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
  - $2.1 \times 10^3$ 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
- Dengue
- 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.
  - 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
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

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 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

(1) Spillover from enzootic cycle

(2) Human urban amplification

(3) Spillback into enzootic cycle

Control of urban transmission

Control of arboreal vectors or modulation of their vectorial capacity; immunization of enzootic NHP hosts

Targeted vaccination of people exposed to enzootic spillover; reduction of exposure to enzootic vectors

Control of urban vectors or modulation of urban vectorial capacity; immunization of urban populations

A. aegypti
A. albopictus

TRENDS in Microbiology
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
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

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

Yap Island, 2007

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

<table>
<thead>
<tr>
<th>Features</th>
<th>Zika</th>
<th>Dengue</th>
<th>Chikungunya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Rash</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Myalgia</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Shock</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
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
Typical newborn head CT scan

- scattered intracranial calcifications
- enlarged ventricles and volume loss
What’s the risk of microcephaly with Zika virus infection?

  - 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 with Zika confirmed cases
- Epi Week 52 2015
- Country limits
- Brazil State Boundaries

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 Triplex 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
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
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

<table>
<thead>
<tr>
<th>Specimen collected within 3 days of onset</th>
<th>PCR</th>
<th>IGM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen collected within 4-7 days of onset</td>
<td>Zika Chik Dengue</td>
<td>Zika Chik Dengue</td>
</tr>
<tr>
<td>Specimen collected within 1-12 weeks of onset</td>
<td></td>
<td>Zika Chik Dengue</td>
</tr>
</tbody>
</table>

History of Travel to area of Zika virus transmission AND Symptomatic
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**

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of Patients</th>
<th>Zika Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;=1 Zika-associated symptom (All)</td>
<td>1,541</td>
<td>182 (11.8%)</td>
</tr>
<tr>
<td>&gt;=1 other symptom (All)</td>
<td>436</td>
<td>8 (1.8%)</td>
</tr>
<tr>
<td>Asymptomatic (ALL)</td>
<td>2557</td>
<td>7 (0.3%)</td>
</tr>
<tr>
<td>Asymptomatic pregnant women</td>
<td>2,425</td>
<td>7 (0.3%)</td>
</tr>
<tr>
<td>&gt;=1 Zika-associated symptom</td>
<td>620</td>
<td>18 (2.9%)</td>
</tr>
<tr>
<td>pregnant women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=1 other symptom</td>
<td>290</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>pregnant women</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Wisconsin Residents Tested

**As of March 8, 2016**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>159</td>
<td>73%</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>58</td>
<td>27%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>217</strong></td>
<td></td>
</tr>
</tbody>
</table>
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).
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
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

- 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

Viral loads: plasma

- 10E6
- 10E5
- 10E4
Urine Viral Loads

Viral loads: pan_urine

Days since Zika virus infection

Viral RNA copies / mL

0 5 10 15 20 25

393422
826226
912116
2 pregnant macaques infected

- Detectable plasma viremia at every timepoint 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

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

- CDC COCA. Zika Virus — What Clinicians Need to Know: http://emergency.cdc.gov/coca/calls/2016/callinfo_012616.asp
- CDC MMWR. Interim Guidelines for Pregnant Women During a Zika Virus Outbreak — United States, 2016: http://www.cdc.gov/mmwr/volumes/65/wr/mm6502e1.htm