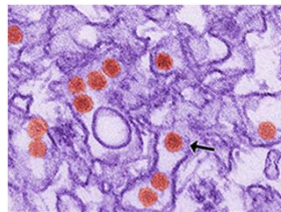




**Wisconsin State
Laboratory of Hygiene**
UNIVERSITY OF WISCONSIN-MADISON



Zika Virus Update
March 21, 2018

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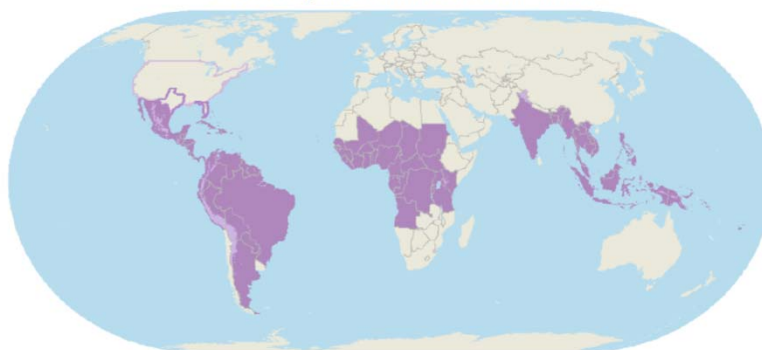
Outline

- Epidemiology
- Transmission
- Clinical Manifestations
- Congenital infection
- Diagnosis and Testing
- Prevention

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World Map of Areas with Risk of Zika



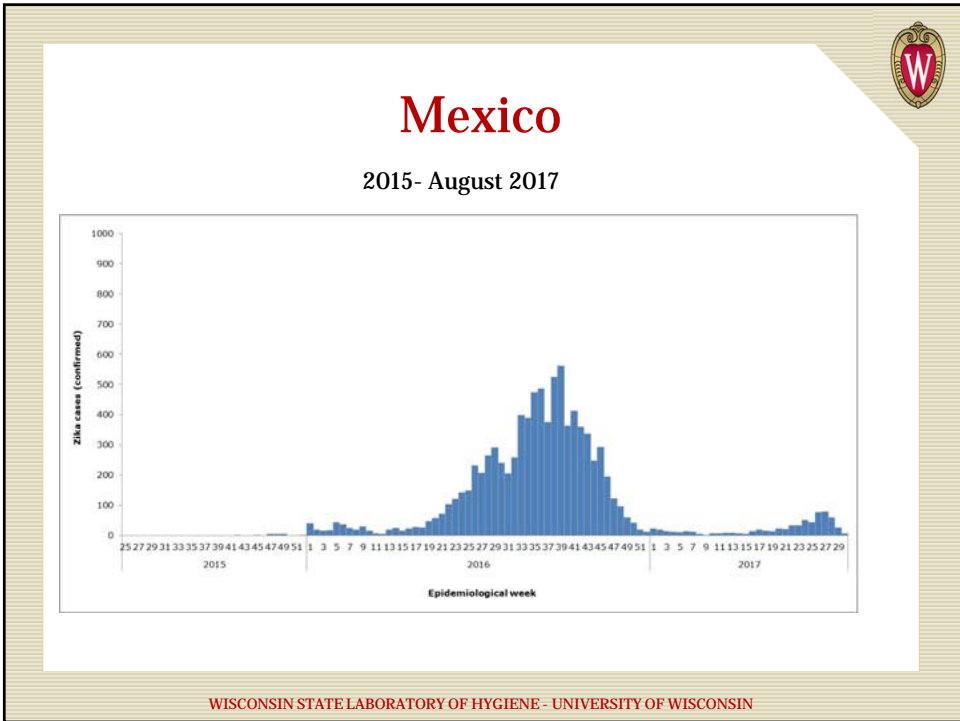
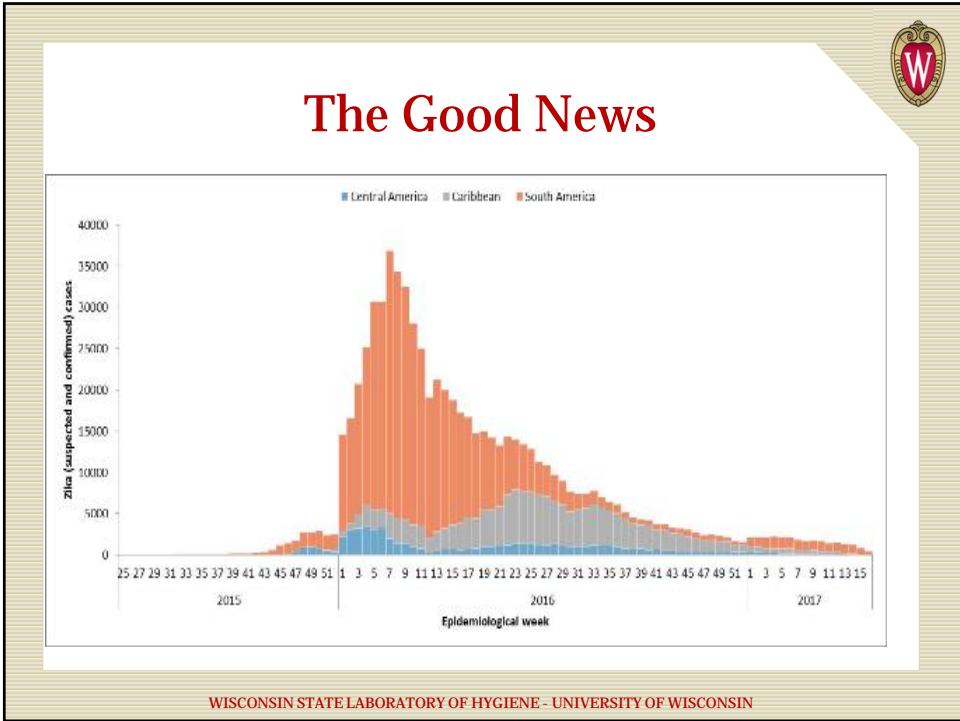
Domestic areas

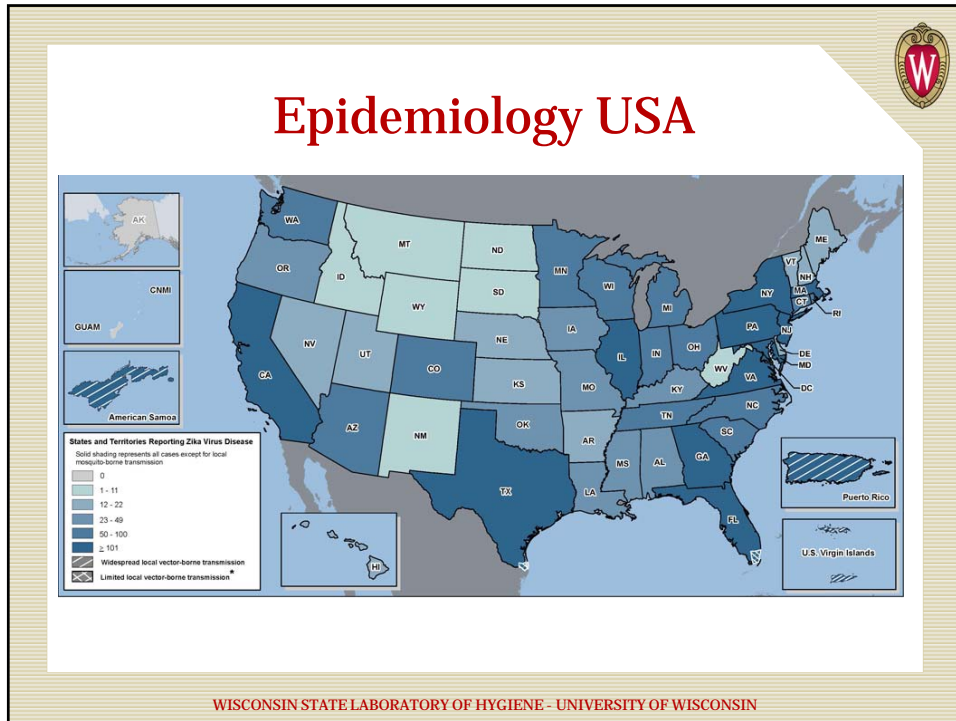
State Reporting Zika: 
No Known Zika: 

International areas

Zika Travel Recommendation:  Low elevation
 High elevation
No Known Zika: 

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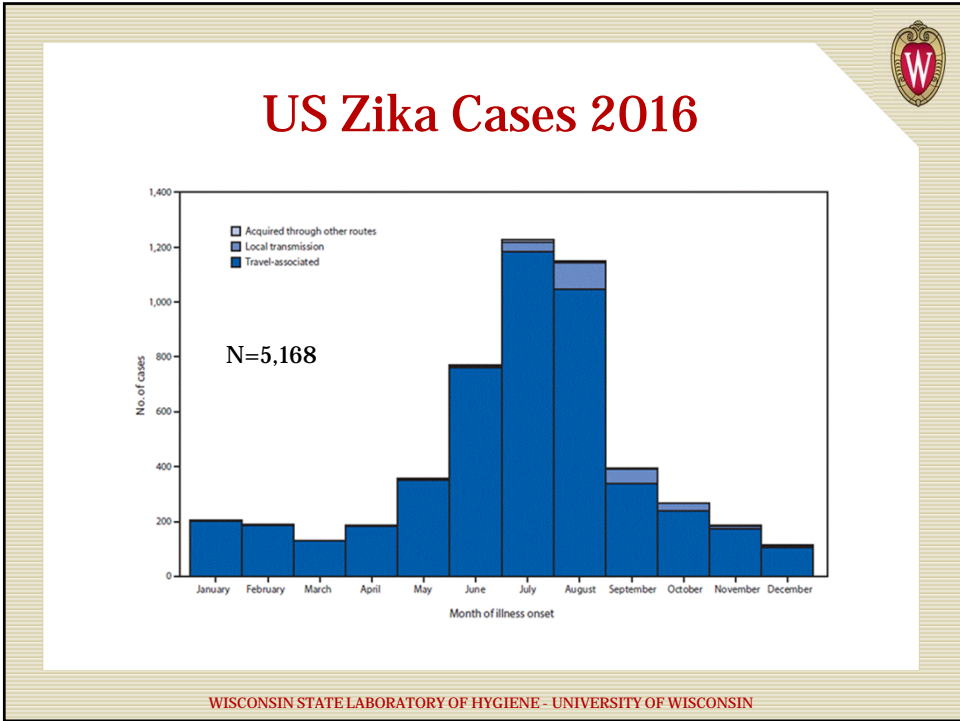



US Symptomatic Zika Cases Reported (2015-March 7, 2018)*

	State	Territories
Travel-Associated	5,389	147
Locally Acquired	229	37,030
Sexual	52	0
Laboratory Acquired	2	0
Unknown	1	0
Total	5,673	37,177
Wisconsin Residents	2	

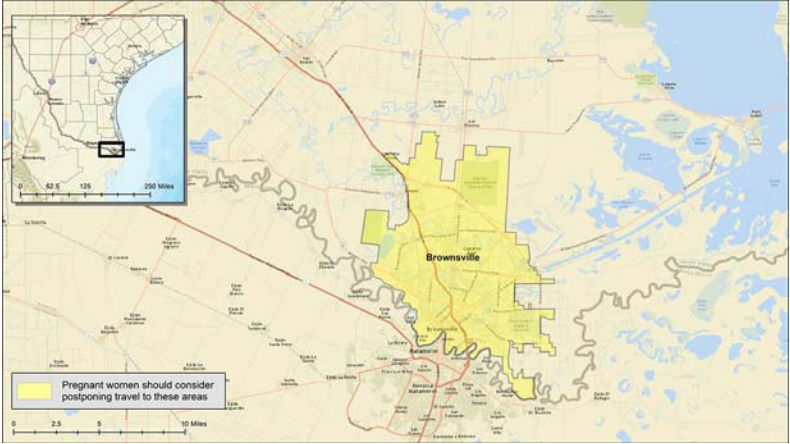
*Zika not nationally reportable in 2015. Reflects cases reported to ArboNET.

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Local Transmission in Texas



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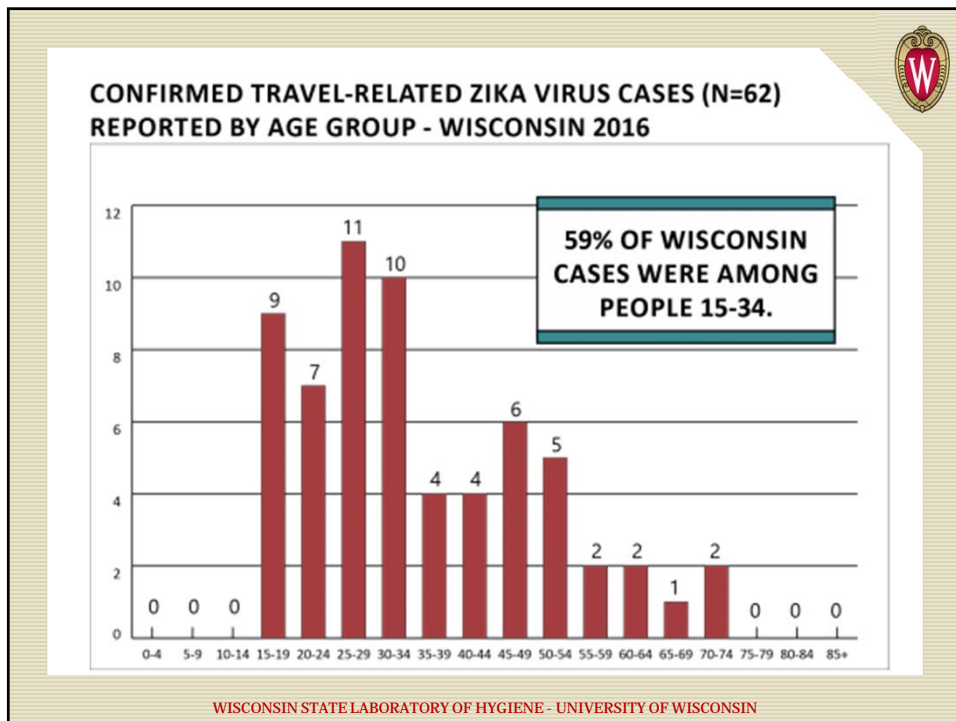
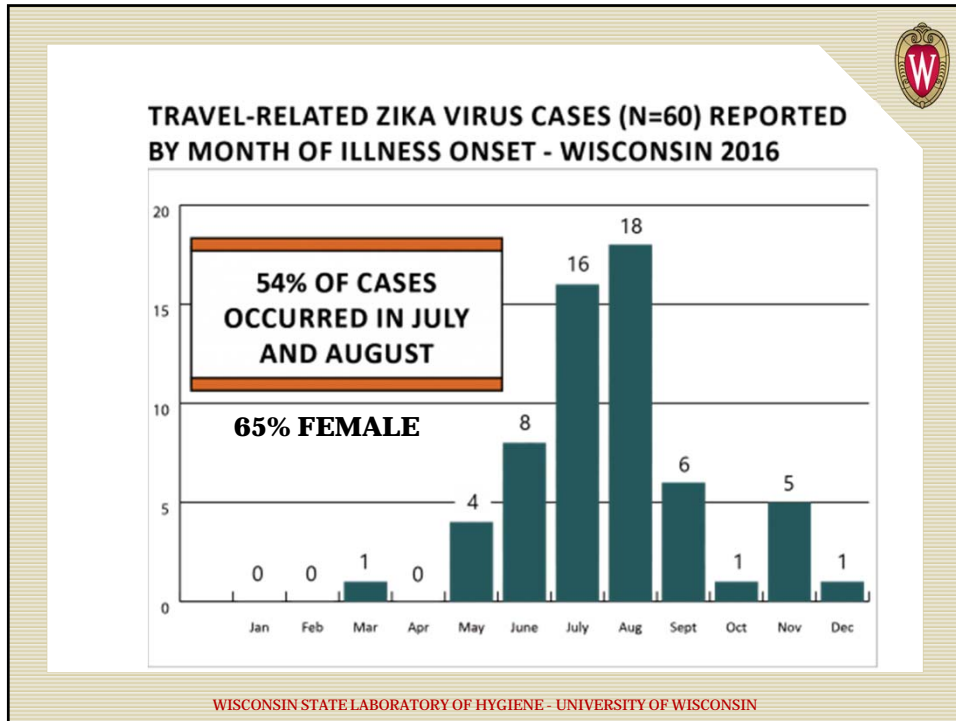
Wisconsin Travel-Related Zika Virus Cases

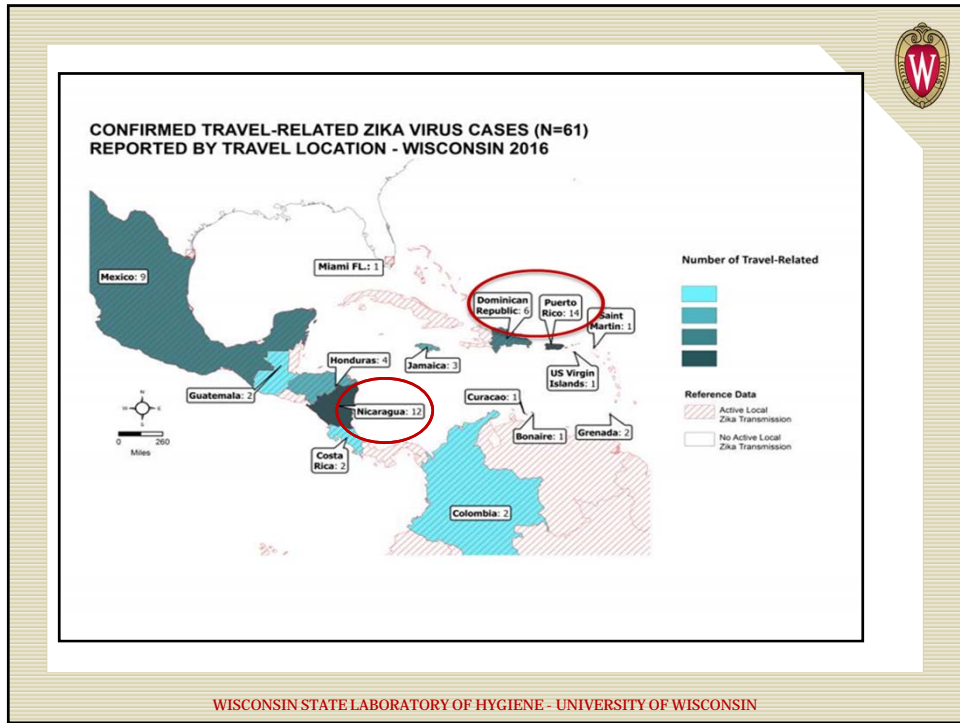
Updated March 14, 2018

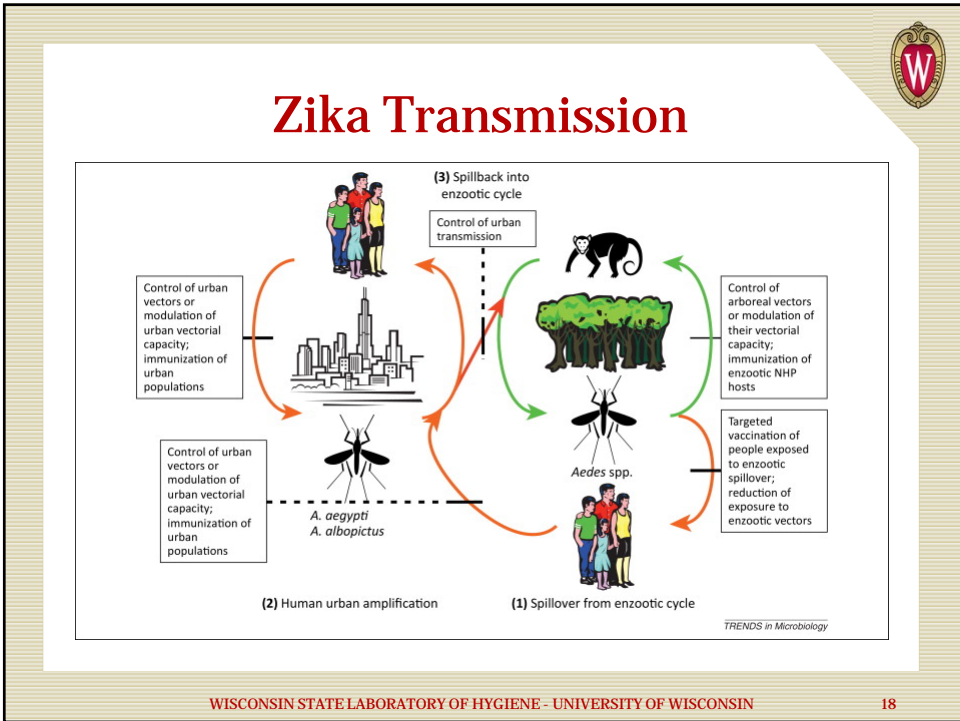
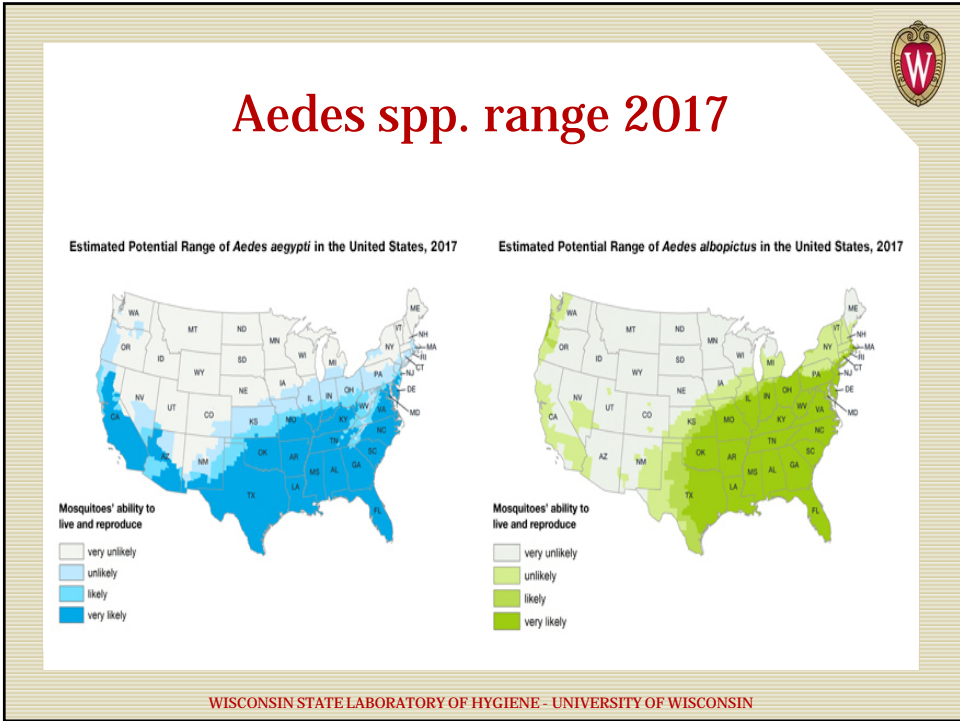
	2016	2017	2018
Confirmed	63	9	0
Probable*	0	0	0
Undetermined Flavivirus, confirmed	1	1	0
Total Tested	1062	948	19


*Probable cases have presumptive positive IgM antibody results without CDC PRNT confirmatory testing

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Other Modes of Transmission in Humans

Maternal-fetal: during pregnancy and time of birth.
Other documented modes of transmission: rare?

Sexual

- Male female:
- 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
 - Screening will go into effect nationwide

Organ transplant

Lab Exposure

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Clinical Disease Course

- Incubation period 2-14 days
- 80% asymptomatic
- Usually mild disease
 - Lasting several days to a week
- Hospitalization uncommon
- Fatalities rare
- Guillain-Barré syndrome reported following suspected Zika virus infection

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Clinical Manifestations in Wisconsin Patients



- Among confirmed cases (n=63):
- 88% reported rash
- 65% reported fever
- 43% reported arthralgia
- 41% reported myalgia
- 37% reported headache
- 27% reported fatigue
- 24% reported conjunctivitis

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Guillain-Barré syndrome-Puerto Rico



- Bilateral flaccid limb weakness attributable to peripheral nerve damage
- GBS in Puerto Rico Jan 1-July 31, 2016
 - 56 suspected cases
 - 34 (61%) with evidence of Zika or other flavivirus infection
 - All hospitalized and treated with immunoglobulin G
 - 21 admitted to ICU
 - 12 required mechanical ventilation
 - 1 died

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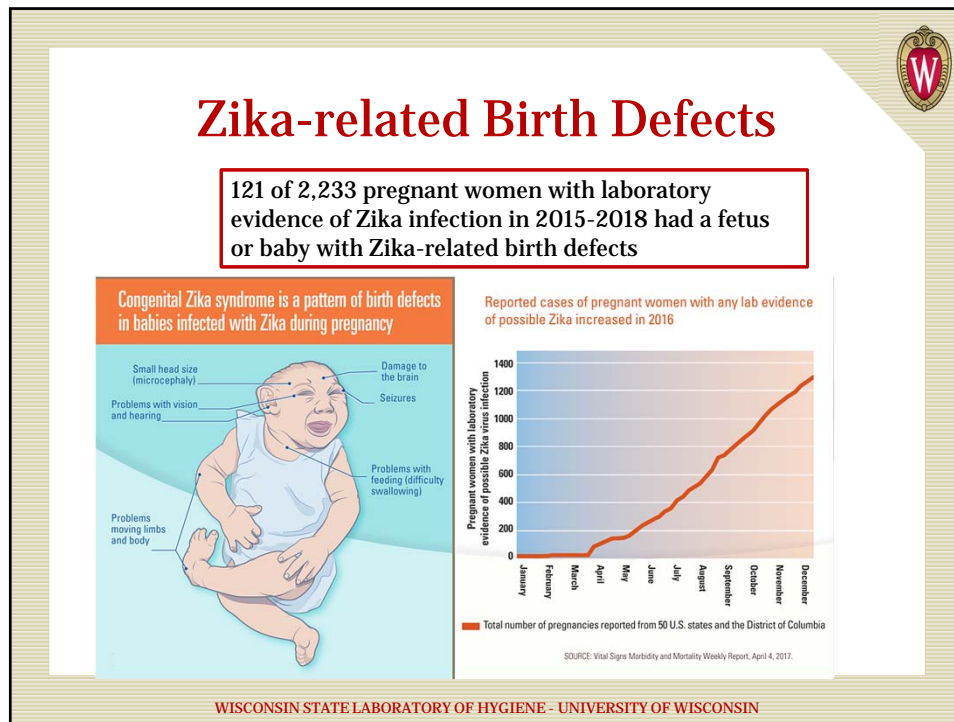
Need to Distinguish Zika from Dengue and Chikungunya

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



Clinical Features: Zika Virus Compared to Dengue and Chikungunya

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




Zika and Associated Birth Defects

- Microcephaly
- Brain atrophy
- Cerebral and intraocular calcifications
- Abnormal formed or absent brain structures
- Cataracts
- Hearing loss
- Joint and limb normalities

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

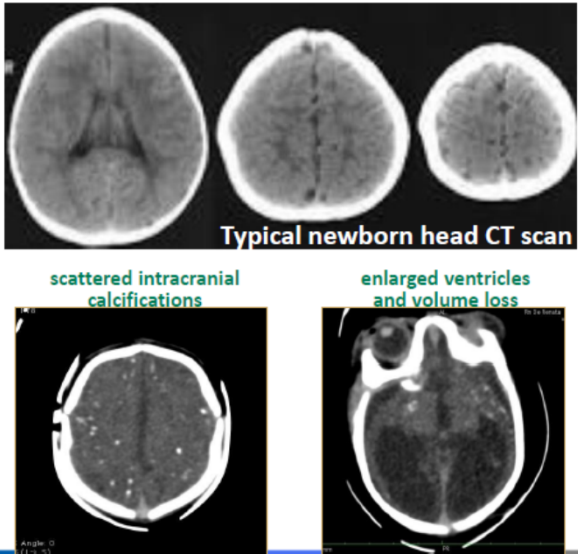
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Typical newborn head CT scan

scattered intracranial calcifications

enlarged ventricles and volume loss



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WSLH Diagnostic Testing

- Real-time PCR
- IgM Serology



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Reverse Transcriptase Real-Time PCR

- **CDC Emergency Use Authorized (EUA)
Trioplex RT-PCR**
 - Zika
 - Dengue 1-4
 - Chikungunya
- **Approved specimen types**
 - Serum
 - Plasma
 - **Blood (EDTA)**
 - CSF
 - Urine----Zika Only
 - Amniotic fluid-----Zika Only; sent to CDC
- **Optimally within 7 days of onset**

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Value of Urine Sample

367 patients with urine and serum submitted

Serum+/Urine +	20
Serum+/Urine -	4
Serum-/Urine+	20

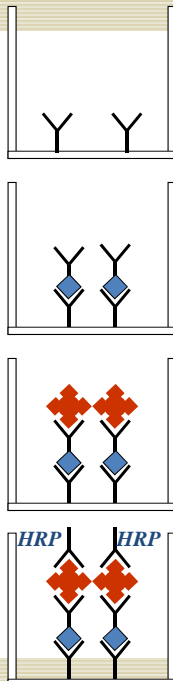
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Prolonged PCR Positivity in Serum

- Puerto Rico study of PCR positivity
 - 10/28 (36%) at 8-15 days
 - 27/129 (21%) at 16-30 days
 - 3/79 (4%) at >60 days
- Other studies show PCR positive from 46 -107 days after symptom onset

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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

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IgM Capture ELISA




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

IgM Capture ELISA Limitations



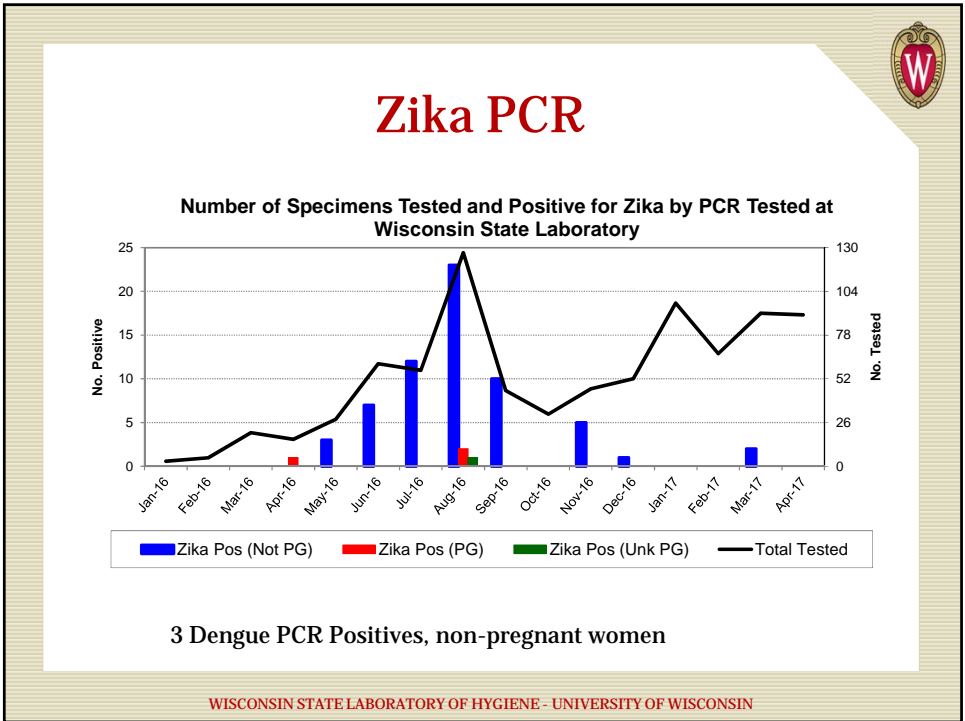
- Cross-reactivity with other flaviviruses
- Difficult to distinguish the infecting virus in people previously infected or vaccinated against a related flavivirus or yellow fever virus
- Anti-dengue virus IgM antibodies cross-react, so positive Zika IgM specimens must be confirmed
 - Plaque reduction neutralization assay (PRNT) performed at CDC

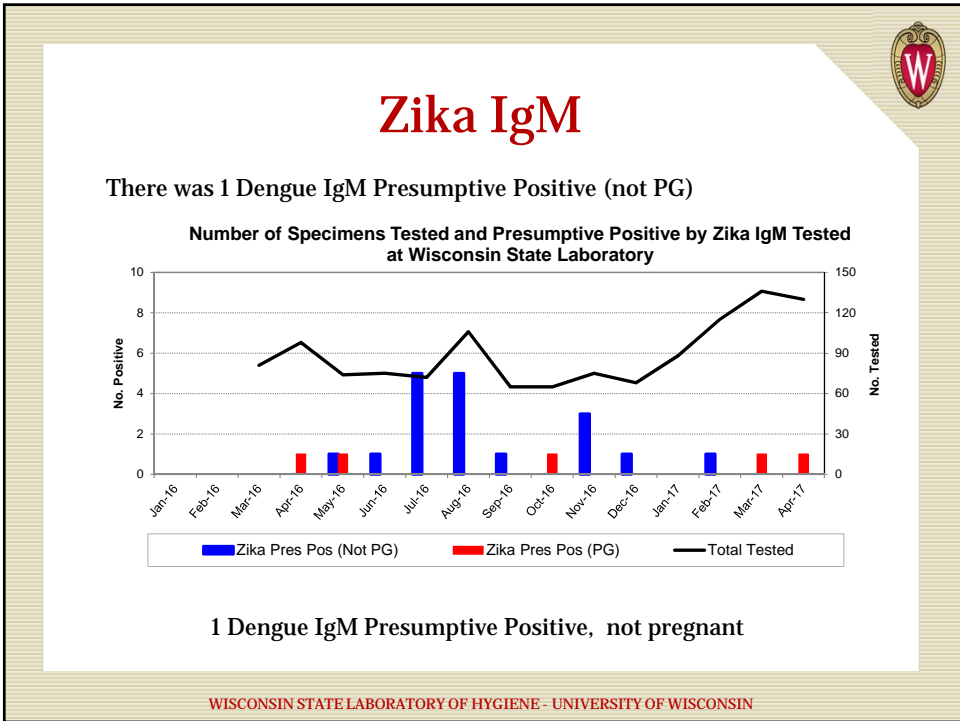
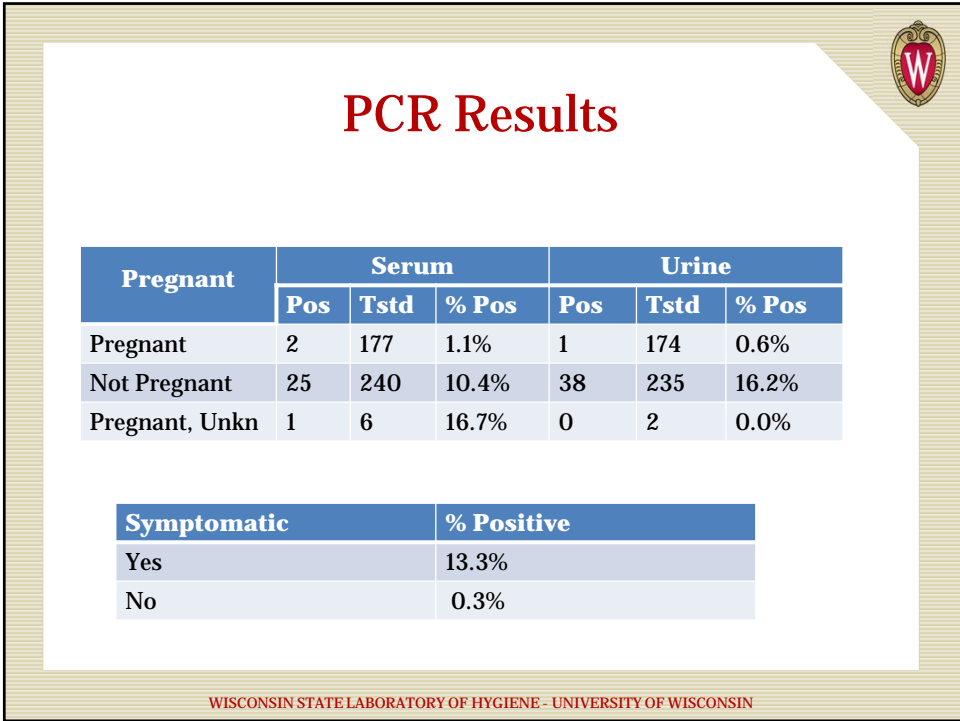


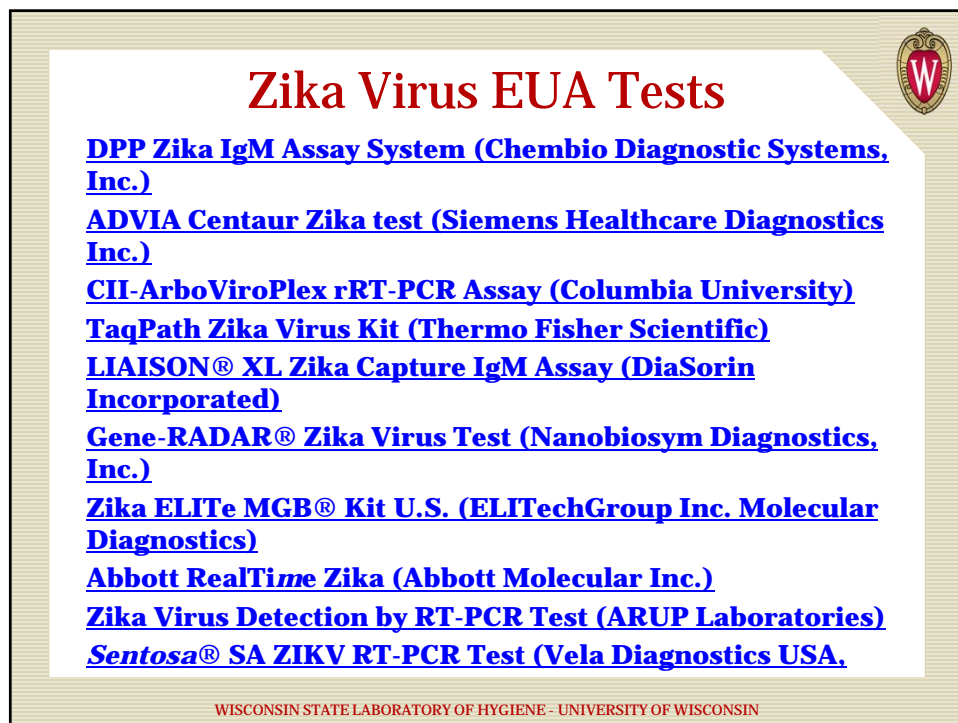
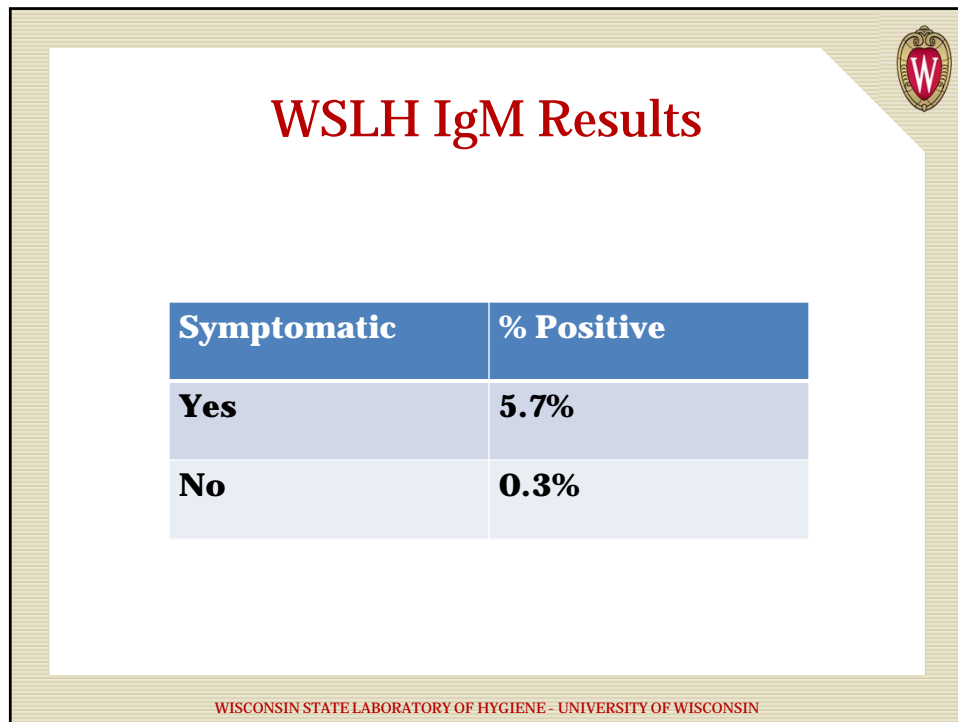
Prolonged IgM Response


- Data that IgM can persist >12 wks in a subset of patients
 - **IgM may not indicate recent infection**
- Persistence
 - Dengue----6 months (155-215 days) after primary infection
 - WNV----1 yr in 50% of patients
 - Zika----Ongoing study shows median of 4 months (8-210 days)

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Zika Virus EUA Tests (cont)

[ZIKV Detect™ IgM Capture ELISA \(InBios International, Inc.\)](#)

[xMAP® MultiFLEX™ Zika RNA Assay \(Luminex Corporation\)](#)

[VERSANT® Zika RNA 1.0 Assay \(kPCR\) Kit \(Siemens Healthcare Diagnostics Inc.\)](#)

[Zika Virus Real-time RT-PCR Test \(Viracor Eurofins\)](#)

[Aptima® Zika Virus Assay \(Hologic, Inc.\)](#)


[RealStar® Zika Virus RT-PCR Kit U.S. \(Altona Diagnostics\)](#)

[Zika Virus RNA Qualitative Real-Time RT-PCR \(Quest Diagnostics Infectious Disease, Inc.\)](#)

[Zika MAC-ELISA \(CDC\)](#)

[Triplex Real-time RT-PCR Assay \(CDC\)](#)

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Promising Microsphere Immunoassay

- Mixture of 7 beads
 - Zika E protein
 - Zika NS1 protein
 - Zika NS5 protein
 - 4 beads with NS1 proteins from DENV 1-4
- 4 hr assay
- Improved specificity compared to MAC ELISA
- Future studies to determine NS1 epitopes that could be used for more specific serologic diagnosis

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Who should be Tested?

- **Symptomatic pregnant women with possible Zika virus exposure**
 - Test concurrently using IgM and NAT ASAP within 12 weeks of symptom onset
- **Asymptomatic pregnant women *with ongoing* possible exposure**
 - Offer NAT 3 times during pregnancy
 - IgM testing no longer recommended

Testing provided fee-exempt at WSLH with WDPH approval

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Who should be Tested?

- **Asymptomatic pregnant women with possible exposure to Zika virus and who have a fetus with prenatal ultrasound findings consistent with congenital Zika infection**
 - NAT and IgM on maternal serum and urine
 - NAT of amnio specimen if being performed for other clinical reasons
- **Asymptomatic pregnant women *without ongoing* possible exposure**
 - Testing not recommended
 - Patient-provider decision
 - Testing in private laboratory

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Who should be Tested?

- **Non-Pregnant Symptomatic Individual**
 - NAT if specimens collected <14 days after onset
 - IgM on NAT negative specimens collected <14 days after onset or on specimens collected >14 days after onset

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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>



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Prevention

- Avoid mosquito bites
 - Clothing
 - Environmental control measures
- Don't travel to areas with ongoing Zika virus mosquito transmission
- Sexual transmission prevention
 - Men—wait at least 6 months after symptom onset or last possible Zika exposure before unprotected sex
 - Women wait at least eight weeks before having unprotected sex

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Prevention Blood Donor Screening 2016-Mar 7, 2018

Viremic Blood Donors	
Territories	No.
American Samoa	0
Puerto Rico	332
U.S. Virgin Islands	0
States	31

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Prevention



Vector Control

- Genetically-modified mosquitoes
 - Oxitec GMO OX513A
 - Field trials conducted in Brazil, Cayman Islands, Panama, and Malaysia
 - Have observed suppression of the targeted mosquitoes
 - Not approved for commercial use
- In August 2016 FDA concluded field trials in Florida will not have significant impacts on the environment

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GMO Mosquitoes



- Gene introduced that stops normal processes in the insect cell and the larva die.
 - Control is species-specific
 - Release only males (>99%)
 - Genes don't spread
 - Genes do not persist in the environment
 - Control gene products are non-toxic and non-allergenic
- GMOs contain a fluorescent marker

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Vaccines



- Live-attenuated vaccine
 - UTMB Galveston and Instituto Evandro Chagas Ministry of Health Brazil
 - One segment of the viral genome deleted
 - 10-nucleoside deletion in the 3' untranslated region
 - Strong immune response and protective in mice

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Vaccines



- NIH plasmid DNA vaccine
 - In Phase 2 clinical trial
 - Encodes for two surface proteins
 - Pre-membrane and envelop proteins
 - IM injection
 - Proteins assemble into particles that mimic Zika virus and trigger an immune response

P. Abbink et al., "Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys," *Science*, doi:10.1126/science.aah6157, 2016.

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Vaccines

- NIH formalin-inactivated virus
 - Protected mice
 - Induced immune response in rhesus macaques
 - Protection was antibody mediated
 - Potential for monoclonal antibody to provide protection for developing fetuses
- NIH adenoviral vector vaccine
 - encodes the pre-membrane and envelop proteins
 - Induce immune response in rhesus macaques

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Thank you!