Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

Presentation Overview

Characteristics of Zika virus
Characteristics of Zika virus infection
Epidemiology and outbreaks
Vector and transmission
Clinical symptoms
Diagnosis and testing
Treatment and prevention
Wisconsin response and preparedness
Disease reporting and investigation
Research at UW-Madison

Some Mosquito Borne Diseases

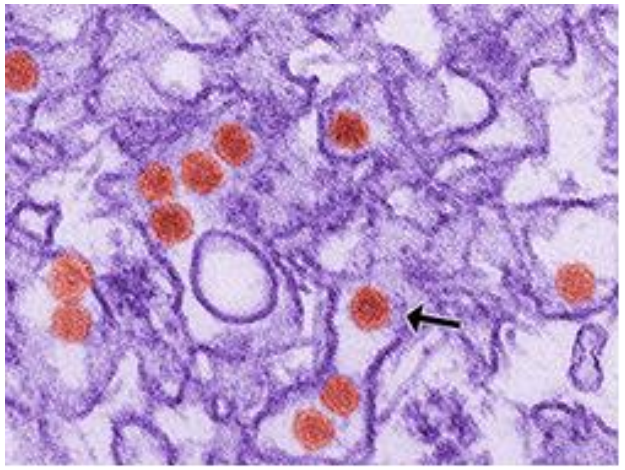
- Chikungunkya
- Denque
- Eastern equine encephalitis
- Filariasis
- Jamestown Canyon virus
- Powassan virus
- Japanese encephalitis
- Malaria
- Rift Valley fever
- St. Louis encephalitis
- Venezuelan equine encephalitis
- Western equine encephalitis
- Yellow fever
- ZIKA

Zika Virus

- First identified in Uganda in 1947
  - From the blood of a sentinel rhesus monkey during a study of sylvanic cycle of yellow fever virus
    - Febrile illness
  - Isolated from mosquitos in 1948
    - *Aedes africanus*

Zika Virus

- Mosquito-borne single-stranded RNA flavivirus
  - 10,794 bases long
  - Closely related to dengue, yellow fever, Japanese encephalitis and West Nile viruses.
  - 40 nm diameter
  - Icosahedral
  - Lipid enveloped
  - Neurotropic



Zika Virus Epidemiology

- Prior to 2007, only sporadic human cases reported from Africa and southeast Asia
  - Many cases and outbreaks likely not recognized
- 2007, first outbreak reported on Yap Island, Federated States of Micronesia
- 2013-2014, >28,000 suspected cases reported from French Polynesia
- May 2015, locally-acquired cases reported in Brazil

Countries and Territories with Active Zika Virus Transmission

Zika Virus in the USA

- Local transmission not yet reported in the continental US
- Zika is a nationally notifiable disease
- As of March 2, 153 travel-associated cases reported to CDC
- Nine pregnant travelers
  - 2 early pregnancy losses
  - 2 elective terminations
  - 1 infant with severe microcephaly
  - 2 healthy infants

Zika Virus in the USA

- With current outbreaks in the Americas, cases will increase
- Imported cases may lead to virus introduction and local spread in some areas of the US

Zika Virus Incidence and Attack Rates

- Yap Island outbreak, 2007 (population 7,391)
  - Infection rate: 73%
  - Symptomatic attack rate among infected: 18%
  - All age groups affected
  - Adults more likely to present for medical care
  - No severe disease, hospitalizations, or deaths

Duffy, M. N Engl J Med 2009

Zika Virus Vectors

- *Aedes* species mosquitoes.
  - *Aedes aegypti* – more efficient vector for humans.
  - *Aedes albopictus* – possible vector.
- Also transmit dengue and chikungunya viruses.
- Aggressive biters with peak feeding at daytime.
- Lay eggs in and around standing water.
- Live indoors and outdoors near households.
- Humans are the primary amplifying host during outbreaks.
- Monkeys are the natural reservoir

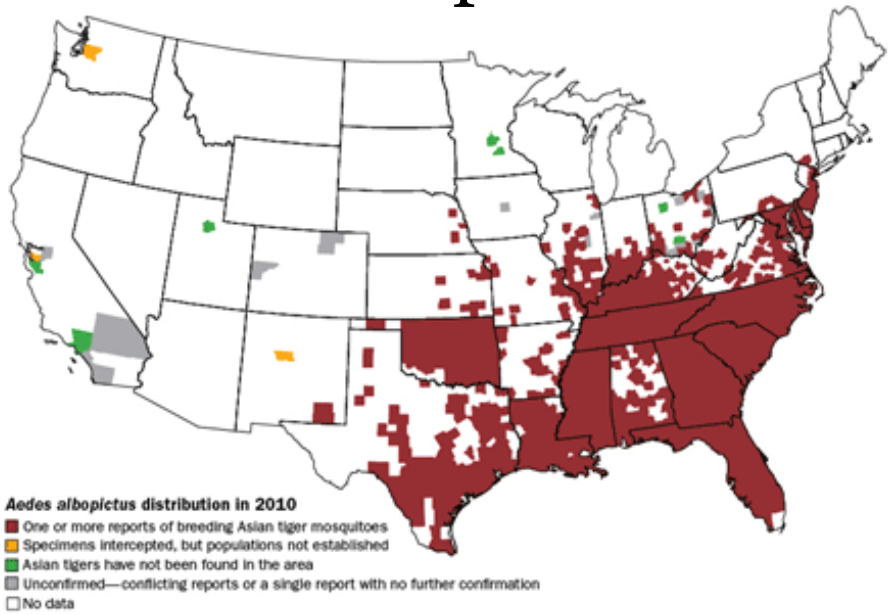
Zika Vectors

Aedes aegypti

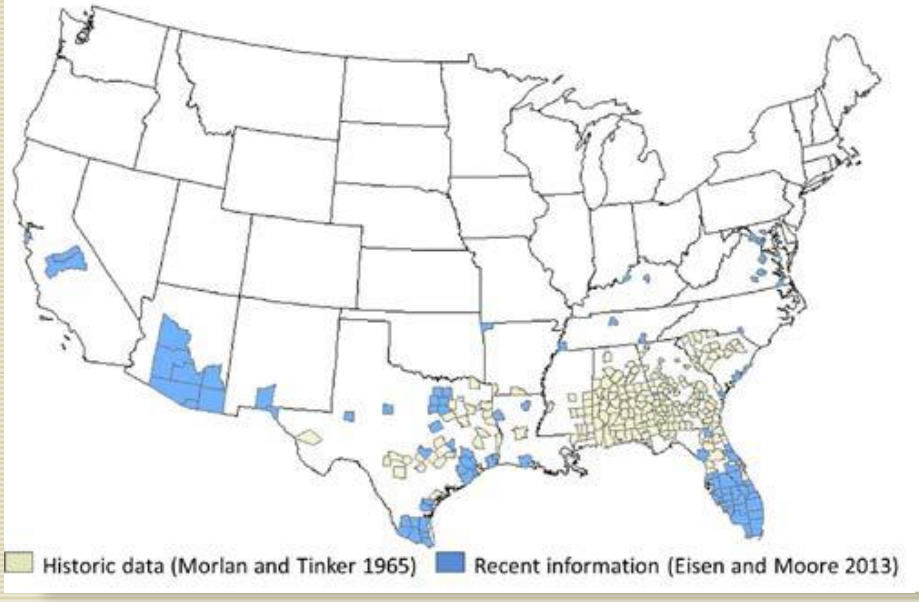
Aedes albopictus

Aedes spp. Distribution

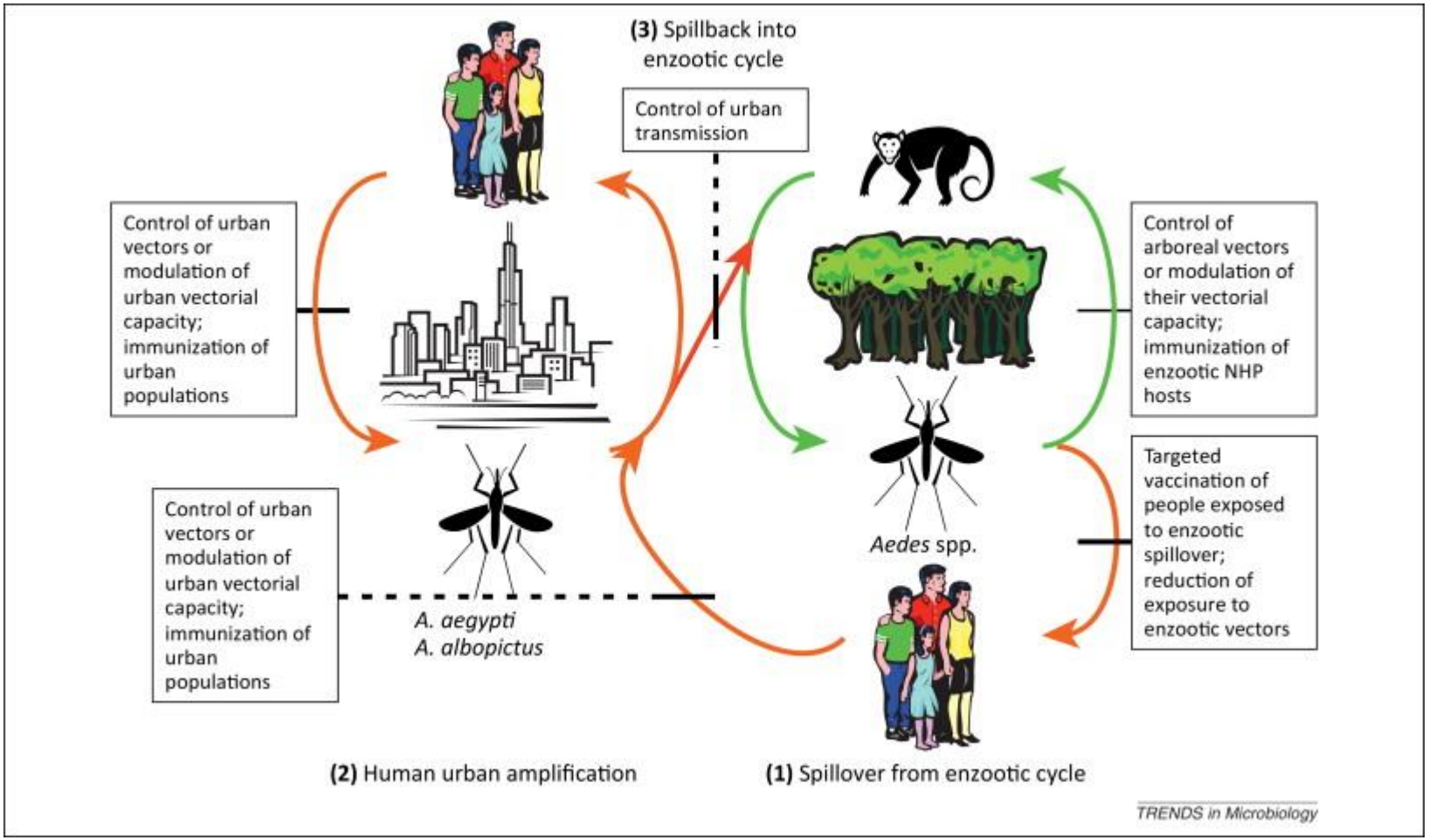
A. albopictus



A. aegypti



Zika Transmission



Other Modes of Transmission

Maternal-fetal: during pregnancy and time of birth.

Other documented modes of transmission: rare?

- Sexual
  - Male to female
  - One report of virus detected in semen at 62 days after onset
- Blood transfusion
  - Reports in Brazil being investigated
  - Deferral for 4 weeks in US
- Laboratory exposure

Theoretical concerns:

- Organ or tissue transplantation
  - Reports in Brazil being investigated
- Breast milk
  - Infectious virus has been detected

What We Don't Know

- How long Zika stays in semen?
- Do asymptomatic men have Zika in semen?
- Can asymptomatic men transmit Zika?
- Can a women transmit Zika to sex partners?
- Can Zika be transmitted through oral or anal sex?
- Does sexual transmission pose a different risk of birth defects than mosquito-borne transmission?

Clinical Disease Course

- Incubation several days to a week
- 80% asymptomatic
- Usually mild disease
  - Lasting several days to a week
- Hospitalization uncommon
- Fatalities rare
- Guillain-Barré syndrome reported following suspected Zika virus infection

Clinical Symptoms

Symptoms	N (n=31)	%
Macular or papular rash	28	90%
Subjective fever	20	65%
Arthralgia	20	65%
Conjunctivitis	17	55%
Myalgia	15	48%
Headache	14	45%
Retro-orbital pain	12	39%
Edema	6	19%
Vomiting	3	10%

Yap Island, 2007

Duffy M. N Engl J Med 2009

Need to Distinguish Zika from Dengue and Chikungunya

- All transmitted by the same mosquitoes with similar ecology
- Dengue and chikungunya can circulate in same area and rarely cause co-infections
- All have similar clinical features
- Important to rule out dengue, as proper clinical management can improve outcome

Treatment

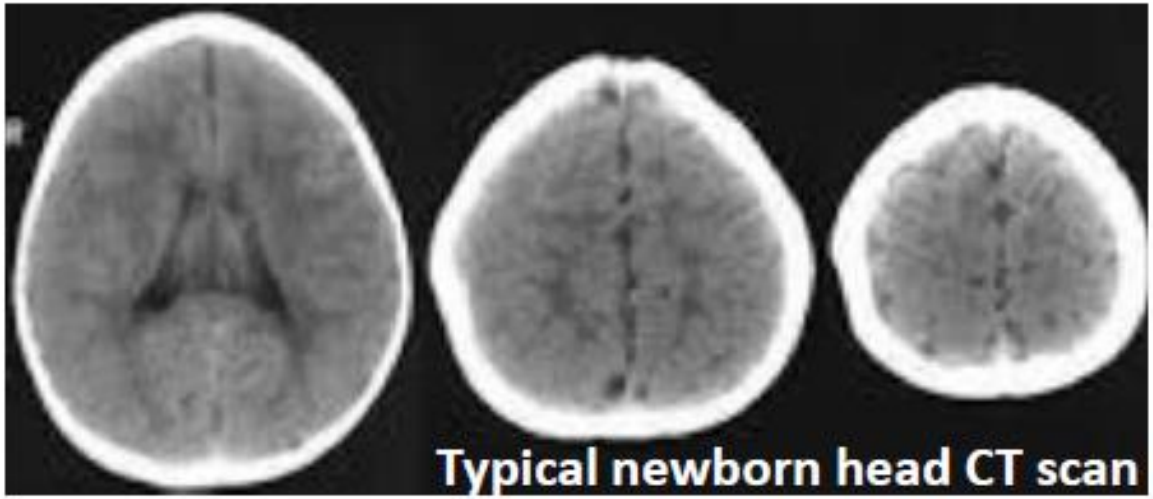
- No specific antiviral treatment is available.
- Treatment is supportive: rest, fluids, and supportive care.
  - Assess for dengue and chikungunya viruses and avoid use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) until dengue is ruled out (to reduce the risk of hemorrhage).
  - Treat pregnant women with acetaminophen.

Clinical Features:
Zika Virus Compared to Dengue and Chikungunya

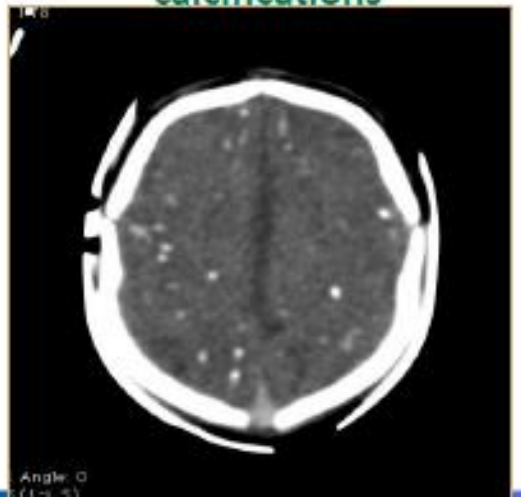
Features	Zika	Dengue	Chikungunya
Fever	++	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-	+	-

Zika and Microcephaly in Brazil

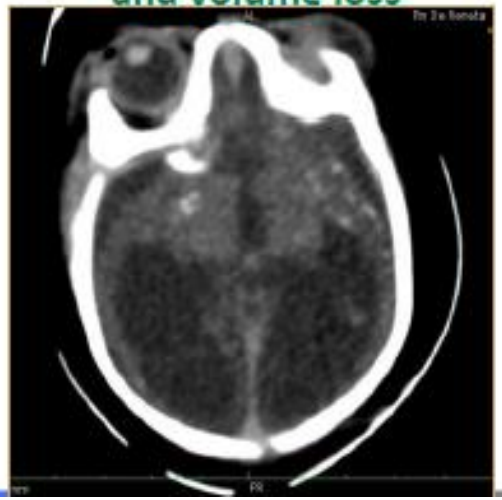
- Zika virus infections may be associated with microcephaly:
  - Considerable increase in the number of infants born with microcephaly has been observed in outbreak in Brazil during 2015.
  - True baseline rate of microcephaly and the association with Zika virus is unknown at this time.
    - Zika virus RNA isolated from several babies born with microcephaly and from fetal losses among women infected during pregnancy.
    - Some of the infants with microcephaly have tested negative for Zika virus.
- Investigations are ongoing.



scattered intracranial calcifications



enlarged ventricles and volume loss



Zika and Associated Birth Defects

- Microcephaly
- Brain atrophy
- Cerebral and intraocular calcifications
- Abnormal formed or absent brain structures
- Cataracts

Evidence not yet definitive

Rates of Microcephaly Over Time: the Americas and the Caribbean

Comparison of the rates of microcephaly in the Americas and Caribbean from 2010-2014 and 2015

Updated as of Epidemiological Week 52 (December 27, 2015 – January 2, 2016)

Microcephaly rates by state in Brazil (cases per 1.000 live births)

- 0.1-1.0
- 1.1-15.0
- 15.1-30.0
- 30.1-45.0
- 45.1-88.6
- Countries

Countries with Zika confirmed cases

- Epi Week 52 2015
- Country limits
- Brazil State Boundaries

0 1.000 2.000 4.000 Km

Data Source: Reported from the IHR National Focal Points and through the Ministry of Health websites.

Map Production: PAHO-WHO AD CHA IR ARO

Source: Pan American Health Organization, Epidemiological update, 17 January 2016

What We Don't Know

- Causal relation between Zika virus and microcephaly and other adverse pregnancy outcomes?
- Full spectrum of impact in affected infants?
- Impact of severity of maternal infection
- Does asymptomatic pose a risk?
- Timing of the infection during pregnancy effect on risk of fetal abnormalities?
- Magnitude of the risk?

Recent Study

- 88 women living in Rio de Janeiro who developed rash within previous 5 days
- Zika RT-PCR on blood and urine
  - 72 (82%) were positive
  - Range of time of infection was 5-35 weeks gestation
  - Fetal ultrasonography in 42 Zika-positive women
    - Abnormalities found in 12 **(29%)**

Brasil, P. et al NEJM March 10, 2016

Recent Study (cont)

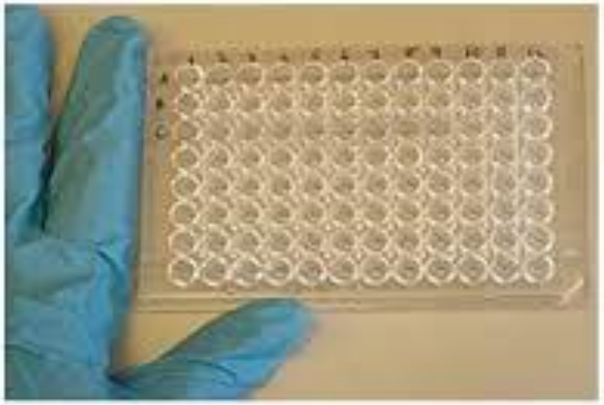
- Adverse findings
  - 2 fetal deaths at 36 and 38 weeks
  - 5 fetuses with in utero growth restriction with or without microcephaly
  - 7 with ventricular calcifications or other CNS lesions
  - 7 with abnormal amniotic fluid volume or cerebral or umbilical artery flow

Laboratory Testing

- Processing of clinical specimens for Zika virus should be performed at a minimum of BSL2 precautions
- Perform a risk assessment to determine if higher levels of biocontainment are required
  - e.g. Suspicion of Chikungunya virus

Diagnostic Testing

- Real-time PCR
- IgM Serology
- Immunohistochemical staining



Reverse Transcriptase Real-Time PCR

- Two targets
  - Screening, broadly reactive target
  - Target specific for the Asian strain

- Waiting for a CDC Emergency Use Authorized triplex PCR
  - Zika, Dengue 1-4, and Chikungunya

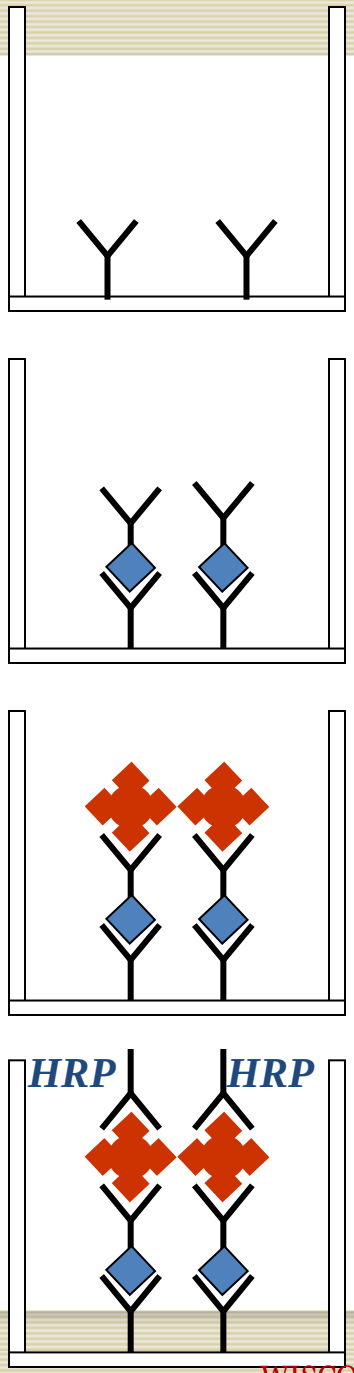
Types of Specimens for Zika Virus PCR Testing

- Serum
- CSF
- Amniotic fluid (collected after 15 weeks gestation)
- Placental and umbilical cord tissues (fixed or frozen)
- Cord blood

WSLH will be testing only serum specimens at this time

IgM Capture ELISA

- CDC EUA assay
- Serum and CSF
  - CSF must be accompanied by a serum specimen
- IgM detectable ≥4 days after illness onset
  - Detectable up to 12 weeks



CDC IgM Capture ELISA

1. Coat With Goat anti-Human IgM
   - 4° Overnight
2. Add Patient Serum @ 1:400
   - 37° 1 Hour
3. Add Zika Antigen
   - 4° Overnight
4. Add HRP anti-Flavivirus McAb
   - 37° 1 Hour
5. Add substrate
   RT 10 min
6. Add stop solution and Read

IgM Capture ELISA

- Difficult to distinguish infecting virus in people previously infected or vaccinated against a related flavivirus
- Anti-dengue virus IgM antibodies cross-react, so positive Zika IgM specimens must be confirmed
  - Plague reduction neutralization assay (PRNT) performed at CDC

Diagnosis and Testing, Wisconsin

- All requests for fee-exempt Zika virus testing must be approved by the DPH. Phone 608-267-9003

- **Criteria for testing**
  - History of travel to an area with localized Zika virus transmission
  - Signs and symptoms within 2 weeks after returning. (Males or Females)
  - Asymptomatic pregnant females who are within 2-12 weeks of return from travel to an area with localized Zika virus transmission

Zika Testing Performed

History of Travel to area of Zika virus transmission AND Symptomatic

	PCR	IGM
Specimen collected within 3 days of onset	Zika Chik Dengue	
Specimen collected within 4-7 days of onset	Zika Chik Dengue	Zika Chik Dengue
Specimen collected within 1-12 weeks of onset		Zika Chik Dengue

Zika Testing Performed

Asymptomatic pregnant patient with history of travel to area of Zika virus transmission AND within 2-12 weeks of return from travel

Perform Only Zika virus IgM

Testing in Infants with Microcephaly or Intracranial Calcifications

- RT-PCR
  - Umbilical cord serum
  - Serum directly from infant within 2 days of birth
  - Maternal serum
  - Placental and cord tissue
  - CSF obtained for other studies
  - Mother's serum, saliva, and urine if not previously tested
- IgM ELISA
- IHC and histopathology on placenta and umbilical cord tissues

Testing in Infants without Microcephaly or Intracranial Calcifications

- If mother's test results positive or inconclusive
  - RT-PCR
  - IgM ELISA
  - IHC

Surveillance in Wisconsin

- Provide fee-exempt testing of Zika virus (also chikungunya and dengue) in travelers with appropriate signs and symptoms within 2 weeks after returning from areas with localized Zika virus transmission. Provide testing for asymptomatic pregnant women.
- Provide funding to assist the Wisconsin State Laboratory of Hygiene to bring on testing.
- Support mosquito surveillance for possible emerging *Aedes* species by collaborating with the University of Wisconsin-Madison, Medical Entomology Laboratory.
- Report all Zika virus confirmed and probable cases in real time to CDC ArboNet via WEDSS and National Electronic Disease Surveillance System (NEDSS).

Disease Reporting and Investigation

- Suspected Zika virus and other arboviral infections are Category II diseases and must be reported to public health within 72 hours: https://www.dhs.wisconsin.gov/disease/diseasereporting.htm
- Follow arboviral management protocol posted on the DHS website. https://www.dhs.wisconsin.gov/publications/p0/p00894.pdf
- Use Arboviral case report form for investigation.
- Disease reported category in WEDSS: Arboviral diseases, Zika virus.

Reminder: when investigation is completed for all arboviral diseases, the local health department should "send to state" for review, leaving the resolution status as "suspect". The DHS epidemiologist will determine if it meets the case definition for confirmed or probable case. Once the case is submitted as a confirmed or probable, it will be sent to CDC via NEDSS within 15-20 minutes.

Prevention

- Vaccines under development
- Avoid exposure to mosquitoes: use air conditioning or window/door screens; wear long sleeves and pants; use permethrin-treated clothing and gear, and Environmental Protection Agency (EPA)–registered repellents when outdoors.
- Pregnant women should consider postponing travel to any areas where Zika virus transmission is ongoing.
- Persons infected with Zika, dengue, or chikungunya viruses should be protected from further exposure to mosquitoes during illness to reduce the risk of local transmission.

Prevention of Sexual Transmission

- Men who reside in or have traveled to an area of active Zika virus transmission who have a pregnant partner **should**
  - Abstain from sexual activity or use condoms during sex
- Men with nonpregnant sex partners **might consider** abstaining or use condoms

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Wisconsin Residents Tested

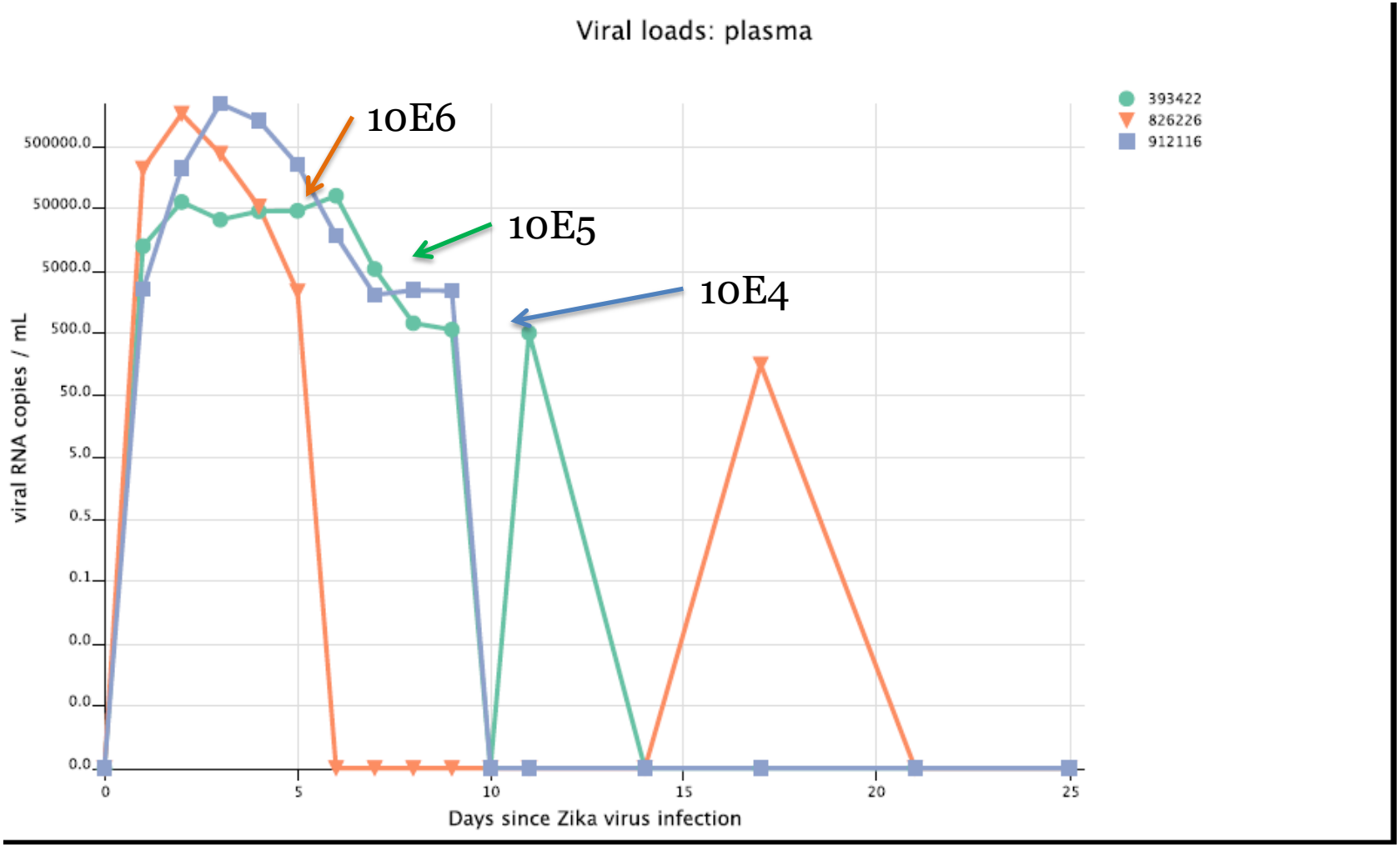
As of March 8, 2016

	Patients	Percentage
Asymptomatic	159	73%
Symptomatic	58	27%
Total	217	

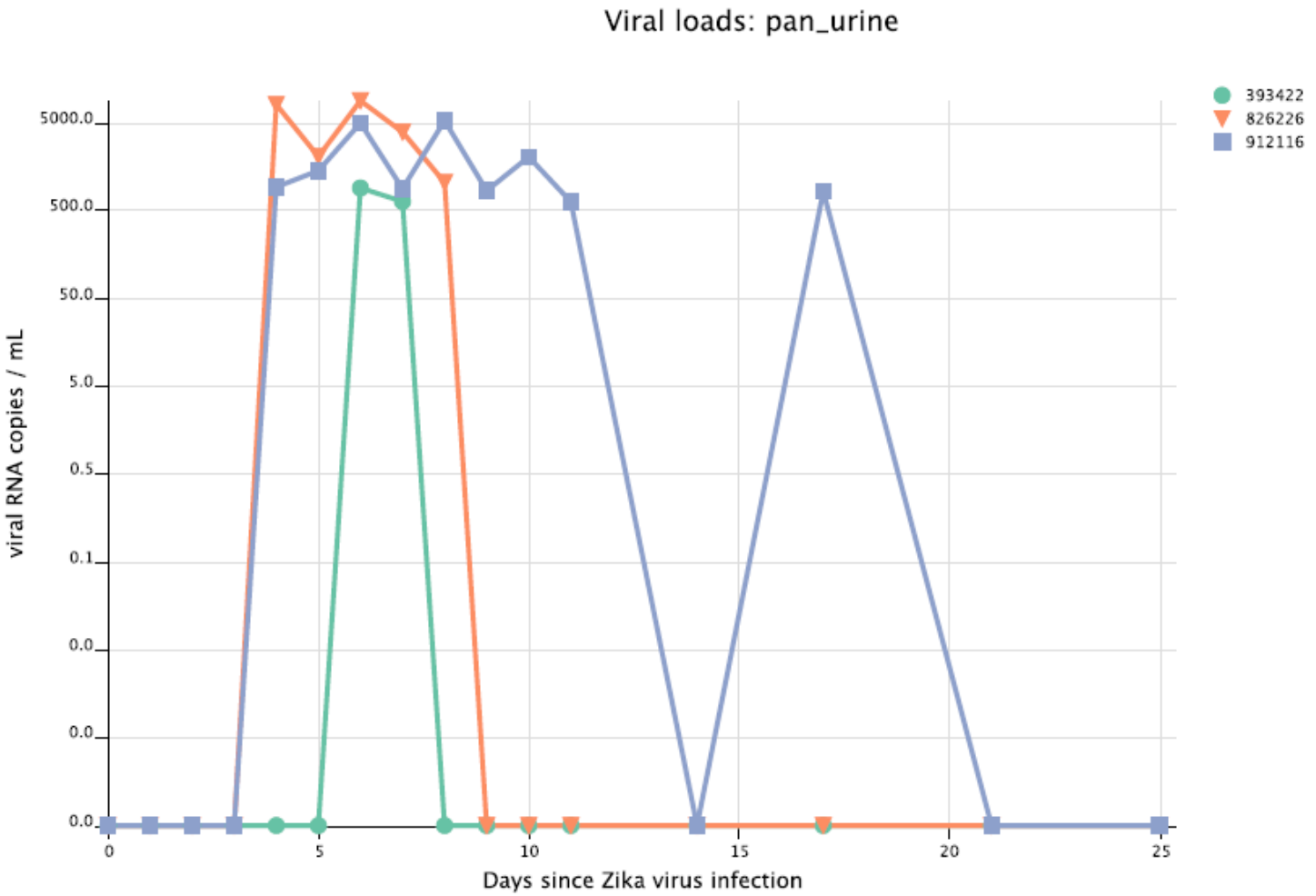
Research at UW Madison

- Drs. Dave O'Connor and Jorge Osorio
  - https://dholk.primate.wisc.edu/project/dho/public/Zika/public/begin.view
- Infected 3 rhesus monkeys subcutaneously with 10^6, 10^5, and 10^4 PFU of Zika virus
- Looking out
  - Viral RNA quantification
  - Blood counts
  - Immunology
  - Blood Chemistry

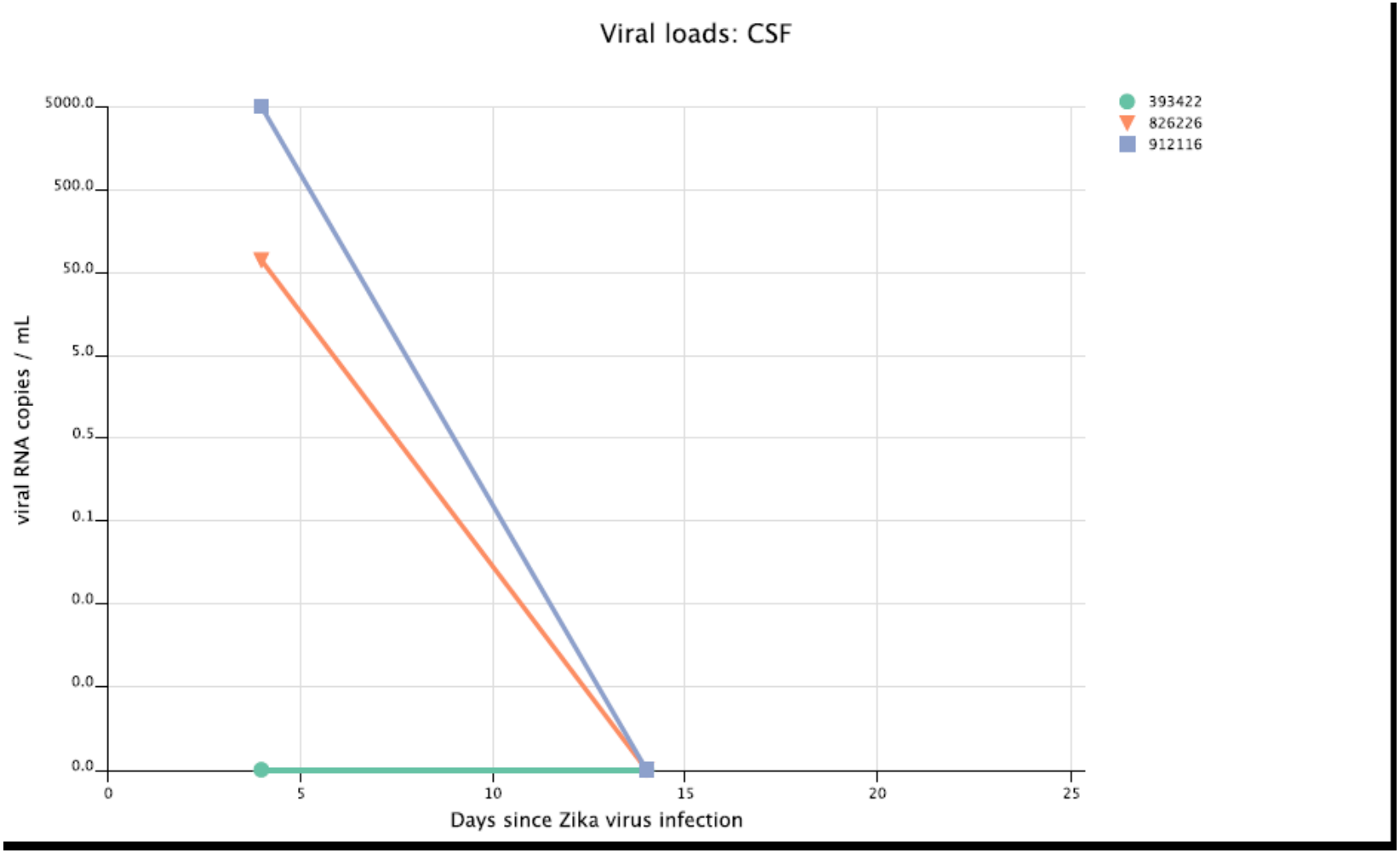
Plasma Viral Loads



Urine Viral Loads



CSF Viral Loads



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