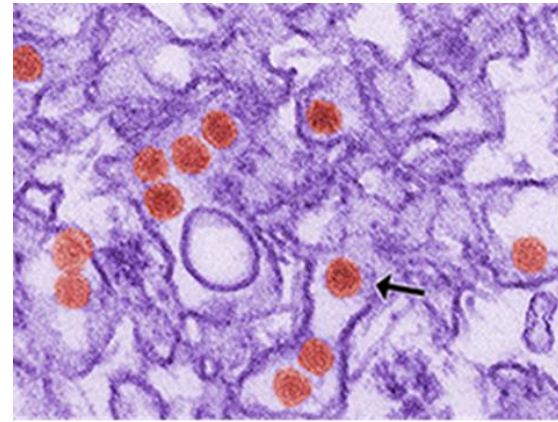




Wisconsin State
Laboratory of Hygiene

UNIVERSITY OF WISCONSIN-MADISON



Zika Virus Update

March 21, 2018

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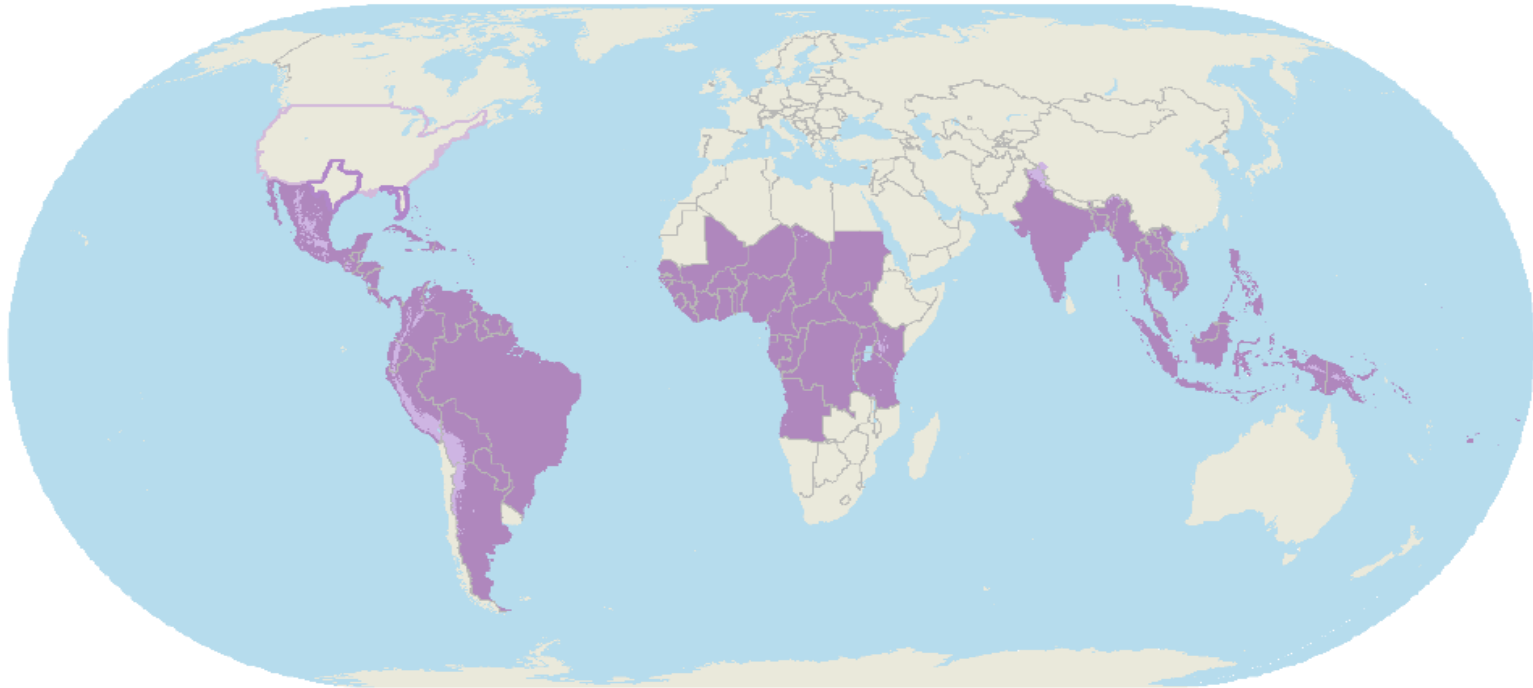


Outline


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




World Map of Areas with Risk of Zika



Domestic areas

State Reporting Zika: 

No Known Zika: 

International areas

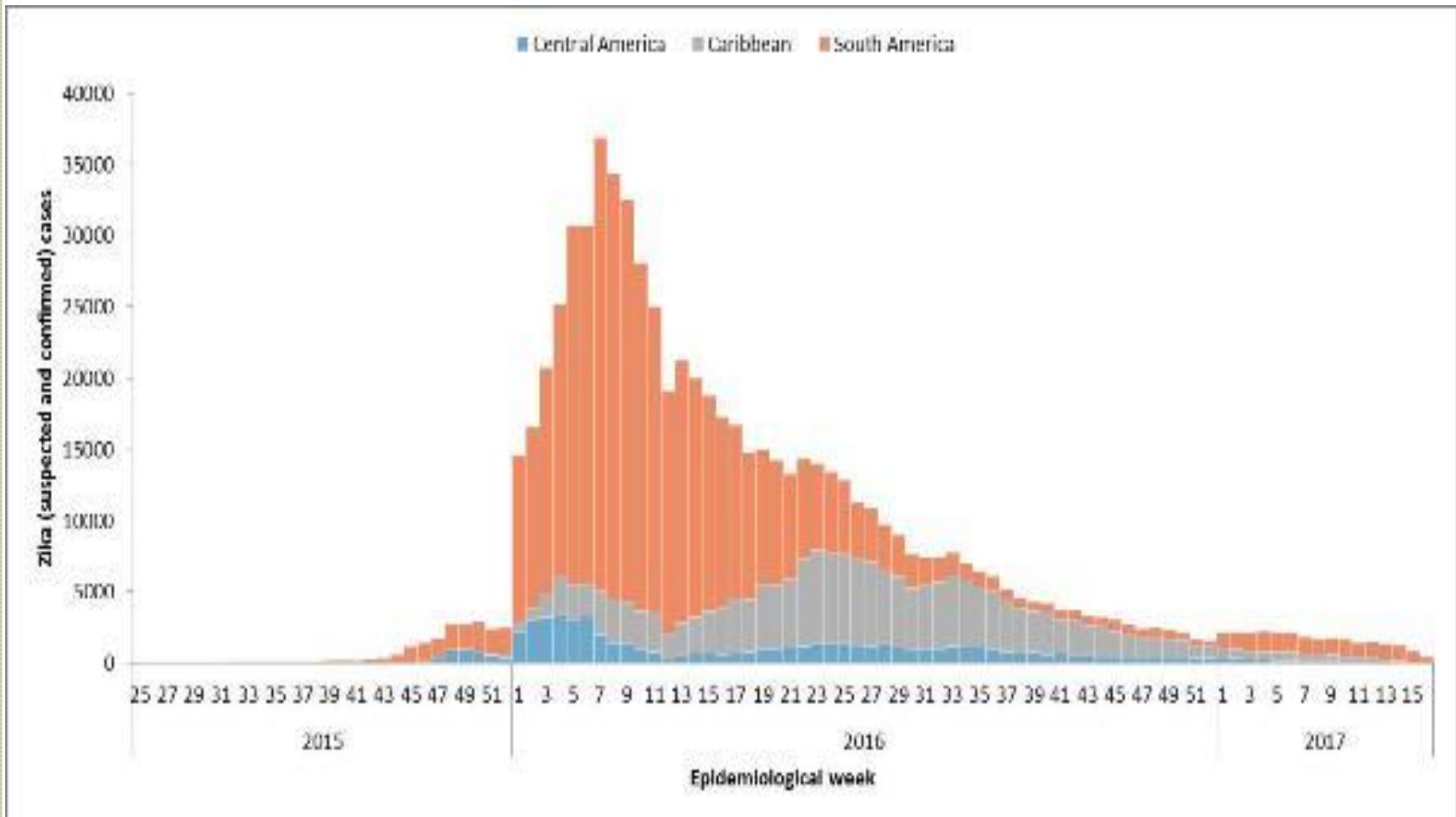
Zika Travel Recommendation:  Low elevation

 High elevation

No Known Zika: 



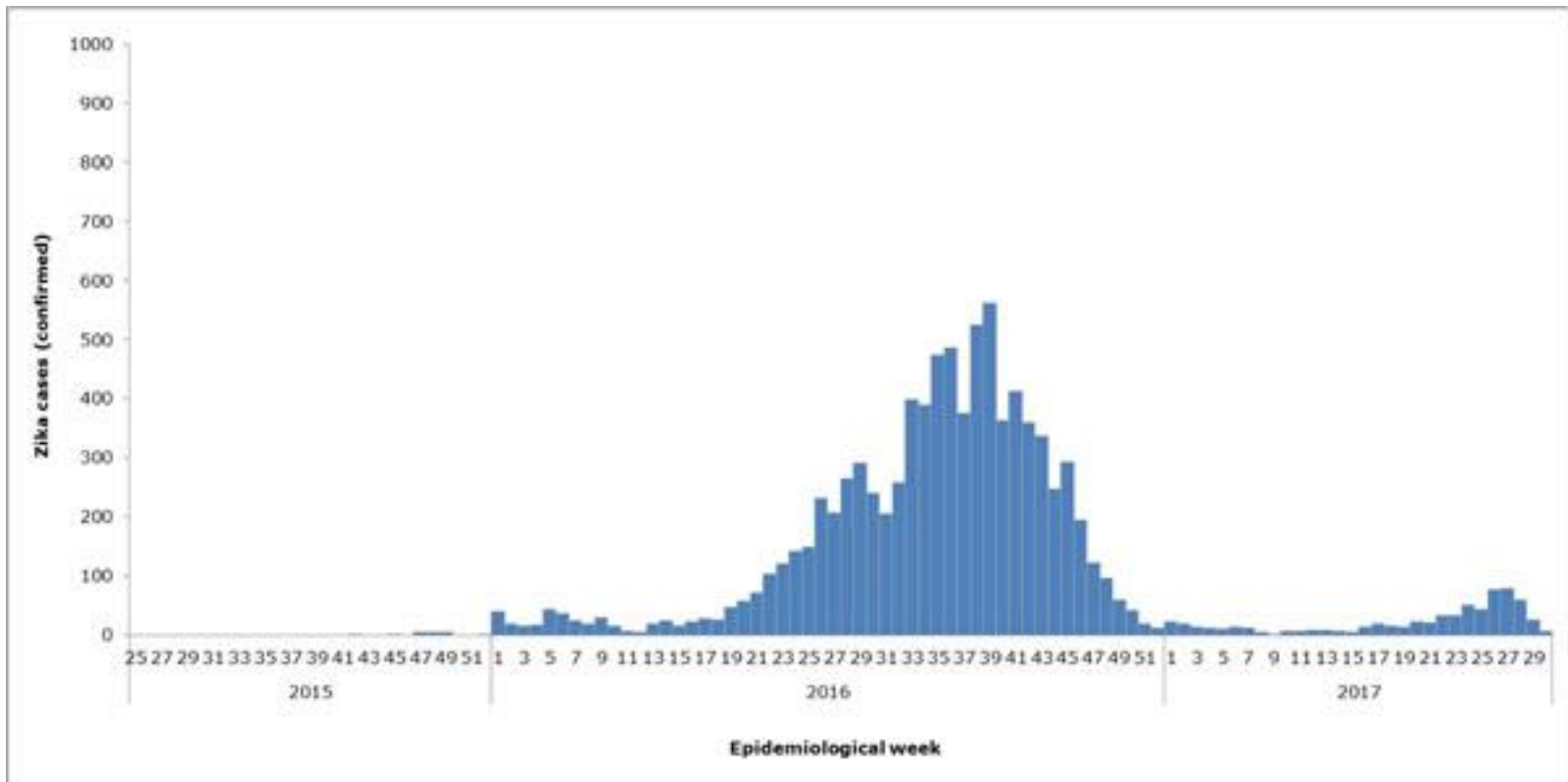
The Good News





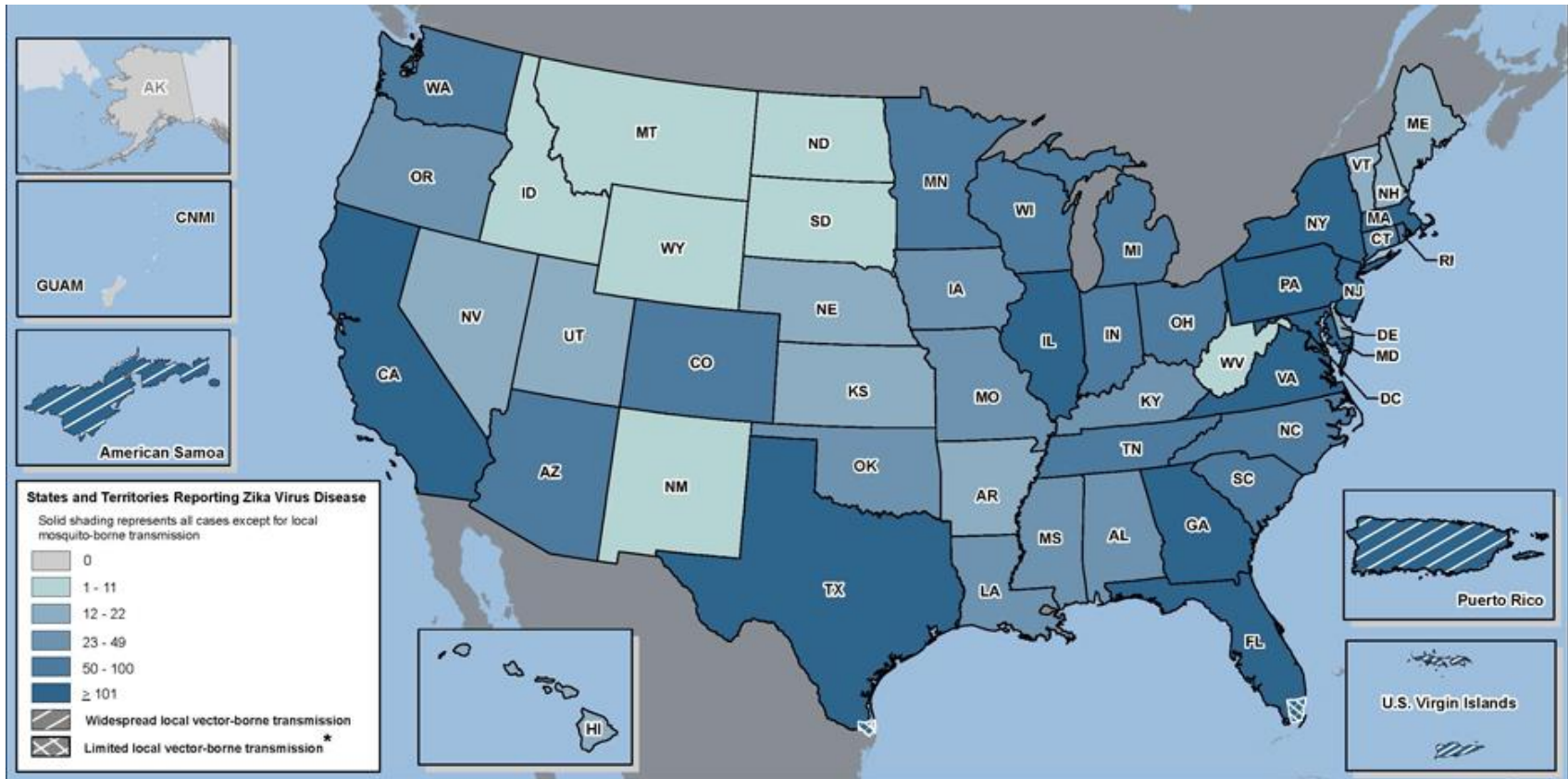
Mexico

2015- August 2017





Epidemiology USA





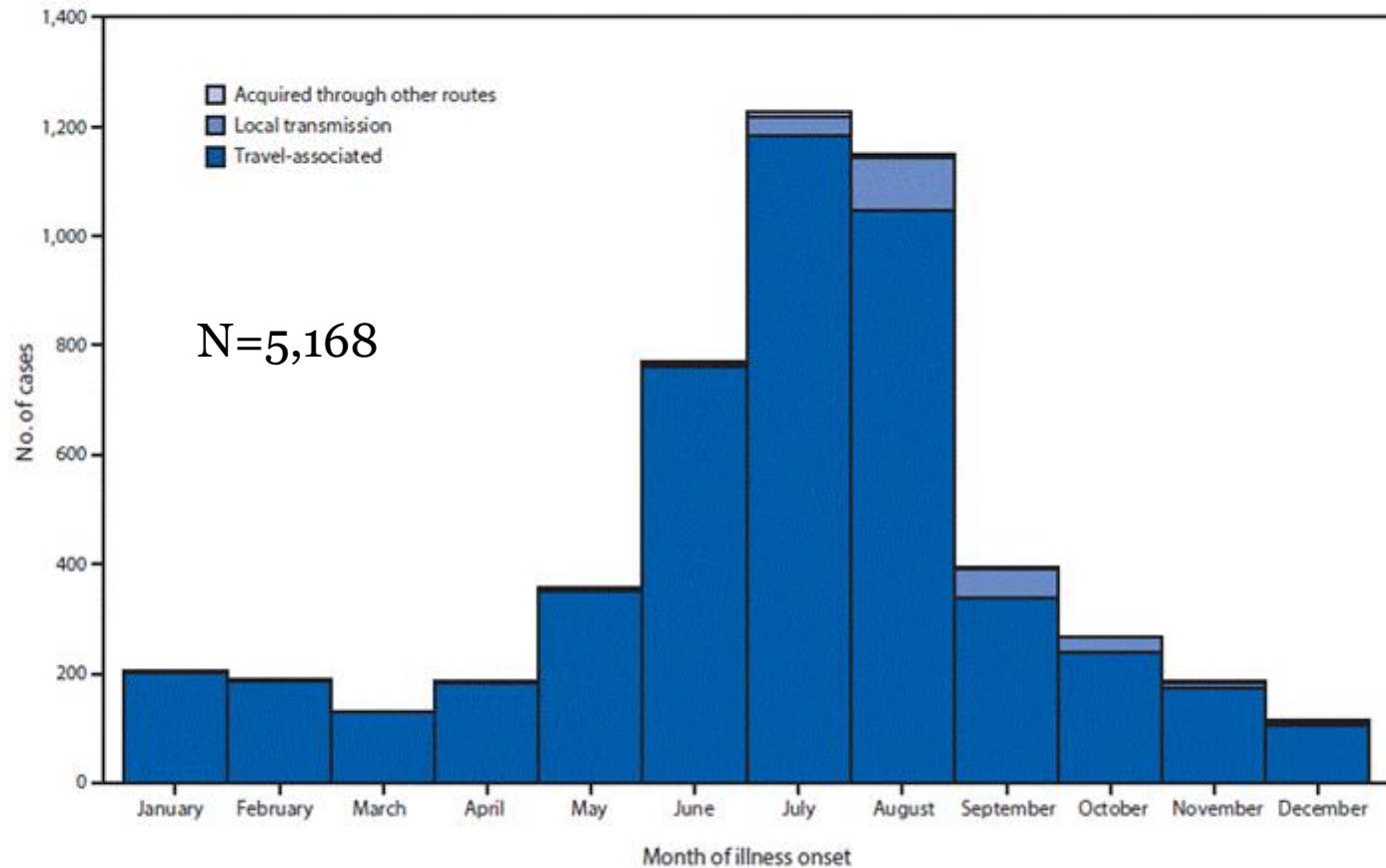
US Symptomatic Zika Cases Reported (2015-March 7, 2018)*

	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.

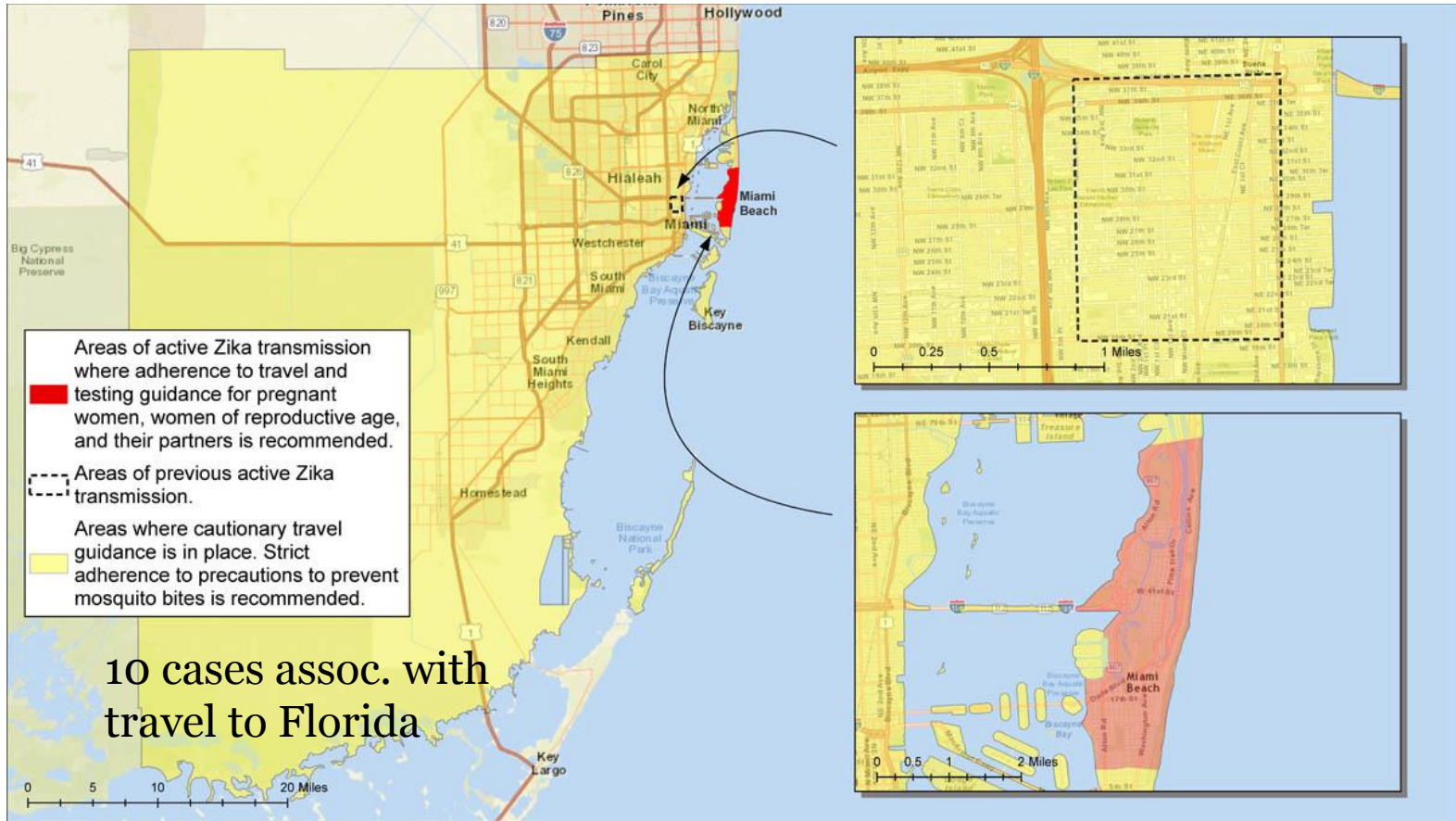


US Zika Cases 2016





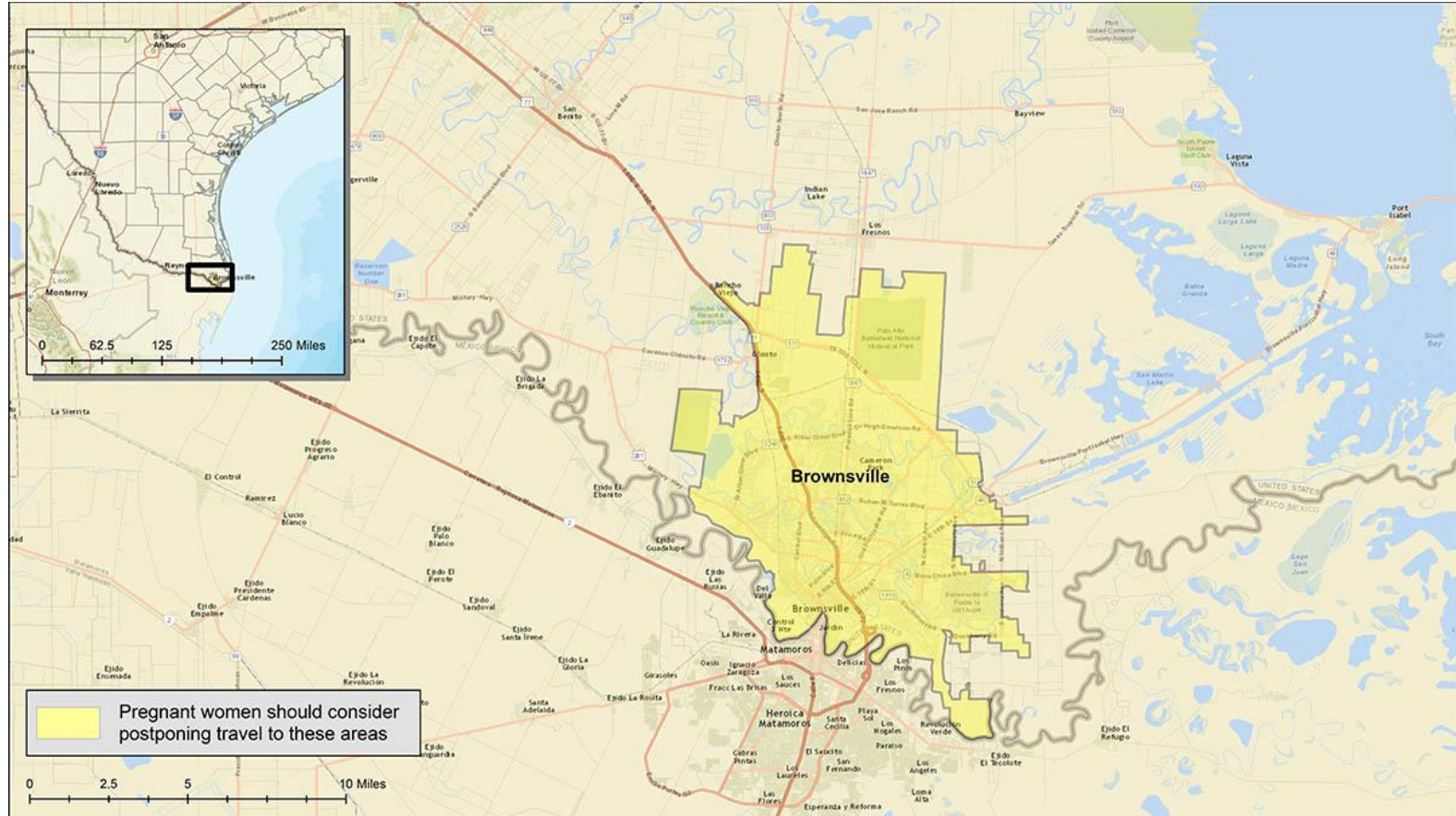
Local Transmission in Florida



Palm Beach and Miami Beach Counties



Local Transmission in Texas





Wisconsin Travel-Related Zika Virus Cases

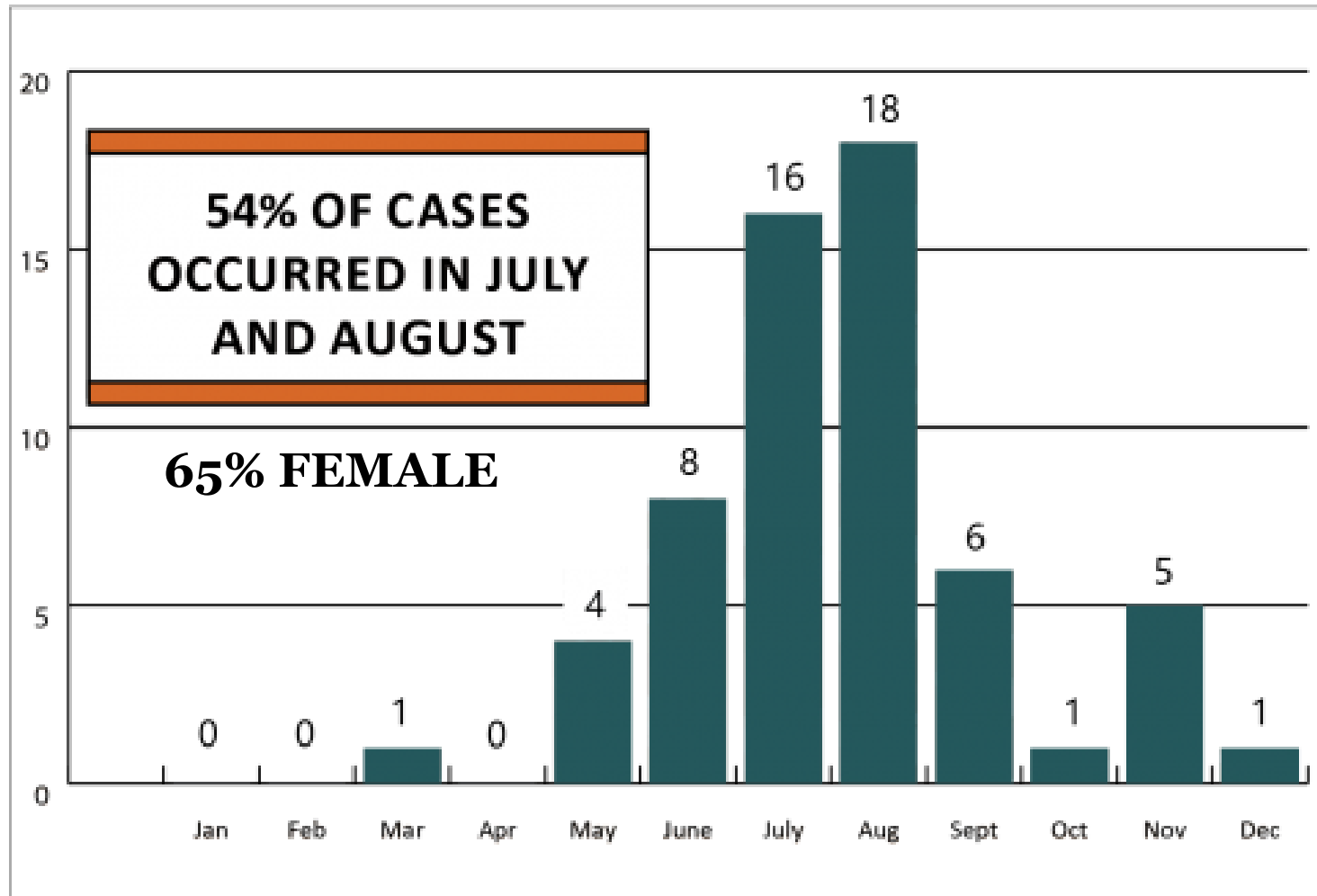
Updated March 14, 2018

	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

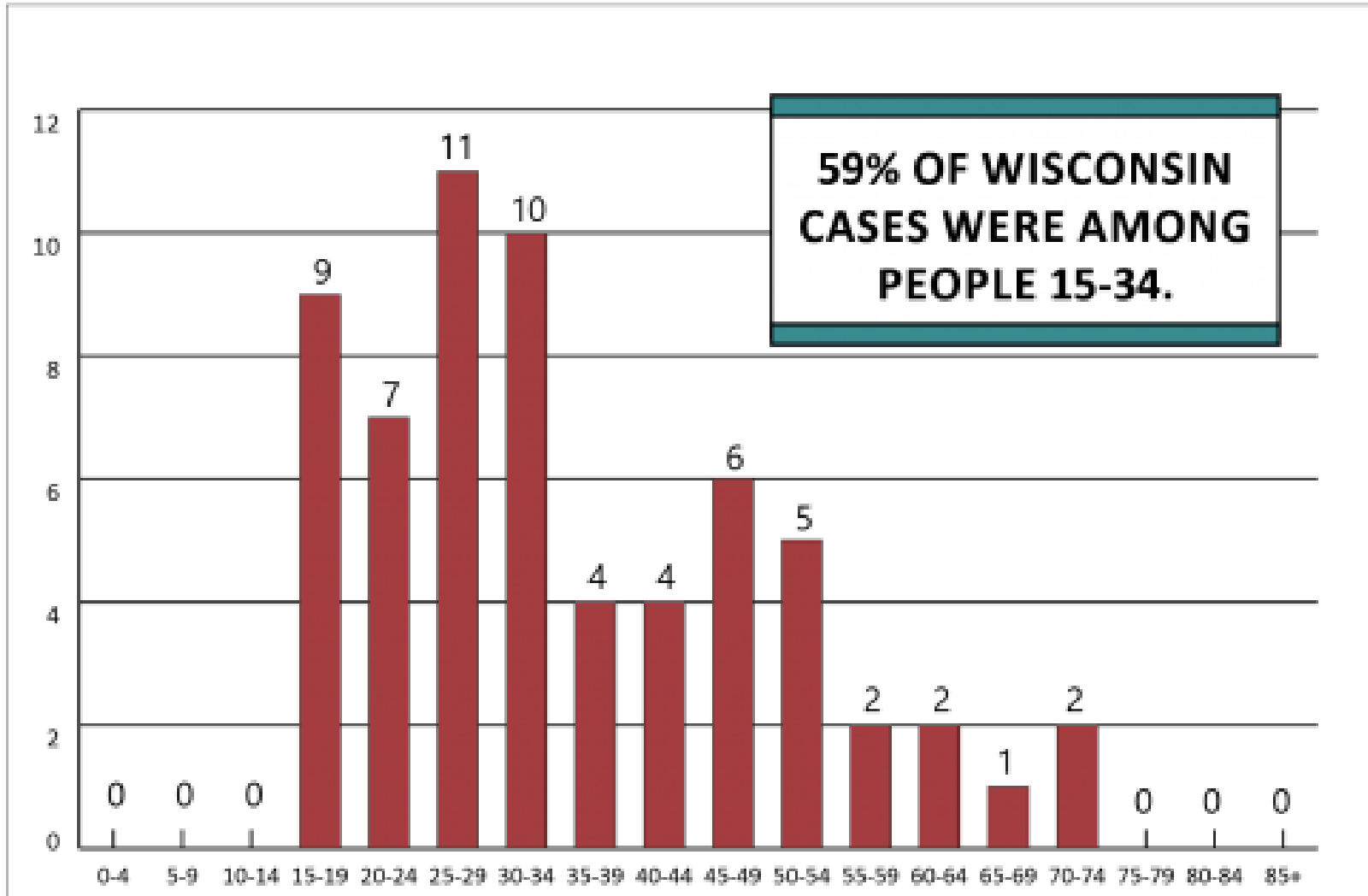


TRAVEL-RELATED ZIKA VIRUS CASES (N=60) REPORTED BY MONTH OF ILLNESS ONSET - WISCONSIN 2016



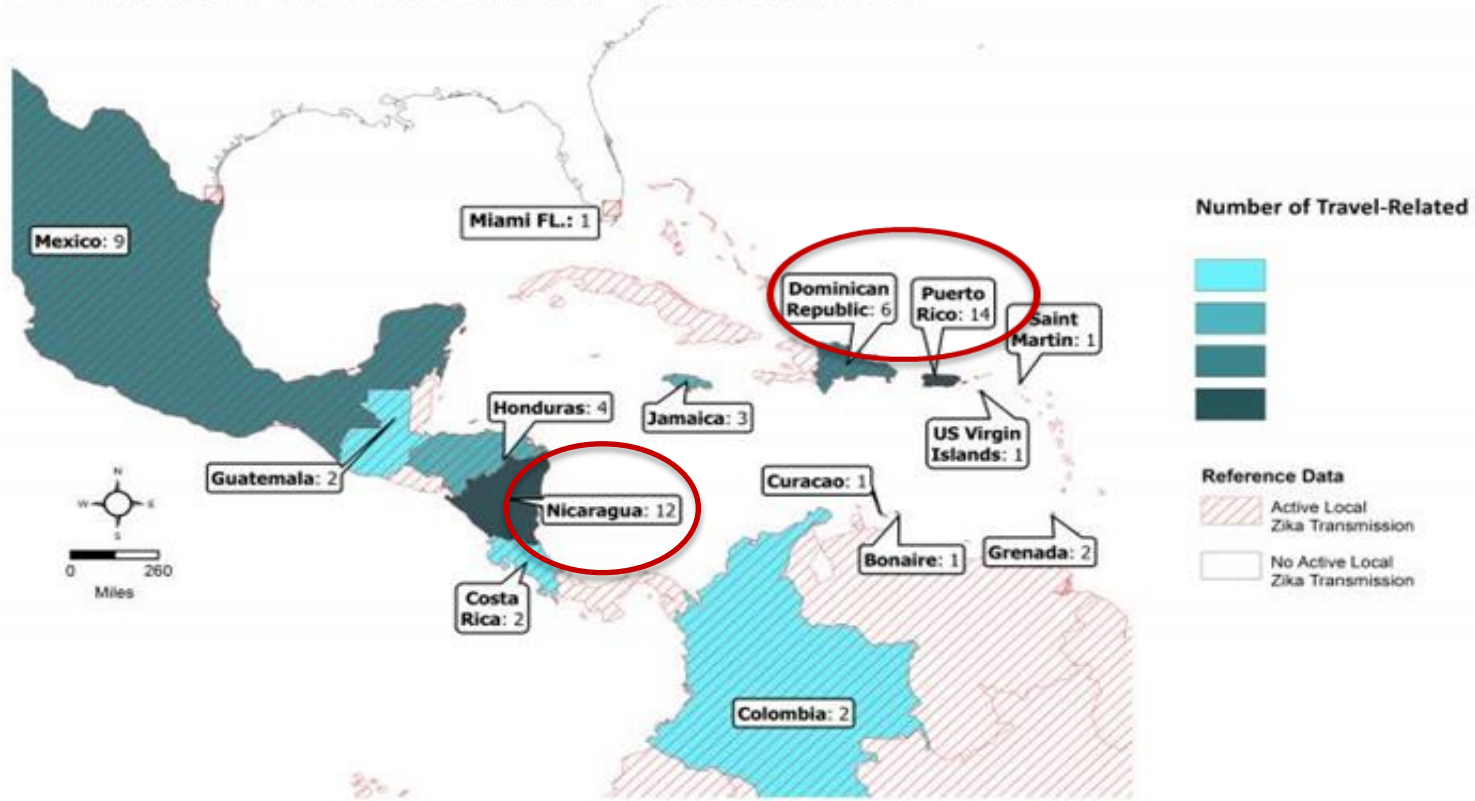


CONFIRMED TRAVEL-RELATED ZIKA VIRUS CASES (N=62) REPORTED BY AGE GROUP - WISCONSIN 2016





CONFIRMED TRAVEL-RELATED ZIKA VIRUS CASES (N=61) REPORTED BY TRAVEL LOCATION - WISCONSIN 2016





Zika Vectors



Aedes aegypti



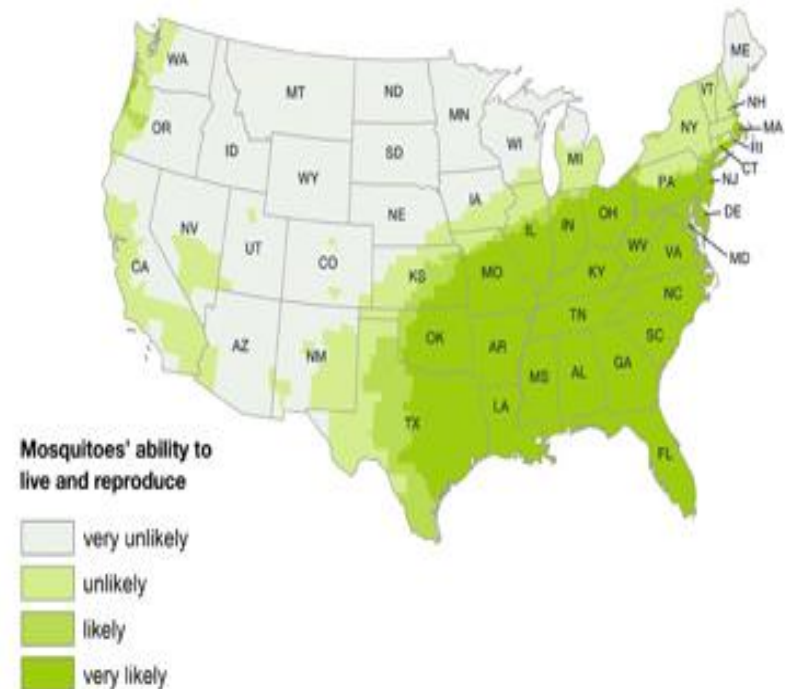
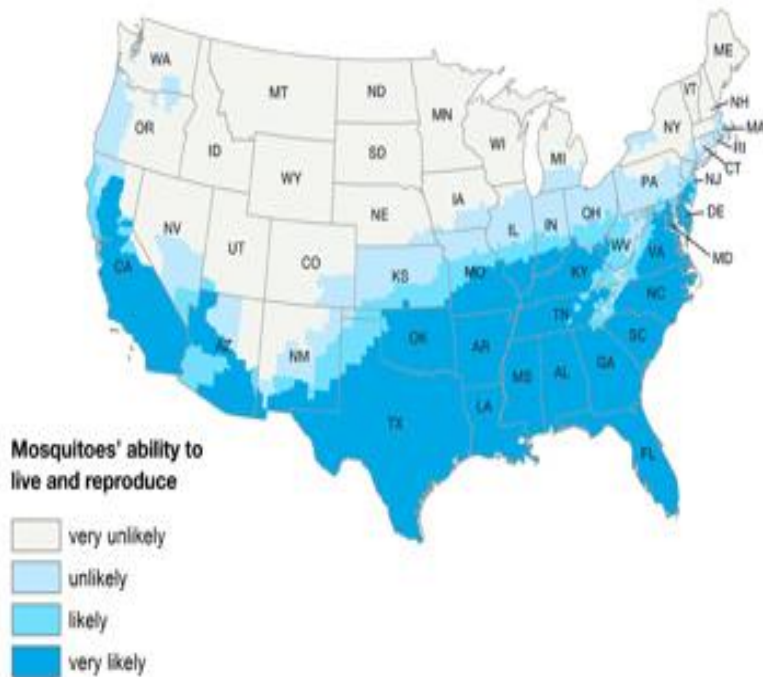
Aedes albopictus



Aedes spp. range 2017

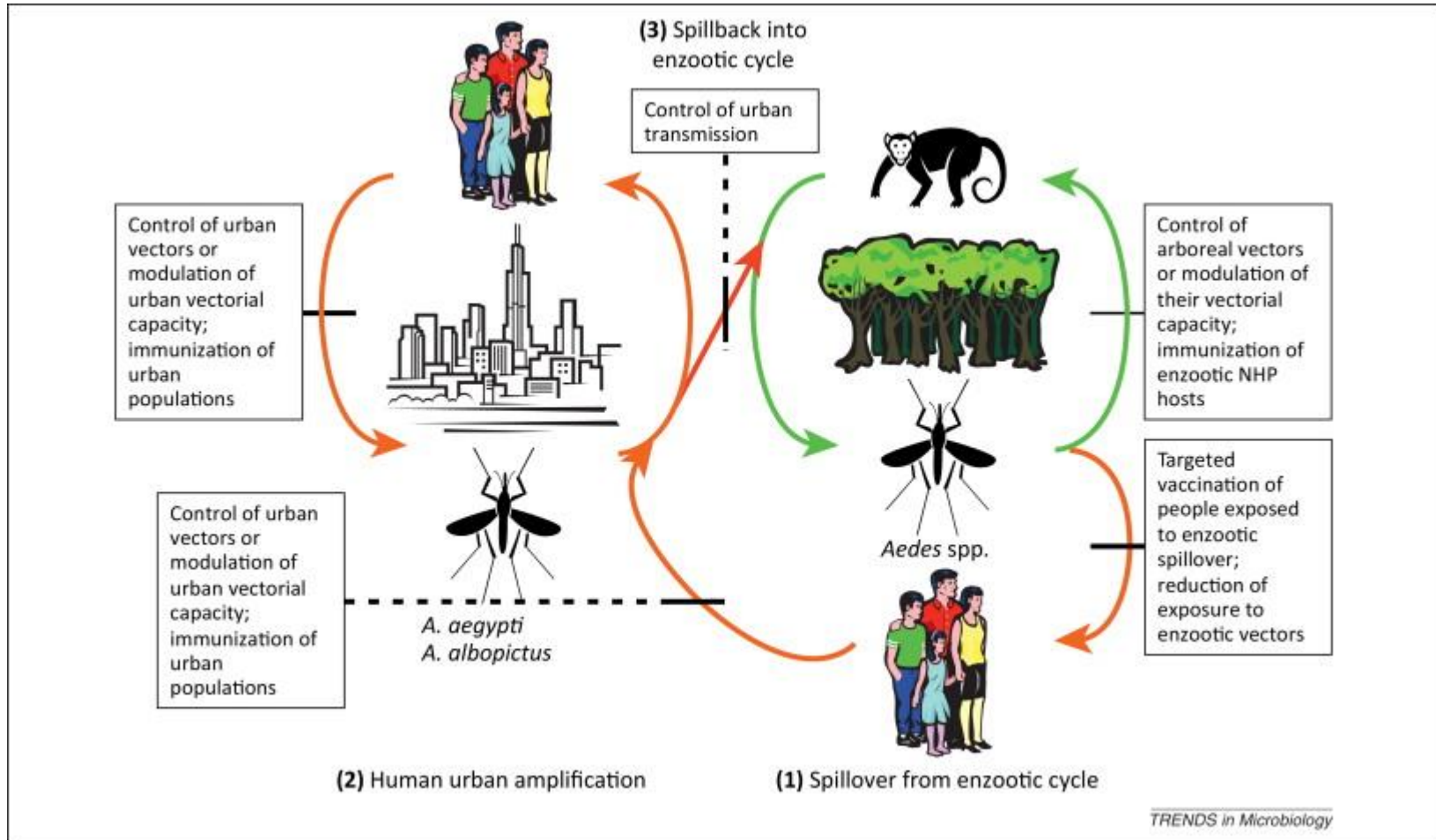
Estimated Potential Range of *Aedes aegypti* in the United States, 2017

Estimated Potential Range of *Aedes albopictus* in the United States, 2017





Zika Transmission





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



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



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



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



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

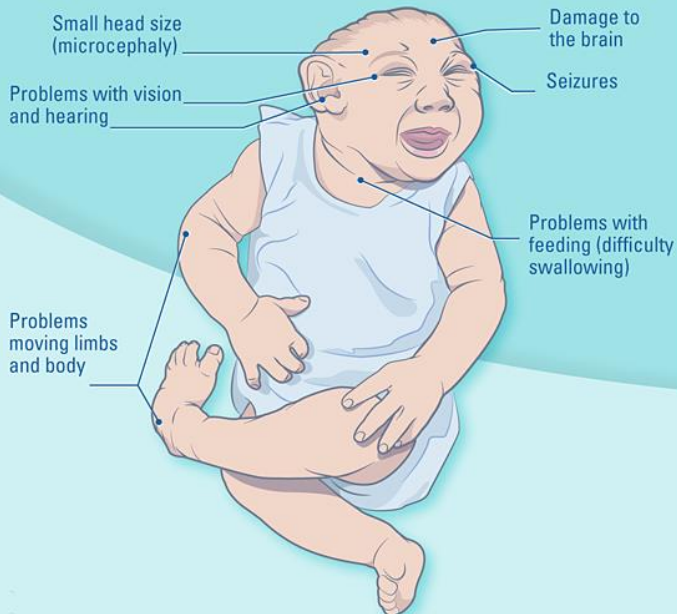
Features	Zika	Dengue	Chikungunya
Fever	++	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-	+	-



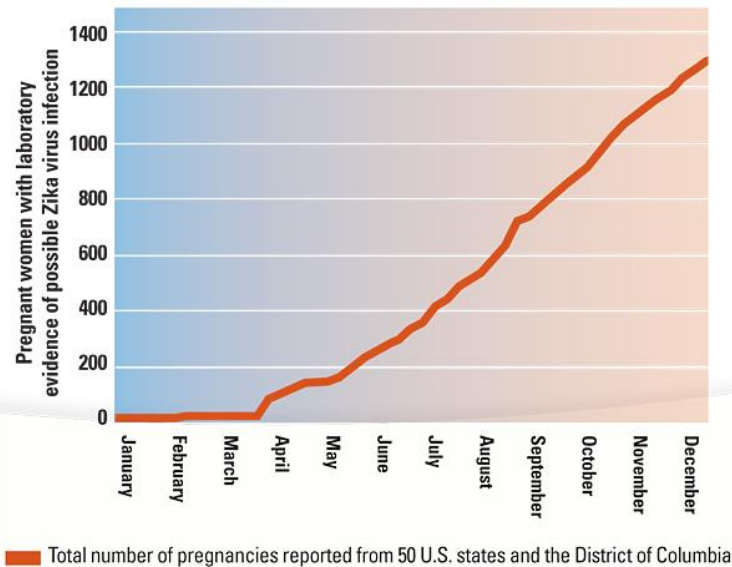
Zika-related Birth Defects

121 of 2,233 pregnant women with laboratory evidence of Zika infection in 2015-2018 had a fetus or baby with Zika-related birth defects

Congenital Zika syndrome is a pattern of birth defects in babies infected with Zika during pregnancy



Reported cases of pregnant women with any lab evidence of possible Zika increased in 2016



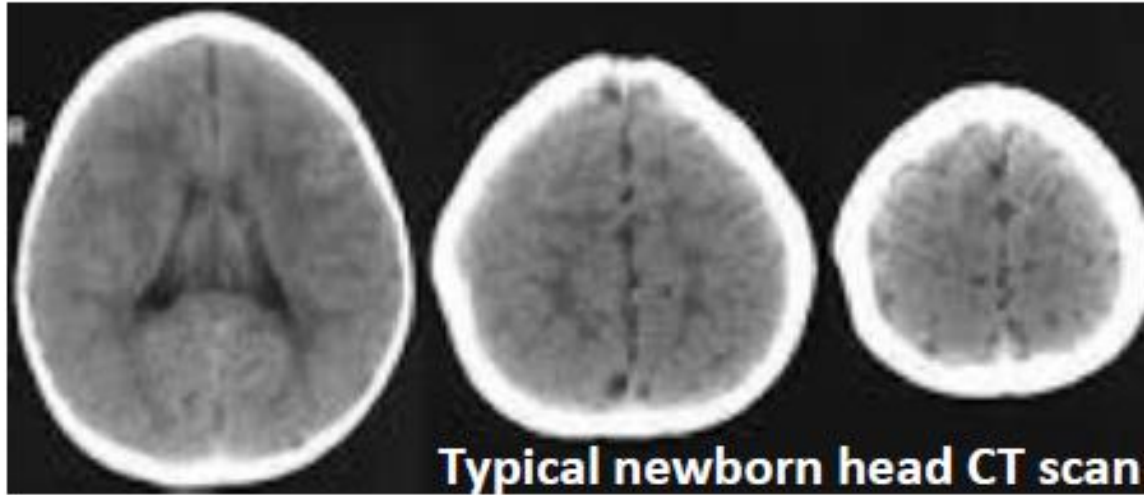
SOURCE: Vital Signs Morbidity and Mortality Weekly Report, April 4, 2017.



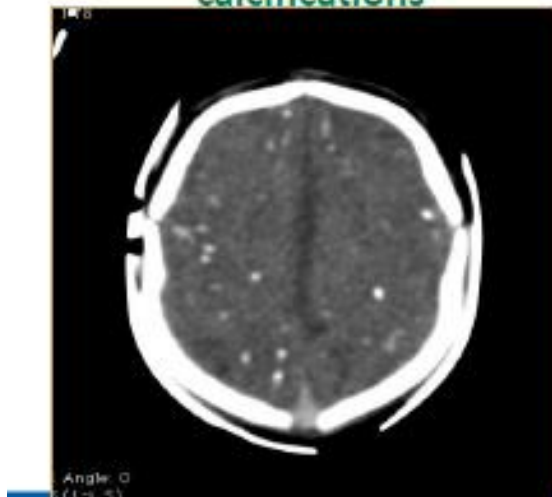
Zika and Associated Birth Defects

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

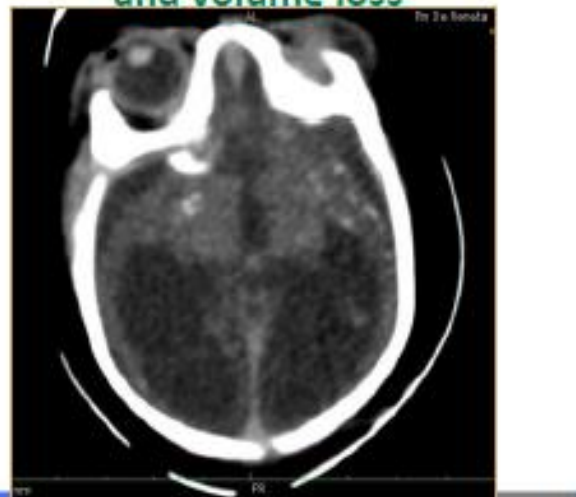
Tip of iceberg? Developmental problems and other effects on the brain?



scattered intracranial calcifications



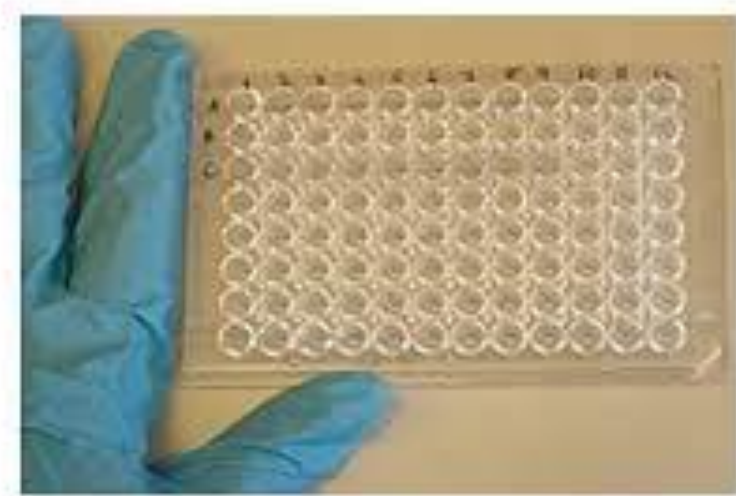
enlarged ventricles and volume loss





WSLH Diagnostic Testing

- Real-time PCR
- IgM Serology



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**



Value of Urine Sample

367 patients with urine and serum submitted

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

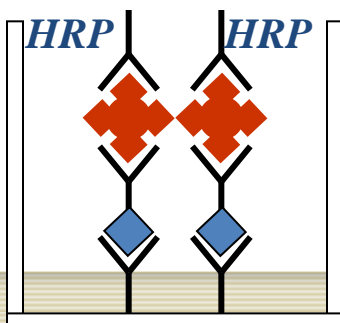
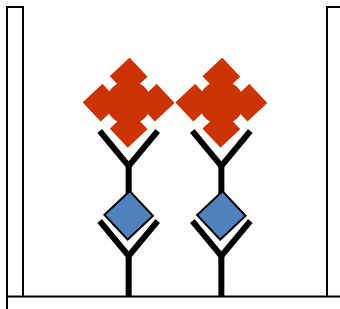
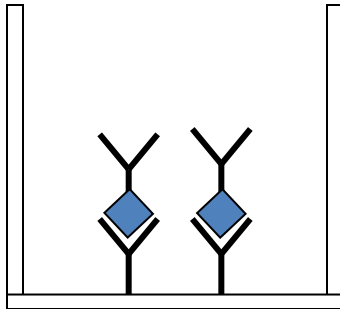
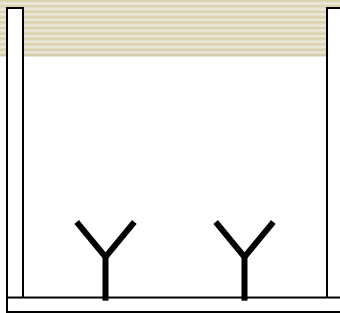


Prolonged PCR Positivity in Serum

- 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



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

- 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



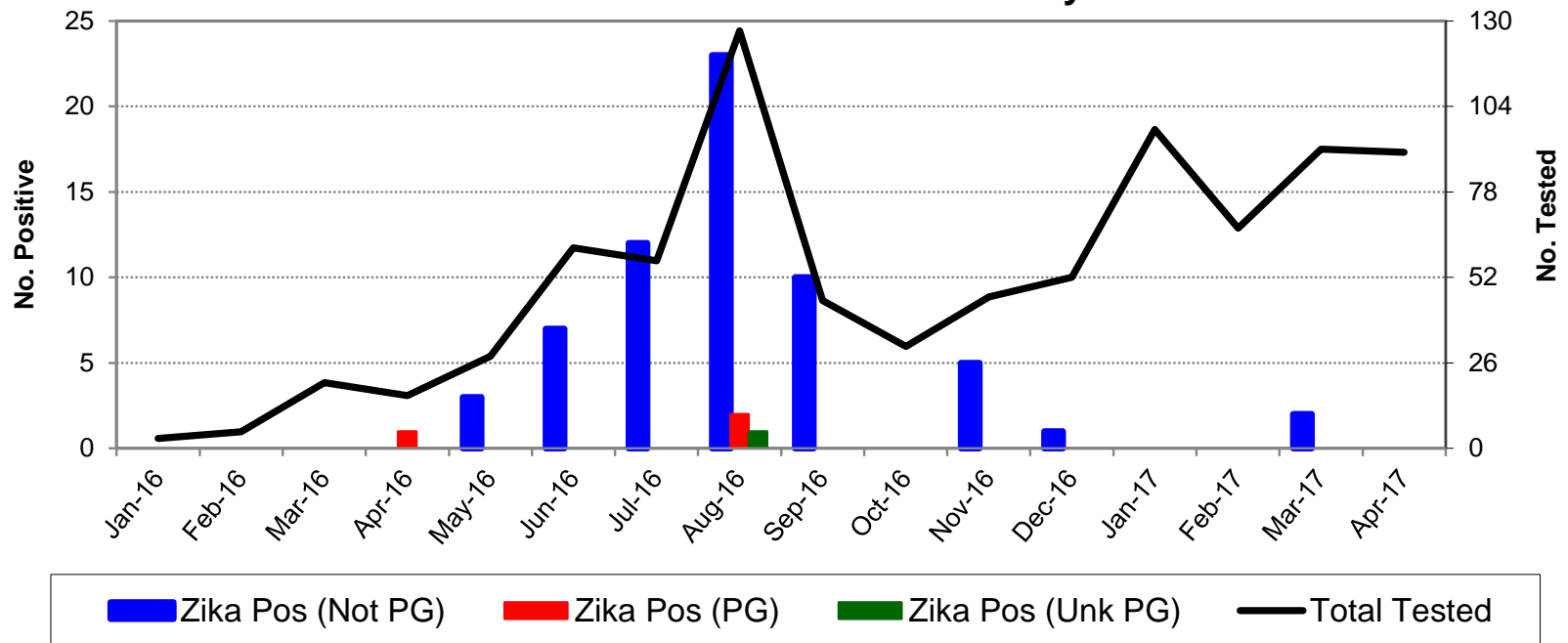
Prolonged IgM Response

- 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)



Zika PCR

Number of Specimens Tested and Positive for Zika by PCR Tested at Wisconsin State Laboratory



3 Dengue PCR Positives, non-pregnant women



PCR Results

Pregnant	Serum			Urine		
	Pos	Tstd	% Pos	Pos	Tstd	% Pos
Pregnant	2	177	1.1%	1	174	0.6%
Not Pregnant	25	240	10.4%	38	235	16.2%
Pregnant, Unkn	1	6	16.7%	0	2	0.0%

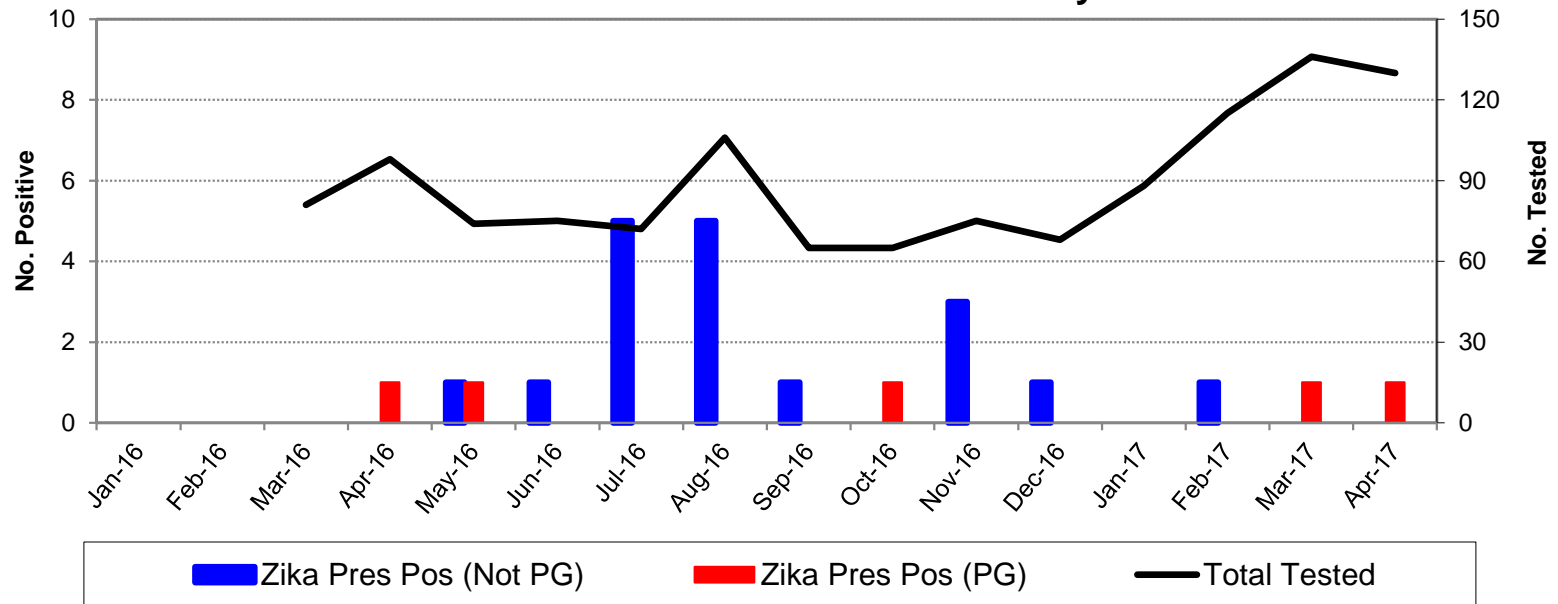
Symptomatic	% Positive
Yes	13.3%
No	0.3%



Zika IgM

There was 1 Dengue IgM Presumptive Positive (not PG)

Number of Specimens Tested and Presumptive Positive by Zika IgM Tested at Wisconsin State Laboratory



1 Dengue IgM Presumptive Positive, not pregnant



WSLH IgM Results

Symptomatic	% Positive
Yes	5.7%
No	0.3%



Zika Virus EUA Tests

[DPP Zika IgM Assay System \(Chembio Diagnostic Systems, Inc.\)](#)

[ADVIA Centaur Zika test \(Siemens Healthcare Diagnostics Inc.\)](#)

[CII-ArboViroPlex rRT-PCR Assay \(Columbia University\)](#)

[TaqPath Zika Virus Kit \(Thermo Fisher Scientific\)](#)

[LIAISON® XL Zika Capture IgM Assay \(DiaSorin Incorporated\)](#)

[Gene-RADAR® Zika Virus Test \(Nanobiosym Diagnostics, Inc.\)](#)

[Zika ELITe MGB® Kit U.S. \(ELITechGroup Inc. Molecular Diagnostics\)](#)

[Abbott RealTime Zika \(Abbott Molecular Inc.\)](#)

[Zika Virus Detection by RT-PCR Test \(ARUP Laboratories\)](#)

[Sentosa® SA ZIKV RT-PCR Test \(Vela Diagnostics USA,](#)



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 \(CDC\)](#)



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



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



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



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



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

- 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



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



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



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



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



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.



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



Thank you!