Zika Virus

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Zika Virus

- **Definition:**
  - Arthropod-borne flavivirus transmitted by mosquitoes
  - The virus is related to other flaviviruses including Dengue Virus, Yellow Fever Virus, and West Nile Virus
  - RNA virus
  - Named after the Ugandan forest where it was first isolated in a rhesus monkey in 1947
The Flavivirus Life Cycle

A. Virus attachment and entry
B. Viral RNA replication on membranes
C. Protein translation, polyprotein processing on membranes
D. Immature virus assembly and budding into ER
E. Mature virus release
F. Virus maturation
G. Furin cleavage of prM

DOI: 10.1016/j.antiviral.2008.05.004
Epidemiology

- Geographic distribution
  - Outbreaks of Zika virus infection have occurred in Africa, Southeast Asia, and the Pacific Islands
  - Ongoing Zika virus outbreak in the Americas
  - From Africa subsequently spread to Southeast Asia, where it was associated with sporadic infections
  - The first major recognized outbreak occurred in the Yap Islands of Micronesia in 2007
    - More than 70 percent of the population ≥3 years of age was infected
Epidemiology

- Geographic distribution
  - Zika virus infection appeared in the Western hemisphere in February 2014 on Chile’s Easter Island
  - The virus continued to be detected there until June 2014
  - Zika virus infection in Brazil was confirmed in May 2015
  - As of February 2016, countries with autochthonous (mosquito-borne) circulation of Zika virus included:
    - Barbados, Bolivia, Brazil, Cape Verde, Colombia, Costa Rica, Curaçao, Dominican Republic, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Nicaragua, Panama, Paraguay, Saint Martin, Samoa, Suriname, Tonga, and Venezuela
Epidemiology

- **Geographic distribution**
  - Zika virus infection has been detected in the United States territories of Puerto Rico, the US Virgin Islands, and American Samoa
  - Local mosquito-borne transmission of Zika virus has been reported in Brownsville, Texas and South Florida in 2016
  - The first case of Zika-related congenital microcephaly in the United States was reported in January 2016 in Hawaii, in a baby born to a woman who had resided in Brazil during her pregnancy
Epidemiology

- Geographic distribution
  - A case of sexually transmitted Zika infection was reported in Texas in February 2016
  - This is the first case of locally acquired Zika infection in the continental US during the 2015-2016 outbreak
  - First Florida sexually transmitted case reported in Pinellas County in July 2017
How Zika virus spread from Africa

1977-78: Pakistan, Malaysia, Indonesia
1947: Discovered in Uganda
2007: Yap, Micronesia
2013: French Polynesia
2015: Brazil

Source: Lancaster University
Areas with Zika Virus

- Areas with active mosquito-borne transmission of Zika virus
  - Prior to 2015, Zika virus outbreaks occurred in areas of Africa, Southeast Asia, and the Pacific Islands

- In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infections in Brazil

- Currently, outbreaks are occurring in many countries: https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika

- Zika virus will continue to spread and it will be difficult to determine how and where the virus will spread over time
Areas with Zika Virus

- **US Territories**
  - Local mosquito-borne transmission of Zika virus has been reported in the Commonwealth of Puerto Rico, the US Virgin Islands, and America Samoa

- **US States local transmission**
  - Brownsville, Texas – advisory lifted 8/29/17
  - South Florida – advisory lifted 6/2/17
  - 80% of cases will not be diagnosed
Florida Zika Case Counts
(As of 8/30/17)

<table>
<thead>
<tr>
<th>Infection Type</th>
<th>Infection Count 2016</th>
<th>Infection Count 2017</th>
</tr>
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<tbody>
<tr>
<td>Travel-Related Infections of Zika</td>
<td>1122</td>
<td>121</td>
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<tr>
<td>Locally Acquired Infections of Zika</td>
<td>285</td>
<td>10</td>
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<tr>
<td>Undetermined</td>
<td>49</td>
<td>30</td>
</tr>
<tr>
<td>Pregnant Women with Lab Evidence of Zika</td>
<td>299</td>
<td>101</td>
</tr>
</tbody>
</table>

*Note: These categories are not mutually exclusive and cannot be added together.*
Zika virus disease and Zika virus congenital infection are nationally notifiable conditions – in Florida, both Zika and Dengue are notifiable upon suspicion (reported when testing is ordered)

US States for January 1\(^{st}\), 2015 – August 23\(^{rd}\), 2017
- Travel-Associated Zika virus disease cases reported: 5150
- Locally acquired vector-borne case reported: 224
- Laboratory acquired cases reported: 1
- Sexually transmitted: 47
- Guillain-Barré syndrome: 13
For January 1st, 2016 – August 23rd, 2017:
- US Territories
  - Locally acquired cases reported: 36,866
  - Travel-associated cases reported: 147
  - Total: 37,013*
- Guillain-Barré syndrome: 45
*Sexually transmitted cases are not reported for US territories because with local transmission of Zika virus it is not possible to determine whether infection occurred due to mosquito-borne or sexual transmission.
Transmission

- Zika virus is transmitted to humans primarily via the bite of an infected Aedes mosquito.
  - Other modes of transmission can also occur.
  - Has been detected in blood, urine, semen, saliva, cerebrospinal fluid, amniotic fluid, and breast milk.
- Zika virus is carried by the Aedes aegyptus mosquito, which lives only in tropical regions; however, the Aedes albopictus mosquito, which lives in temperate regions, is also capable of transmission.
  - Aedes mosquitoes can also transmit Dengue and Chikungunya viruses.
  - Aedes mosquitos bite during the daytime as well as at twilight; they breed in standing water.
Global distribution of Aedes mosquitoes

Aedes aegypti and Aedes albopictus can spread the Zika virus if infected with it

Aedes aegypti mosquito

Probability of occurrence
- Highest
- Lowest

Aedes albopictus mosquito

Probability of occurrence
- Highest
- Lowest

Predicted global distribution of each species based on statistical distribution models

Source: ELife 2015
Zika virus transmission cycle

First infected Aedes mosquito
- In utero transmission
- First infected person
- Liver infection
- Infected red blood cells
- Second infected person
- Second infected Aedes mosquito
- Plasmodium Gametocytes

Zika can be transmitted through blood, but this is an infrequent mechanism. The virus has also been isolated in semen, but person-to-person sexual transmission is unconfirmed.

Source: PAHO/WHO
Transmission

- Maternal-fetal transmission can result in congenital infection
- Transmission of Zika virus infection through breastfeeding has not yet been observed
- Documented reports of sexual transmission
  - Appears to be an infrequent mechanism for Zika virus transmission
  - Further study of the duration of the viral persistence in semen is needed
    - One report described detection of Zika virus in semen 62 days after onset of febrile illness, when it was no longer detectable in blood
- Zika virus is transmissible via blood products
Signs and Symptoms of Zika Virus

- About 1 in 5 people infected with Zika virus become ill
- It can be a mild to moderate disease
- Clinical manifestations of Zika virus infection occur in approximately 20-25 percent of patients
  - Acute onset of low-grade fever with maculopapular rash, arthralgia (notably small joints of hands and feet), or conjunctivitis (non-purulent)
  - Clinical illness is consistent with Zika virus disease if two or more of these symptoms are present
- In Florida, rash is most commonly reported, followed by fever (but 30% of cases are afebrile)
Signs and Symptoms of Zika Virus

- Other commonly reported clinical manifestations include myalgia, headache, retro-orbital pain and asthenia
  - Rarely: abdominal pain, nausea, diarrhea, ulcerations of mucous membranes and pruritus
- Other manifestations: malaise, swelling of the extremities, headache, retro-orbital pain, mouth sores, questionable pericarditis? And questionable epididymitis?
- The incubation period is not known, but is likely to be a few days to a week - typically occur approximately 2 to 12 days after the mosquito vector bite
- Once infected, thought to be protected for life
Zika virus transmission cycle

Symptoms

- Fever
- Rash
- Joint pain
- Conjunctivitis (red eyes)

Person infected with Zika virus → Possible transmission from mother to baby during pregnancy

Infected mosquito bites another person and transmits virus

Aedes mosquito bites infected person

Zika can be transmitted through blood, but this is an infrequent mechanism. The virus has also been isolated in semen, but person-to-person sexual transmission is unconfirmed.

Source: PAHO/WHO
Diagnosis of Zika Virus

- **Individuals at risk**
  - **Suspected case:**
    - Should have relevant epidemiologic exposure (residence in or travel to an area where mosquito-borne transmission of Zika virus infection has been reported)
    - Unprotected sexual contact with a person who meets these criteria
  - **Probable case:**
    - Suspected case with IgM antibody against Zika virus and relevant epidemiologic exposure
  - **Confirmed case:**
    - Person with laboratory confirmation of Zika virus infection, either by detection of viral RNA or antigen in serum
Diagnosis of Zika Virus

- Definitively established via reverse-transcription polymerase chain reaction (RT-PCR) for Zika viral RNA or Zika virus serology

- Detection of Zika IgM antibody and Zika virus 90 percent plaque-reduction neutralization test (PRNT90) titer ≥20 and PRNT90 titer ratio ≥4 compared with other flaviviruses
Diagnosis of Zika Virus

- For individuals within the first seven days after onset of symptoms:
  - Diagnosis may be established via RT-PCR of serum for detection of Zika virus RNA
    - RT-PCR is positive only for a brief window (three to seven days) when the infected person has viremia
      - Negative results cannot exclude infection
    - RT-PCR testing for Dengue virus and Chikungunya virus should also be pursued
Diagnosis of Zika Virus

- For individuals four or more days after the onset of symptoms:
  - Diagnosis may be established by Zika virus serologic testing
    - Zika virus IgM and neutralizing antibody titers that are ≥4-fold higher than dengue virus neutralizing antibody titers in serum
    - Testing is considered inconclusive if Zika virus neutralizing antibody titers are <4-fold higher than dengue virus neutralizing antibody titers.
  - For individuals presenting four to seven days after onset of symptoms
    - Both RT-PCR and serology should be performed
A need for improved diagnostics

- Multiplex or singleplex PCR based assays
- Sensitive and specific
- Limited window in serum or plasma
- Extend RNA window urine and whole blood
- Other molecular testship assays
  - Antigen detection
  - NS1 and others
  - Capture an antigen with a Mab that is specific to Zika antigen → does not detect DENV antigen
Reduce Barriers to Testing, Screening and Counseling

- Commercially available Zika testing
- No need for testing approval from the Health Department
- Florida continues to offer free testing to all pregnant women
- Health Department can assist providers with testing / or counsel on testing
Increase Access to Specialized Health Care Services

- Florida Head Start
- Early Head Start
- Healthy Start
- Florida Birth Defects Registry
There is no vaccine to prevent or specific medicine to treat Zika infections. No specific treatment.

Management consists of symptomatic treatment:
- Rest
- Drinking fluids to prevent dehydration
- Acetaminophen to relieve fever and pain
- Avoid aspirin and other non-steroidal anti-inflammatory medications until Dengue diagnosis is ruled out
- Prevent mosquitoes bites during the first week of the illness
  - During the first week of infection, Zika virus can be found in the blood
  - An infected mosquito can then spread the virus to other people
Zika therapeutics

- Possible use for women who are planning pregnancy → prophylaxis during pregnancy to protect the fetus
- Can help reduce viral load → reduce transmission and prevent complications
- FDA pregnancy category B → safest
6,000 Drugs screened

- 3 classes of lead compound effective in vitro identified
  - 1 caspace inhibitors
  - cycling dependent kinase inhibitors
  - Niclosamide
- NS2B/ NS3
  - Viral protease inhibitors
  - Viral polymerase inhibitors
- Reduced viremia and delayed morbidity as well as mortality in mice
Monoclonal antibodies against Zika

- During pregnancy ➔ prophylaxis or treatment
- Women planning pregnancy ➔ prophylaxis
- Panels of Mab generated in multiple labs and they look promising in 3 clinical studies
Strategies to Reduce Zika Affected Pregnancies

- **Mosquito protection**
  - “Drain and cover” messaging

- **Personal protective measures:**
  - Preventing mosquito bites by wearing long sleeves and long pants
  - Using insect repellent
  - Staying indoors as feasible (with air conditioning, window/door screens, and/or mosquito nets to minimize contact between mosquitoes and people)

- **Awareness of travel advisories / areas of Zika infections**
Prevention of Zika Virus

- Environmental control measures include identification and elimination of potential mosquito breeding sites
  - Mosquito larvae breed in standing water
  - Domestic water tanks should be covered so that mosquitoes cannot enter, and drains that allow stagnant or standing water should be eliminated
The Zika virus

Pregnant women are at risk in Latin America and the Caribbean

**Symptoms**
- Fever, headaches
- Conjunctivitis
- Ganglion cysts
- Rash
- Joint and muscle pain

**Affected countries**
- Honduras
- Guatemala
- Haiti
- Puerto Rico
- Martinique
- Panama
- Colombia
- Ecuador
- Brazil
- Suriname
- French Guiana
- Guyana

**Mosquito Aedes aegypti**

Investigators are probing an apparent link between the Zika virus and a rise in microcephaly in Brazil

- Cases: 3,900
- In 2014: 147

*Source: OMS, OPS*
Microcephaly and Zika Virus

- Between March 2015 and February 2016
  - More than 4000 cases of microcephaly have been reported among newborns born to Brazilian mothers with Zika virus infection
    - This represents a 20-fold increase in microcephaly as compared with previous years
  - No birth defects were reported at the time of the outbreak in the Yap Islands of Micronesia in 2007
    - Perhaps due to the relatively small size of the population?
There is no evidence to suggest that pregnant women are more susceptible to Zika virus infection or experience more severe disease during pregnancy.

The greatest risk of microcephaly and malformations appears to be associated with Zika infection during the first trimester.

The rate of vertical transmission and the rate with which infected fetuses manifest complications is unknown.

No developmental complications have been observed in otherwise healthy neonates, infants, or children with postnatal Zika virus infection or exposure.
Microcephaly and Zika Virus

- Exam findings of infants and fetuses
  - A report including 35 Brazilian infants with microcephaly described findings on computed tomography and transfontanellar cranial ultrasonography
    - These included widespread brain calcifications
      - Mainly in the periventricular, parenchymal, and thalamic areas and in the basal ganglia
    - Ventricular enlargement secondary to cortical/subcortical atrophy was frequently observed
Microcephaly and Zika Virus

- **Exam findings of infants and fetuses**
  - Zika virus was detected in brain tissue via reverse transcriptase polymerase chain reaction (RT-PCR) testing and electron microscopy
    - No virus or pathologic changes were detected in other organs, suggesting strong neurotropism of Zika virus
  - Ocular involvement has been observed among infants with congenital infection due to presumed Zika virus exposure, including macular atrophy and optic nerve abnormalities
Pregnant woman with history of travel to an area with Zika virus transmission

Pregnant woman reports clinical illness consistent with Zika virus disease during or within 2 weeks of travel

Test for Zika virus infection

Positive or inconclusive test for Zika virus infection
Fetal ultrasound to detect microcephaly or intracranial calcifications
Offer amniocentesis for Zika virus testing

Negative test(s) for Zika virus infection
Fetal ultrasound to detect microcephaly or intracranial calcifications
Either finding present

Either finding present
Consider amniocentesis for Zika virus testing

Either finding develops

Pregnant woman does NOT report clinical illness consistent with Zika virus disease during or within 2 weeks of travel

Fetal ultrasound to detect microcephaly or intracranial calcifications

Either finding present
Consider amniocentesis for Zika virus testing

No findings present

Consider serial ultrasounds to detect development of microcephaly or intracranial calcifications

Either finding develops
Zika Virus and Neurological diseases

- Guillain Barre syndrome
  - Frequency unknown 1/ 5,000 infections
  - Increasing evidence of association with Zika infections
  - As of October 5, 2016, 19 countries and territories report an increase in GBS

- Other syndromes- unclear associations
  - Encephalitis
  - Acute disseminated encephalomyelitis
  - Anterior myelitis
  - Ocular abnormalities
  - Hearing loss
Several countries in the Americas have reported unusual increases in cases of Guillain-Barré syndrome (GBS) in parallel with the ongoing Zika virus outbreak.

During the Zika virus outbreak in French Polynesia (2013 to 2014):
- 74 patients had presented neurologic or autoimmune syndromes after the manifestation of symptoms consistent with Zika virus infection
- 42 were classified as GBS
Blood transfusion and Zika Virus

- Zika virus is transmissible via blood products
- Prior recommendations:
  - Deferral of blood donors for one month following Zika Virus infection/exposure
  - Individuals who have donated blood and subsequently develop symptoms consistent with Zika virus infection within 14 days should notify the donation side so the product can be quarantined
Blood transfusion and Zika Virus

- Current recommendations Feb 2016:
  - The Red Cross:
    - Individuals who have visited areas where mosquitoes are actively spreading the virus need to wait 28 days before they donate blood
Blood transfusion and Zika Virus

Current recommendations Feb 2016:

- The FDA:
  - Broadened that deferral policy to include anyone who has had symptoms suggestive of a Zika infection in the prior 4 weeks
  - Anyone who has had sexual contact with a person who has traveled to, or lived in, an area with active Zika transmission in the prior 3 months
Transplant and Zika Virus

- There is no routine screening policy for organ donors or recipients
  - There are reports of possible Zika virus transmission by blood transfusion and it is probable that infection may also be transmitted by organ transplantation.

- This virus has a longer duration of viremia compared with other flaviviruses
  - Might be reasonable to screen the donors coming from areas affected by the outbreak, with probable exposure in the previous 10-14 days.
Transplant and Zika Virus

- The impact of immunosuppression on the natural history of Zika virus infection is unknown

- Donor characterization includes a full recent travel history
  - When the potential donor has travelled to Latin America or other affected areas, the SNOD should inquire whether the donor had been bitten by mosquitos and about any associated illness
  - Should be documented on the Donor Characterization Form

- Recipient clinicians should balance the risk and possible consequences of a donor-transmitted infection and the risks of harm by declining the organ
Genetically engineered/modified mosquitoes and Zika Virus

- In mid-2012, British biotech company Oxitec released the super bugs with the aim of reducing the overall mosquito population that spreads Dengue fever, Zika virus and Chikungunya in Northeast Brazil.

- The first cases of Zika in the Americas were reported last May after spending decades working its way from Africa through Asia.

- The aim of Oxitec’s GM program was to release only male Aedes mosquitoes into the wild and they would in turn produce offspring with their virus carrying female counterparts.
Genetically engineered/modified mosquitoes and Zika Virus

- This offspring would then die off before breeding age due to the GM coding in their genes, as long as the antibiotic tetracycline wasn't present, which would override the GM DNA

- Because of a genetic change in their DNA, they will live only four days—and their offspring won’t ever develop past the larval stage

- There is already a known survival rate of up to 5% in these GMM regardless of the presence of tetracycline
Genetically engineered/modified mosquitoes and Zika Virus

- This antibiotic, which is often used to treat teenage acne, can be found in nature too, showing up in soil, surface water, and food, with some research stating that the GMM survival rate could potentially increase to 15%.
How GM mosquitoes work

Life cycle of wild male and female mosquitoes produces blood-sucking females.

1. Male and female mate
2. Female lays eggs
3. Eggs hatch into larva
4. Larva develops into pupa
5. Pupa grows into adult, female adult sucks blood and transmits virus

Introduction of GM males breaks this cycle as faulty gene causes offspring to prematurely die.

1. GM male with sterile gene and wild female mate
2. Female lays eggs that contain sterile gene
3. Eggs hatch into larva that die before developing into pupa

More GM males are created in the lab by adding tetracycline to larvae to allow development.

5. Pupa grows into adult, researchers have more supplies of GM males to release into the wild.
Vaccines:

- Considerations for Zika vaccine
- Effect of prior flavivirus immunity
- Vaccine induced GBS
- Will vaccine prevent viremia? → importance for pregnancy
  - Currently human challenge study being considered
- Can the vaccine be developed in timely fashion?
Who’s the vaccine for?

- Initially when there is limiting supply of the vaccine
- Women of childbearing age and their sexual partners
- Travelers (men or women) to epidemic/ endemic areas
- Commercial sex workers in epidemic/ endemic areas
Broadly neutralizing activity of Zika virus

- If there is a vaccine developed against a single strain of Zika or lineage of Zika – would the vaccine be protective against diverse strains?
“Phase 2B study” Zika DNA vaccine candidate

- VRC705
- Phase 2B randomized placebo control trial to evaluate the safety and immunogenicity of a Zika virus DNA vaccine
- Started in January 2017
- 30 plus sites in the US, Caribbean, Central and South America
- Enroll 2400 candidates
- Can go up to 5000 if the end point not achieved
Other vaccine studies

- Vaccine Inovio
  - 40 subjects in the US and Canada fully enrolled - Phase I study
    Started in June 2016

- Phase 2 recruiting in PR
  - 160 subjects

- 1:1 vaccine / placebo

- Study in Dengue virus seropositive adults
WRAIR Zika virus purified inactivated vaccine plus aluminum

- Four studies to be started
- Accelerated vaccine schedule: 4 schedules - week 0, 0 and 1, 0 and 2, 0 and 4
- 3 other phase 1 trials initiated
- SLU VTEU → flavivirus naïve
- PR → Dengue experienced
- WRAIR → Yellow Fever Virus and Japanese Encephalitis Virus vaccinated
Zika virus pathogenesis: outstanding questions?

- What is the risk of Zika virus infection?
- How likely is viral transmission from mother to fetus?
- How does gestational state impact fetal outcome?
- What maternal factors contribute to fetal outcome?
- How does Zika virus access the fetal compartment?
- How significant is Zika virus sexual transmission?
- Can Zika virus cause neurological damage in adults?
- Why is the virus that used to be quite benign, now causing severe disease in this outbreak?
- Host immunologic or genetic contributions?
- Virus strain changes?
- Interactions with DENV antibody responses?
Antibody Dependent Enhancement (ADE)

- Prior DENV infection does not protect against ZIKV

- Could DENV antibodies enhance ZIKV?

- ADE is concentration dependent and ADE can be demonstrated for many flaviviruses in vitro→ only DENV antibody known to have human disease relevance
Are contemporary ZIKV strains more virulent than historical ones?

» N154 glycosylation varies between ZIKV strains and this glycosylation contributes to enhance tissue invasiveness of contemporary ZIKV strains

» Study with mice: 5-6 weeks old mice

» 2 types of strains of Zika

» Contemporary clinical strain or prototype strain

» They found that mice lacking IFN and “alpha beta” are susceptible to ZIKV disease AND

» ZIKV infection is lethal in mice that lack IFN Alpha-beta signaling
IFN AR1 blocking Mab increases SIKV viremia in mice

- Treat mice with IFN AR1 blocking Mab → infect with ZIKV (contemporary strain) → measure viremia and weight loss

- Conclusion:
  - Mice treated with IFN AR1 blocking Mab develop significantly higher viremia but no weight loss, diseases or lethality
  - Mice can develop viremia even in the absence of disease signs
  - Ab treatment allows for trans-placental transmission and ocular disease
Current USF Grants

University of South Florida, $2,458,995

• USF Integrated Clinical Trial Network structuring and enhancement of for execution of Zika virus vaccine and diagnostic clinical trials - $1,117,413
• Cellular and molecular mediators of Zika virus replication and mechanisms of transmission - $1,141,582
• Rapid identification of natural products with antiviral activity against Zika - $200,000
Questions?
Sources

- Sexton, Daniel J. MD; Hirch, Martin S.MD; Lockwood, Charles J. MD; Zika virus infection; Uptodate August 23rd, 2017