To view an archived recording of this presentation please click the following link: http://pho.adobeconnect.com/p9jw6f446so/

For the most up to date Zika virus infection information, please visit our website.

Please scroll down this file to view a copy of the slides from the session.

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Zika Virus Infection: Epidemiology, Prevention, Diagnosis and Pregnancy Management—Latest Developments

PHO Grand Rounds March 22, 2016
Doug Sider MD, FRCPC
Jonathan Gubbay MBBS, FRCPC
Kellie Murphy MD, FRCSC
Zika Virus: Talk Outline

• Review virology and clinical features
• Summarize the epidemiology of Zika virus, including spread to the Americas
• Discuss new associations with Zika virus
  • Congenital infection resulting in microcephaly, cerebral malformations
  • Guillain–Barré syndrome
• Review laboratory testing guidelines for Zika virus
• Discuss the approach to the pregnant patient potentially exposed to Zika virus
• Outline prevention strategies and key areas for further study
Zika Virus Virology

- *Flaviviridae* family, genus *Flavivirus*.
  - Other flavivirus examples: WNV, dengue, yellow fever virus, Japanese encephalitis.

- Spondweni serocomplex
  - Closest neighbour: Spondweni virus

- Three lineages based on NS5 sequence:
  - East African, West African, Asian

10,794bp genome
Translated into a 3,419aa protein

- Single stranded RNA (+) virus
- Enveloped, spherical,
- Approx. 50nm diameter
- Icosahedral symmetry

Early History of Zika Virus

• First detected in rhesus monkeys in Zika forest, Uganda in 1947
• First reported in humans in Nigeria in 1954
• Transmitted by many Aedes mosquitos, including Ae. Africanus, Ae. luteocephalus, Ae. henselli, Ae. Aegypti
  • Ae. albopictus, Gabon 2007
COMMUNICATIONS

ZIKA VIRUS

(I). ISOLATIONS AND SEROLOGICAL SPECIFICITY

BY

G. W. A. DICK,

The National Institute for Medical Research, London

S. F. KITCHEN,

Formerly staff member of the Division of Medicine and Public Health, The Rockefeller Foundation, New York, U.S.A.

AND

A. J. HADDOW,

Formerly staff member of International Health Division, The Rockefeller Foundation, New York, U.S.A.

(From the Virus Research Institute, Entebbe, Uganda.)
Transmission and Incubation Period

- Transmitted by *Aedes aegypti* mosquitos
- Incubation period: 3 to 12 days (may be up to 14 days)
- Usually causes a mild, self limiting illness
  - Maculopapular rash
  - Low grade fever
  - Conjunctivitis
  - Arthralgia

Possible human to human transmission of Zika

- Sexual transmission (several confirmed cases)
  - Virus detectable in semen longer than in blood. Case reports:
    - RNA detected in semen up to 62 days post illness onset (EID Feb 11, 2016)
    - Semen viral load 100,000 x that of blood (March 3, 2016)
- Blood donation (several reports under investigation)
- Organ donation (theoretical)
- Virus detectable in urine/saliva, no cases of transmission
- RNA detected in breast milk, no cases of transmission. One publication (March 1, 2016) documenting culture of virus from breast milk.

Sources:
How Zika Virus spread around the world

• First autochthonous cases in the Americas in February 2014 on Easter Island
Asian lineage detected in French Polynesia, 2014 and Suriname, 2015

Anticipating the international spread of Zika virus from Brazil

Isaac Bogoch, Oliver J Brady, Moritz UG Kraemer, Matthew German, Marisa I Creatore, Manisha AKulkarni, John S Brownstein, Sumiko RMekaru, Simon I Hay, Emily Groot, Alexander Watts, *Kamran Khan
khank@smh.ca


From Jan 1, 2007 to 16 March 2016:
- Zika virus transmission documented in 59 countries/territories
Since 2014, autochthonous transmission in 33 countries/territories in the Americas

From Jan 1, 2007 to 16 March 2016, Zika virus transmission documented in 59 countries/territories; 48 since Jan 1, 2015. Latest to report: Cuba, Dominica.

Autochthonous transmission in 33 countries/territories in the Americas

France, Italy and USA have had locally acquired cases, likely through sexual transmission

Emergence in Brazil

• Late 2014: Cluster of cases of febrile rash in Northeast Region
  • May 2015: Diagnosis of Zika confirmed by RT-PCR
• Estimated 0.4 to 1.3 million Zika cases since outbreak began.

Emergence in Brazil, Reports of Microcephaly

- From 22 October 2015 to 12 March 2016—6480 cases microcephaly and/or CNS malformation, including 182 deaths.
  - Vs. an average of 163 microcephaly per year nationwide, 2001 to 2014.
  - Prevalence in 15 states with lab-confirmed Zika (2.8 cases per 10,000 live births) significantly exceeds that in 4 states without Zika (0.6 cases per 10,000)

- Investigated 2212 cases to date—863 potentially associated with Zika virus infection.

- Among 462 cases investigated as of Feb 6:
  - 421 had radiological findings compatible with congenital infection
  - 41 had lab confirmation of Zika virus infection

Sources:
Emergence in Brazil, Reports of Microcephaly

- Among 6480 suspected cases, 182 deaths after birth or during pregnancy:
  - 40 confirmed as microcephaly or CNS malformation potentially linked to Zika virus infection; 18 discarded, 124 still under investigation.
- French Polynesia reported 19 cases CNS malformation (including 8 microcephaly) in children between March 2014 and May 2015.
  - National average 0 to 2 cases /year.
- 1 imported case each in USA, Slovenia
- Cape Verde investigating a case of microcephaly
  - (reported 14 March, 2016)

Zika Virus and Guillain–Barré syndrome

- 12 countries/territories reporting 2015-16 increases in GBS and/or lab-confirmed ZIKV in GBS cases.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Country/Territory/Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported increase in incidence of GBS cases, with no GBS case confirmed with Zika virus infection</td>
<td>Colombia, Honduras</td>
</tr>
<tr>
<td>Reported increase in incidence of GBS cases, with at least one GBS case with confirmed Zika virus infection</td>
<td>Brazil, El Salvador*, French Polynesia, Suriname, Venezuela (Bolivarian Republic of)</td>
</tr>
<tr>
<td>No increase in GBS incidence reported, with at least one GBS case with confirmed Zika virus infection</td>
<td>French Guiana, Haiti*, Martinique, Panama, Puerto Rico</td>
</tr>
</tbody>
</table>

*GBS cases with previous history of Zika virus infection were reported by the International Health Regulations (2005), National Focal Point in United States of America.

Case-control study of French Polynesia GBS outbreak (Feb. 29 2016)
  • 42 GBS cases compared with age/sex-matched hospitalized non-Zika controls (n=98) and age/sex-matched Zika non-GBS controls (n=70)

Results:
  • Significant morbidity from GBS:
    • Generalized muscle weakness (74%), inability to walk (44%), facial palsy (64%)
    • 16 (38%) admitted to ICU - median LOS 51 days
    • 12 (29%) required respiratory assistance
  • Strong association of GBS with ZIKV infection (OR 59.7  p< .0001)
    • No association with previous/current dengue infection
    • 37 (88%) with GBS experienced transient illness a median 6 days (IQR 4-10) before onset of neurological symptoms.

Results

Laboratory testing:

• 41/42 (98%) with GBS and 35/98 (36%) of controls had anti-Zika IgM and/or IgG
  • 39 (93%) with GBS had Zika IgM vs 17% of controls

• Zika neutralizing antibodies:
  • in all 42 (100%) with GBS and only 54 (56%) of 98 in the control group

• Risk of GBS 24/100,000 Zika infections
  • 30/100,000 C. jejuni
  • 17.2 /1,000,000 influenza-encoded encounters
  • 1.03/1,000,000 influenza vaccinations

NEWS

Zika virus is a global public health emergency, declares WHO

February 1, 2016

Anne Gulland
London

Zika virus and microcephaly: why is this situation a PHEIC?

When the Director-General of WHO declared, on Feb 1, 2016, that recently reported clusters of microcephaly and other neurological disorders are a Public Health Emergency of International Concern (PHEIC),1 it of them had been working for the past months with the WHO Regional Office in the Americas on the Zika virus outbreaks, and before that on those caused by the dengue and chikungunya viruses. During one country

Zika Virus Laboratory Testing
Testing Modalities Available

• Molecular detection: real-time RT-PCR
• Serology
  • ELISA IgM
  • Plaque reduction neutralization titre (PRNT)
• Antigen detection
  • Immunohistochemistry (CDC only)
  • Immunochromatographic tests
• Virus Culture
Proposed Laboratory Based Case Definitions For Diagnosis: Specific Case Classifications
Confirmed

- Clinical correlation and one of more of the following laboratory criteria:
  - Isolation of virus from, or detection of specific viral antigen or nucleic acid in, tissue, blood, urine, CSF, or other body fluid
  - Viral IgM antibodies in serum and the identification of confirmatory virus-specific neutralizing antibodies in the same or a later specimen
  - A demonstrated seroconversion or diagnostic rise (4-fold or greater change) in virus-specific neutralizing antibody titers in paired sera

In development, PHAC and Canadian Public Health Laboratory Network
Zika Virus Real-time RT-PCR

- NML using a CDC in-house protocol (PHOL commenced testing March 14)
  - 2 PCRs: a. junction of E and M genes; b. E gene
- Positive test (both targets detected) is confirmatory
- Supplementary NS5 gene PCR/sequencing when required
- Useful for detecting viral RNA during acute infections.
- May be present in fetal/congenital infection.
- Can be performed on serum, whole blood, urine, CSF, tissue

Zika Virus Real-time RT-PCR

- Specimens should be collected as soon as possible after symptom onset, but no later than 10 days following onset of illness.
  - Virus can be detected by PCR in serum up to 7 days, and in urine up to 10 days following symptom onset.
  - PCR sensitivity maximized if specimens are collected earlier in the course of illness.
  - A negative PCR result does not rule out Zika virus infection.

- Has been detected in semen when serum is PCR-negative.
  - Not recommended to test semen for diagnosis/clinical management.
Zika virus serology

• Acute serology should be collected at the time of initial presentation
  • IgM antibody develops at ≥4 days after symptom onset, lasts 2-12 weeks.

• Convalescent serology should be collected at least 2 – 3 weeks after the initial (acute) serology specimen is collected.

• Absence of IgM at 2 to 12 weeks post symptom onset (or post departure from endemic area in asymptomatic pregnant) makes infection unlikely (but does not rule it out).
Zika virus IgM serology

- Zika virus IgM reactive specimens are considered indicative of a recent **flavivirus** infection.

- IgM antibodies against Zika virus, dengue virus, and other flaviviruses including West Nile virus, have strong cross reactivity in serological assays.

- Current assays cannot reliably distinguish between Zika and dengue virus infections.
  - IgM reactive specimens will be further investigated by neutralization assays (PRNT).
Zika virus plaque reduction neutralization testing (PRNT)

- PRNT can also cross react among different flaviviruses.
- PRNT is run in parallel to PRNTs to other relevant flaviviruses the patient may have been exposed to (e.g., dengue virus).
- Zika PRNT reactive specimens with a Zika titre ≥4-fold that to other flaviviruses (e.g. dengue) will be considered confirmed seropositive for Zika virus.
  - Those with titres <4 fold that of comparator flaviviruses will be considered inconclusive for Zika virus seropositivity.

Zika virus testing: Specimen Requirements

• Specimen types and minimum volume required:

• Serology +/- molecular testing from blood, sera or CSF: two tubes (when possible), each containing 2 to 5 ml blood in serum separator tubes (SST) or 1.0 ml serum; or 1ml of CSF*.

• Additional Molecular testing (Real time PCR): 5ml of urine; 400 µl of amniotic fluid* or CSF*; tissue*. Any of these specimens must be submitted in a tightly sealed sterile container.
Zika Virus

Important notice:

The availability and recommendations for Zika testing may change as the outbreak evolves. Please return to this page for the latest information for testing for Zika virus in Ontario. This page was last updated March 14, 2016.

All samples submitted for testing must be accompanied by a separate Public Health Ontario Laboratory General Test Requisition for each sample type collected. All fields on each requisition must be completed. In addition, fill in the Mandatory Information Intake Form for Zika Virus Testing, which requests the following mandatory information:

a. Order relevant Zika virus testing as directed in the Testing Guidance Table
b. Country (or countries) visited
c. Dates of travel (arrival to and departure from affected country)
d. Relevant symptoms
e. Date of symptom onset (omit if pregnant asymptomatic)
f. Date of sample collection
g. History of receiving any flavivirus vaccine (e.g., Japanese encephalitis vaccine, yellow fever vaccine) or previous flavivirus infection (e.g., West Nile virus, dengue virus)
h. If female, pregnancy status (Y/ N/ unknown).
   i. If pregnant, is patient asymptomatic?
   ii. If fetal or neonatal ultrasound performed, describe findings (normal, fetal microcephaly, or CNS calcification)

Alternatively, the mandatory information can be documented on the Public Health Ontario Laboratory General Test Requisition
Mandatory Information Intake Form

Go to [http://www.publichealthontario.ca](http://www.publichealthontario.ca) frequently for updates

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### Mandatory Information:

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patient Name and Date of Birth</td>
</tr>
<tr>
<td>2</td>
<td>Country(s) visited</td>
</tr>
<tr>
<td>3</td>
<td>Dates of travel</td>
</tr>
<tr>
<td>4</td>
<td>Date of onset of symptoms</td>
</tr>
<tr>
<td>5</td>
<td>Symptoms (list all relevant)</td>
</tr>
<tr>
<td>6</td>
<td>Date of collection</td>
</tr>
<tr>
<td>7</td>
<td>History of receiving any flavivirus vaccine or prior flavivirus infection</td>
</tr>
<tr>
<td>8</td>
<td>Pregnancy status (Yes/No/NA)</td>
</tr>
</tbody>
</table>

### Additional Information if available regarding fetal or neonatal ultrasound:

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fetal microcephaly (Yes/No/NA)</td>
</tr>
<tr>
<td>2</td>
<td>CNS calcification (Yes/No/NA)</td>
</tr>
</tbody>
</table>

### Completed by:

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of healthcare provider</td>
</tr>
<tr>
<td>Signature/Date</td>
</tr>
</tbody>
</table>
## Testing Guidance

### Testing directions and algorithms for patients suspected of Zika infection, according to patient category

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Tests to order</th>
<th>Zika Virus Testing Algorithm</th>
<th>Comments</th>
</tr>
</thead>
</table>
| A. Currently symptomatic patients with illness compatible with acute Zika virus infection | 1. Zika virus serology and PCR  
2. Chikungunya serology and PCR  
3. Dengue serology and PCR | Zika virus PCR at PHOL  
Zika virus PCR and serology at NML  
Chikungunya/dengue serology at PHOL | 1. Zika virus can be detected by PCR in serum up to 7 days, and in urine up to 10 days following symptom onset. PCR sensitivity will be maximized if specimens are collected earlier in the course of illness. |
| B. Non-pregnant patients who have recovered from a Zika-like illness (currently asymptomatic) | 1. Zika virus testing is not indicated  
2. Order other testing as clinically indicated | No Zika virus testing will be performed. | 1. Patients who have recovered from a self-limiting illness suggestive of Zika virus infection do not require testing for the virus.  
2. Specimens from this patient group will not be accepted for Zika virus testing; testing for other pathogens will occur as ordered on the test requisition.  
3. See the PHAC guidelines for further information on management of asymptomatic patients, including prevention of sexual transmission after travel. |
| C. Non-pregnant asymptomatic patients who have never experienced a Zika-like illness | 1. Zika virus testing is not indicated  
2. Order other testing as clinically indicated | No Zika virus testing will be performed. | 1. Specimens from this patient group will not be accepted for Zika virus testing; testing for other pathogens, if indicated, will occur as ordered.  
2. See the PHAC guidelines for further information on management of asymptomatic patients, including prevention of sexual transmission after travel. |
| D. Asymptomatic pregnant patients | 1. Zika virus serology | Specimens will be forwarded to NML for Zika virus serology. | 1. Serology is the appropriate test for asymptomatic pregnant patients. PCR is not recommended in asymptomatic patients as they are unlikely to be viremic at the time of testing.  
2. A negative Zika IgM at 2 to 12 weeks following the last potential exposure indicates that infection is unlikely, though does not exclude it.  
3. See the PHAC guidelines for further information on management of asymptomatic pregnant patients, including if diagnosed with Zika virus infection. |
| E. a. Confirmed maternal Zika virus infection during pregnancy, or b. Risk factors for maternal Zika virus infection in pregnancy and suspected fetal anomaly on antenatal ultrasound (e.g., | 1. Zika virus serology on the mother in scenario a.  
2. Consider amniocentesis (see comments) | Specimens will be forwarded to NML for Zika virus serology. | 1. Amniotic fluid can be tested for Zika virus by PCR, and will be forwarded to NML for testing. Decisions around performing amniocentesis should be made after review by a fetal medicine specialist with expertise in congenital infections. See the PHAC guidelines for further information. |

---

If any queries, contact Public Health Ontario Laboratories Customer Service Centre at 416-235-6556 or 1-877-604-4567
Testing directions and algorithms for patients suspected of Zika infection

Currently symptomatic patients with illness compatible with acute Zika virus infection

• Tests to order:
  1. Zika virus PCR and serology
  2. Chikungunya PCR and serology
  3. Dengue PCR and serology
Testing directions and algorithms for patients suspected of Zika infection (cont’d)

Asymptomatic pregnant patients

• Tests to Order
  • Zika virus serology

• Comments:

  1. Serology is the appropriate test for asymptomatic pregnant patients. PCR is not recommended in asymptomatic patients as they are unlikely to be viremic at the time of testing.

  2. A negative Zika IgM at 2 to 12 weeks following the last potential exposure indicates that infection is unlikely, though does not exclude it.
Testing directions and algorithms for patients suspected of Zika infection (cont’d)

Nonpregnant asymptomatic patients who have never experience a Zika-like illness, or recovered from a Zika – like illness

• Laboratory Testing
  1. Zika virus testing is not indicated
  2. Order other testing as clinically indicated

• Comments
  • See the PHAC guidelines for further information on management of asymptomatic patients, including prevention of sexual transmission after travel.
14 instances of suspected sexual transmission
- All males symptomatic or recently symptomatic
- 2 cases laboratory confirmed (Zika PCR positive)
- 4 probable cases (Zika IgM pos., PRNT pending)
- 8 still under investigation
- 2 excluded

Sources:
Zika Virus Commercial Assays

• Euroimmun Anti-Zika Virus ELISA (IgM)

Anti-Zika Virus ELISA (IgM)
Test instruction

<table>
<thead>
<tr>
<th>ORDER NO.</th>
<th>ANTIBODIES AGAINST</th>
<th>IG CLASS</th>
<th>SUBSTRATE</th>
<th>FORMAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI 2668-9601 M</td>
<td>Zika virus</td>
<td>IgM</td>
<td>Ag-coated microplate wells</td>
<td>96 x 01 (96)</td>
</tr>
</tbody>
</table>

Indication: The ELISA test kit provides a semiquantitative assay for human antibodies of the immunoglobulin class IgM against Zika virus in serum or plasma for the diagnosis of Zika virus.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with specific NS1 antigen of Zika virus. In the first reaction step, diluted patient samples are incubated in

Anti-Zika Virus ELISA (IgG)
Test instruction

<table>
<thead>
<tr>
<th>ORDER NO.</th>
<th>ANTIBODIES AGAINST</th>
<th>IG CLASS</th>
<th>SUBSTRATE</th>
<th>FORMAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI 2668-9601 G</td>
<td>Zika virus</td>
<td>IgG</td>
<td>Ag-coated microplate wells</td>
<td>96 x 01 (96)</td>
</tr>
</tbody>
</table>

Indication: The ELISA test kit provides a semiquantitative or quantitative assay for human antibodies of the immunoglobulin class IgG against Zika virus in serum or plasma for the diagnosis of Zika virus.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with specific NS1 antigen for Zika virus. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG (also IgA and IgM) antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Hamburg, January 27, 2016 The RealStar® Zika Virus RT-PCR Kit 1.0 is an in vitro diagnostic assay based on real-time Reverse Transcriptase/Polymerase Chain Reaction (RT-PCR) technology, for the identification of Zika virus. RealStar® Kits are reliable CE-IVD marked assays for detection and quantification of various viruses, bacteria and parasites. The assays meet all requirements of the IVD Directive 98/79/EC. With the development of the RealStar® Zika Virus RT-PCR Kit 1.0, altona Diagnostics enlarges its panel of CE-IVD marked ready-to-use kits for tropical pathogens.

Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO (originally issued 10 February 2016)

Zika Virus Commercial Assays

- Zika Virus ICT test

Biocan Diagnostics Inc.

www.rapidtest.ca

Address:
55A & 55B Fawcett Road
Coquitlam BC
V3K6V2
Canada

WHO PREQUALIFICATION TEAM:
DIAGNOSTICS

World Health Organization

Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO (originally issued 10 February 2016)

http://www.who.int/diagnostics_laboratory/eual-zika-virus/160211Invitation_to_rmx_of_Zika_virus_diagnostics_v2.pdf?ua=1
Management of the Pregnant Patient
Child with microcephaly
Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?

- First case “series”
- 2 cases from Paraiba state, Brazil
- Both cases:
  - Maternal blood negative Zika
  - Amniocentesis positive for Zika
- No description of maternal symptoms
- Genotype Zika of Asian origin


PublicHealthOntario.ca
• Case 1:
  • Diagnosed at 30+1 GA
  • EFW 21%, HC < 2.6 SD below normal

• Cranial imaging:
  • Normal lateral ventricles
  • Brain atrophy
  • Intracranial calcifications
  • Absent corpus callosum
  • Cerebellar vermian dysgenesis
  • Enlarged cisterna magna

Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?

• **Case 2**
  
  • Diagnosed at 29+2 GA  
  • EFW 19%, HC < 3.1 SD below normal  

• **Cranial imaging:**
  
  • Markedly asymmetric cerebral hemispheres, displaced midline  
  • Unilateral ventriculomegaly  
  • Thinning brain parenchyma  
  • Absent corpus callosum and loss of thalami  
  • Intracranial calcifications  
  • Bilateral cataracts

Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?

25 European volunteer based in Natal Brazil

- At 13 wk GA developed fever, MSK pain, retroocular pain, M-P rash
  - Positive Zika virus
- U/S at 14 and 20 weeks normal
- Returned to Europe at 28 weeks
- U/S at 29 weeks question of abnormalities
- U/S at 32 weeks:
  - IUGR < 3%; HC < 2%
  - Thick calcified placenta
  - Moderate ventriculomegaly
  - Cerebellar diameter < 2%
  - Blurred parenchyma
  - Intracranial calcifications
  - Decreased FM
  - Normal anatomy otherwise, normal Dopplers
- Interrupted pregnancy at 32 weeks

Autopsy

- Fetal weight 1470g (5%), HC 26cm (1%)
- Microcephaly only external anomaly
- Brain abnormalities
  - Complete agyria & internal hydrocephalus of lateral ventricles
  - Numerous variable sized calcifications in cortex & subcortical white matter
  - Diffuse astrogliosis & activated macrophages throughout
  - Brain stem & spinal cord degeneration of long descending tracts
- Brain tissue + Zika RT PCR, negative other flaviviruses, TORCH, parvo

Zika Virus Infection in Pregnant Women in Rio de Janeiro – Preliminary Report

• September 2015–February 2016, ongoing

• Inclusion:
  • Pregnant women w/ rash within last 5 days

• No fetal malformations or medical conditions prior to enrollment

• Serum & urine samples for Zika

• Weekly phone follow up

• U/S at <20, 20-30 and >30wks GA

<table>
<thead>
<tr>
<th>Variable</th>
<th>ZIKV-Positive Women (N=72)</th>
<th>ZIKV-Negative Women (N=16)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age — yr</td>
<td>29 (26–34)</td>
<td>28 (26–33)</td>
<td>0.94†</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>17–46</td>
<td>20–36</td>
<td></td>
</tr>
<tr>
<td><strong>Other family members ill — no./total no. (%)</strong></td>
<td>36/64 (56.2)</td>
<td>5/16 (31.2)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Partner ill — no./total no. (%)</strong></td>
<td>12/57 (21.1)</td>
<td>1/16 (6.2)</td>
<td>0.27</td>
</tr>
<tr>
<td>Use of repellent — no./total no. (%)</td>
<td>19/47 (40.4)</td>
<td>3/10 (30.0)</td>
<td>0.73</td>
</tr>
<tr>
<td>History of dengue — no./total no. (%)</td>
<td>22/70 (31.4)</td>
<td>9/16 (56.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>Socioeconomic status — no./total no. (%)‡</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Income ≤2× minimum wage</td>
<td>24/65 (36.9)</td>
<td>2/13 (15.4)</td>
<td>0.20</td>
</tr>
<tr>
<td>Income &gt;2 to ≤5× minimum wage</td>
<td>26/65 (40.0)</td>
<td>5/13 (38.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Income &gt;5× minimum wage</td>
<td>15/65 (23.1)</td>
<td>6/13 (46.2)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Week of gestation at time of infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>20 (14–26)</td>
<td>17 (10–23)</td>
<td>0.60†</td>
</tr>
<tr>
<td>Range</td>
<td>5–38</td>
<td>7–39</td>
<td></td>
</tr>
<tr>
<td><strong>Distribution — no. (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4 to ≤13 wk</td>
<td>17/72 (23.6)</td>
<td>5/16 (31.2)</td>
<td>0.53</td>
</tr>
<tr>
<td>&gt;13 to ≤26 wk</td>
<td>38/72 (52.8)</td>
<td>8/16 (50.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt;26 to ≤39 wk</td>
<td>17/72 (23.6)</td>
<td>3/16 (18.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>Variable</td>
<td>ZIKV-Positive Women (N = 72)</td>
<td>ZIKV-Negative Women (N = 16)</td>
<td>P Value*</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------------</td>
<td>------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms — no./total no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash§</td>
<td>72/72 (100.0)</td>
<td>16/16 (100.0)</td>
<td>0.47†</td>
</tr>
<tr>
<td>Any</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median duration</td>
<td>4</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2–14</td>
<td>2–60</td>
<td></td>
</tr>
<tr>
<td>Macular</td>
<td>37/72 (51.4)</td>
<td>8/16 (50.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Maculopapular</td>
<td>32/72 (44.4)</td>
<td>2/16 (12.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Other</td>
<td>3/72 (4.2)</td>
<td>6/16 (37.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Pruritus</td>
<td>69/72 (95.8)</td>
<td>14/15 (93.3)</td>
<td>0.54</td>
</tr>
<tr>
<td>Arthralgia or arthritis</td>
<td>46/72 (63.9)</td>
<td>7/16 (43.8)</td>
<td>0.16</td>
</tr>
<tr>
<td>Conjunctival injection</td>
<td>42/72 (58.3)</td>
<td>2/15 (13.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Headache</td>
<td>38/72 (52.8)</td>
<td>9/16 (56.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Fatigue or malaise</td>
<td>35/72 (48.6)</td>
<td>7/16 (43.8)</td>
<td>0.79</td>
</tr>
<tr>
<td>Retro-orbital pain</td>
<td>34/69 (49.3)</td>
<td>5/16 (31.3)</td>
<td>0.27</td>
</tr>
<tr>
<td>Myalgia</td>
<td>30/72 (41.7)</td>
<td>8/16 (50.0)</td>
<td>0.59</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>29/72 (40.3)</td>
<td>1/15 (6.7)</td>
<td>0.015</td>
</tr>
<tr>
<td>Localized</td>
<td>15/29 (51.7)</td>
<td>0/1</td>
<td>1.00</td>
</tr>
<tr>
<td>Generalized</td>
<td>14/29 (48.3)</td>
<td>1/1 (100.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>27/58 (46.6)</td>
<td>4/10 (40.0)</td>
<td>0.75</td>
</tr>
<tr>
<td>Edema</td>
<td>23/64 (35.9)</td>
<td>4/16 (25.0)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>ZIKV-Positive Women (N=72)</th>
<th>ZIKV-Negative Women (N=16)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>20/72 (27.8)</td>
<td>2/16 (12.5)</td>
<td>0.34</td>
</tr>
<tr>
<td>Duration &lt;24 hr</td>
<td>12/20 (60.0)</td>
<td>0/2</td>
<td>0.20</td>
</tr>
<tr>
<td>Duration ≥24 to &lt;72 hr</td>
<td>8/20 (40.0)</td>
<td>2/2 (100.0)</td>
<td>0.20</td>
</tr>
<tr>
<td>Median temperature (range) — °C</td>
<td>37.6 (37.5–38.0)</td>
<td>38.2 (37.5–39.0)</td>
<td>1.00†</td>
</tr>
<tr>
<td>Photophobia</td>
<td>17/72 (23.6)</td>
<td>5/16 (31.2)</td>
<td>0.53</td>
</tr>
<tr>
<td>Anorexia</td>
<td>16/72 (22.2)</td>
<td>2/16 (12.5)</td>
<td>0.51</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16/72 (22.2)</td>
<td>5/16 (31.2)</td>
<td>0.52</td>
</tr>
<tr>
<td>Nausea or vomiting</td>
<td>15/72 (20.8)</td>
<td>6/16 (37.5)</td>
<td>0.20</td>
</tr>
<tr>
<td>Bleeding, petechia, or enanthema</td>
<td>15/72 (20.8)</td>
<td>2/16 (12.5)</td>
<td>0.73</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>9/71 (12.7)</td>
<td>1/16 (6.2)</td>
<td>0.68</td>
</tr>
<tr>
<td>Dizziness or light-headedness</td>
<td>8/71 (11.3)</td>
<td>1/16 (6.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Respiratory symptoms: coryza, cough, or sore throat</td>
<td>5/72 (6.9)</td>
<td>3/16 (18.8)</td>
<td>0.16</td>
</tr>
<tr>
<td>Dysuria</td>
<td>1/69 (1.4)</td>
<td>0/16</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Pregnancy Outcomes in +ZIKV

- 2/72 → Early pregnancy loss
- 42/70 (60%) → U/S
- 12/42 (29%) → abnormal U/S or Doppler
  - Intrauterine death - 2
  - IUGR -5
  - CNS abnormalities -7
  - Abn AFI or Doppler -7
  - Additional malformation -1

Pregnancy Outcomes

12 Abnormal U/S:

- 2 IUFD → stillbirth
- 6 in utero
- 4 live births
  - 1 severe microcephaly, cerebral calcifications, global cerebral atrophy, macular hypoplasia/scarring
  - 1 severe IUGR, oligohydramnios, placental insufficiency, macular hypoplasia
  - 1 IUGR, anhydramnios → emergent delivery → lethargy, poor suck reflex, normal growth measures at birth → doing well
  - 1 IUGR → SGA in NICU

2 Normal U/S + Delivered: Both normal neonatal exam

Conclusions of Prospective Cohort Study

- Distinctive clinical features:
  - Conjunctivitis, lymphadenopathy, pruritic maculopapular rash
  - No respiratory symptoms
  - Fever unreliable in case definition

- Most concerning:
  - Healthy ♀, no other risk factors
  - Microcephaly not an isolated finding
  - *29% abnormality rate, *4.8% IUFD rate

- Supports link between maternal ZIKV infection and fetal-placental abnormalities

Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study

Simon Cauchemez, Marianne Besnard, Priscillia Bompard, Timothée Dub, Prisca Guillemette-Artur, Dominique Eyrolle-Guignot, Henrik Salje, Maria D Van Kerkhove, Véronique Abadie, Catherine Garel, Arnaud Fontanet*, Henri-Pierre Mallet*

- Retrospectively analysed serological & surveillance data to estimate the weekly probability of Zika infection during the epidemic
- Searched medical records for cases of microcephaly
- Models were made to assess associated risk of Zika virus & microcephaly & to assess timing of risk of Zika virus exposure in pregnancy

Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study

- Outbreak October 2013 to April 2014
- 66% of general population infected
- 8 cases of microcephaly identified
  - 7 cases from March to July 10, 2014
- Timing of cases best explained by risk of first trimester exposure
- Risk of microcephaly associated with Zika was 95 cases per 10,000 women in the first trimester (approx 1%)

Limitations of data

- Historic rates of microcephaly in Brazil 0.5 per 10,000
  - Well below expected 1-2 cases per 10,000
  - ? Under ascertainment of microcephaly in Brazil prior to outbreak
  - Current rate 20 per 10,000

- Head circumference not routinely measure on newborns prior to November 2015

- Not all have serology for Zika, rash non-specific
  - ? Recall bias, misclassified as Zika infection

- No comment on other features of congenital infections
  - HSM, rash, chorioretinitis, calcification

Available from: http://www.cdc.gov/mmwr/volumes/65/wr/mm6503a1.htm
PublicHealthOntario.ca
Pregnancy recommendations
Pregnant Women Advised to Consider Postponing Travel to Areas where Zika Virus Transmission is Ongoing

ACOG Update on Zika Virus

January 16, 2016

ACOG is reviewing this information in consultation with CDC, and other subject matter experts to inform its membership. Check ACOG’s Practice Advisories and Immunization for Women websites for updates in the coming days.

CDC has issued an official health advisory and travel alert (Level 2—Practice Enhanced Precautions) for people traveling to regions and certain countries where Zika virus transmission is ongoing: Brazil, Colombia, El Salvador, French Guiana, Guatemala, Haiti, Honduras, Martinique, Mexico, Panama, Paraguay, Suriname, Venezuela, and the Commonwealth of Puerto Rico. This list is being updated frequently and further spread to other countries in the region is likely. Please check the Pan American Health Organization website for an up to date list.

For additional details, visit CDC's health advisory and travel alert pages. This is a rapidly evolving issue and ACOG and SMFM will continue to update members as information becomes available.
Protect yourself from bites:

1. Cover up:
   - Wear light-coloured, long-sleeved, loose fitting, tucked-in shirts, long pants, shoes or boots (not sandals), and a hat.

2. Use insect repellent on exposed skin
   - It is recommended that adults use repellents that contain DEET (20-30%) or icaridin (20%).
   - It is recommended that children 6 months to twelve years of age use repellents that contain icaridin (20%). As a second choice, this age group can use repellents with age-appropriate DEET concentrations as per label.
   - If bites cannot be avoided using a physical barrier, consider use of up to 10% DEET or 10% icaridin for infants under six months of age.

3. Protect living areas from mosquito entry:
   - Stay in a well-screened or completely enclosed air-conditioned room.
   - Reduce your risk in work and accommodation areas by closing eaves, eliminating holes in roofs and walls and closing any other gaps.

4. If mosquito entry into living quarters cannot be otherwise prevented (e.g. by screening):
   - Use a bed net (e.g. for sleeping or resting inside), preferably treated with insecticide.
   - Netting can also be used to protect children in playpens, cribs, or strollers.
   - Bed nets will also provide protection against diseases like malaria.

5. Apply a permethrin insecticide to clothing and other travel gear for greater protection
   - Although permethrin is not available in Canada, travel health clinics can advise you how to purchase permethrin and pre-treated gear before or during your trip.
   - Permethrin-treated clothing is effective through several washes.
   - Always follow label instructions when using permethrin.
   - Do not use permethrin directly on skin.
Centers for Disease Control algorithm

Pregnant woman with history of travel to an area with ongoing Zika virus transmission


Test for Zika virus infection

Positive or inconclusive for Zika virus infection

Consider serial fetal ultrasounds
Consider amniocentesis for Zika virus testing

Negative for Zika virus infection

Fetal ultrasound to detect microcephaly or intracranial calcifications

Microcephaly or intracranial calcifications present

Retest pregnant woman for Zika virus infection
Consider amniocentesis for Zika virus testing

Microcephaly or intracranial calcifications not present

Routine prenatal care

Source:
http://www.cdc.gov/mmwr/volumes/65/wr/mm6502e1.htm
Evaluation of neonate

- Newborn to a mother with confirmed Zika or displays signs of congenital Zika virus
  - Referral to Pediatric Infectious Disease Specialist

- At delivery:
  - Neonate serum (PCR & serology)
  - PCR placenta
  - PCR of CSF if obtained

- CBC and liver enzymes
- Hearing & ophthalmologic examination
- Head ultrasound
- Serial neurodevelopment examinations
Additional Recommendations

• For **men** who have traveled to a Zika endemic area, symptomatic or not, who have pregnant partners, should use condoms with sexual activity for the **remainder** of pregnancy

• For **women** who have traveled to a Zika endemic area, symptomatic or not, should avoid conception for **2-3 months**

• For **men** who have traveled to a Zika endemic area, symptomatic or not, should avoid conception for **6 months**
Pope suggests contraceptives could be used to slow spread of Zika
Thu February 18, 2016

(CNN) Pope Francis suggested that contraceptives may be used to prevent the spread of the Zika virus, despite the church's longstanding ban on most forms of birth control.

"Avoiding pregnancy is not an absolute evil," the Pope says.

Case #1

- 32yo women at 22 wks GA
  - Returned from her grandmother’s funeral in Venezuela one week ago
  - A few mosquito bites, no history of illness

- Should she be screened?
Case #1

- Yes!
- Serology testing
Case #1

• Serology test was performed
  • Elisa IgM positive
  • Plaque reduction neutralization titre (PRNT) positive

• How should she and her pregnancy be managed?
Case #1

• Referral to Maternal Fetal Medicine Specialist or Reproductive Infectious Diseases Specialist

• Ultrasound to evaluate fetal growth q 4 wks

• Consider amniocentesis
Case #2

- 37 year old woman at 29 weeks GA
  - Relocated from Guatemala to Canada, one month ago, secondary to Zika scare
  - Recalls numerous mosquito bites

- Should she be screened?
Case #2

• Yes!

• Serology testing

• Results
  • Elisa IgM negative
  • Plaque reduction neutralization titre (PRNT) negative

• How should she and her pregnancy be managed?
Case #2

• Yes!

• Serology testing

• Results
  • Elisa IgM negative
  • Plaque reduction neutralization titre (PRNT) negative

• How should she and her pregnancy be managed?
  • Back to routine care
  • Consider additional USGs at 29-31 and 34-36 weeks
Case #3

• 27yo women at 16 weeks GA
  • Returned from her “Babymoon” in Martinique 6 weeks ago
  • A few mosquito bites, no history of illness

• Serology testing
  • Elisa IgM positive
  • Plaque reduction neutralization titre (PRNT) Inconclusive

• Management?
Case #3

• Referral to Maternal Fetal Medicine Specialist or Reproductive Infectious Diseases Specialist

• Ultrasound to evaluate fetal growth q 4 wks

• Consider amniocentesis
Prevention Strategies (mosquito control, immunization)
What are We Learning about Mosquitos/Vector Control?

- **Aedes aegypti** and **albopictus** susceptible to infection but low competent vectors\(^1\)
  - Rapidity of transmission likely due to immunologically naïve population + intense mosquito exposure

- **Culex quinquefasciatus** could be a competent vector\(^2\), close relative of **Culex pipiens** (WNV vector)
  - Brock University studies of Ontario mosquito vectors, especially **Ae. japonicus**
  - If found to be competent vector, substantial changes in risk, vector control and public education

- Risk of transmission in **Aedes**-affected countries <1% at altitudes >2,000 metres\(^3\)

- Neighbouring US jurisdiction mosquito surveillance control plans, e.g. New York State Zika kits for pregnant women – larvicide, insecticide, condoms

Sources:
- Reuters, March 16, 2016
- Cetron M. Revision to CDC’s Zika Travel Notices: Minimal likelihood for mosquito-borne Zika virus transmission at elevations above 2,000 meters. MMWR Morb Mortal Wkly Rep. 2016;65(10):267-8. Available from: [http://www.cdc.gov/mmwr/volumes/65/wr/mm6510e1.htm](http://www.cdc.gov/mmwr/volumes/65/wr/mm6510e1.htm)
What’s the risk of Zika coming to your city?

Mosquito Vector Control

• Personal protection measures → acceptability? compliance? effectiveness?

• Release of genetically-modified adult male mosquitoes:
  • WHO-supported
  • Evidence of impact on mosquito populations
  • How much of a reduction is necessary to reduce/eliminate risk?
  • Replacement of *Aedes aegypti* with *Aedes albopictus* or other competent mosquito vectors?
  • Impact on incidence and complications, especially pregnancy-related?
  • Scale up, sustainability, cost-effectiveness?
  • Potentially part of the answer, not THE answer
Mosquito Vector Control (cont’d)

- Larviciding → myriad breeding sites
- Adulticiding:
  - Magnitude of applications
  - Reduction of adult mosquito populations
  - Impact on disease transmission/impacts?
  - Cost/sustainability
- Eradication of breeding sites:
  - El Nino and rainfall patterns
  - Scale of what would be required, given *Aedes* breeding capacity
  - Huge community capacity/compliance
- Re-introduce DDT → indoor use to reduce environmental impacts
Sources:
Brazil Communications Company – EBC. Available from:
Brazil Communications Company – EBC. Available from:
National Post article February 18 2016

From Ebola to Zika, the Winnipeg virology lab solving all the world’s disease problems lately

ZIKV Vaccines

Options for vaccine development:

• Inactivated whole virus
• Subunit vaccines
• Live attenuated →
  • ?? with pregnant women
• Chimeras, using existing viral platforms
• DNA vaccines → virus-like particles
Other Flavivivirus Vaccines

• Dengue vaccine (Sanofi-Pasteur) → chimeric tetravalent dengue vaccine, live attenuated Yellow Fever virus 17D strain with structural genes replaced with the corresponding dengue structural genes

• WNV investigational vaccine (HydroVax-001) → novel, hydrogen peroxide-based process to inactivate virus

• Japanese equine encephalitis → inactivated Vero cell culture-derived, adsorbed vaccine

• Yellow fever → live attenuated vaccine
Issues in ZIKV Vaccine Development

• At least 18 months before vaccines could be tested in large-scale trials (WHO Feb 16 2016)

• Challenges of developing/testing a vaccine for women in reproductive years/pregnant:
  • Outcomes of Zika in pregnancy not established/fully characterized
  • Ethical challenges—risks vs. benefits mother/fetus, consent
  • Research design—vaccination prior to/early pregnancy
  • Baseline outcome rates (e.g., miscarriage) across geographies
  • Data monitoring

• Time needed for animal models/studies and Phase 1, 2 and 3 trials

• Association of GBS with ZIKV infection → Phase 3 and 4 detection/monitoring
Issues in ZIKV Vaccine Development (cont’d)

• WHO Emergency Assessment and Listing procedure for the use of experimental products during an epidemic:
  • accelerated assessment process
  • established during the Ebola epidemic
  • ensure products meet acceptable levels of quality, safety and efficacy, even if evaluation fast-tracked

• Regulatory approvals, manufacturing and distribution

• Funding, education, infrastructure, delivery, Phase 4 capacity
Intensified research on Zika and neuro/GBS/microcephaly

- Standardize and enhance surveillance
- Standardize case definition for “congenital Zika syndrome”
- Genetic sequences/clinical effects of different ZIKV strains
- Neuropathology of microcephaly
- Case-control/cohort studies in recently-affected areas
- Causative factors and/or co-factors for complications
- Animal models for experimental studies

Natural history of Zika infections

- Rates/consequences of asymptomatic infections
- Persistence of viral excretion

Rapid sharing of clinical, virological and epi data
WHO IHR Emergency Committee Recommendations

Vector control

- Mosquito surveillance
- Insecticide resistance
- Aggressive promotion/implementation
- Strengthen long term vector control capacity

Clinical care

- Pregnant women—counselling and monitoring
- Care for congenital consequences, neurological/GBS outcomes

Diagnostic tests, treatments, vaccines, novel vector control strategies