

# Zika and Pregnancy: What We Know So Far

September, 2016

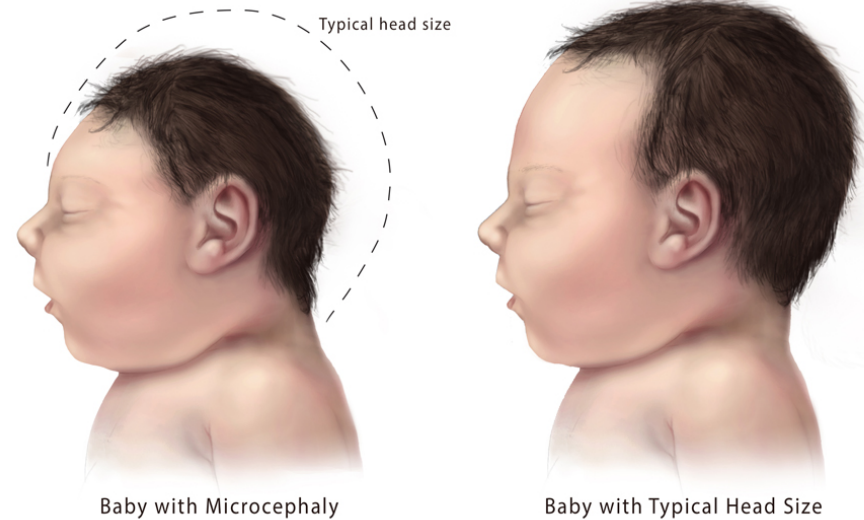
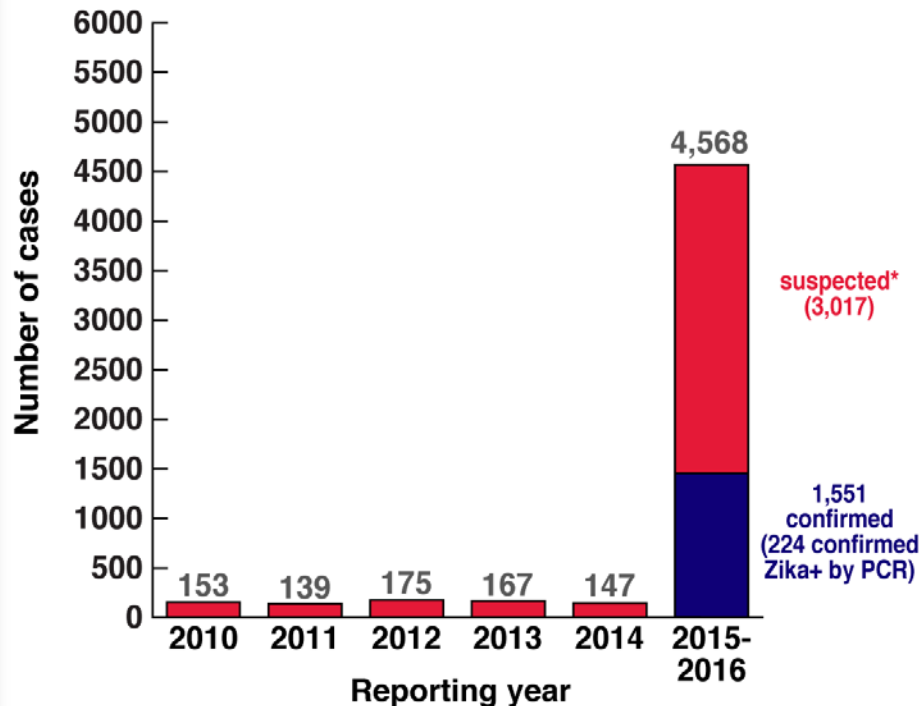


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# Brazil Zika Outbreak

- May 2015: First infection in Brazil
- October 2015: increase in microcephaly

Microcephaly cases in Brazil 2010-14;  
suspected/confirmed cases 2015-2016



\*does not include 3,262 cases investigated and discarded

Source: Brazilian MOH; data as of 6/4/2016.

# Guidance from other Viral Infections?

- Well-established risks and effects of maternal infection with rubella and CMV
- Both with greater impact with 1<sup>st</sup> trimester infection but still impact later
  - Congenital rubella in 90% of 1<sup>st</sup> Δ infections
  - CMV: 30% infection risk across pregnancy, with greater risk of severe impact with 1<sup>st</sup> Δ infection
- US prevalence of microcephaly: 6 cases per 10,000 live births (range: 2-12)
  - With Zika, risk of developmental brain abnormalities will be greater than risk of microcephaly

# Microcephaly: the *most apparent* marker?

- Microcephaly is a very specific diagnosis, and typically unusual as an isolated finding: initially seen in *newborns*
  - On ultrasound, typically defined as HC < 3<sup>rd</sup> %ile for GA
- Microcephaly became an *early trigger* to search for Zika association, but spectrum of disease became apparent
  - Microcephaly can occur as a result of a **fetal brain disruption sequence**: this appears to be pathology of Zika infection
- Polynesia outbreak in 2014: higher rates of fetal CNS abnormalities in some women who tested positive for Zika, though none had had symptoms (*Euro Ctr Dis Prev Control 2015*)

# Fetal Brain Anomalies

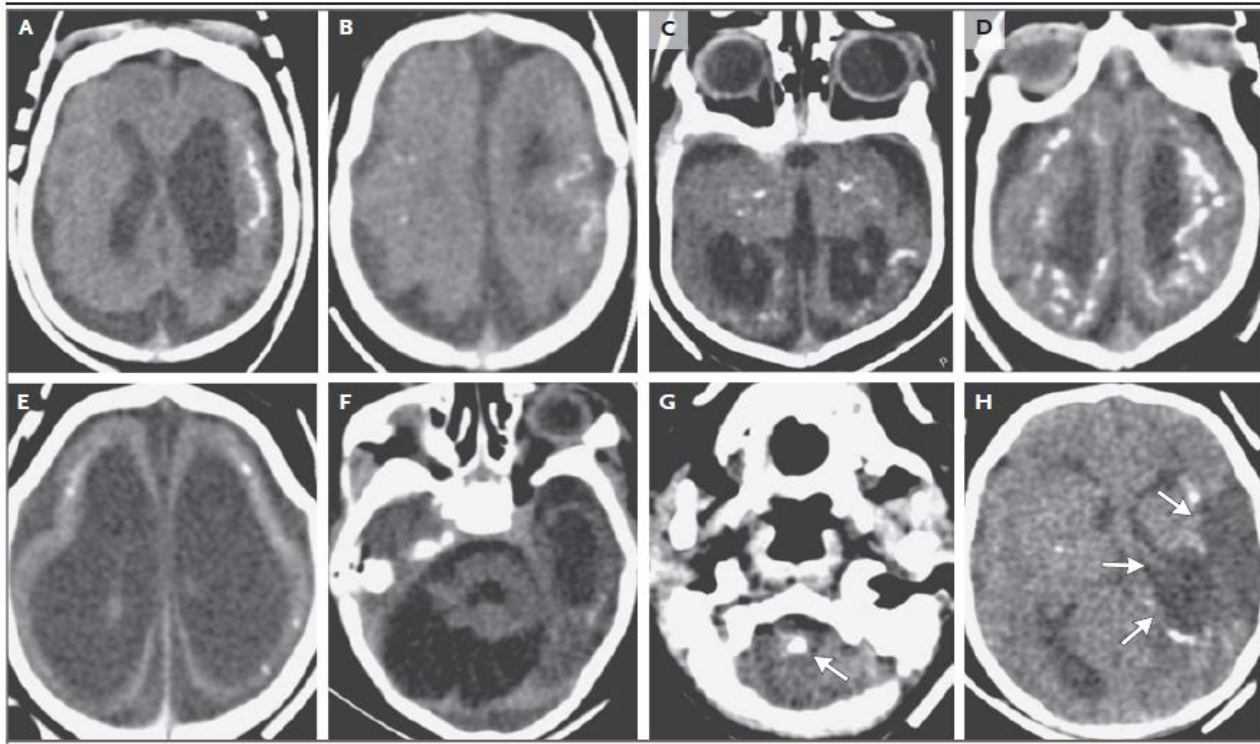
- Microcephaly
- Hydrocephalus/hydranencephaly
- Absent structures: (CC, pons, cerebellar vermis)
- Neuronal migration disorders (lissencephaly)
- Fetal brain disruption sequence
- Cerebral calcifications
- Brain asymmetry



# CT Scans Reveal Extensive Abnormalities

23 infants with microcephaly in Pernambuco, Brazil

*The NEW ENGLAND JOURNAL of MEDICINE*



- Intracranial calcifications
- Global cortical hypogyration
- Ventriculomegaly
- Global cerebellar hypoplasia



Original Investigation

# Ocular Findings in Infants With Microcephaly Associated With Presumed Zika Virus Congenital Infection in Salvador, Brazil

Bruno de Paula Freitas, MD; João Rafael de Oliveira Dias, MD; Juliana Prazeres, MD; Gielson Almeida Sacramento, BS; Albert Icksang Ko, MD; Maurício Maia, MD, PhD; Rubens Belfort Jr, MD, PhD

- 29 infants with microcephaly
  - 79% with suspected Zika
    - 18 in first trimester
  - 29% with ocular findings
    - Bilateral macular and perimacular lesions
    - Optic nerve abnormalities

Figure 4. Fundus Photographs of a 1-Month-Old Infant

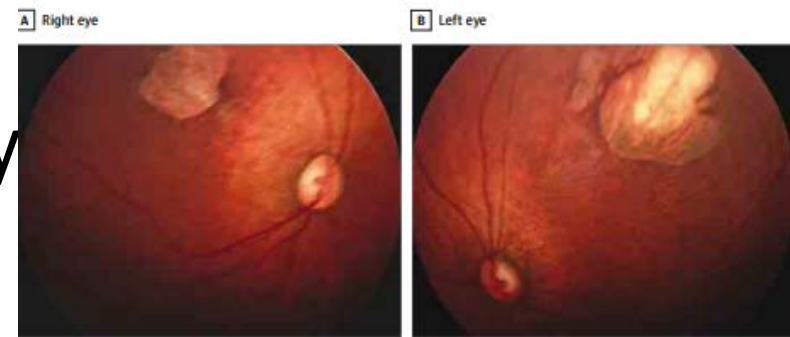
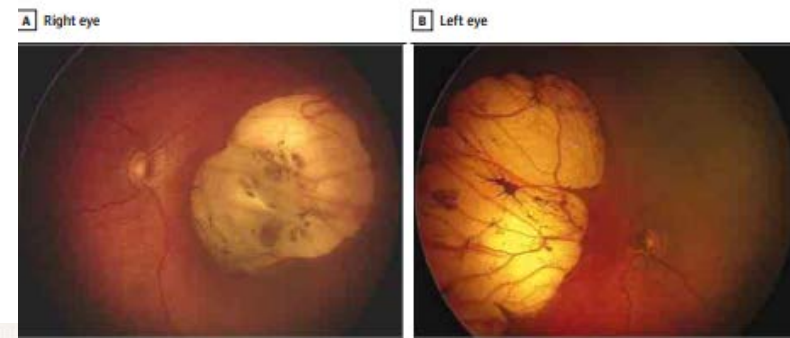
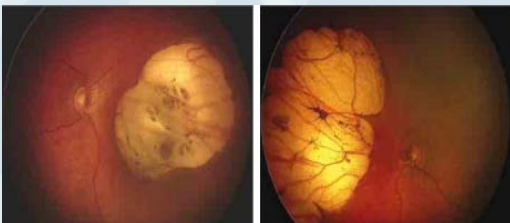
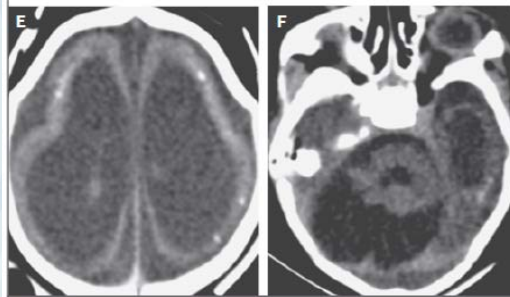
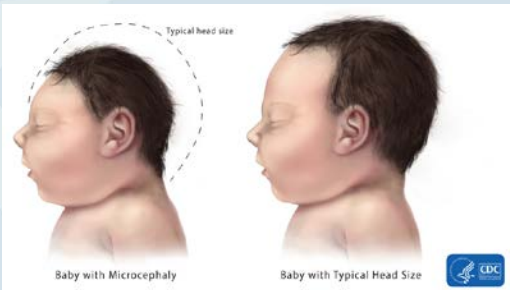


Figure 5. Fundus Photographs of a 20-Day-Old Infant



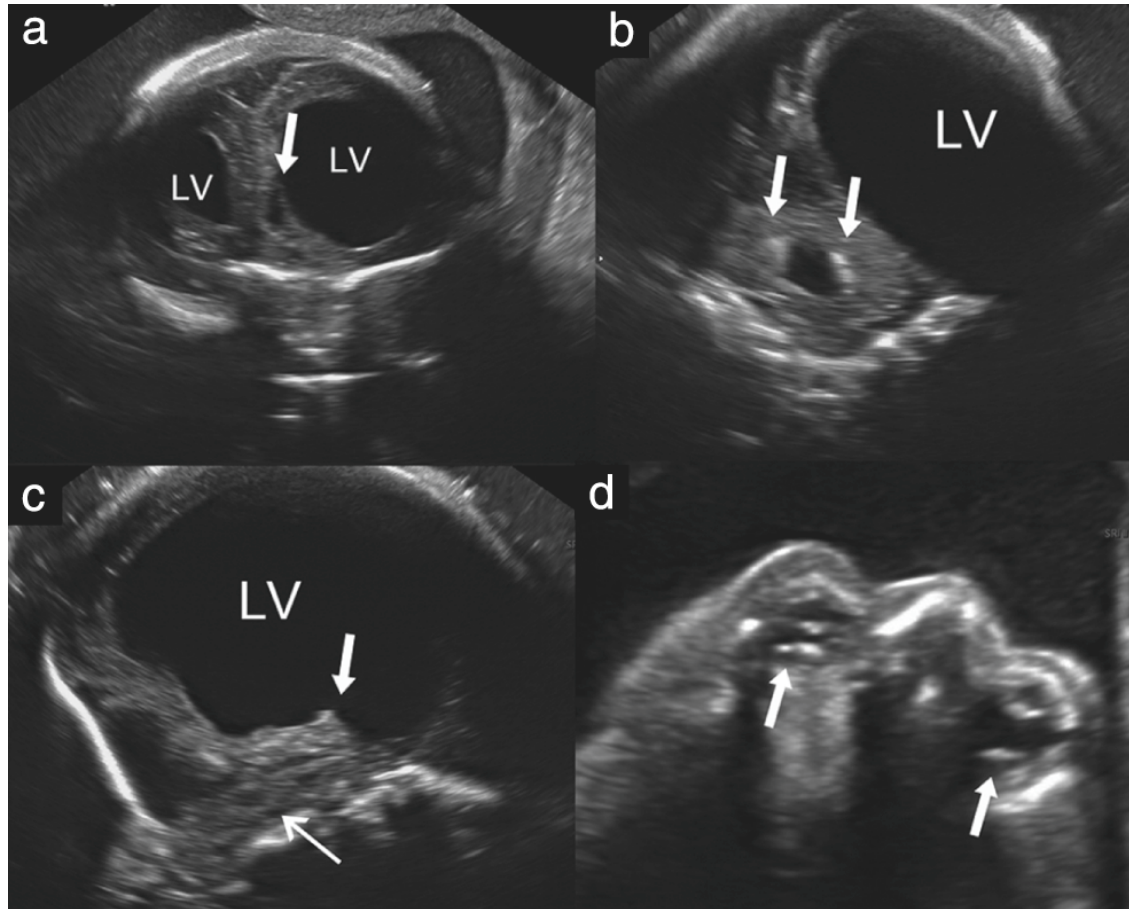
# Zika Associated Pregnancy Outcomes

- Fetal loss/miscarriage, stillbirth
- Fetal growth abnormalities
- Fetal brain anomalies
  - Microcephaly
  - Ventriculomegaly
  - Intracranial calcifications
- Eye abnormalities
- Neurologic
  - Hypertonia
  - Arthrogryposis
  - Seizures
  - Neurobehavioral anomalies

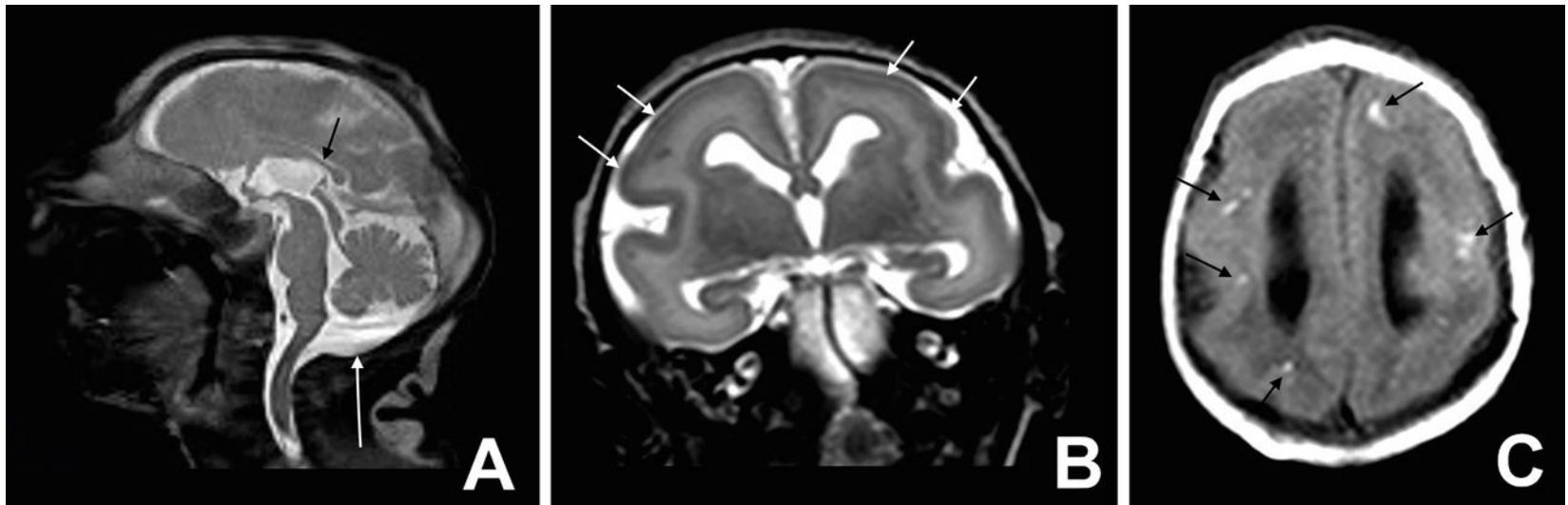




## Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?

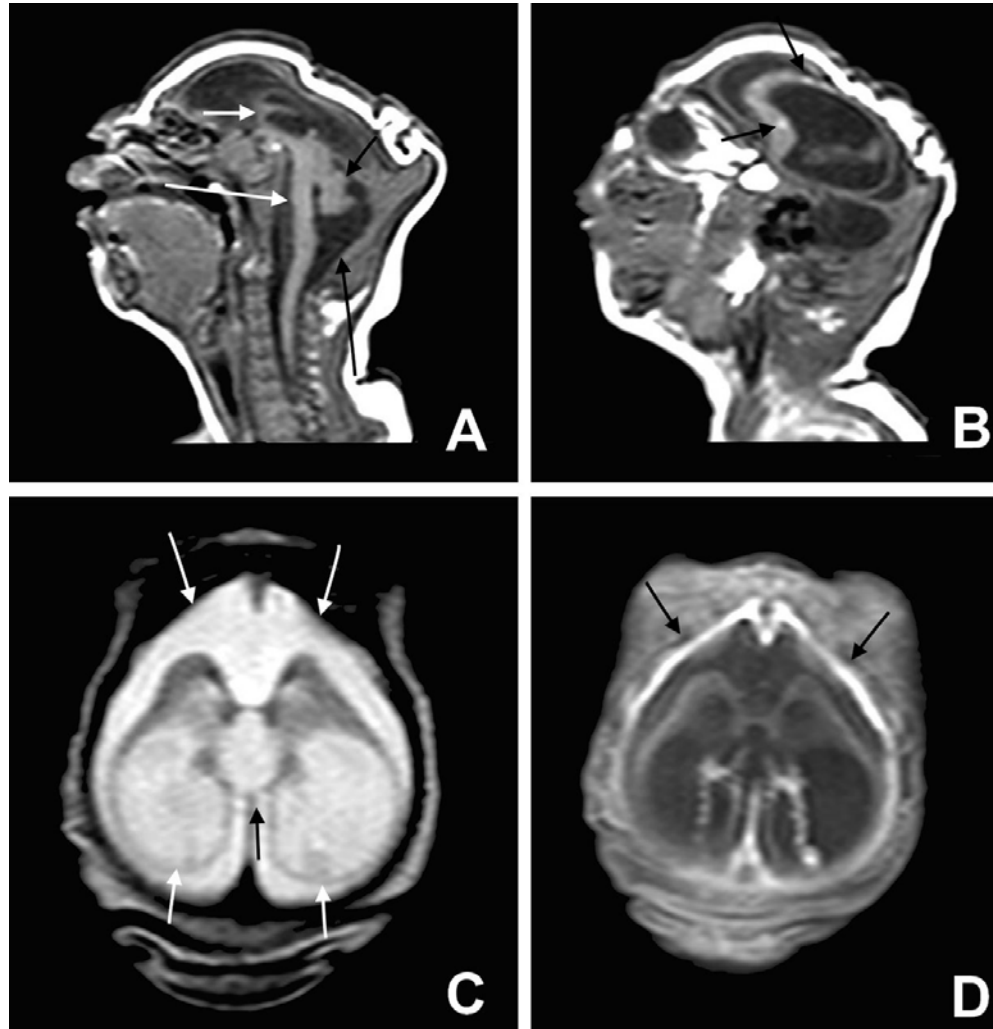


**Fig 4 Microlissencephaly.**



Maria de Fatima Vasco Aragao et al. *BMJ*  
2016;353:bmj.i1901

**Fig 3 Severe microcephaly.**



**Maria de Fatima Vasco Aragao et al. BMJ  
2016;353:bmj.i1901**

# Prospective NEJM series, Brazil: Background

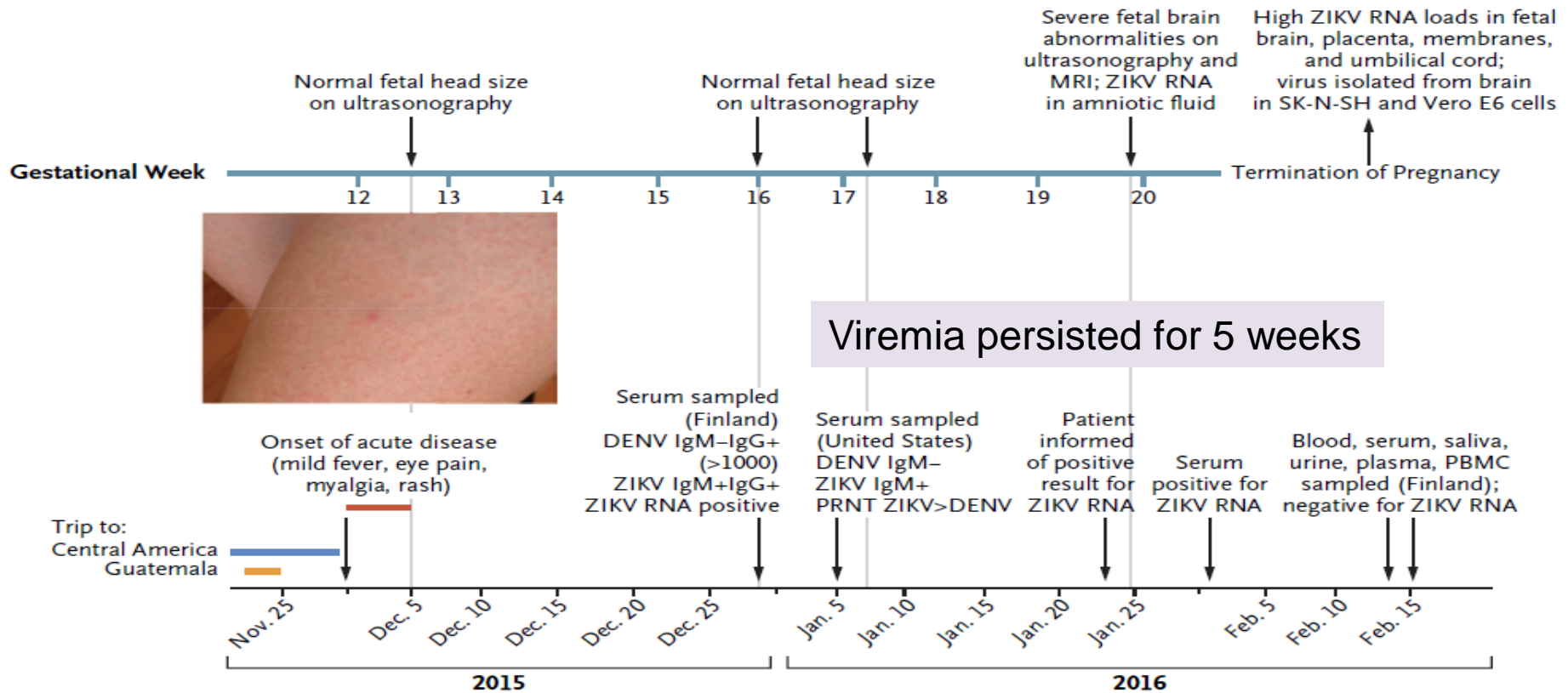
- Brazil group had been conducting surveillance for dengue in population of Rio de Janeiro since 2007
  - In 2015, noted increase in dengue-like illness with rash, coinciding with surge of similar cases in NE Brazil → ID as Zika
- Study cohort: 88 symptomatic pregnant women (9/15-2/16)
  - 82% (72/88) tested positive for ZIKV (PCR) in blood, urine, or both
  - Serial U/S done: 20-30+ weeks
  - Timing of infection: 5-38 weeks of gestation

# Prospective NEJM series, Brazil: Results

- Of 72 women with PCR-positive test results
  - > 50% reported an ill family member; **21%: partner had been ill**
- 2 women miscarried in 1<sup>st</sup> Δ; 42 (60%) of others had u/s
  - 28 women declined u/s: either too far or fear of finding anomalies
- **Abnormal u/s results seen in 12/42 (29%) pts with Zika infx**
  - No abnormalities seen in any of the 16 Zika-negative women
  - IUGR in 5/12 fetuses (42%), with or without microcephaly
  - Cerebral calcifications in 4/12, other CNS anomalies in 2 fetuses
  - 2 IUFDs @ 30 and 38 wks: women infected at 25 and 32 wks



# Pregnancy: Prolonged Viremia



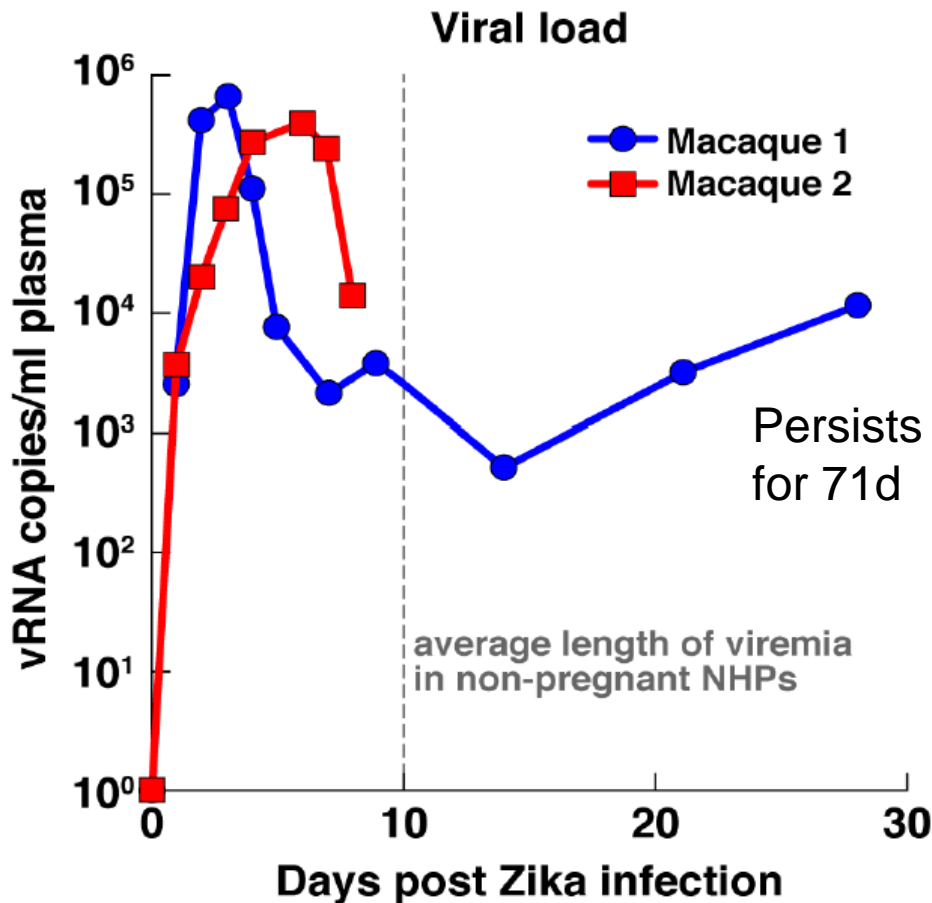
**Figure 1. Timeline of Symptoms and Radiographic and Laboratory Studies.**

This timeline highlights the symptoms of Zika virus (ZIKV) infection in the mother (bottom row) and the corresponding radiographic and laboratory findings in the fetus (top row). The inset photograph shows the mother's rash at the time of the onset of the acute illness. DENV denotes dengue virus, MRI magnetic resonance imaging, PBMC peripheral-blood mononuclear cells, and PRNT plaque-reduction neutralization test.

# Prolonged Detection of Zika Virus in RNA in Pregnant Women

- US Zika Pregnancy Registry
- Prolonged Zika virus RNA detection
  - 4 symptomatic pregnant women up to 46 days after symptom onset
  - 1 asymptomatic pregnant woman 53 days after exposure
- Both symptomatic and asymptomatic pregnant women may have prolonged detection of Zika virus RNA in serum

# Pregnancy: Prolonged Viremia



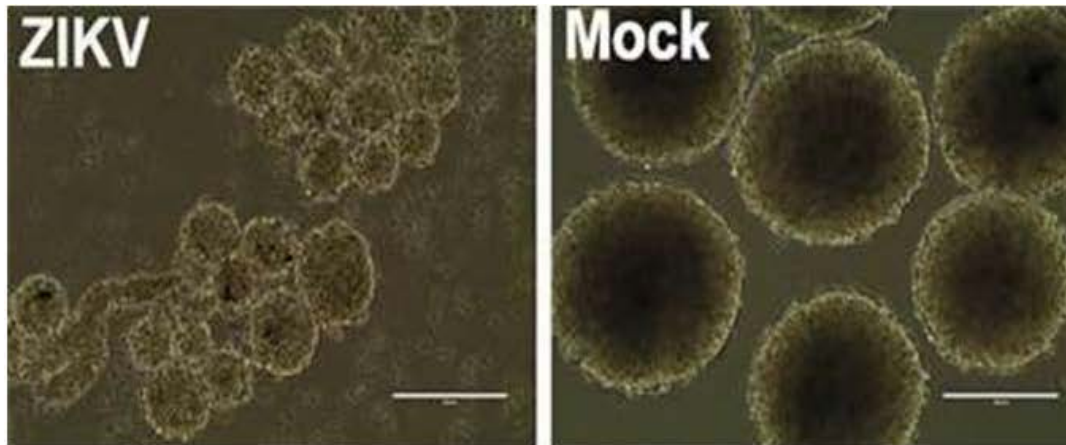
## Virus is likely from fetus

- Virus in plasma, not urine, suggesting the maternal immune system was able to contain virus replication

# Proposed Mechanism for Zika's Impact

- ZIKV directly infects human cortical neural progenitor cells with high efficiency, resulting in stunted growth, transcriptional dysregulation and cell death

Garcez et al, Science 4/14/16  
Tang et al, Cell Stem Cell 4/4/16  
Cugola et al, Nature Res Letter 5/11/16



Human neurospheres infected with the Brazilian Zika virus after 96 hours. Compared to mock-infected controls, the neurospheres show dramatic cell death with arrested growth, resulting in significantly reduced size. Credit: UC San Diego Health

## Recent Research: Zika Mechanisms and Possible Pharmacologic Inhibition

- ZIKV efficiently targets human neural progenitor cells (hNPCs) and negatively impacts their growth
  - Since hNPCs drive development of human cortex, provides potential mechanism for ZIKV-induced microcephaly
  - Research model: brain “organoids”, used as 3D models of early human brain: ZIKV infection reduces thickness of neuronal layers and overall ↓ in organoid size
- Team used “drug repurposing screens” to assess a variety of potential candidate drugs, using finding that ZIKV-induced cell death increases **capsase-3** levels as a marker (*protease enzymes playing essential roles in cell death*)



## Early Thoughts: Drug Candidates?

- Xu group: looked at potential modifiers of ZIKV replication and of cell death
  - Emricasan – pan-capsase inhibitor, currently in phase 2 trials for reduction of liver injury from HCV: can inhibit cell death in ZIKV/brain studies but not replication
  - Niclosamide – currently used antihelminthic: known to inhibit other flaviviruses (Japanese enceph), potential for inhibition
- Onorati et al: looked at current antivirals' potential
  - Found that nucleoside analogs could inhibit ZIKV replication in neuro stem cells and protect against cell death
  - Primary candidate: anti-HCV meds (another flavivirus) – sofosbuvir showed potential in initial studies

# Long Term Pregnancy Outcomes: Evolving

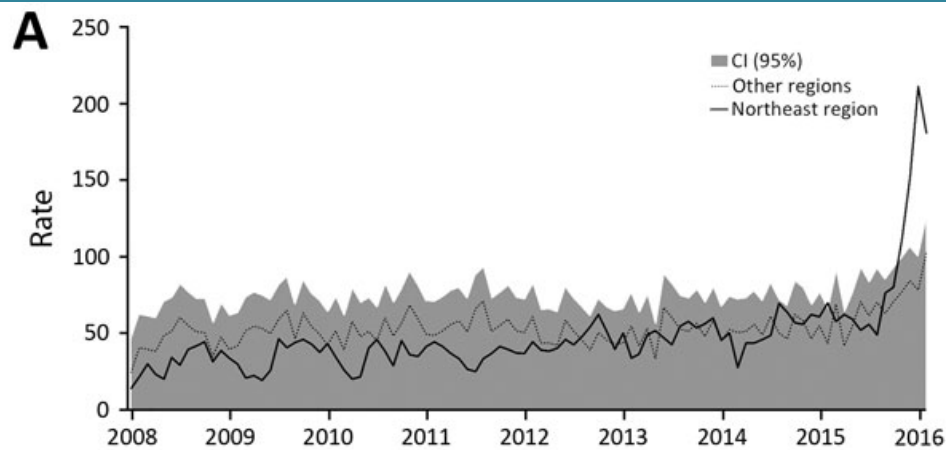
- Emerging reports and series of long-term functional motor and sensory abnormalities
  - van der Linden et al, *BMJ* 8/16: 7 infants with microcephaly and abnormal MRI: also with arthrogryposis: neurologic not muscular
  - Pestorius et al, CDC, 8/4/16: “late-onset microcephaly” in series from Brazil: normal head size at birth, abnormal by 6 months
- Anticipate a spectrum of outcomes?
  - Developmental delay
  - Intellectual impairments
  - Mental disorders – autism, schizophrenia, etc
  - Motor abnormalities

# Zika-Related Arthrogryposis

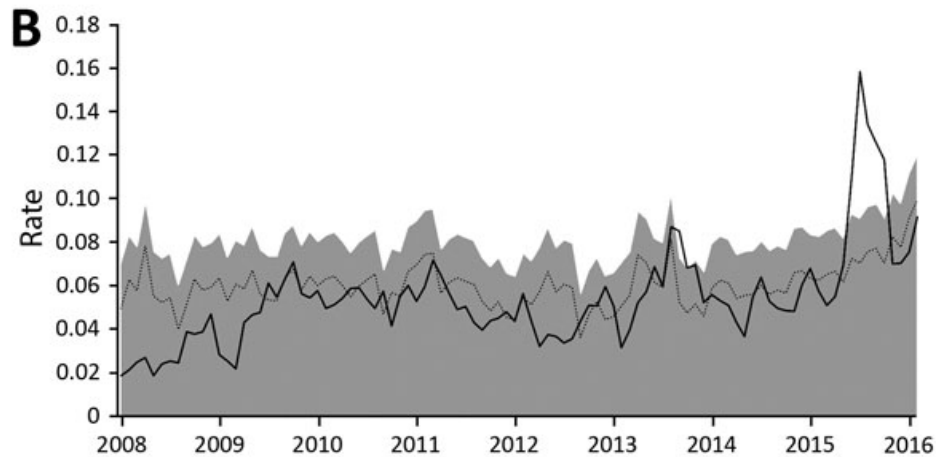


# Increased Hospitalizations for Neuropathies in Brazil

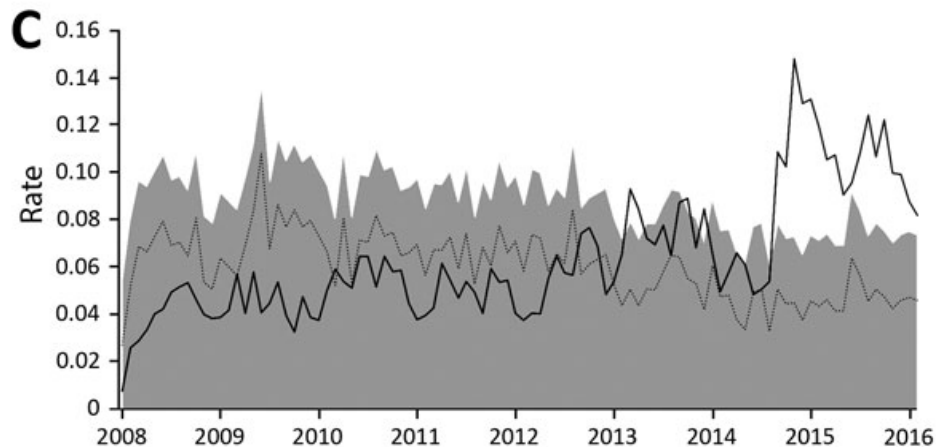
- Recent study reported on rates of hospital admissions in Brazil for pediatric/congenital neurologic issues
  - Tracked hospital records by ICD-10 coding for “congenital malformations of the nervous system” from 1/08-2/16
- Stable mean rate until Nov 2015, when increase seen from 40 to 170 hospitalizations /100,000 live births
  - RR 4.2 (95% CI 3.8-4.6)
- Significant increases seen not just in malformations but for encephalitis, myelitis, and encephalomyelitis as well



A. Congenital malformations of nervous system per 100,00 live births



B. Guillain-Barre syndrome per 100,000 residents



C. Encephalitis, myelitis, encephalomyelitis per 100,000 residents



# Experimental Model for Zika Infection in Pregnancy

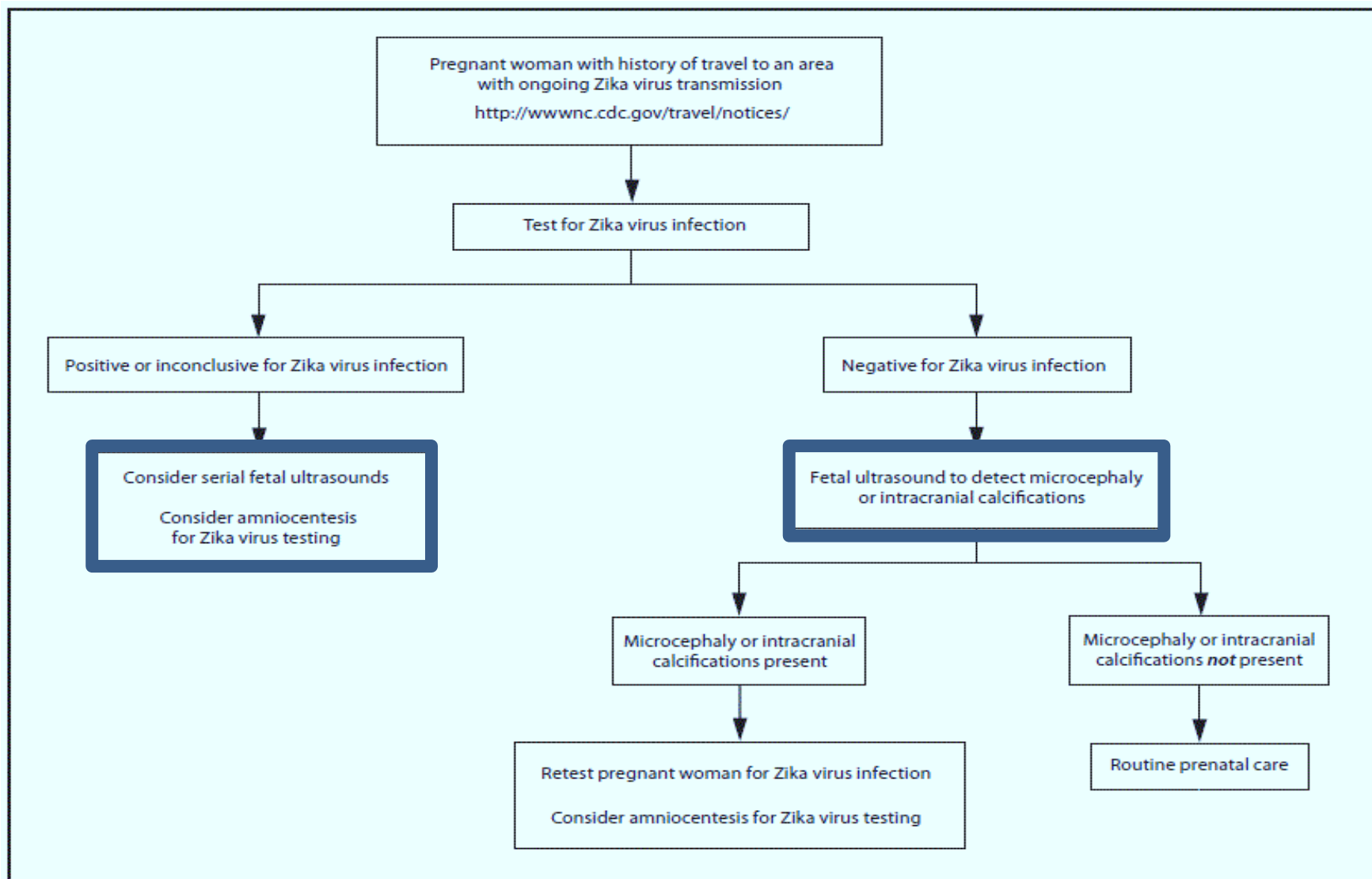
- Researchers at U of Wash described a primate model
  - Demonstrated development of fetal brain lesions within 10 days of ZIKV infection of a pregnant macaque at a GA comparable to 28 weeks of human pregnancy
  - Pregnant animal had no ZIKV symptoms
  - Weekly ultrasound showed lag of fetal head measurements that ultimately fell to  $< 3$  SD below normal
  - 1<sup>st</sup> MRI done 10 days after infection and showed dense foci surrounding lateral ventricles in brain
  - Fetal autopsy showed ZIKV-RNA in brain and diffuse brain injury
- Potential model for evaluation of prevention and therapeutic interventions

# Zika – Education and Testing

# What do we tell our pregnant patients?

- How much fetal risk with confirmed maternal infection?
  - Based on current data, **range may be as high as 29%**
  - Rates are derived from methodologically diverse studies
- Despite earlier reports, recent data suggest later GA at infection does not exclude potential adverse impact
- Pregnant women **should not travel** to areas with active Zika transmission
- If in an area with transmission, protection and prevention strategies are important – **and repellent for 3 weeks after return from these areas**
  - DEET, picaridin most effective --- both fine for use during pregnancy

# Testing algorithm for a pregnant woman possible Zika exposure



# Zika Testing – Newest CDC guidelines

- Expanded testing for pregnant women
  - Attempt to increase the proportion of pregnant women with Zika infection who receive definitive dx
  - Expand testing for women in areas with ongoing transmission (IgM once/trimester), with PCR for symptoms
  - Recognizing risks of sexual transmission regardless of whether sexual partner who traveled to risk area had symptoms or not → also ask partner travel history
  - Recognizing longer time for viremia in some pregnant women compared to nonpregnant adults

## Zika Testing – What Testing should be done?

- PCR done for all pts with symptoms within **2 weeks after onset of symptoms**: test blood AND urine
- PCR for asymptomatic: only in pregnant women with exposure in past 2 weeks (including sex w/ male or female partner who traveled)
  - If PCR on pregnant woman negative, still do antibody testing **2-12 weeks after exposure**
- All other pregnant patients with exposure (including sexual contact with traveler): testing for **Zika-specific IgM antibodies**
  - Typically develop toward the end of the first week of illness
  - **Testing in asymptomatic patients no earlier than 2 weeks after exposure (no later than 12 weeks) : TAT 2-3 weeks**
  - If Ab (+), then further testing done to confirm and PCR



# Sexual Transmission: What We Don't Know

- For how long after the infection semen/vaginal fluids can infect a sexual partner
  - *Lancet* 6/7/16: transmission through semen 34-41 d after infection
  - *Lancet* 8/2016: Zika RNA found in semen after 90 days
  - *Eurosurveillance* 8/11/16: RNA (+) in urine up to 91 days and in semen 134 days after sx
- If Zika can be transmitted through saliva or other body fluids
- Transmission risk/duration after ***asymptomatic*** infection

# Sexual Partner concerns/guidelines

- Sexual transmission of Zika virus can occur
  - Male/female, female/male, male/male all reported
- Pregnant women whose male partners have or are at risk for Zika virus infection should consider using condoms or abstaining from sexual intercourse – ***duration of pregnancy***

## Suggested timeframe to wait before trying to get pregnant

<b>Possible exposure via recent travel or sex without a condom with a man infected with Zika</b>		
	<b>Women</b>	<b>Men</b>
<b>Zika symptoms</b>	Wait at least 8 weeks after symptoms start	Wait at least 6 months after symptoms start
<b>No Zika symptoms</b>	Wait at least 8 weeks after exposure	Wait at least 8 weeks after exposure. Talk with your healthcare provider
<b>People living in areas with Zika</b>		
	<b>Women</b>	<b>Men</b>
<b>Zika symptoms</b>	Wait at least 8 weeks after symptoms start	Wait at least 6 months after symptoms start
<b>No Zika symptoms</b>	Talk with doctor or healthcare provider	Talk with doctor or healthcare provider

# Zika's Additional Impact on OBGYN Care

- Tissue/organ donation – including egg/sperm donors
  - Donors are **ineligible for 6 months** if dx'd with ZKVD, in an area with active transmission, or had sex with a male partner with either of those risks
  - Also applies to umbilical cord blood and placenta
  - ***FDA 3/1/16, affirmed by ASRM 3/4/16***
- Blood donation
  - Major impact on blood bank capabilities: for US, greatest impact in Puerto Rico (*also Miami*) -- no reported cases via transfusion, but local blood collections stopped 3/1 pending PCR
  - Investigational use of Zika PCR for PRBCs/WB (Apr 4)
  - FDA statement (2/16/16): 4 week waiting period for potential donors -- after illness, travel, or sexual contact

# Zika Immunity -- Issues

- Presumption has been that Zika infection confers immunity
  - Viremia is transient, resulting in response with neutralizing antibodies
- If true, prior infection would prevent risks for a future pregnancy
- No IgG testing exists, and IgM duration limited

## New data on immunity: animal model

- Researchers infected 8 primates with ZIKV (including 2 pregnant –mid 1<sup>st</sup> Δ): all (+) RNA 1 day post-infection
- ZIKV-neutralizing antibodies detected by 21 days after infection in all animals
- Rechallenge with ZIKV 10 weeks after initial infection produced no detectable viral replication (**immunity**)
- Of note, pregnant animals were viremic significantly longer after infection: **mean 45 vs 10 days for nonpreg**
  - Amnios on both monkeys ZIKV-negative
  - Potential impact of 1<sup>st</sup> trimester exposure, comparable to Driggers et al, NEJM 4/2016? (*or.... fetal infection?*)



Aug 4, 2016

Cite as: Abbink *et al.*, *Science*  
10.1126/science.aah6157 (2016).

# Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys

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Zika virus (ZIKV) is responsible for a major ongoing epidemic in the Americas and has been causally associated with fetal microcephaly. The development of a safe and effective ZIKV vaccine is therefore an urgent global health priority. Here we demonstrate that three different vaccine platforms protect against ZIKV challenge in rhesus monkeys. A purified inactivated virus vaccine induced ZIKV-specific neutralizing antibodies and completely protected monkeys against ZIKV strains from both Brazil and Puerto Rico. Purified immunoglobulin from vaccinated monkeys conferred passive protection in adoptive transfer studies. A plasmid DNA vaccine and a single-shot recombinant rhesus adenovirus serotype 52 vector expressing ZIKV prM-Env also elicited neutralizing antibodies and completely protected monkeys against ZIKV challenge. These data support the rapid clinical development of ZIKV vaccines for humans.

# Zika Resources

- CDC Zika website: [www.cdc.gov/zika](http://www.cdc.gov/zika)
- ACOG's Zika webpage: [www.acog.org/zika](http://www.acog.org/zika)
- CA Dept of Public Health webpage for health care professionals
  - [www.cdph.ca.gov/HealthInfo/discond/Pages/ZikaInformationforHealthProfessionals.aspx](http://www.cdph.ca.gov/HealthInfo/discond/Pages/ZikaInformationforHealthProfessionals.aspx)