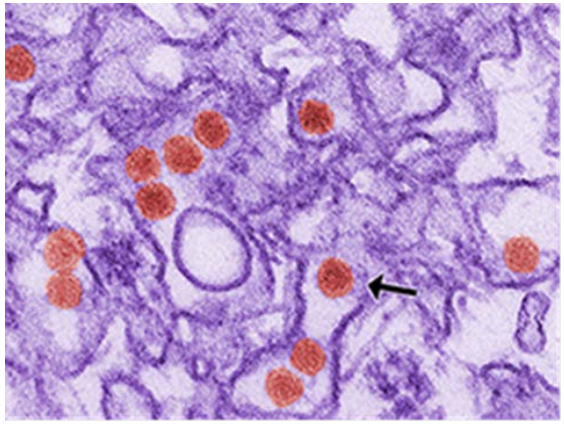
Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON



Zika Virus Update: What We are Learning

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Presentation Overview

- Case Presentation
- Characteristics of Zika virus
- Epidemiology and outbreaks
- Vectors and other modes of transmission
- Clinical symptoms
- Diagnosis and testing
- Treatment and prevention
- Wisconsin response and preparedness
- Research at UW-Madison

The Patient

- 33-year-old woman
- 11 weeks pregnant
- Presented with
  - Ocular pain
  - Myalgia
  - Mild fever which lasted for 5 days
  - Rash developed second day of fever
- Husband reported similar symptoms

History

- Travel
- Had been traveling the week before
- Guatemala
- Belize
- Mexico
- Onset 1 day after return home
- Reported mosquito bites, particularly in Guatemala
- Vaccinated against yellow fever virus 10 years earlier

Serology

- Serology----4 weeks after onset
- **Dengue** virus
  - IgG Pos, IgM Neg
- **Chikungunya** virus
  - IgG Neg, IgM Neg
- **Zika** virus
  - IgG Pos, IgM Pos
    - Acute or recent Zika virus infection

What about the fetus?

- Ultrasound at 16 and 17 weeks
- No evidence of microcephaly or intracranial calcifications.
- During 16 to 20 weeks
  - Decrease in head circumference
  - From 47th percentile at 16 weeks to 24th percentile at 20 weeks
- Zika virus detected in mother's serum at 16 weeks gestation
  - Closely related to Guatemalan strain

The Fetus

- Ultrasound at 19 weeks gestation
  - Abnormal intracranial anatomy
- MRI at 20 weeks gestation
  - Diffuse atrophy of the cerebral mantle with other abnormalities
- Pregnancy terminated at 21 weeks
- Maternal serum positive for Zika RNA on the day before termination
  - 2.1 x 10^3 copies/ml
- Mother also positive at 4 weeks and 10 weeks after clinical onset
- PCR negative one day after termination

Some Mosquito Borne Diseases

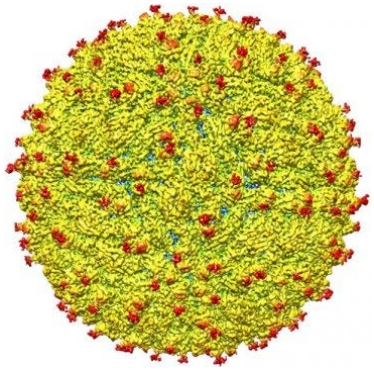
- Chikungunkya
- Denque
- Eastern equine encephalitis
- Filariasis
- Jamestown Canyon virus
- Powassan virus (tickborne)
- Japanese encephalitis
- Malaria
- Rift Valley fever
- St. Louis encephalitis
- Venezuelan equine encephalitis
- Western equine encephalitis
- Yellow fever
- ZIKA

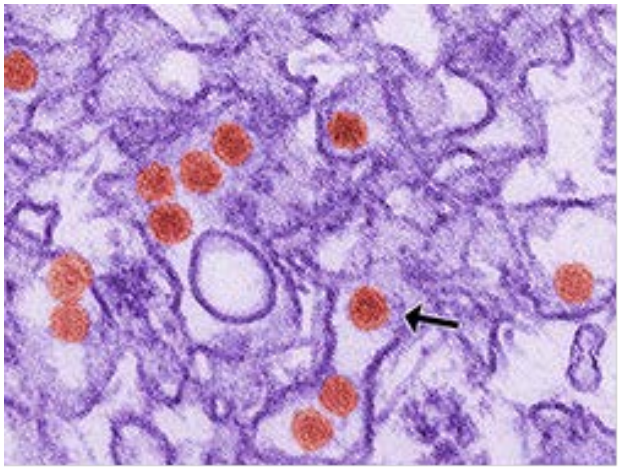
Zika Virus

- First identified in Uganda in 1947
  - From the blood of a sentinel rhesus monkey during a study of sylvatic 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
  - Closely related to dengue, yellow fever, Japanese encephalitis and West Nile viruses.
  - 4o 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

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

Countries and Territories with Active Zika Virus Transmission

http://www.cdc.gov/zika/geo/active-countries.html

Zika Virus in the USA

- Local transmission not yet reported in the continental US
- Zika is a nationally notifiable disease
- As of April 13, **358 travel-associated** cases reported to CDC
- **31 pregnant**
  - Of the first 9 reported
    - 2 early pregnancy losses
    - 2 elective terminations
    - 1 infant with severe microcephaly
    - 2 healthy infants
    - 2 still pregnant

Zika Virus Vectors

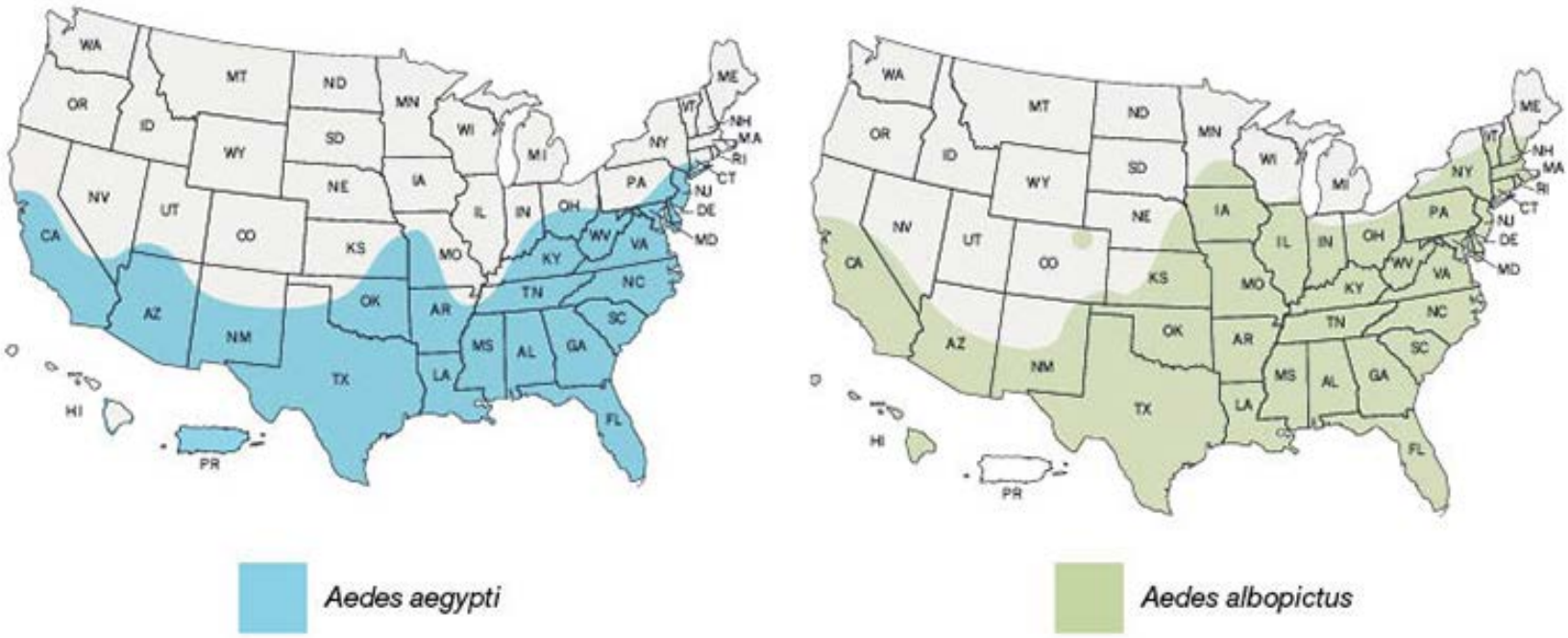
- *Aedes* species mosquitoes.
  - *Aedes aegypti* – more efficient vector for humans.
  - *Aedes albopictus* – possible vector.
- Also transmit dengue and chikungunya viruses.
- Aggressive biters with peak feeding during daytime.
- Lay eggs in and around standing water.
- Live indoors and outdoors near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

Zika Vectors

Aedes aegypti

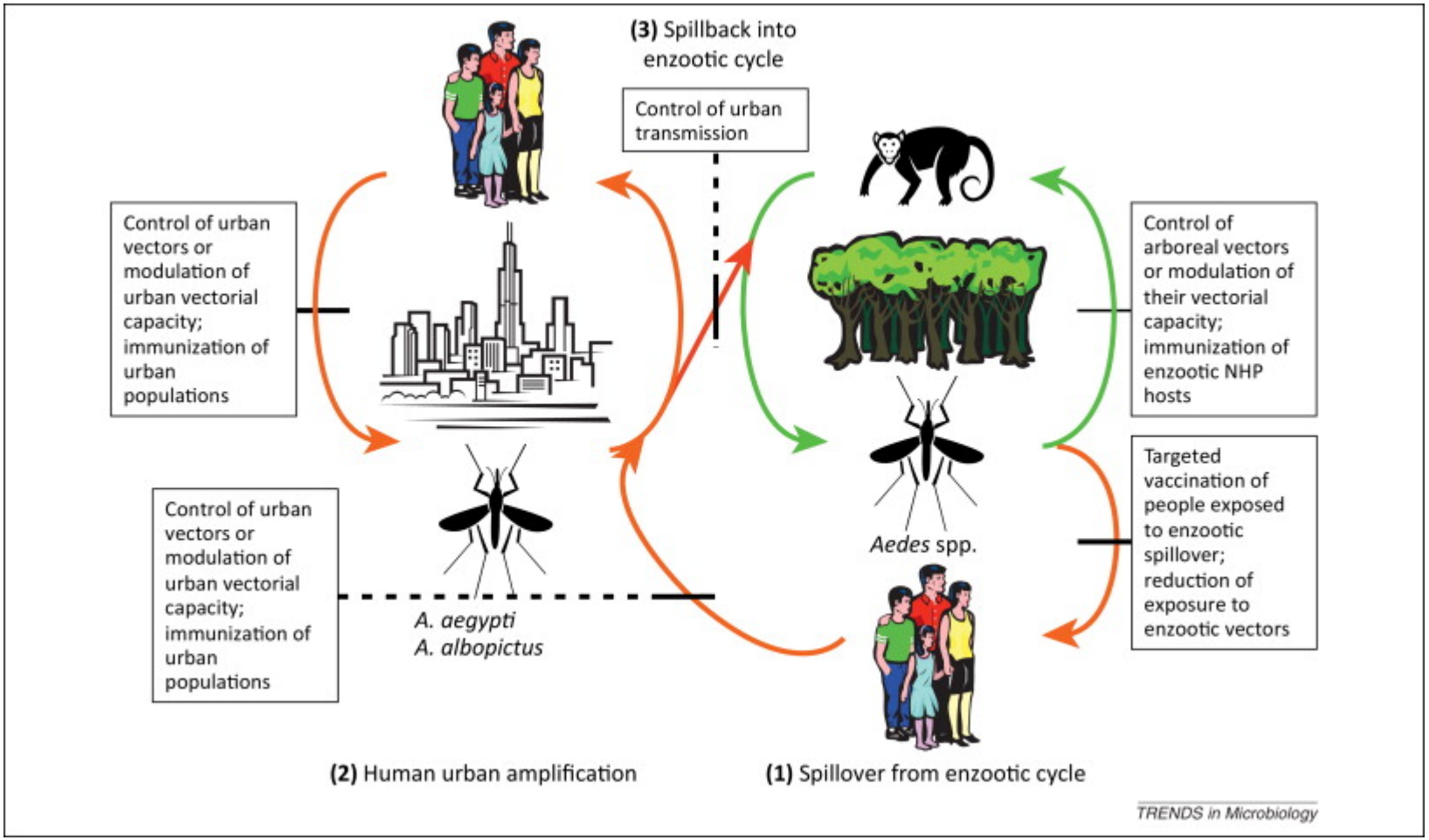
Aedes albopictus

Aedes spp. Distribution



CDC estimated ranges, 2016

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
- Male to male

Blood transfusion

- Reports in Brazil being investigated
- Deferral for 4 weeks in US
  - Suspension of blood donations in Puerto Rico
- Roche Zika PCR assay approved for screening March 30th
  - Now being used in Puerto Rico

Laboratory exposure

Theoretical concerns:

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

Sexual Transmission USA

- Documented sexual transmission from infected men to female sex partners
  - At least 6 cases reported
  - Involved vaginal sex
  - All males were symptomatic
- Male-to-male sexual transmission (MMWR , 65/No.14 April 15, 2016)
  - Symptomatic man to his nontraveling male partner via anal sex

Sexual Transmission USA Male-to-Male

- Had anal sex 1 day before and 1 day after symptom onset (fever, rash, conjunctivitis)
- On Day 7, partner developed symptoms
  - fever, myalgia, headache, lethargy, malaise, conjunctivitis, arthritis

Sexual Transmission USA

TABLE. Reverse transcription polymerase chain reaction (RT-PCR) and serologic testing of serum from patients A and B — Texas, January 2016

Patient	Days after symptom onset	ZIKV RT-PCR	ZIKV IgM*	DENV IgM	CHIK IgM	ZIKV PRNT†	DENV-1 PRNT†	DENV-2 PRNT†
Patient A	14	Negative	Positive	Positive	Negative	>20,480	>20,480	5,120
Patient B	4	Negative	ND	ND	ND	160	<10	<10
Patient B	7	Negative	Positive	Positive	Negative	2,560	10	<10

Abbreviations: CHIKV = chikungunya virus; DENV-1 or 2 = dengue virus serotype type 1 or 2; IgM = immunoglobulin M; ND = not done; PRNT = plaque-reduction neutralization test; ZIKV = Zika virus.

* IgM antibody capture-enzyme linked immunosorbent assay.

† Serum dilution-plaque reduction neutralization test, titers of neutralizing antibodies to ZIKV, DENV-1, and DENV-2.

RT-PCR----Patient A and B semen at Day 24 and 17 days negative at CDC. Patient A equivocal at Dallas County Health and Human Services.

Urine and saliva were negative

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

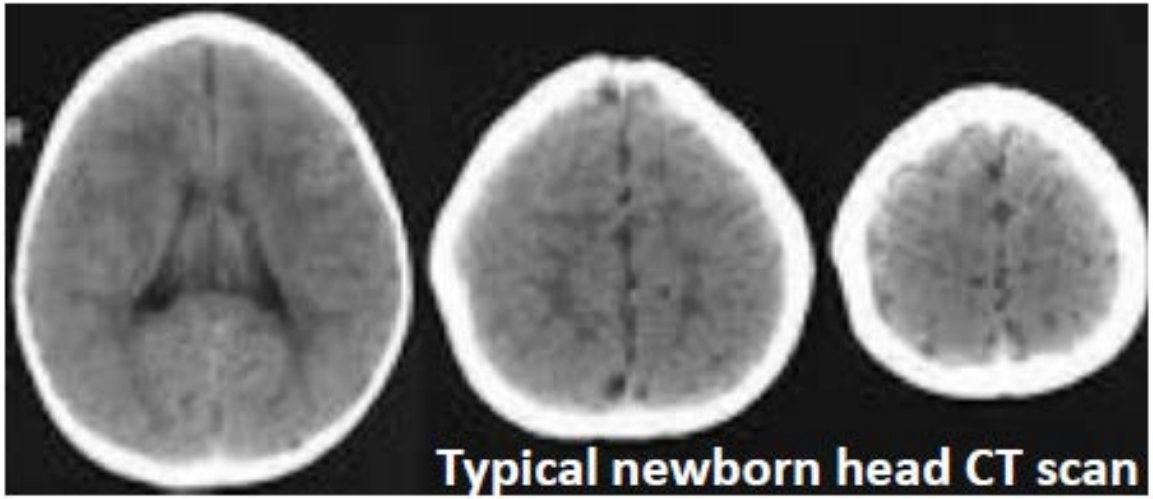
- No specific antiviral treatment is available.
- Treatment is supportive: rest, fluids, and supportive care.
  - Assess for dengue and chikungunya viruses and avoid use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) until dengue is ruled out (to reduce the risk of hemorrhage).
 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

Clinical Features:
Zika Virus Compared to Dengue and Chikungunya

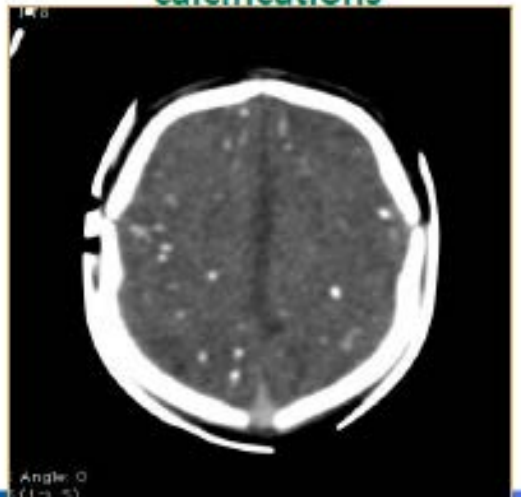
Features	Zika	Dengue	Chikungunya
Fever	++	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-	+	-

Zika and Microcephaly in Brazil

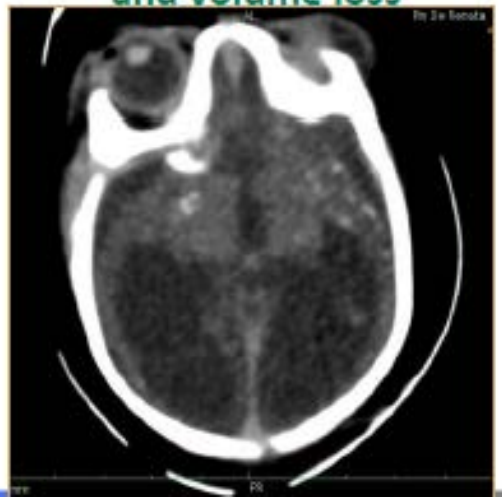
- Zika virus infections may be associated with microcephaly:
  - Increase in number of infants born with microcephaly during the outbreak
  - Zika virus RNA detected from several babies born with microcephaly and from fetal losses among women infected during pregnancy.
- CDC concludes Zika is a cause of microcephaly and other severe fetal brain defects
  - NEJM April 13, 2016



scattered intracranial calcifications



enlarged ventricles and volume loss



What's the risk of microcephaly with Zika virus infection?

- Retrospective study of the French Polynesian outbreak, 2013-2015
  - 66% of the population were infected
  - Baseline prevalence of microcephaly was two cases per 10,000 neonates
  - Risk of microcephaly associated with Zika virus infection was 95 cases per 10,000 women infected in the first trimester

Zika and Associated Birth Defects

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

Tip of iceberg? Developmental problems and other effects on the brain?

Time of infection and impact on fetus

- First trimester and early second trimester
  - Microcephaly
  - Other congenital brain anomalies
- Later in pregnancy
  - Poor intrauterine growth
  - Fetal death
- Perinatal
  - Hearing loss
  - Poor growth

Complications Keep on Coming

- Guillain-Barre
- Acute disseminated encephalomyelitis (ADEM)
  - Brazilian study (Amer. Academy of Neurology 2016 Annual Meeting, April 19, 2016)
  - Describes 6 patients
    - 2 with ADEM
    - 4 with Guillain-Barre
    - Neurologic complications 0-15 days after Zika symptoms
    - Blood and CSF positive for Zika virus

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

- Full spectrum of impact in affected infants?
- Impact of severity of maternal infection
- Does asymptomatic pose a risk?
- The effect of the time of the infection during pregnancy 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%)**

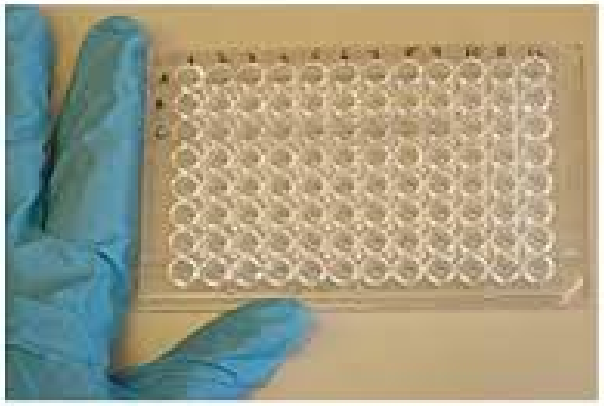
Brasil, P. et al NEJM March 10, 2016

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

Diagnostic Testing

- Real-time PCR
- IgM Serology
- Immunohistochemical staining



Laboratory Testing

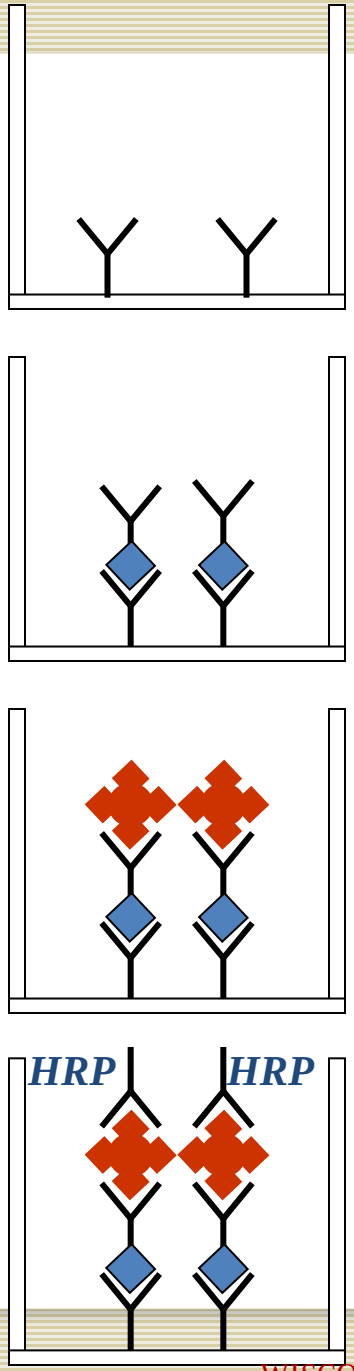
- Processing of clinical specimens for Zika virus should be performed at a minimum of BSL2 precautions
- Perform a risk assessment to determine if higher levels of biocontainment are required
  - e.g. Suspicion of Chikungunya virus

Reverse Transcriptase Real-Time PCR

- CDC EUA Trioplex RT-PCR
  - Zika
  - Dengue 1-4
  - Chikungunya
- Approved specimen types
  - Serum
  - CSF
  - Urine----Zika Only
  - Amniotic fluid-----Zika Only
- Must be collected within 7 days of illness onset

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 or yellow fever
- 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
    - Placenta
    - Cord tissue

Zika Virus Test Results in USA January 3—March 5, 2016

4534 persons tested

	No. of Patients	Zika Confirmed
>=1 Zika-associated symptom **(All)**	1,541	182 (11.8%)
>=1 other symptom **(All)**	436	8 (1.8%)
Asymptomatic **(ALL)**	2557	7 (0.3%)
Asymptomatic **pregnant women**	2,425	7 (0.3%)
>=1 Zika-associated symptom **pregnant women**	620	18 (2.9%)
>=1 other symptom **pregnant women**	290	3 (1%)

Wisconsin Residents Tested

As of March 8, 2016

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

Surveillance in Wisconsin

- Provide fee-exempt testing of Zika virus (also chikungunya and dengue) at WSLH for travelers that meet DPH testing criteria
- 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

Prevention---Vaccines

- Several vaccines under development
  - NIAID vaccine possibly ready for human phase 1 trials later this year, but will take several years to get to market
    - Focus on pregnant women, and women of childbearing age
    - DNA-based vaccine
  - Bharat Biotech vaccine
    - Killed, purified virus
    - Recombinant DNA-based vaccine
  - Inovio Pharmaceuticals
    - DNA-based vaccine
  - NIH and several private companies
    - Live attenuated vaccines

- Avoid exposure to mosquitoes: use air conditioning or

Other Means of Prevention

- Avoid exposure to mosquitoes:
  - use air conditioning or window/door screens
  - wear long sleeves and pants
  - use permethrin-treated clothing and gear
  - use 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 CDC Recommendations

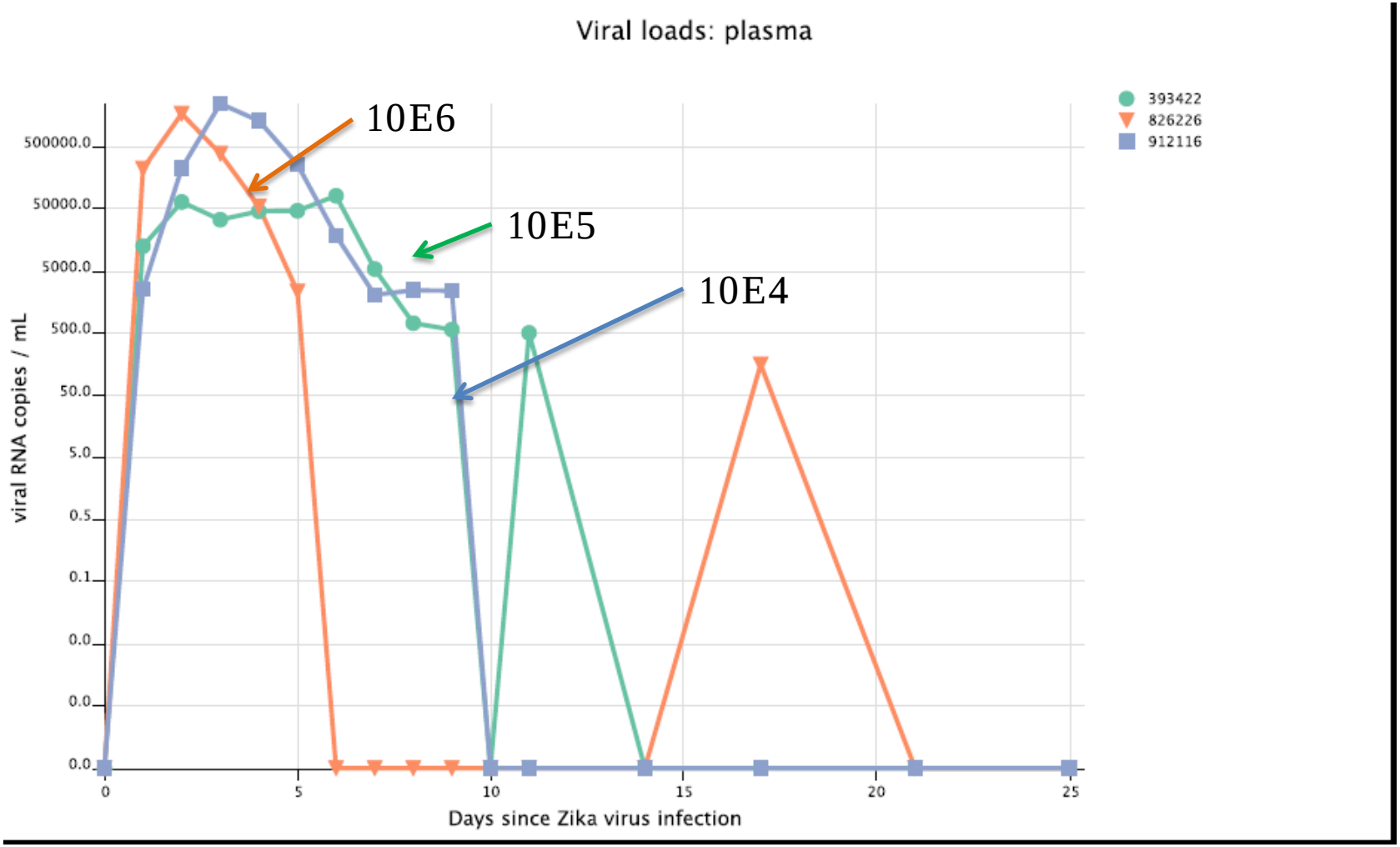
- Men who reside in or have traveled to an area of active Zika virus transmission who have a pregnant partner **should**
  - Abstain from sexual activity or use condoms for the duration of the pregnancy
- Men with confirmed Zika infection or symptoms with non-pregnant sex partners **should consider** abstaining or using condoms at least 6 months after illness onset
- Asymptomatic men-----at least 8 weeks

MMWR/March 25, 2016/Vol. 65

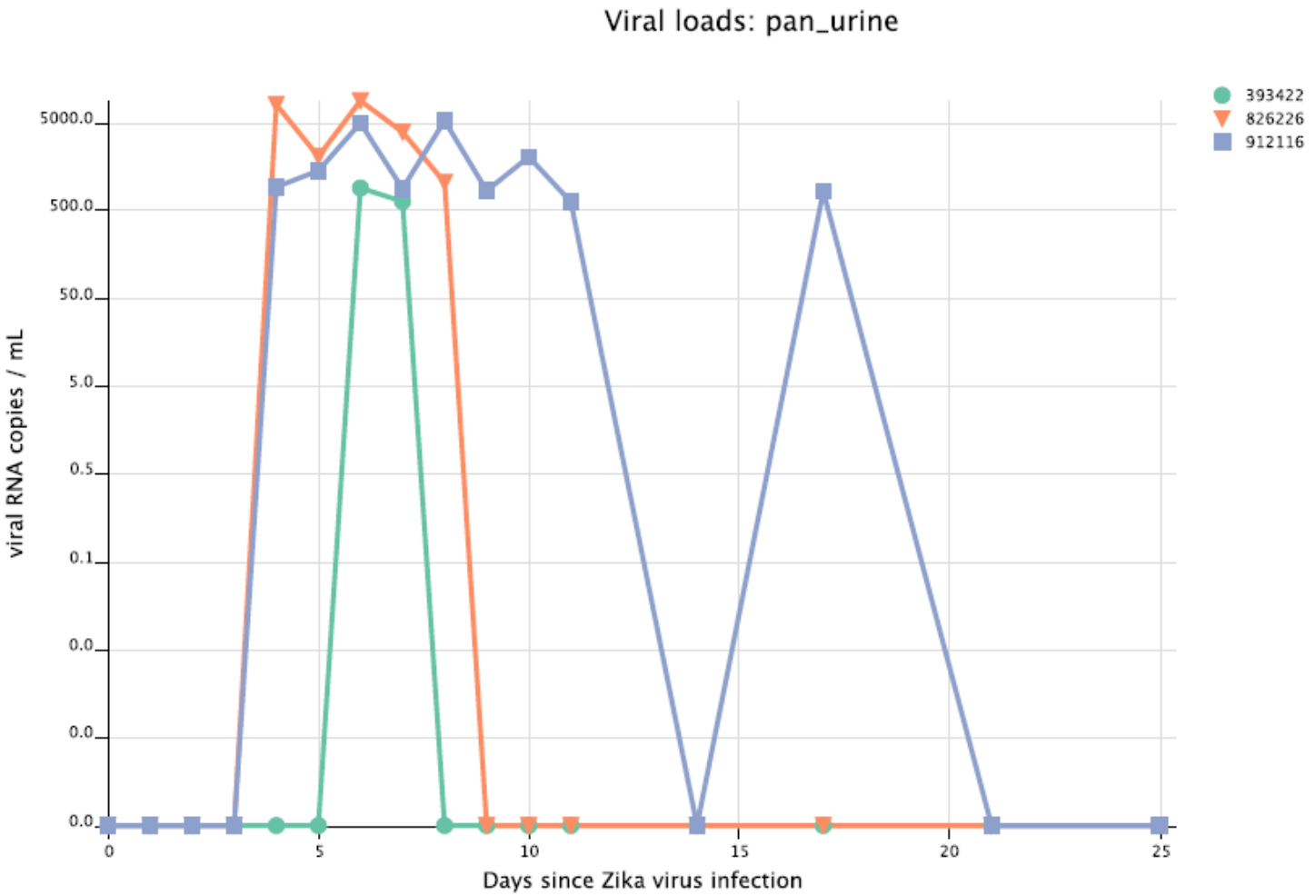
Research at UW Madison

- 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

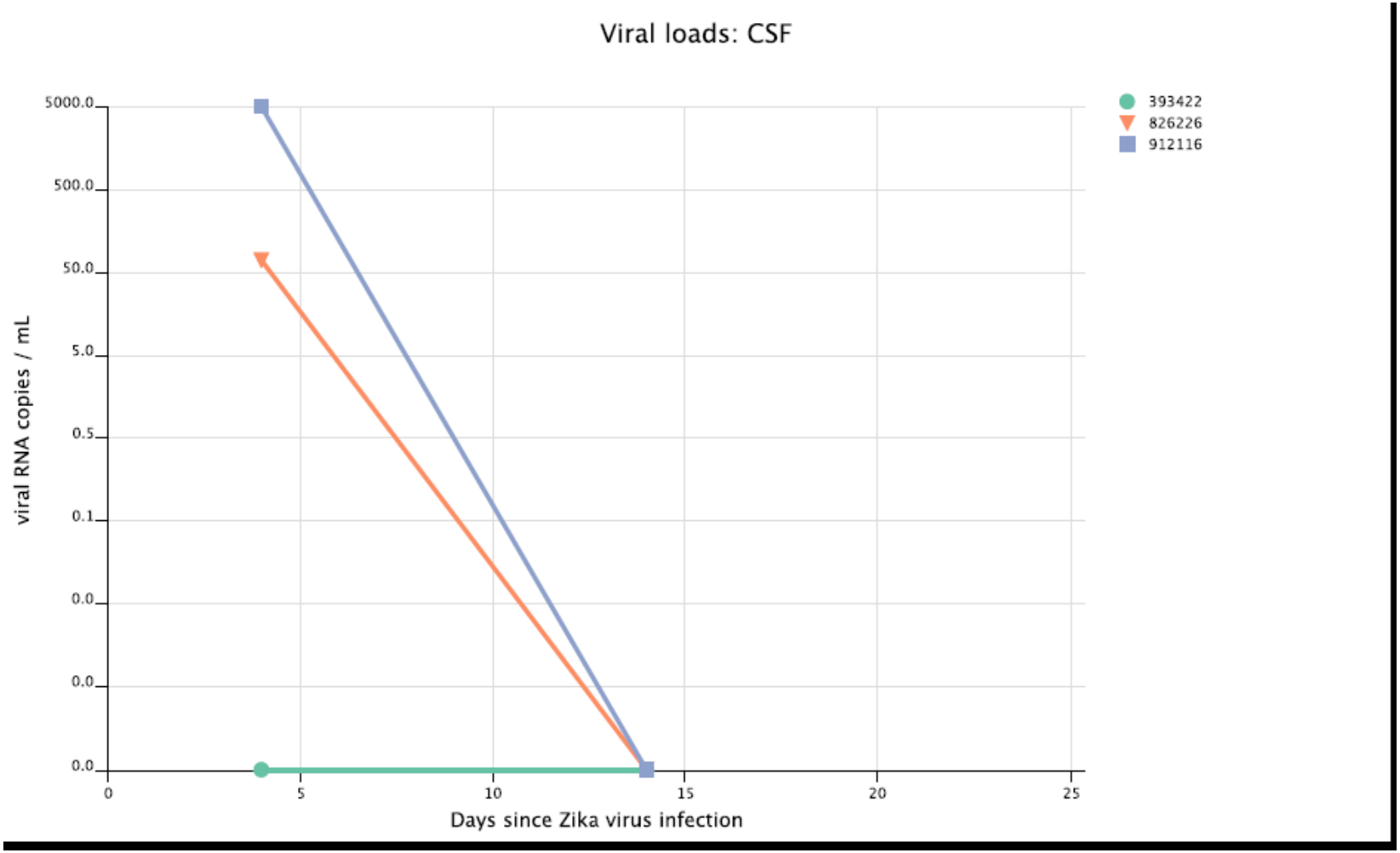
Plasma Viral Loads



Urine Viral Loads



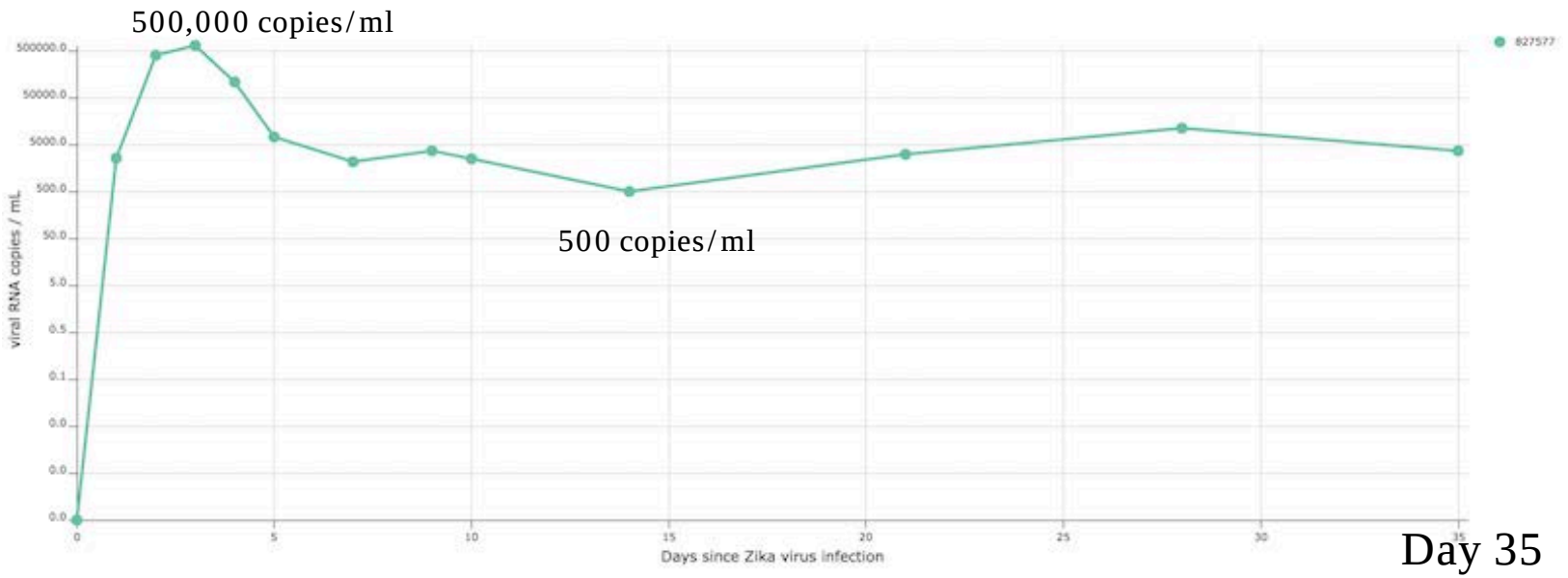
CSF Viral Loads



Infection of Pregnant Macaques

- 2 pregnant macaques infected
  - Detectable plasma viremia at evry timepooint tested
    - 1 remains viremic at 35 days post-infection
    - 1 at day 14
  - Evidence suggest that virus in maternal plasma is coming from the fetus
    - Virus not detected in urine
    - Viral variants detected at day 7 completely distinct from those during “prolonged” viremia

Viremia in Pregnant Macaque



Day 35

https://zika.labkey.com/announcements/OConnor/download.view?entityId=dd79299f-e2ea-1033-b64a-39ba30458cd8&name=Screen%20Shot%202016-04-12%20at%2010.06.51%20AM.png

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