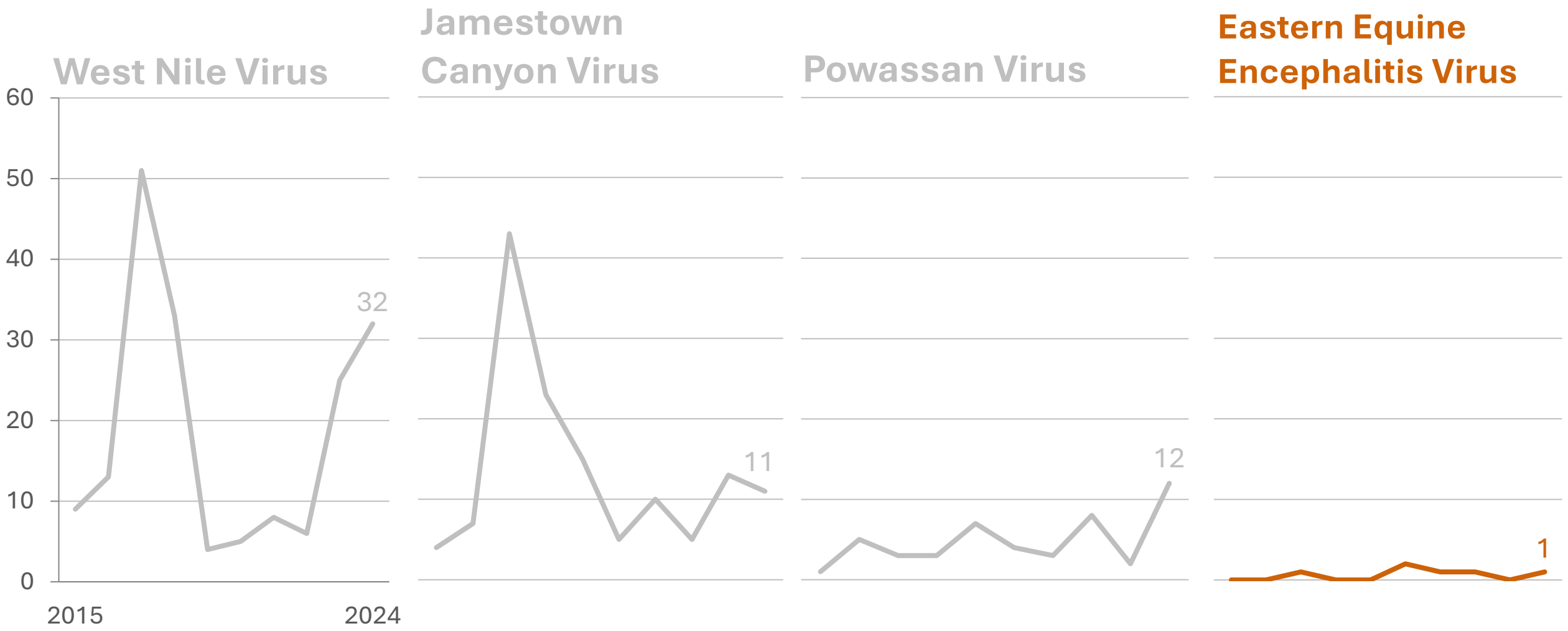
Arboviral Diseases in Wisconsin (2015–2024)



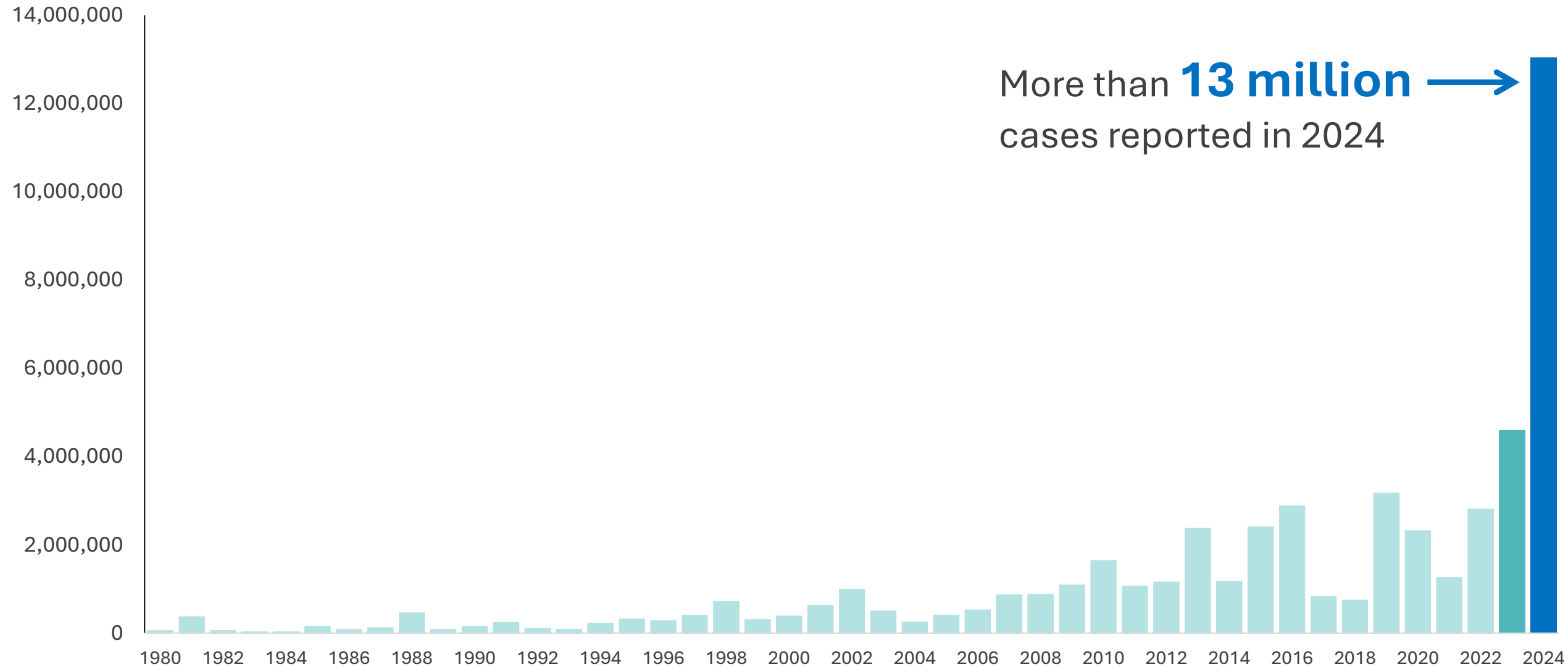
Arboviral Diseases in Wisconsin (2015–2024)



Arboviral Diseases in Wisconsin (2015–2024)

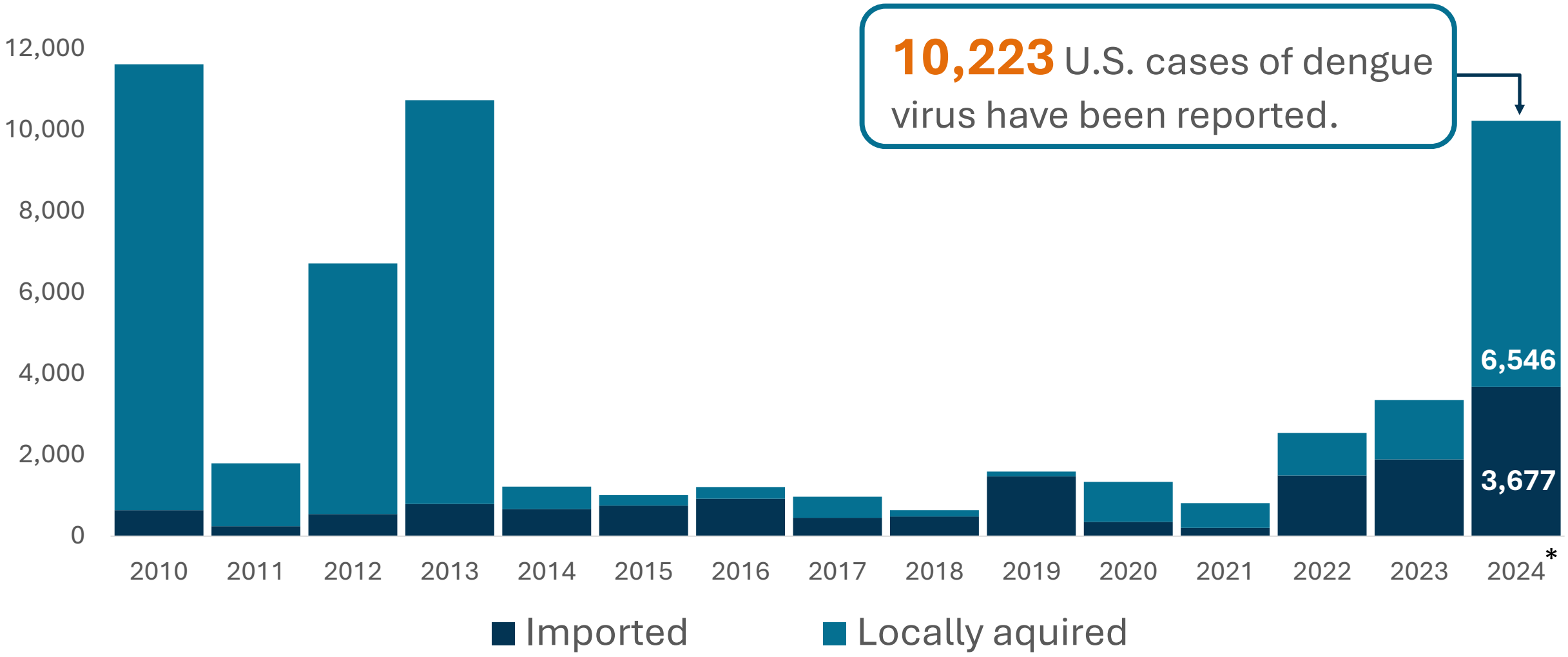


Dengue Cases in the Americas, 1980-2024*



*Data from PAHO PLISA Health Information Platform for the Americas

Dengue Outbreak – U.S. Cases



*CDC ArboNET data as of May 20, 2025

Locally Acquired Dengue – U.S. Cases

Most of the **6,546** locally acquired cases occurred in **Puerto Rico**.

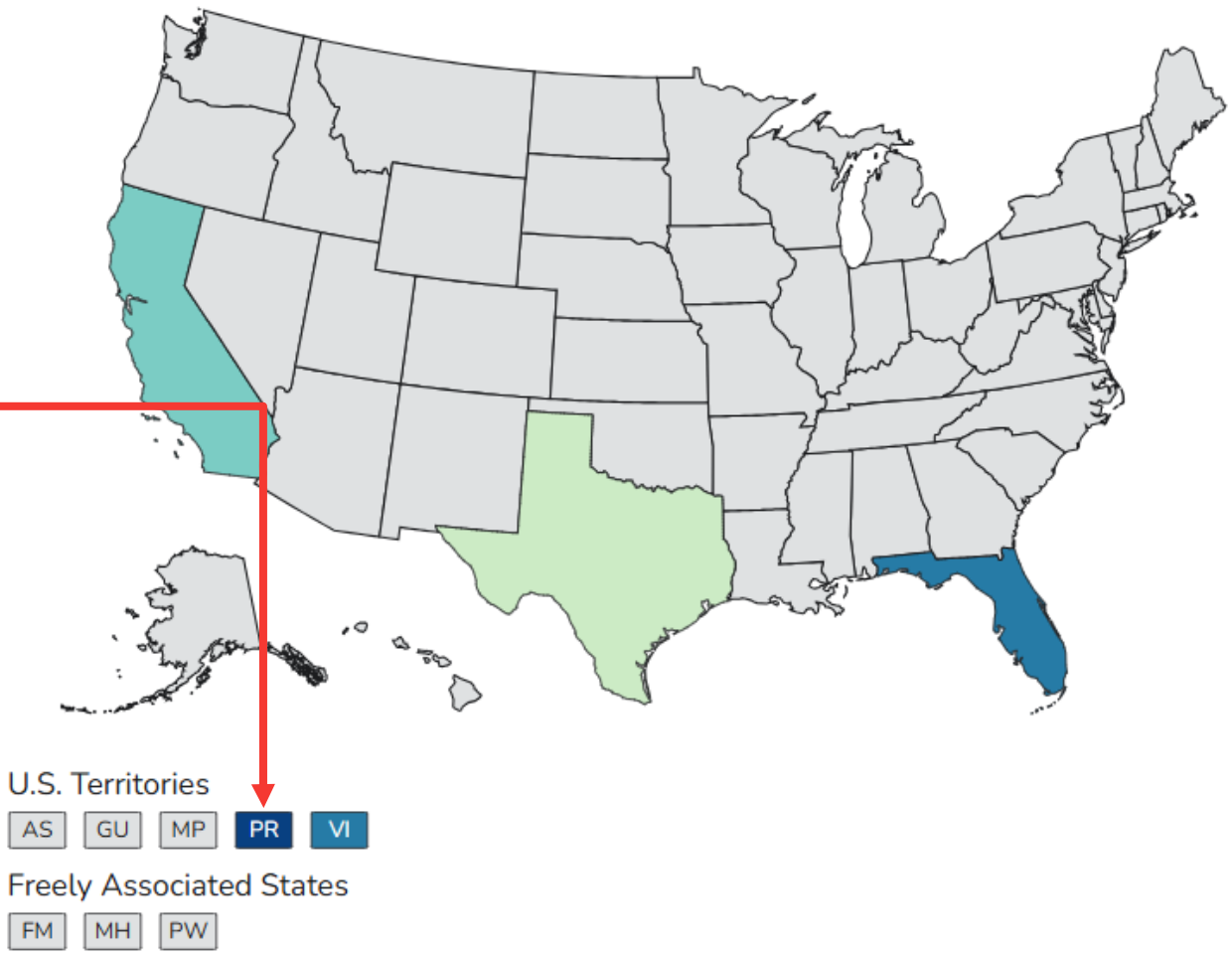
Puerto Rico: 6,238

Virgin Islands: 205

Florida: 83

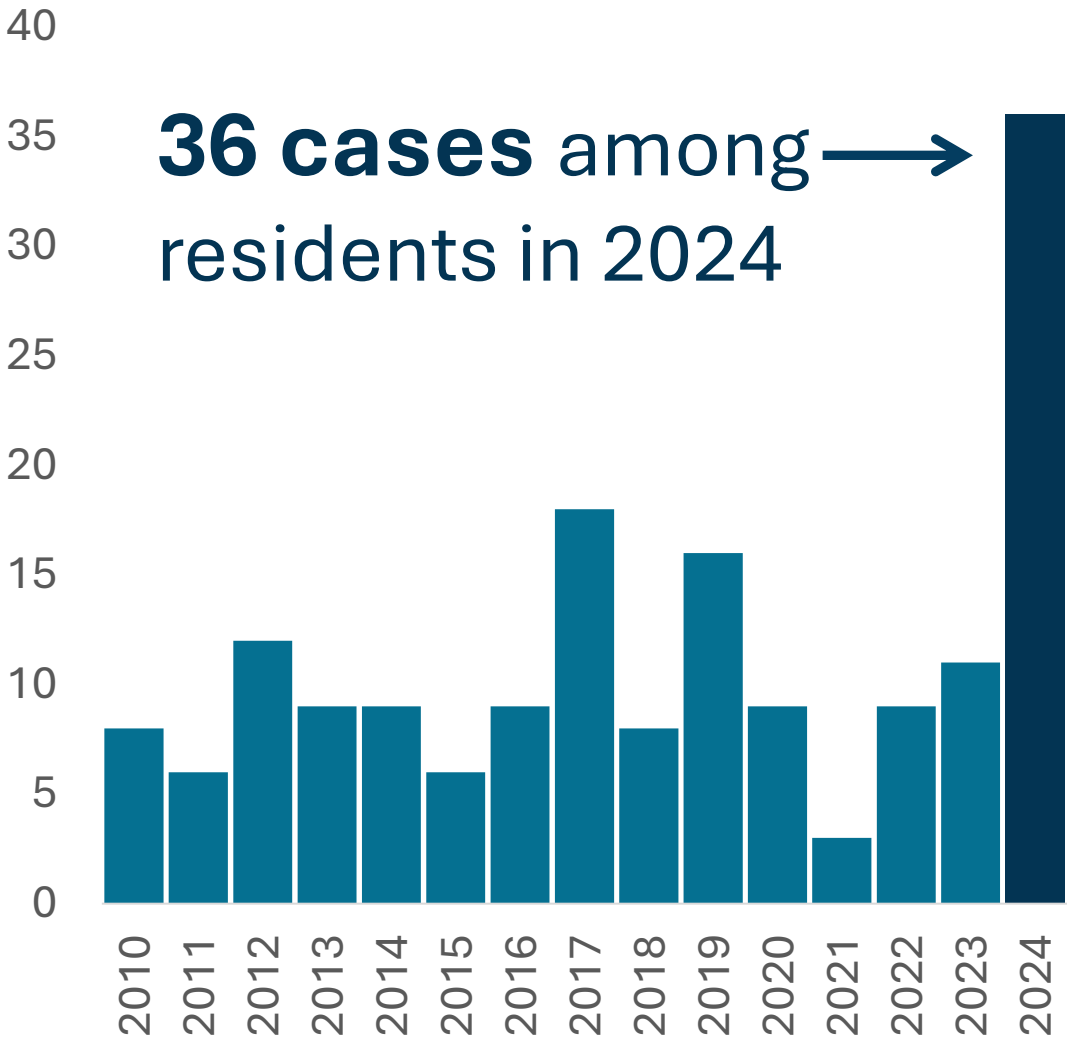
California: 18

Texas: 2



*CDC ArboNET data as of May 20, 2025

Dengue Outbreak – Wisconsin Cases



36 cases among residents in 2024

Travel destinations of Wisconsin case-patients

Oropouche Virus

Mosquito (right) and biting midge (left)
Image source: CDC

Oropouche virus is spread primarily by **biting midges** (“no-see-ums”) and some mosquitoes.

No cases of sexual transmission have been reported, but the virus has been **detected in semen**.

Vertical transmission is possible and is linked to reports of fetal loss, stillbirth, and congenital anomalies (for example, microcephaly).

Oropouche Virus Disease

Common symptoms include **fever, severe headache, chills, muscle aches, and joint pains** after an incubation period of 3–10 days.

Initial illness duration of 2–7 days. **60% of people have recurrent symptoms** days to weeks later.

Oropouche Virus Disease

About **4% of patients will develop neurologic disease.** Oropouche virus infection may increase risk of Guillain-Barré syndrome.

There is **no specific treatment or vaccine** for Oropouche. Management includes rest, hydration, medications to control fever and pain, and monitoring for possible complications.

Oropouche Virus Epidemiology

No human disease cases

Recent human disease cases

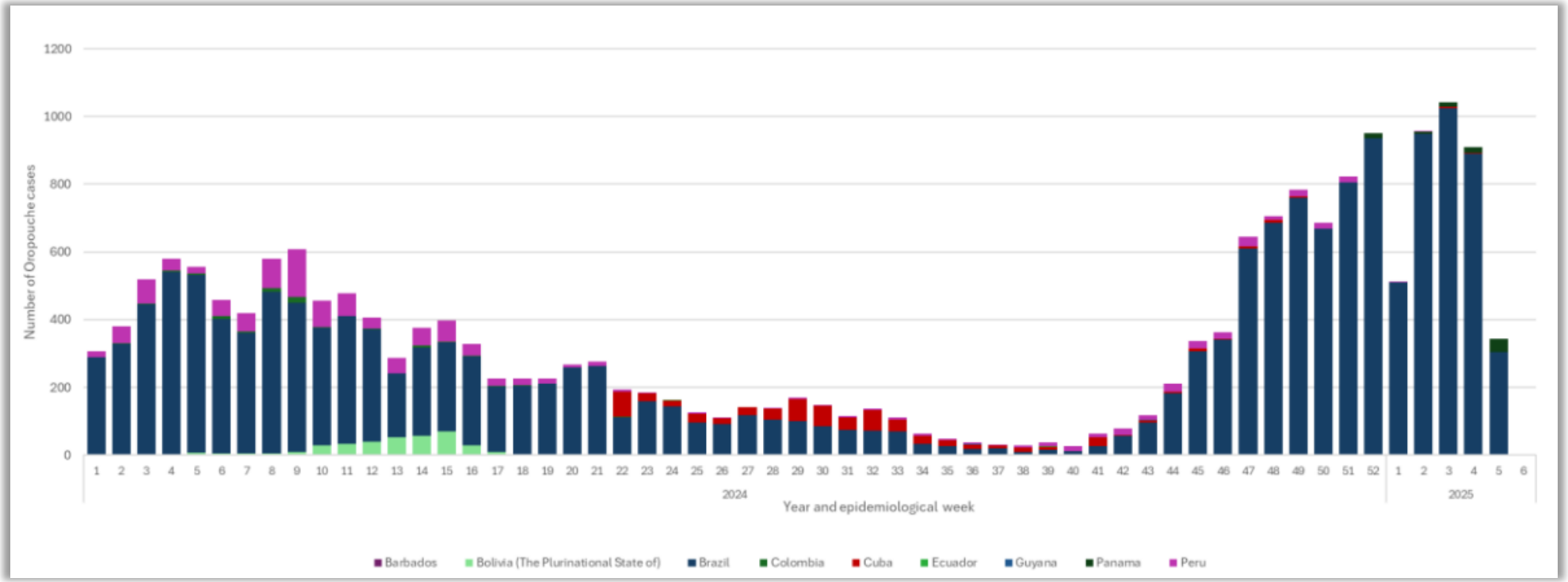
Previous human disease cases

www.cdc.gov/oropouche/data-maps/countries-and-territories-at-risk-for-oropouche.html

Oropouche Virus Cases, 2024-2025

109 cases of **travel-related** Oropouche have been reported in the **U.S.** as of May 20, 2025. **1** case reported in a Wisconsin resident.

20,004 cases of Oropouche have been reported in the **Americas** as of Feb. 11, 2025.



https://www.paho.org/sites/default/files/2025-02/2025-feb-10-epi-update-oropoucheeng-final_0.pdf

First Oropouche Virus Case in Wisconsin

WISCONSIN DEPARTMENT of HEALTH SERVICES

DHS Health Alert Network

Wisconsin Health Alert #64: First Imported Case of Oropouche Virus Disease in a Wisconsin Resident

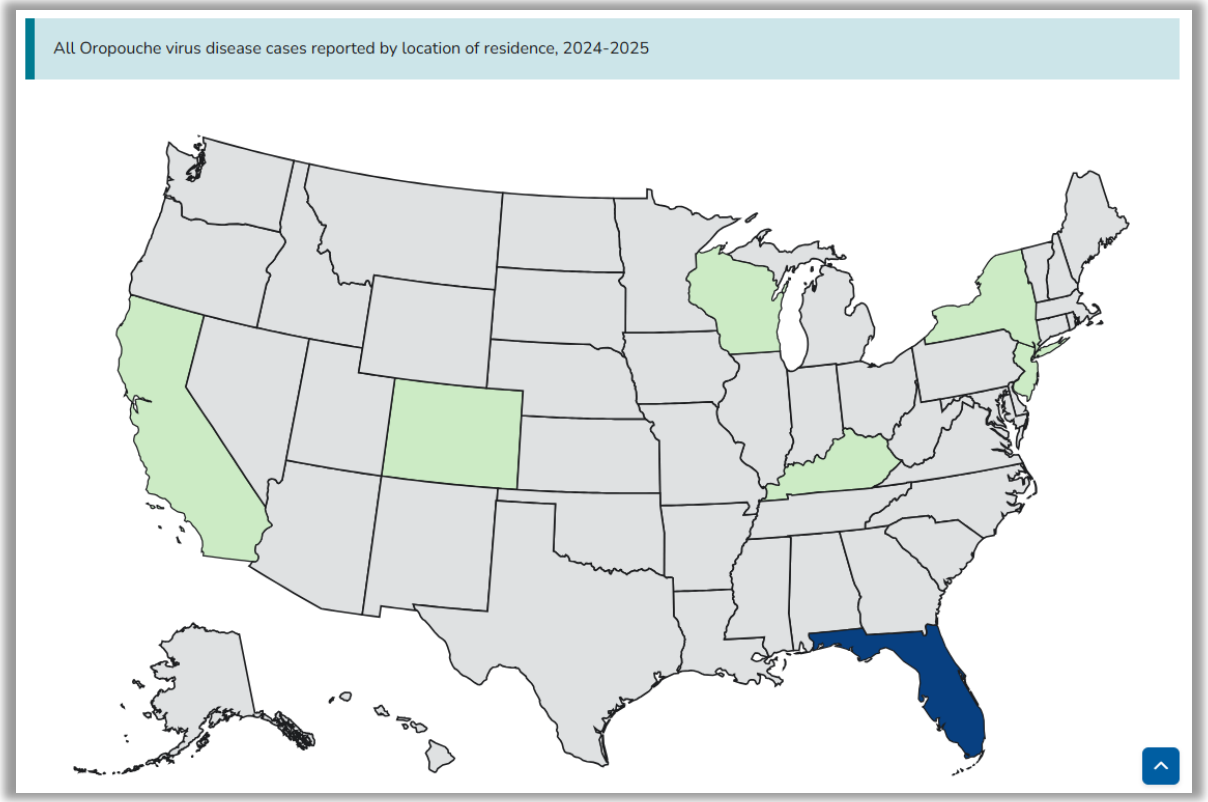
Bureau of Communicable Diseases

March 14, 2025

Key Points

- The Wisconsin Department of Health Services (DHS) reports **a case of neuroinvasive Oropouche virus (OROV) disease in a Wisconsin resident who traveled to Panama prior to illness onset.**
- This is the first case of OROV identified in a Wisconsin resident, and the first case of OROV disease in the U.S. in 2025.
- OROV continues to cause outbreaks in parts South and Central America, and the Caribbean with 3,765 confirmed cases reported during the first four weeks of 2025.
- During 2024, a total of 108 imported cases of OROV disease were reported in the U.S., including two neuroinvasive disease cases. More than 16,000 locally acquired cases of OROV disease were reported in the Americas in 2024, including four deaths and several cases of vertical transmission associated with fetal death or congenital abnormalities.
- Countries reporting locally acquired (autochthonous) cases since January 1, 2024, include Barbados, Bolivia, Brazil, Colombia, Cuba, Ecuador, Guyana, Panama, and Peru.

1 case of imported Oropouche virus reported in Wisconsin.



Level 1 Travel Health Notice: **The Americas**

- All travelers should take steps to **prevent bug bites**.
- All travelers should consider **using condoms or not having sex** during travel and for 6 weeks after returning from travel.

https://wwwnc.cdc.gov/travel/notices/level1/oropouche-in-the-americas

Level 2 Travel Health Notice:
Espírito Santo, Brazil
Darién Province, Panama

→ Pregnant people should **avoid non-essential travel** to these locations.

https://wwwnc.cdc.gov/travel/notices/level2/oropouche-brazil-panama

Arboviral Disease Diagnostics

Domestic Arboviral Diseases

Domestic Arboviruses: Clinical Evaluation

Consider arboviral disease in patients with acute febrile or neurologic illness with possible recent exposure to **ticks(≤5 weeks)** or **mosquitoes (≤14 days)**.

Many arboviruses can also rarely spread through **blood transfusion**, **organ transplantation**, or **exposures in a laboratory setting**.

Obtaining a thorough patient history to evaluate risk factors can aid in diagnosis.

Domestic Arboviruses: Clinical Presentation

Mild disease presentation:

- Fever
- Malaise or fatigue
- Myalgias or arthralgias
- Headache
- Nausea, vomiting
- Diffuse maculopapular rash

Neuroinvasive disease presentation:

- Meningitis
  - Fever
  - Headache
  - Nuchal rigidity
- Encephalitis
  - Fever
  - Acutely altered mental status
  - Seizures
  - Focal neurologic deficits
  - Movement disorders

Domestic Arboviruses: Neuroinvasive Disease

Cerebrospinal fluid (CSF) findings typically include lymphocytic pleocytosis, mildly elevated protein levels, normal glucose levels.

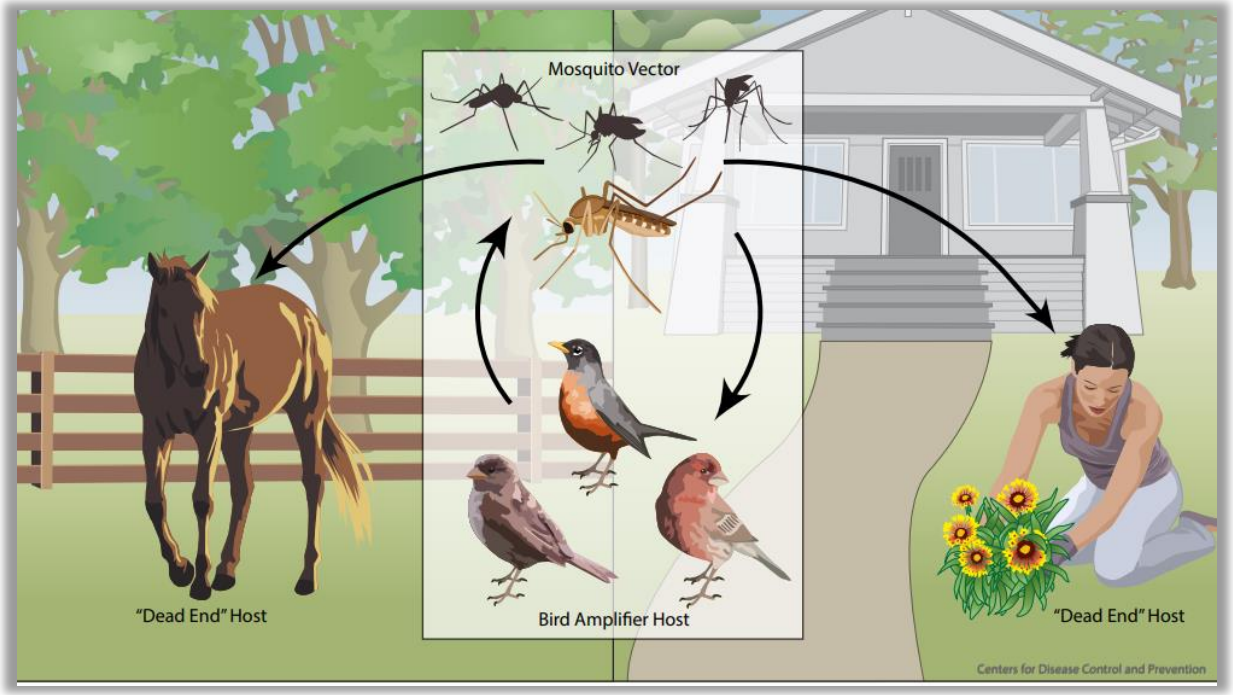
Brain imaging is frequently normal.

Risk factors:

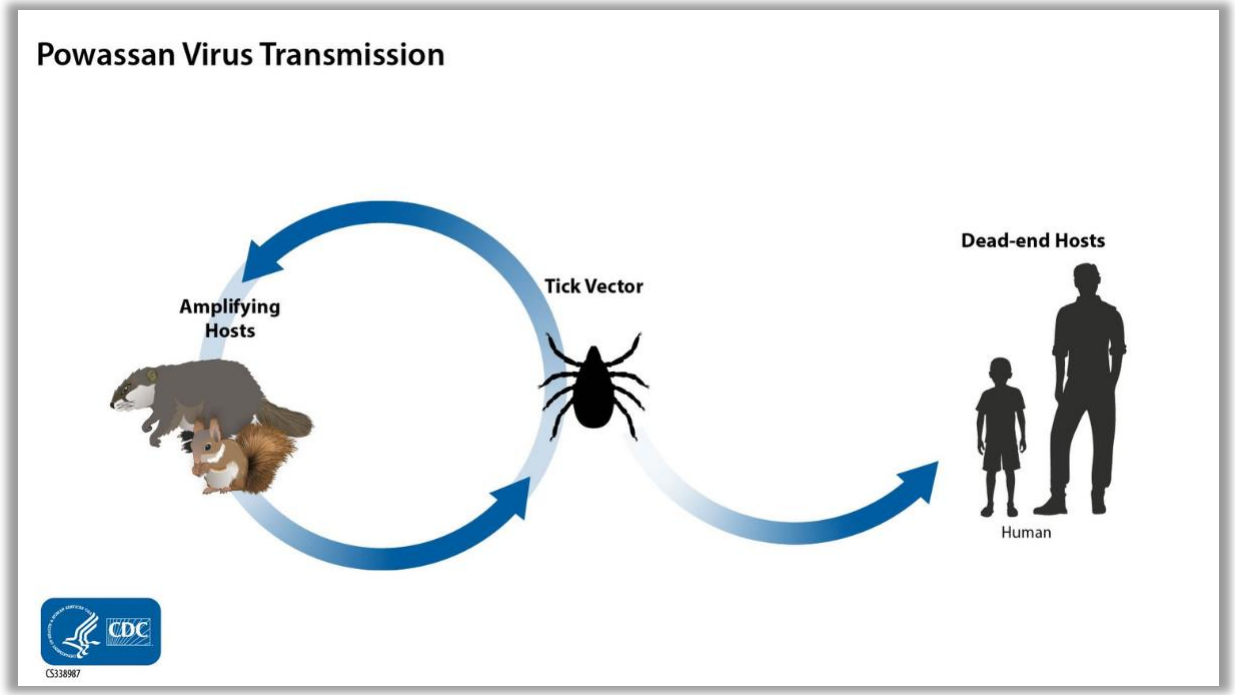
- Older age (for example, West Nile, Jamestown Canyon)
- Younger age (for example, La Crosse, eastern equine encephalitis, Powassan)
- Immune suppression

Other causes of aseptic meningitis and encephalitis should also be considered (for example, herpes simplex viruses, enteroviruses).

Domestic Arboviruses are **Zoonotic**. Humans Do Not Serve as Amplifying Hosts.

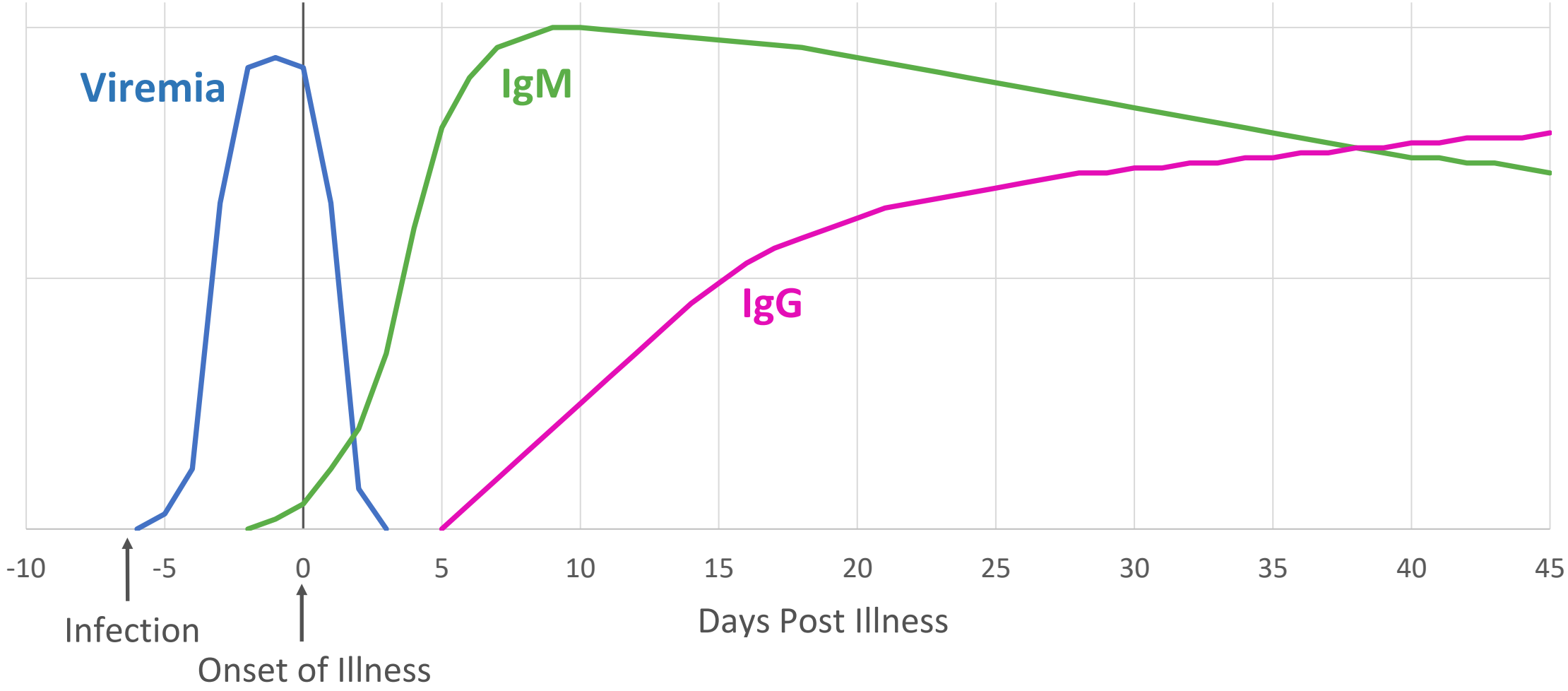


West Nile Virus



Powassan Virus

Timing of **West Nile Virus** Viremia and Antibody Response



Domestic Arboviruses: Diagnostic Testing

For **immunocompetent** patients, **serologic testing** is the primary method for diagnosis. **Molecular assays (RT-PCR)** are typically not useful in **immunocompetent** patients.

Use **IgM antibody** testing of serum or CSF collected 3–90 days after illness onset.

- False positive IgM results can occur (cross-reactivity, non-specific reactivity).
  - **Plaque-reduction neutralization tests (PRNTs)** can help determine the specific infecting virus.

Domestic Arboviruses: Diagnostic Testing

IgM antibody testing of serum or CSF

- Detectable **IgM antibodies** can sometimes **persist for weeks to months** after infection.
  - PRNTs can confirm acute infection by demonstrating a fourfold or greater change in neutralizing antibody titer between acute and convalescent sera.
- Negative IgM results in a specimen collected within the first **8 days** of illness **does not rule out infection**.
  - Repeat IgM testing on a later sample may be useful.

Domestic Arboviruses: Diagnostic Testing

IgG antibody testing is not appropriate for diagnosing an acute arboviral disease.

- Detectable **IgG antibodies** persist for **many years** following symptomatic or asymptomatic infection.
- The presence of **IgG antibodies** alone is only evidence of previous infection.

Domestic Arboviruses: Diagnostic Testing

Immunocompromised patients may have prolonged viremia and delayed or absent antibody response, **molecular testing** may be preferred.

RT-PCR testing of acute serum, CSF, or tissue specimens

- Positive PCR results can confirm an arboviral infection.
- Negative PCR results cannot rule out infection.
- Limited commercial availability.

PCR assays are unlikely to detect viral RNA in **immunocompetent** patients.

Domestic Arboviruses: Diagnostic Testing

If an arboviral disease is clinically suspected, **concurrent testing for all circulating endemic arboviruses should be considered**.

Wisconsin State Laboratory of Hygiene offers an **Arbovirus IgM Antibody Panel** (test code SS02201):

- *West Nile virus IgM*
- *St. Louis encephalitis virus IgM*
- *Eastern equine encephalitis virus IgM*
- *Jamestown Canyon virus IgM*
- *La Crosse virus IgM*
- *Powassan virus IgM*

Positive IgM specimens are automatically **reflexed to PRNT** at CDC.

Imported Arboviral Diseases

Imported Arboviruses: Clinical Evaluation

Consider **travel associated arboviral diseases** in patients with acute febrile, hemorrhagic, or neurologic illness **with recent (≤14 days of onset) travel** to risk areas.

Consider **vaccination history**, especially for chikungunya, dengue, yellow fever, and Japanese encephalitis.

Obtaining a thorough patient history to evaluate risk factors can aid in diagnosis.

Imported Arboviruses: Clinical Presentation

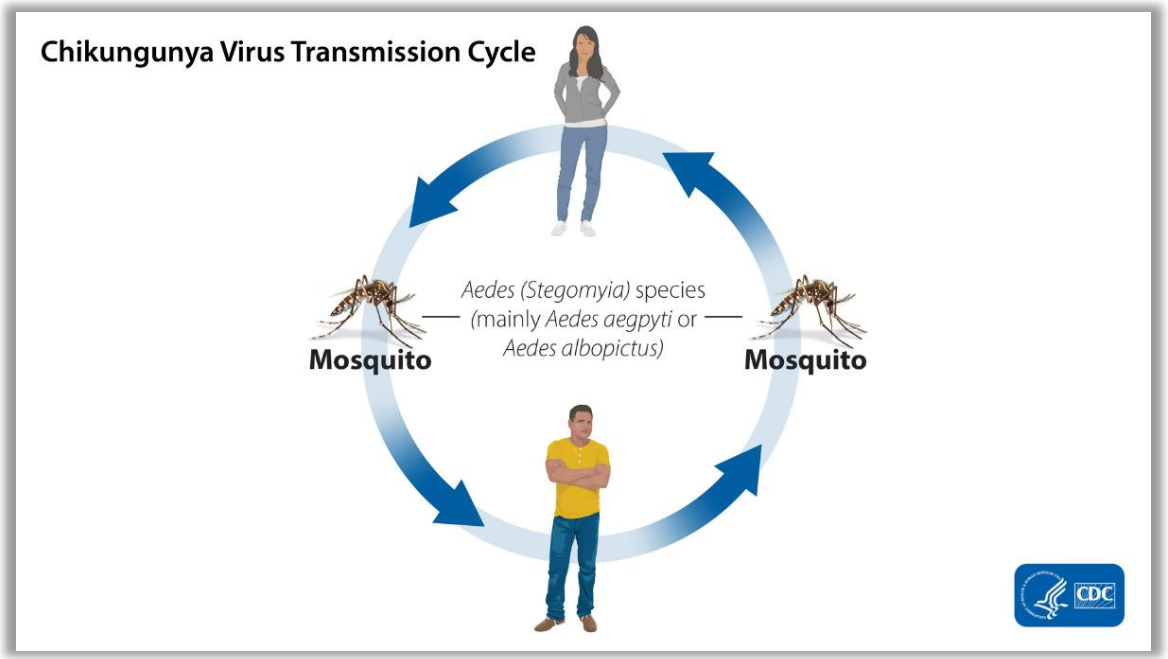
Mild disease presentation:

- Fever
- Headache
- Myalgias or arthralgias
- Retro-orbital pain
- Maculopapular rash
- Leukopenia
- Lymphopenia
- Thrombocytopenia

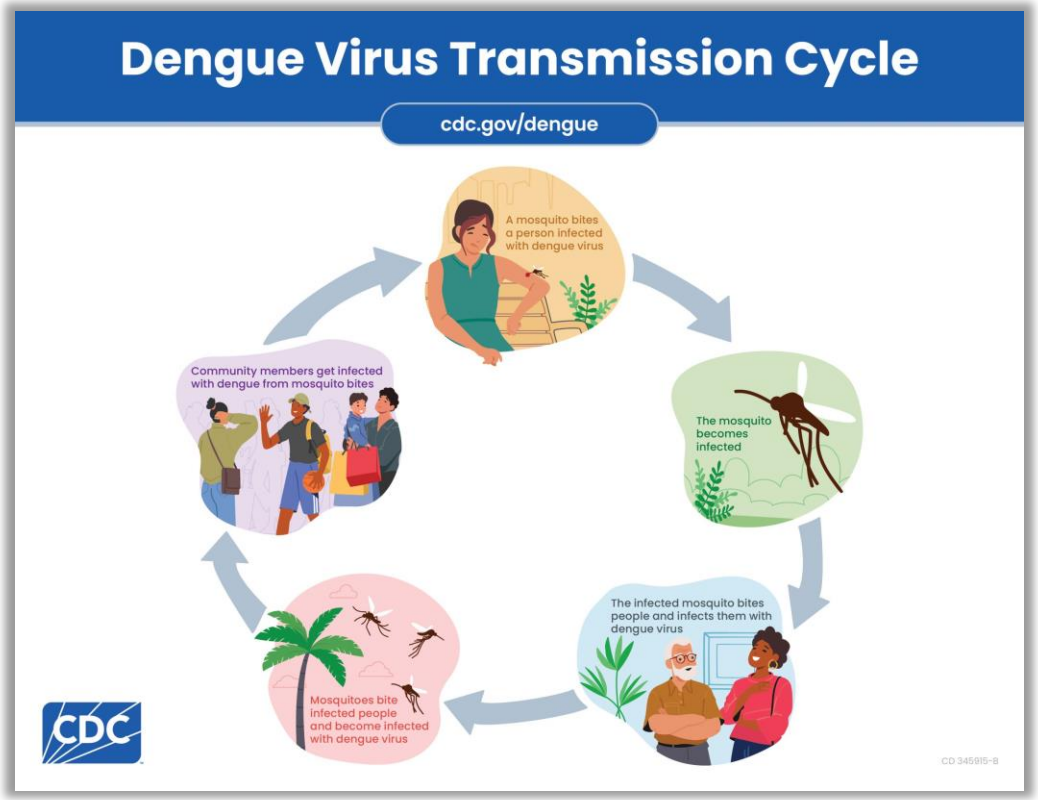
Severe disease presentation:

- Hemorrhagic disease (for example, dengue)
  - Severe bleeding
  - Fluid accumulation
  - Respiratory distress
- Neurologic disease (for example, Oropouche)
  - Meningitis
  - Encephalitis

Humans are the Primary Hosts of Most Travel Associated Arboviruses.

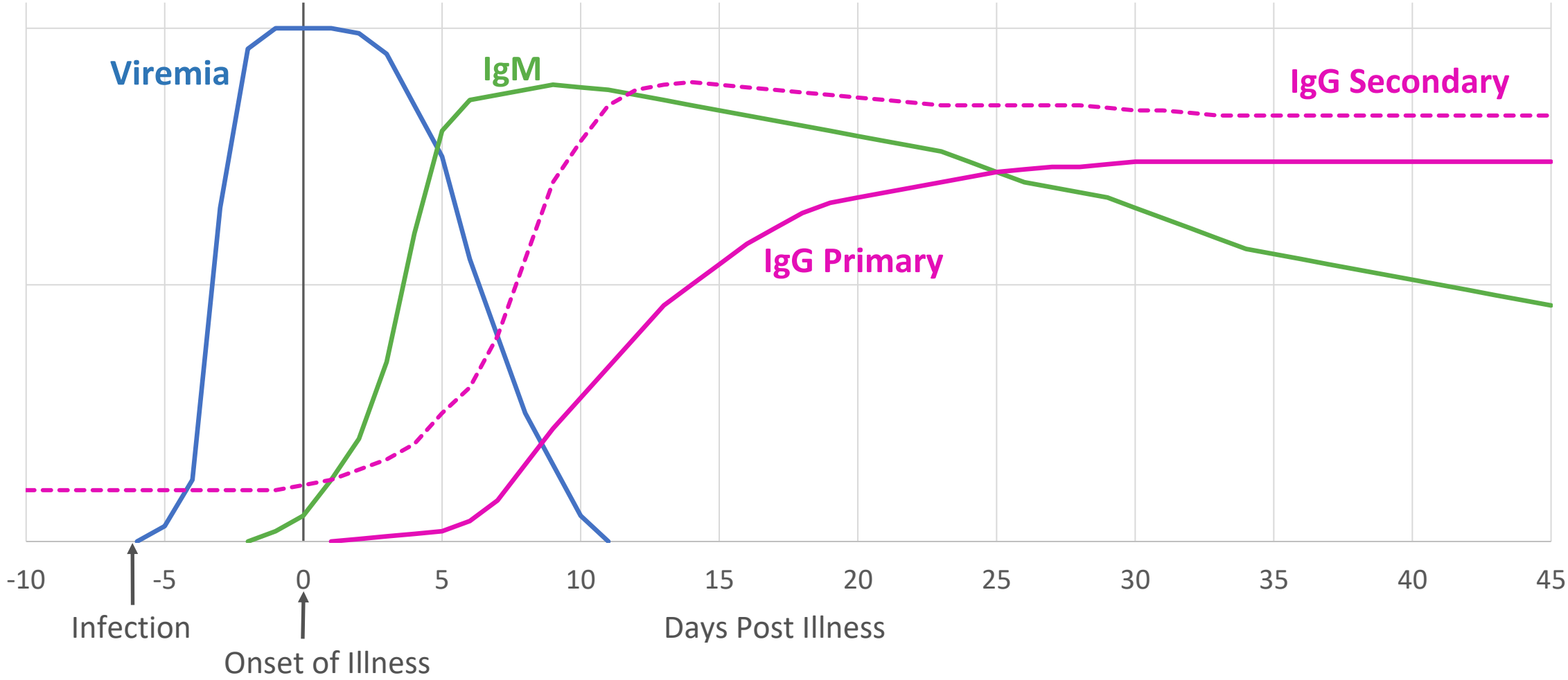


Chikungunya Virus

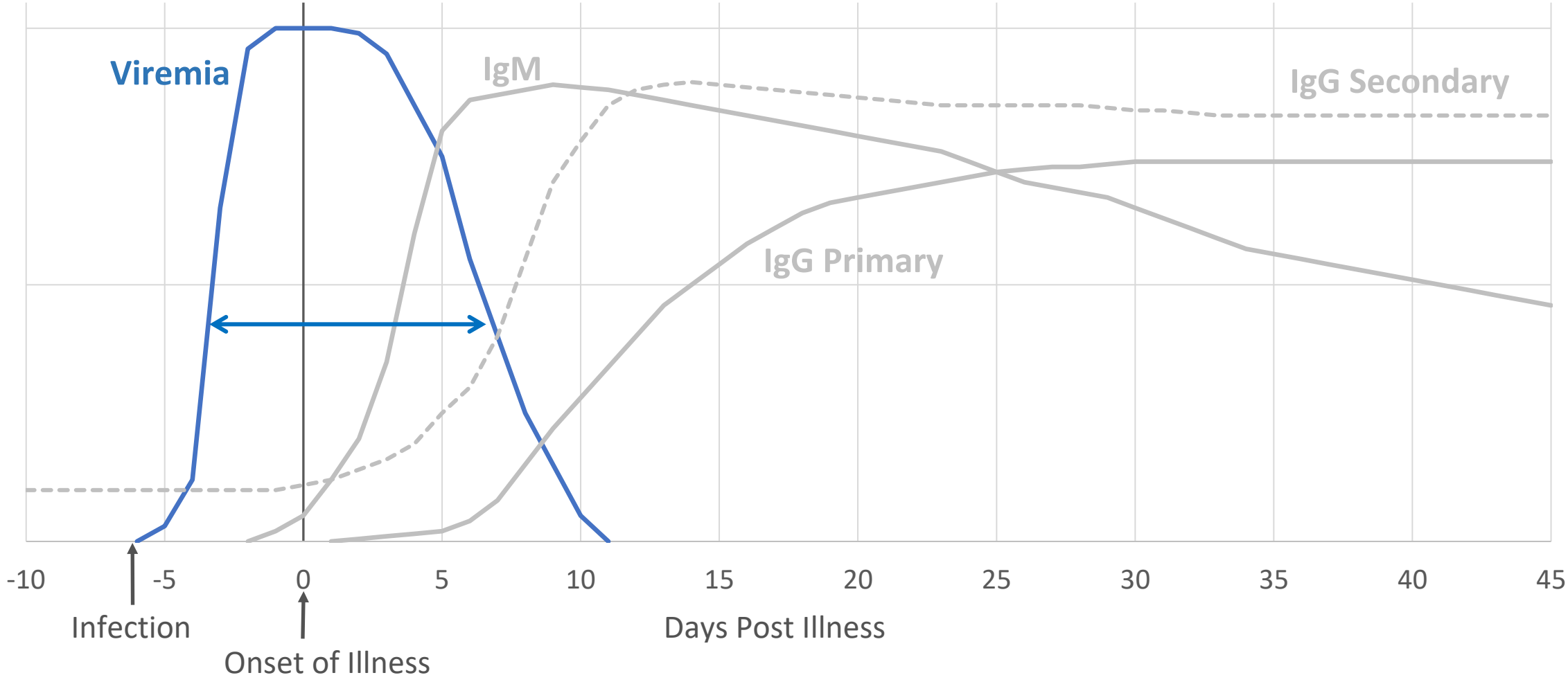


Dengue Virus

Timing of **Dengue Virus** Viremia and Antibody Response



Timing of **Dengue Virus** Viremia and Antibody Response



Imported Arboviruses: Diagnostic Testing

For all patients, test recommendations depend on the **timing of specimen collection** and the **suspected arboviral infection**.

Acute phase illness (first 7 days)

- **RT-PCR** alone (*Oropouche, Zika, chikungunya*)
- **RT-PCR** and **IgM antibody** (*dengue*)
- **NS1 antigen** and **IgM antibody** (*dengue*)

After day 7 of illness

- **IgM antibody** and/or **PRNT**
- **RT-PCR** becomes less sensitive

Imported Arboviruses: Diagnostic Testing

Depending on travel location and clinical presentation, **concurrent testing for co-circulating arboviruses should be considered**.

Wisconsin State Laboratory of Hygiene offers:

Arbovirus PCR Panel (test code SS02310)

- Dengue virus PCR
- Chikungunya virus PCR
- Zika virus PCR

Dengue IgM antibody (test code SS02272), **reflexed to PRNT** at CDC.

Diagnostic Testing for Dengue

For patients with suspected acute dengue, both **molecular** or **antigen** and **serologic** diagnostic tests are recommended.

After seven days of illness, **IgM ELISA** is recommended as the primary diagnostic test.

Day of specimen collection post symptom onset	Assay(s)
0–7	**RT-PCR** and **IgM antibody**, or **NS1 antigen** and **IgM antibody**
7–14	**IgM antibody**, **RT-PCR**, or **NS1 antigen** can also be considered
>14	**IgM antibody**

Oropouche Testing Guidance

Who should be tested?

Patients who have been in an area with documented or suspected Oropouche virus circulation within 2 weeks of ***initial*** symptom onset, and in the absence of a more likely clinical explanation, meet one of the following clinical criteria:

Oropouche Testing Guidance

Who should be tested?

Patients who have been in an area with documented or suspected Oropouche virus circulation within 2 weeks of ***initial*** symptom onset, and in the absence of a more likely clinical explanation, meet one of the following clinical criteria:

- Fever or chills or two or more of the following: headache, myalgia, arthralgia, retro-orbital pain, or generalized rash; **or**
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction; **and**
- Tested negative for other possible diseases, in particular dengue.

Oropouche Testing Guidance

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Patients who have been in an area with documented or suspected Oropouche virus circulation within 2 weeks of ***initial*** symptom onset, and in the absence of a more likely clinical explanation, meet one of the following clinical criteria:

- Fever or chills or two or more of the following: headache, myalgia, arthralgia, retro-orbital pain, or generalized rash; **OR**
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction; **AND**
- Tested negative for other possible diseases, in particular dengue.

All Oropouche virus testing must be approved by DHS.

Contact Bureau of Communicable Diseases: **608-267-9003, dhsdphbcd@dhs.wisconsin.gov**

Diagnostic Testing for Oropouche

CDC currently offers both **RT-PCR** and **plaque reduction neutralization test (PRNT)**.

Day of specimen collection post symptom onset	Assay
0–7	RT-PCR
6–7	PRNT, if RT-PCR is negative
>7	PRNT

→ Collection of both **acute** and **convalescent** serum specimens from **pregnant patients** may be necessary to confirm a recent infection.

Case Study 1: Cross-Reactive Antibodies

73-year-old female patient with fever, photophobia, headache, fatigue, diarrhea, altered mental status, ataxia, slurred speech with onset in May.

- West Nile virus (WNV) IgG positive, WNV IgM negative in a day 15 serum from a reference lab.

DHS requested residual serum from reference lab for arbovirus IgM panel testing at WSLH.

- Powassan (POW) IgM positive, all other arboviral IgM results negative.
- POW infection confirmed via positive PRNT at CDC.

→ **Confirmed case of neuroinvasive Powassan virus.**

Case Study 2: Persistent Detectable IgM

24-year-old female patient with fever, photophobia, fatigue, nausea, vomiting, altered mental status, CSF pleocytosis with onset in July.

- Jamestown Canyon virus (JCV) IgM positive and JCV PRNT positive in a day 3 serum.
- PCR positive for herpes simplex virus in CSF.

DHS requested a convalescent serum for repeat JCV PRNT.

- JCV PRNT titers were stable (no significant change).
- The patient's JCV infection was likely not acute and not contributing to illness.

→ **Not reported as a case of Jamestown Canyon virus.**

Case Study 3: Limitations of PCR Testing

31-year-old male patient with recent travel to Mexico presents with fever, chills, headache, myalgias, arthralgias, unknown onset date.

- Dengue, chikungunya, and Zika PCR all negative in serum at WSLH.

- Follow-up revealed serum was collected on day 10 of illness.
- DHS requested dengue and chikungunya IgM on residual day 10 serum.
- Dengue IgM and PRNT positive, confirming acute dengue infection.

→ **Confirmed case of dengue fever.**

Case Study 4: Non-specific Reactivity

19-year-old female patient with recent travel to Panama presents with a 3-week history of recurrent fevers, chills, headache, fatigue, new onset of nuchal rigidity, tremors, ataxia.

- Chikungunya (CHIK) IgM positive, dengue IgM negative in a day 20 serum from a reference lab.

DHS requested residual serum from reference lab for chikungunya and Oropouche PRNT.

- CHIK PRNT was negative, Oropouche PRNT was positive.
- Initial CHIK IgM result was a false positive.

→ **Probable case of neuroinvasive Oropouche virus.**

Reminders for Providers and Laboratories

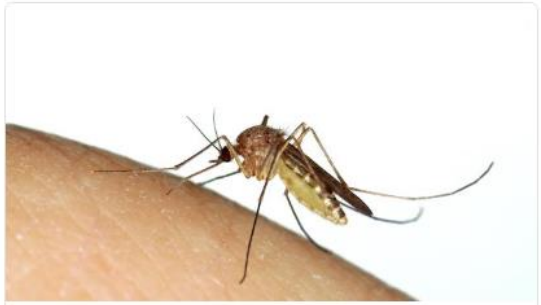
- Several **serologic** and **molecular** testing options are available commercially or at public health laboratories.
- **Limited availability** of some assays for rare, emerging arboviral diseases (for example, Jamestown Canyon, Powassan, Oropouche, yellow fever viruses).
- Serologic assays can be difficult to interpret and can sometimes **complicate diagnosis**.

Reminders for Providers and Laboratories

For assistance with arboviral disease testing, please contact:

- DHS Bureau of Communicable Diseases: **608-267-9003** or **dhsdphbcd@dhs.wisconsin.gov**
- WSLH Communicable Disease Customer Service: **800-862-1013**

DHS Resources



About West Nile Virus

Learn more about West Nile virus, including signs and symptoms, treatment, and resources.

Learn more about West Nile virus

Data

Maps and charts with West Nile virus data.

View the number of reported cases of West Nile virus

Reporting and Surveillance

Local or Tribal health departments and health care providers can learn about disease reporting, testing, treatment, and more.

Find information for health care professionals

Mosquito Bite Prevention

Tips to prevent mosquito bites.

Learn about the steps you should take to "fight the bite"

West Nile virus and other arbovirus testing at the Wisconsin State Laboratory of Hygiene

If West Nile virus infection is suspected, concurrent testing for other domestic arboviruses should be considered, since clinical features of these viruses overlap. The Wisconsin State Laboratory of Hygiene (WSLH) offers an arbovirus IgM antibody panel, which includes IgM tests for West Nile, St. Louis encephalitis, Jamestown Canyon, La Crosse encephalitis, Eastern equine encephalitis, and Powassan viruses.

The WSLH arbovirus IgM antibody panel screens for evidence of infection through IgM antibody-capture enzyme-linked immunosorbent assay or microsphere immunoassay performed on serum or CSF. The WSLH will automatically forward specimens with positive IgM results to CDC for confirmatory PRNT.

The arbovirus IgM antibody panel is available at the WSLH either fee-for-service (no prior approval needed) or fee-exempt (prior approval needed).

Providers may request fee-exempt arbovirus IgM testing for patients who:

- Would not otherwise have access to testing for financial reasons (for example, lack insurance coverage).

Public health officials may request fee-exempt arbovirus IgM testing for patients with:

- Evidence of an arboviral infection who are part of a public health investigation.

To request fee-exempt testing at WSLH, contact a DPH vectorborne disease epidemiologist at 608-267-9003 or email DHSDPHBCD@dhs.wisconsin.gov prior to specimen submission.

Instructions for collection and shipping of clinical specimens to WSLH:

- Submit 2-5 mL of serum and/or ≥1 mL of CSF in sterile, screw-capped vials on cold packs using a WSLH kit #22 or equivalent within five days of collection. If shipping more than five days after collection, freeze specimen at -20°C and ship on dry ice. It is recommended that a paired serum specimen be submitted with a CSF specimen. Specimens should be triple packages as a Category B Biological Substance (include UN3373 label).
- Fee-exempt testing submissions must include a WSLH Enhanced Wisconsin Arbovirus Surveillance form provided by a DPH vectorborne disease epidemiologist in addition to the WSLH Requisition Form B.
- Fee-for-service testing submissions must include a WSLH CDD Requisition Form B. Please contact the WSLH Clinical Orders at 800-862-1088 or 608-224-4275 to obtain the WSLH CDD Requisition Form B and to order specimen shipping kits.
- It is essential that the lab requisition form be as complete as possible, including patient name, city, date of birth, specimen type, submitting agency, onset date, signs and symptoms, collection date, and recent travel history. **Note: Testing for West Nile virus may be delayed on specimens missing the above data until information is available.**

[West Nile Virus: Reporting and Surveillance]

CDC Resources

Clinical Testing and Diagnosis for West Nile Virus Disease

KEY POINTS

- Patients with suspected West Nile virus (WNV) disease should first be tested for WNV-specific immunoglobulin (Ig)M antibodies in serum and/or cerebrospinal fluid (CSF).
- In some cases, positive IgM results should be confirmed by neutralizing antibody testing at a state public health laboratory or CDC.
- Reverse transcription-polymerase chain reaction (RT-PCR) should be considered in patients with immunocompromising conditions.

Antibody Testing

Laboratory diagnosis is generally accomplished by testing of serum or CSF to detect WNV-specific IgM antibodies. Immunoassays for WNV-specific IgM are available commercially and through state public health laboratories.

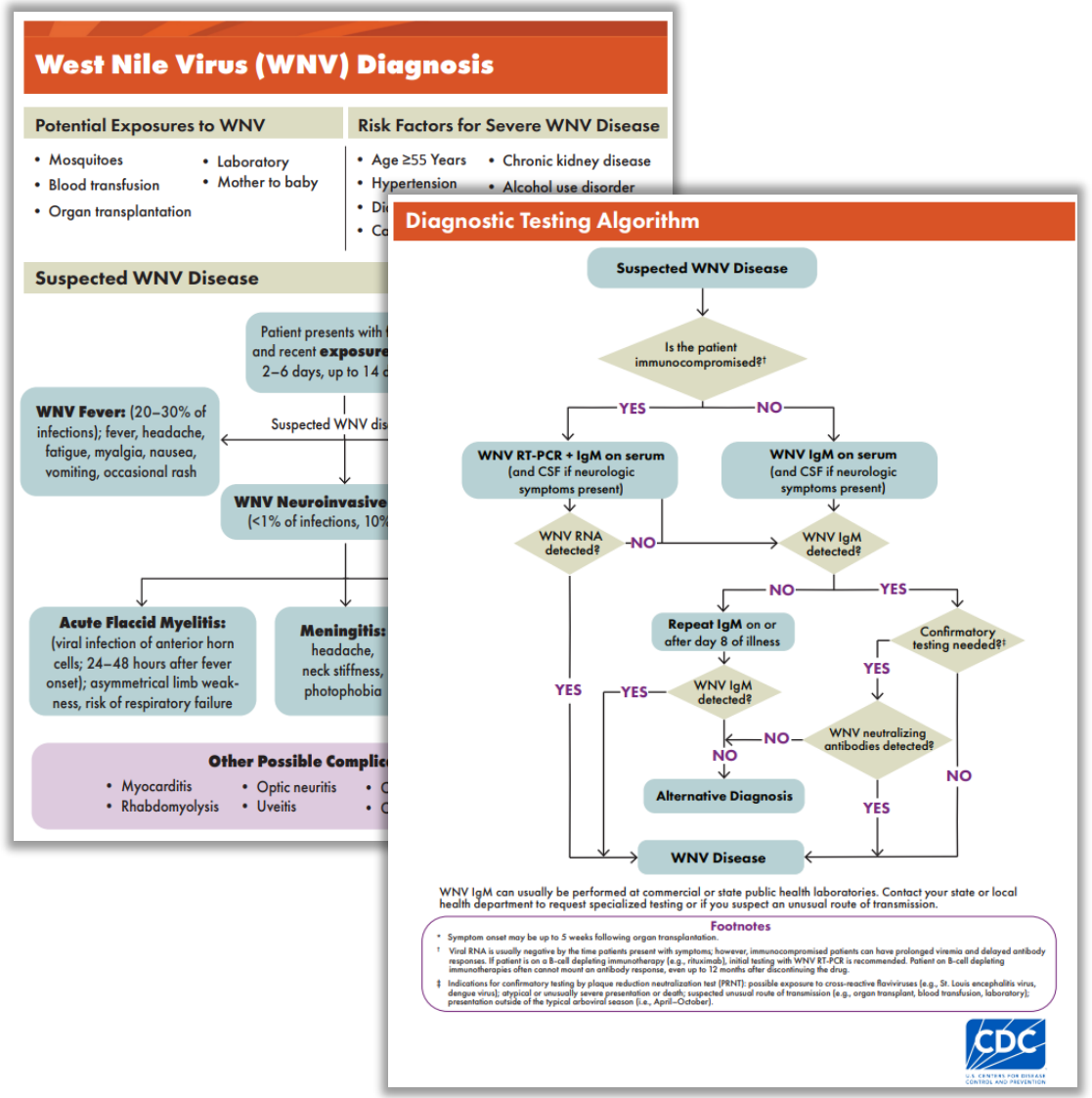
RELATED PAGES

Diagnostic Testing Algorithm

Clinical Signs and Symptoms

Treatment and Prevention

[Clinical Testing and Diagnosis for West Nile Virus Disease]



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