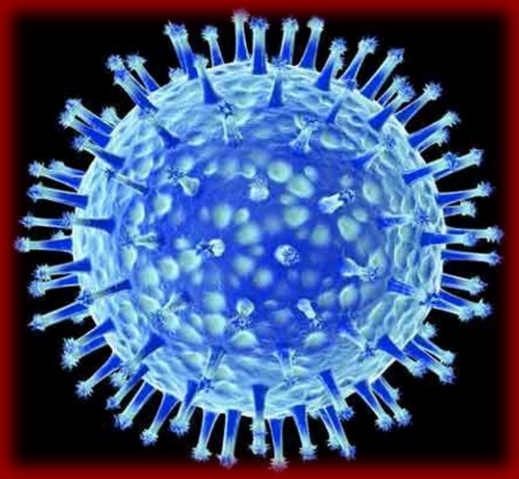
Zika Virus



Division of Infectious Disease
Department of Internal Medicine
Global Health Program Conference
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Zika Virus

- ZIKV is a mosquito-borne ssRNA flavivirus from *Flaviviridae* family, Spondweni serocomplex
  - Dengue Virus
  - West Nile Virus
  - Japanese Encephalitis
  - Yellow Fever
- Initially identified in 1947 : the Zika Forest in Uganda in the Rhesus macaque population
- For most of the past 60 years, Zika remained obscure, confined to a narrow equatorial belt running across Africa and into Asia

Zika Virus

- Most recent, in a series of arthropod - borne viral diseases migrating to the Western Hemisphere in the last 20 years:
  - Dengue (outbreaks over decades, more aggressively in the 1990s)
  - WNV: 1999
  - Chikungunya: 2013

- Pattern of disease emergence?
  - Remote ecologic niches increasingly being perturbed
  - Problems of inner-city crowding / poor sanitation
  - Constant international travel
  - Decreased traditional vector control measures due to expense & public resistance

Transmission

- Decades ago, researchers noted:
  - Enzootic Zika virus spread was linked to human activity
  - *Aedes*-transmitted Zika epizootics tended to follow *Aedes*-transmitted chikungunya epizootics and epidemics
- An analogous pattern began in 2013, when chikungunya spread, and Zika later followed

Transmission

- With the exception of WNV, the other arboviruses that recently reached the Western Hemisphere have been transmitted by *Aedes* mosquitoes, especially *A. aegypti*

Transmission

- Cause for concern: The possibility that Zika may adapt to transmission by *A. albopictus*, a much more widely distributed mosquito found in at least 32 states in the US

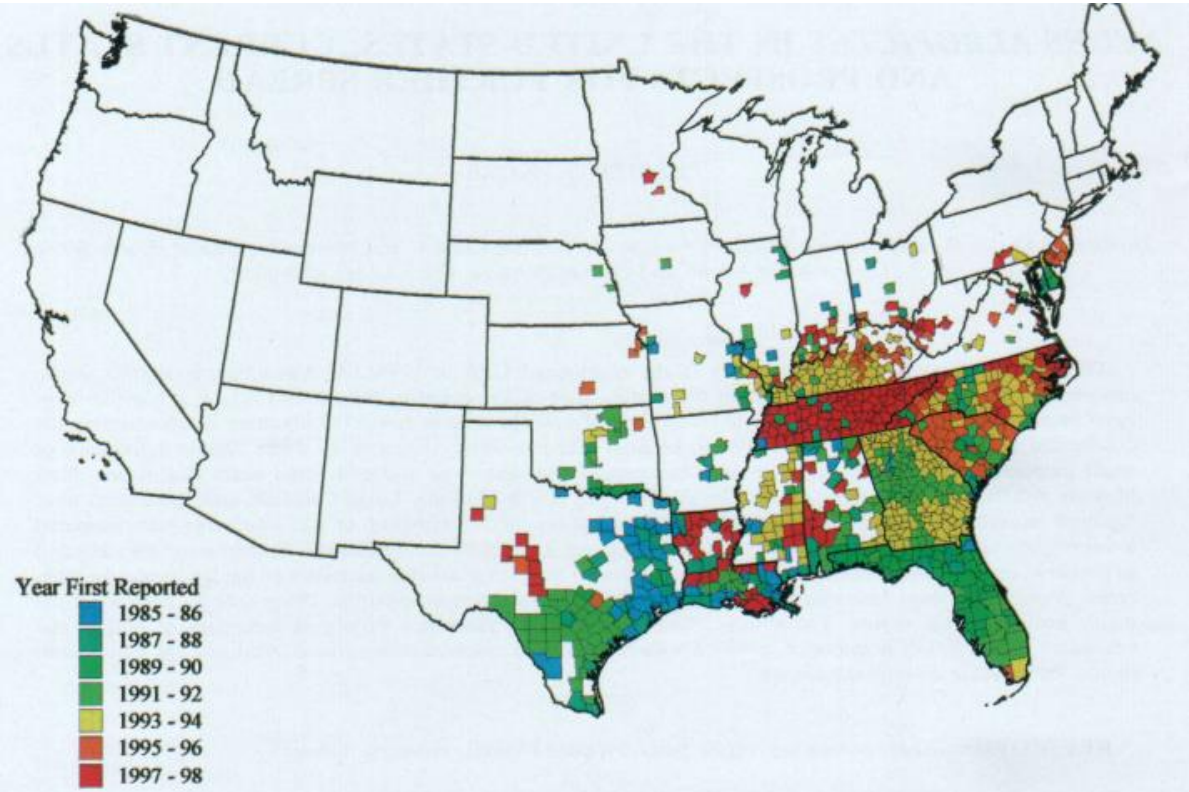

Fig. 1. U.S. counties that have reported *Aedes albopictus* infestations, by year of discovery. Two-year classes were used to simplify the map.

Journal of the American Mosquito Control Association, 15(2):221–227, 1999

Transmission/Incubation Period

- Aedes genus mosquitoes:
  - *Aedes aegypti*
  - *Aedes albopticus*
  - *Aedes hensilii*
  - *Aedes africanus*
  - *Aedes polynensis*
  - *Aedes furcifer*
  - *Aedes taylori*
  - *Aedes luteocephalus*

- Non human primates
- Blood transfusion (3% asymptomatic donors - 2013)
- Perinatal transmission (transplacental/during delivery)
- Sexual transmission (present in semen for 2 weeks after recovery
- Incubation period varies from 3 to 12 days

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PLOS | NEGLECTED TROPICAL DISEASES

Molecular Evolution of Zika Virus during Its Emergence in the 20th Century

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Abstract

Zika virus (ZIKV) is a mosquito-borne flavivirus first isolated in Uganda in 1947. Although entomological and virologic surveillance have reported ZIKV enzootic activity in diverse countries of Africa and Asia, few human cases were reported until 2007, when a Zika fever epidemic took place in Micronesia. In the context of West Africa, the WHO Collaborating Centre for Arboviruses and Hemorrhagic Fever at Institut Pasteur of Dakar (http://www.pasteur.fr/recherche/banques/CRORA/) reports the periodic circulation of ZIKV since 1968. Despite several reports on ZIKV, the genetic relationships among viral strains from West Africa remain poorly understood. To evaluate the viral spread and its molecular epidemiology, we investigated 37 ZIKV isolates collected from 1968 to 2002 in six localities in Senegal and Côte d'Ivoire. In addition, we included strains from six other countries. Our results suggested that these two countries in West Africa experienced at least two independent introductions of ZIKV during the 20th century, and that apparently these viral lineages were not restricted by mosquito vector species. Moreover, we present evidence that ZIKV has possibly undergone recombination in nature and that a loss of the N154 glycosylation site in the envelope protein was a possible adaptive response to the *Aedes dalzieli* vector.

Citation: Faye O, Freire CCM, Iamarino A, Faye O, de Oliveira JVC, et al. (2014) Molecular Evolution of Zika Virus during Its Emergence in the 20th Century. PLoS Negl Trop Dis 8(1): e2636. doi:10.1371/journal.pntd.0002636

Editor: Brian Bird, Centers for Disease Control and Prevention, United States of America

Received March 4, 2013; **Accepted** November 27, 2013; **Published** January 9, 2014

Funding: This study was made possible by the Institute Pasteur of Dakar in Senegal, FAPESP (Fundação de Amparo a Pesquisa do Estado de Sao Paulo, Brazil) projects #00/04205-6 (VGDN program), #08/17013-6 and #10/19341-4. CCMF has a CAPES studentship, AI (project #12/04818-5) and JVCdO received FAPESP scholarships and PMAZ holds a CNPq-PQ grant. Funding was also provided by NIH grant R01-AI069145. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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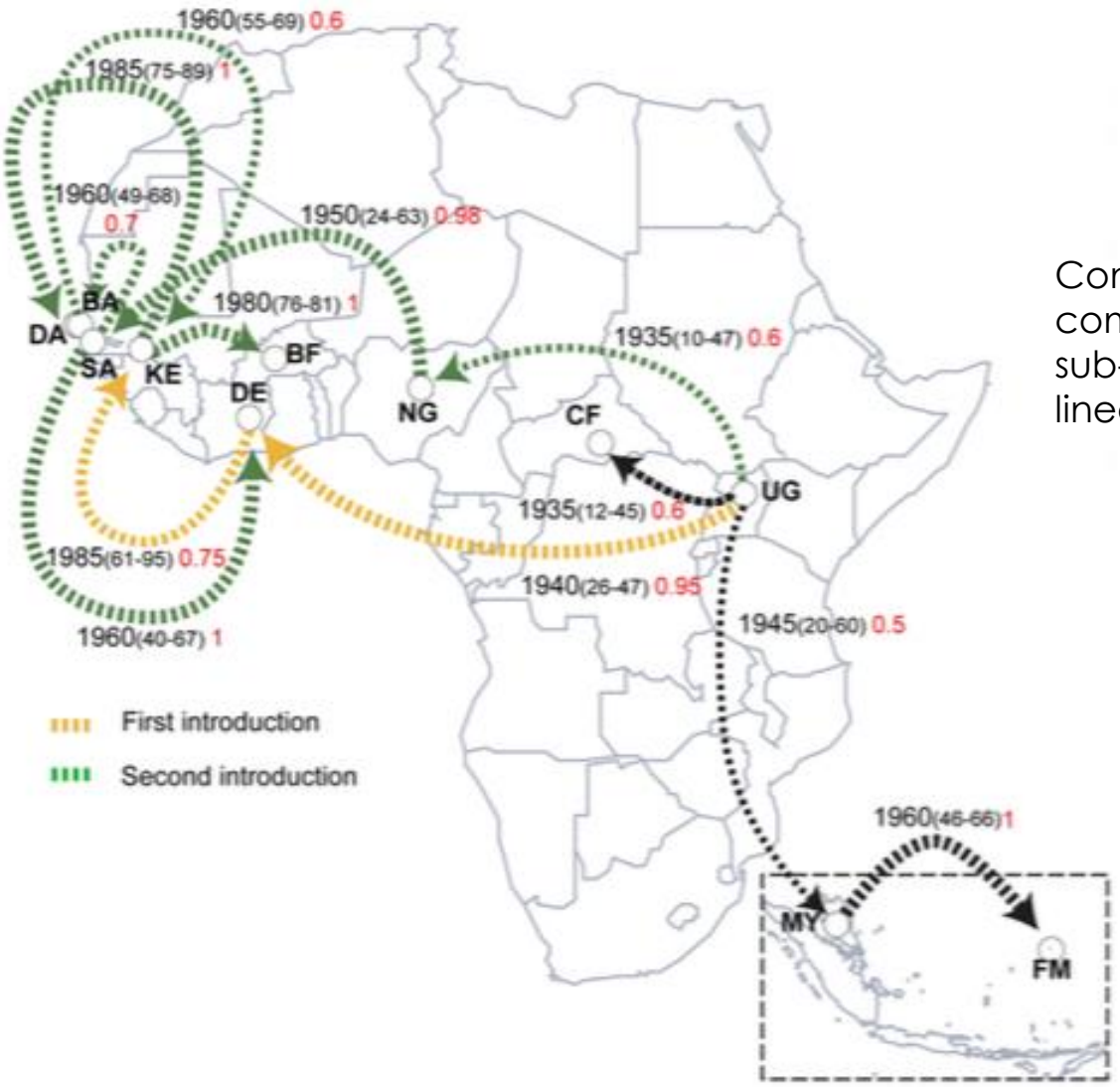
☯ These authors contributed equally to this work.



Comprehensive genetic comparison has revealed sub-clades reflecting 3 main lineages:

- African (2)
- Asian (1)

Figure 2. Geographic spread of ZIKV in Africa and Asia. The directed lines connect the most probable sources and target localities of viral lineages (shown by arrows), with widths proportional to the posterior probabilities and values shown in red. Only plausible routes with probabilities above 50% are shown. The distinct introductions into Senegal and Côte d'Ivoire were represented by different colors. The estimated time to the most recent common ancestor of strains from different countries are shown with 95% posterior time intervals in parenthesis and could be interpreted as the oldest possible year of introduction of that lineage at that locality.
doi:10.1371/journal.pntd.0002636.g002

Epidemiology

- Fist identified in Uganda 1947
- First human case identified in Nigeria 1954
- 7 cases in Java, Indonesia in 1977
- 900 cases in Yap Island, Micronesia in 2007
- 8,750 suspected cases and 383 confirmed in French Polynesia and New Caledonia between 2013-2014
- First case reported in the Americas in February 2014 Easter Island (Chile)
- May 2015 cases reported in Brazil
- October 2015 cases reported in Colombia
- November 2015 in Suriname

Institut Pasteur

Microbes and Infection xx (2015) 1–4

Epidemiology

- Americas
- Pacific Islands
- Africa

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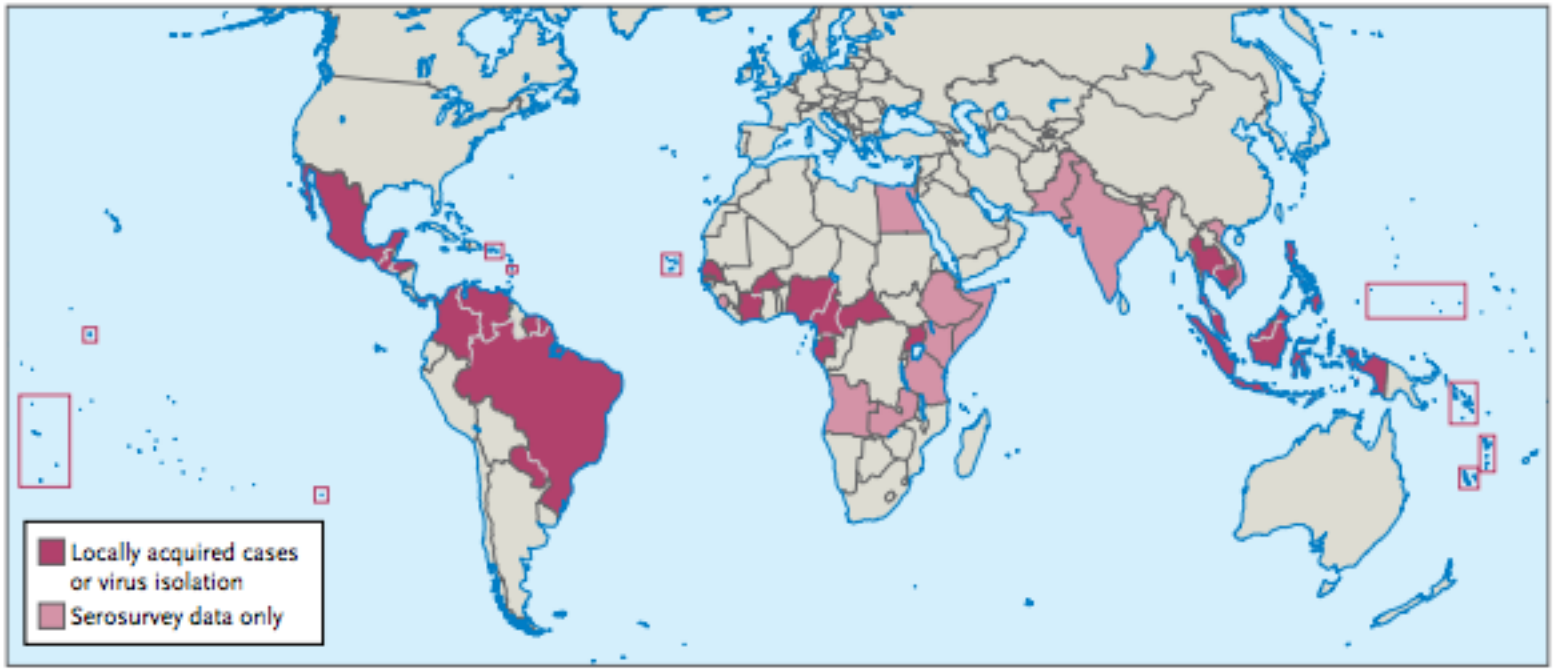
The NEW ENGLAND JOURNAL of MEDICINE



Countries with Past or Current Evidence of Zika Virus Transmission (as of December 2015).

For countries with serosurvey data only, evidence of Zika virus transmission is derived from studies that detected Zika virus antibodies in healthy people. Outlined areas, all with locally acquired cases or virus isolation, include Cape Verde, Cook Islands, Easter Island, Federated States of Micronesia, French Polynesia, Martinique, New Caledonia, Puerto Rico, Solomon Islands, and Vanuatu. Data are from the Centers for Disease Control and Prevention (http://www.cdc.gov/zika).

Clinical Presentation

- About 1 in 5 people infected with ZikV become ill
- The most common symptoms of ZikV are fever, maculopapular rash, joint pain, conjunctivitis.
- Other common symptoms include muscle pain and headache.
- The illness is usually mild with symptoms lasting for several days to a week.
- Zika virus usually remains in the blood of an infected person for a few days but it can be found longer in some people (2-7 days)
- Severe disease requiring hospitalization is uncommon.
- Deaths are rare.

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Diagnostic dilemma

- Dengue and chikungunya result in similar clinical pictures & have both been epidemic in the Americas, confounding clinical diagnosis
- When multiple arboviruses are cocirculating, specific viral diagnosis, if available, can be important in anticipating and managing complications
  - e.g. avoidance of NSAIDs in Dengue or watching for hemoconcentration that can herald Dengue hemorrhagic shock
- PCR can reliably distinguish the three viruses

Comparison of the symptoms, morbidity and mortality, clinical management, sequelae and vectors for dengue virus (DENV) versus Zika virus (ZIKV), 2015. (Table by Erin Archer Kelser).

	Dengue virus (DENV)	Zika virus (ZIKV)
Signs and symptoms	- Maculopapular rash, myalgias and arthralgias, conjunctivitis - Temp >40 °C, plus 2 of the following: severe headache, retro-orbital pain, myalgias and arthralgias, nausea, vomiting [21] - Can have 3 phases: 1)the acute febrile phase, 2) on day 3–7, a critical (plasma leak) phase that often presents with defervescence and capillary permeability, an increase in hematocrit and drop in platelets, bleeding, shock, organ failure and respiratory distress, congestive heart failure, 3) a recovery (reabsorbtion) phase [20,21] - Symptoms usually 2–7 days [21]	- Maculopapular rash, myalgias and arthralgias, conjunctivitis - Temp <38.5 [1] - -joint swelling (especially hands and feet), headache, retro-orbital pain [1–4,14] - Self-limiting, with symptoms 2–7 days [1,2,4,14]
Morbidity and mortality	- Suspected 390 million infected/year - Approx 1/4 infected symptomatic - Approx 500,000 hospitalizations/year where 2.5% die (approx 12,500 deaths/year) [22]	- # cases not yet known - Approx 1/4 of infections believed to be symptomatic [1,7,11] - Hospitalization is rare [1–7]
Clinical management	- No NSAIDS because of risk of bleeding - Close assessment of hydration, bleeding status and signs of organ failure or respiratory distress - If severe/hemhorragic disease, see detailed WHO guidelines [20,21]	- Symptom management with analgesics and anti-pyretics [7,14] NSAIDs acceptable if dengue ruled out [14]
Long-term effects and sequelae	- Long-term effects documented up to 2 years after illness [17] - Link to Guillan-Barre, encephalitis and other neuro syndromes, autoimmune disease [17,18]	- No long-term effects known. - Possible link to Guillain-Barre, encephalitis, other neuro syndromes, autoimmune diseases, especially if previously infected with DENV [2,4,11]
Primary vectors	Aedes aegypti, *Ae*. Albopictus [21,22]	Multiple *Aedes* spp [1,5,6,9,11].

Microbes Infect. 2015 Dec 17. pii: S1286-4579(15)00259-2. doi: 10.1016/j.micinf.2015.12.003

Diagnosis

- Primarily based on detection of viral RNA from clinical specimens in acutely ill patients.
- The viremic period appears to be short, allowing for direct virus detection during the first 3–5 days after the onset of symptoms (serum, urine or saliva)
- ZIKV RNA has been detected in urine up to 10 days after onset of the disease.
- From day five post onset of fever, serological investigations can be conducted by detection of Zika-specific IgM antibodies and confirmation by neutralization, seroconversion or four-fold antibody titer increase of Zika specific antibodies in paired serum samples.
- Serological results should be interpreted according to the vaccination status and previous exposure to other flaviviral infections.

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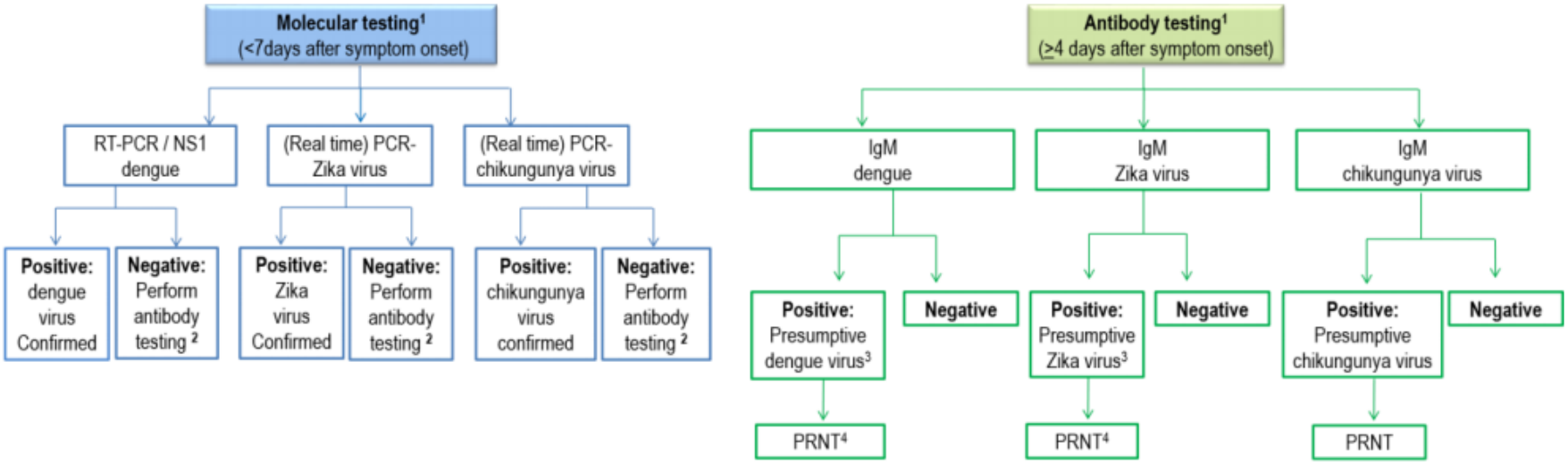
Tiered algorithm for arbovirus detection for suspected cases of chikungunya, dengue, or Zika (Testing only performed if travel history indicates travel to affected area.)



- [1] **Due to extensive cross-reactivity in flavivirus serological assays, for samples collected <7 days post illness onset, molecular detection should be performed first.**
- [2] Perform if sample ≥4 days after symptom onset
- [3] Extensive cross-reactivity would be expected in samples from DENV/ZIKV circulation areas. A positive IgM assay with either antigen should be confirmed by using PRNT against both ZIKV and DENV as well as any other flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).
- [4]PRNT should include any flavivirus (eg. SLEV, ZIKV, WNV, etc.) that might be found in that geographic area (including travel areas).

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Diagnosis

- Zika, Chikungunya, and Dengue virus RT-PCR, IgM ELISA, and plaque reduction neutralization tests (PRNT) are performed at CDC.
- To determine the appropriate testing algorithm and interpret results, please provide:
  - date of illness onset
  - dates of specimen collection
  - specimen type
  - description of clinical illness
  - travel history
  - *Flavivirus* vaccination history
  - contact information for the submitter.
- Testing will primarily be performed on serum or CSF but other specimen types, including urine, amniotic fluid, and tissues, can be submitted for evaluation of the utility of these specimen types.

Potential Association between Zika Virus and Congenital Malformations

- Materno-fetal transmission has been demonstrated for several Flaviviruses (Dengue, West Nile) associated to premature birth, congenital defects and microcephaly
- Microcephaly when due to an infection, is usually caused by transplacental infection occurring early during pregnancy.
- Microcephaly:
  - Occipital frontal circumference of the head is 2 SD below the mean for age, sex and race.
  - When the fetal brain does not increase normally in size, the sutures close prematurely.
  - Other infections: Syphilis, Toxoplasmosis, Rubella, CMV and HSV


Typical head size
Baby with Microcephaly
CDC

Mechanisms for Microcephaly

- The pathological properties of Zika were first described in 1952, when Dick et al. demonstrated viral tropism to the brain in intraperitoneally infected mice and an increase in viral titers over several days. This research suggested the virus could cross the blood brain barrier.
- The research findings were complemented in 1972 by Bell and colleagues who observed the progression of disease in directly infected mouse brains.
- Based on their observations, the virus infected both neurons and glia, producing a variety of intracytoplasmic inclusions, which they termed, "virus factories."
  - endoplasmic reticulum
  - nucleus
  - mitochondria

Jheng JR, Ho JY, Horng JT. ER stress, autophagy, and RNA viruses. Front Microbiol 2014;5:388.

Mechanisms for Microcephaly

- Autophagy. a cellular process designed to ensure cell homeostasis through entrapment and eventual degradation of unwanted cellular material.
- This mechanism is also used to fight viral infections, although the efficiency is varied as a result of viral regulatory mechanisms.
- In the case of flavivirus infection, interactions between the virus and the Endoplasmic Reticulum induce autophagy.

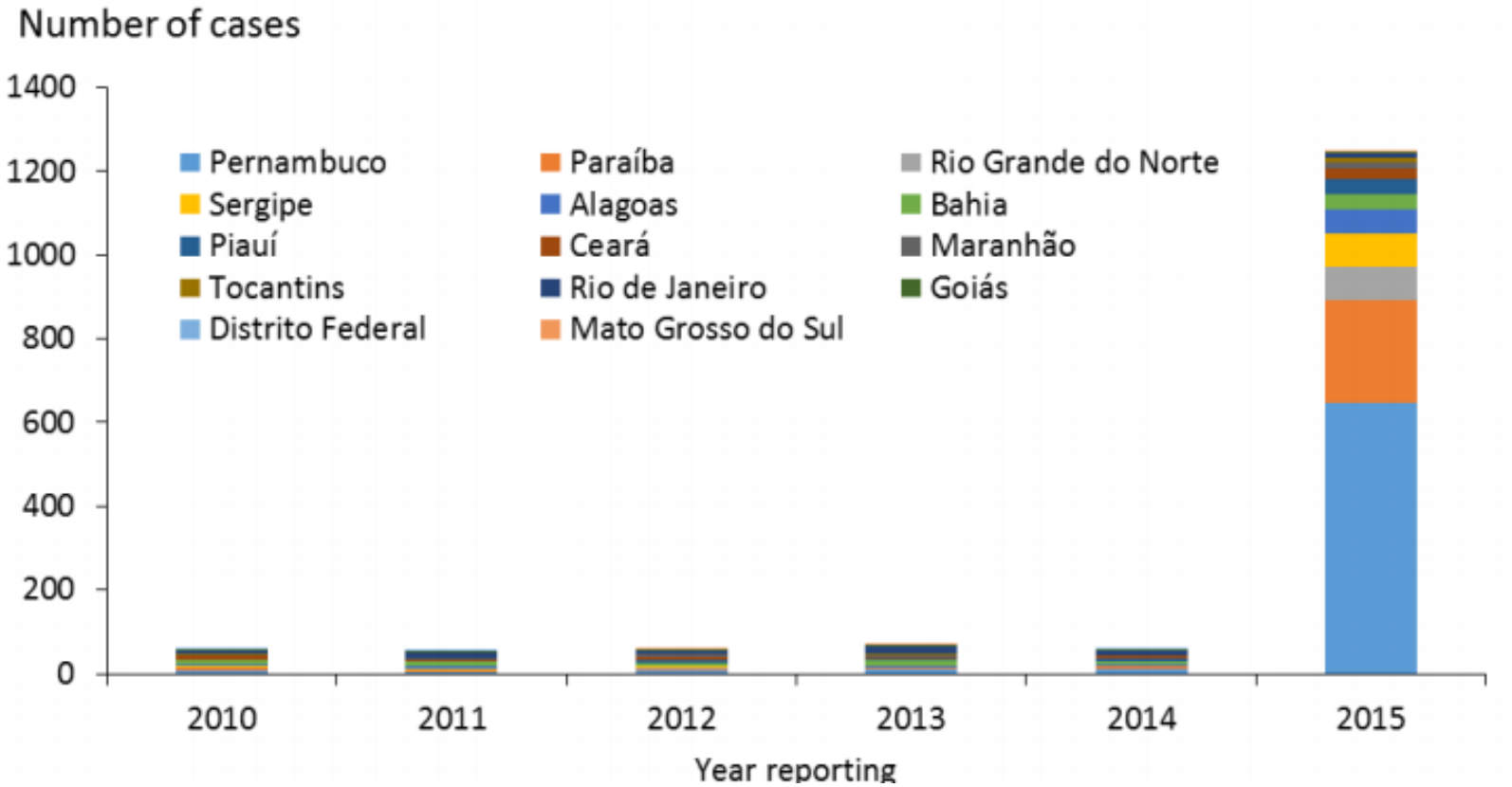
Dick GW. Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg 1952;46:521-34.

Mechanisms for Microcephaly

- One of the causes of microcephaly involves abnormal function of centrosomes :
  - Mitosis
  - Migration
  - Polarity
- Effects of infection:
  - delay in mitosis
  - increase in apoptosis
  - improper neural stem cell orientation
  - premature neuronal differentiation
  - decrease in progenitor cells
- The overall effect reduces the formation of brain matter leading to the reduced brain size indicative of microcephaly.

Dick GW. Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg 1952;46:521-34.

Figure 3. Notified cases of microcephaly in Brazil from 2010 to 2015, with 14 states under investigation, as of 28 November 2015



Ministério da Saúde (Brazil). Ministério da Saúde divulga novos dados de microcefalia [Internet]. 2015 [cited 2015 Nov 30]. Available from: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/noticias-svs/21020-ministerio-da-saude-divulga-novos-dados-de-microcefalia.

Table 1. Summary of number of microcephaly cases per 1 000 live births reported annually in the fourteen Brazilian states that investigate microcephaly; 2010–2014; 2015 data as of 28 November

State	2009–2013[a]	2010–2014[b]		2015, as of 28 November		
	Yearly average number of live births (LB)	Yearly average number of microcephaly cases	Number of microcephaly per 1 000 LB	Number of microcephaly cases	Number of microcephaly cases per 1 000 LB*	Ratio between 2015/2010–2014
Pernambuco	140 264	8.6	0.06	646	4.61	77
Paraíba	47 998	4.2	0.09	248	5.17	57
Rio Grande do Norte	47 698	1.8	0.04	79	1.66	42
Sergipe	34 477	1.6	0.05	77	2.23	45
Alagoas	44 331	3.4	0.08	59	1.33	17
Bahia	211 660	10.6	0.05	37	0.17	3
Piauí	48 989	3	0.06	36	0.73	12
Ceará	128 112	6.6	0.05	25	0.2	4
Rio de Janeiro	219 876	12.4	0.06	13	0.06	1
Tocantins	24 586	1.2	0.05	12	0.49	10
Maranhão	119 069	3	0.03	12	0.1	3
Goiás	90 559	3	0.03	2	0.02	0.7
Distrito Federal	43 935	2.6	0.06	1	0.02	0.3
Mato Grosso do Sul	41 421	0.8	0.02	1	0.02	1
Total	**1 242 975**	**62.8**	**0.05**	**1248**	**1**	**20**

Ministério da Saúde (Brazil). Ministério da Saúde divulga novos dados de microcefalia [Internet]. 2015 [cited 2015 Nov 30]. Available from: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/noticias-svs/21020-ministerio-da-saude-divulga-novos-dados-de-microcefalia.

Ultrasound Obstet Gynecol 2016; **47**: 6–7
Published online in Wiley Online Library (wileyonlinelibrary.com).

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

We have examined recently two pregnant women from the state of Paraiba who were diagnosed with fetal microcephaly and were considered part of the 'microcephaly cluster' as both women suffered from symptoms related to Zika virus infection. Although both patients had negative blood results for Zika virus, amniocentesis and subsequent quantitative real-time polymerase chain reaction[4], performed after ultrasound diagnosis of fetal microcephaly and analyzed at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, was positive for Zika virus in both patients, most likely representing the first diagnoses of intrauterine transmission of the virus. The sequencing analysis identified in both cases a genotype of Asian origin.

In Case 1, fetal ultrasound examination was performed at 30.1 weeks' gestation. Head circumference (HC) was 246 mm (2.6 SD below expected value) and weight was estimated as 1179 g (21st percentile). Abdominal circumference (AC), femur length (FL) and transcranial Doppler were normal for gestational age as was the width of the lateral ventricles. Anomalies were limited to the brain and included brain atrophy with coarse calcifications involving the white matter of the frontal lobes, including the caudate, lentostriatal vessels and cerebellum. Corpus callosal and vermian dysgenesis and enlarged cisterna magna were observed (Figure 1).

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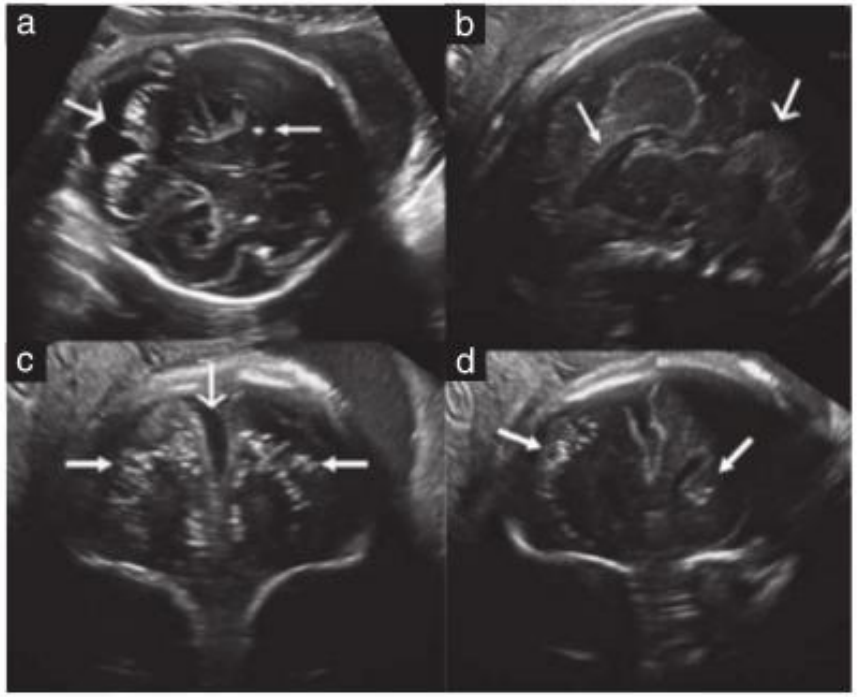

Figure 1 Case 1: (a) Transabdominal axial ultrasound image shows cerebral calcifications with failure of visualization of a normal vermis (large arrow). Calcifications are also present in the brain parenchyma (small arrow). (b) Transvaginal sagittal image shows dysgenesis of the corpus callosum (small arrow) and vermis (large arrow). (c) Coronal plane shows a wide interhemispheric fissure (large arrow) due to brain atrophy and bilateral parenchymatic coarse calcifications (small arrows). (d) Calcifications are visible in this more posterior coronal view and can be seen to involve the caudate (arrows).

Ultrasound Obstet Gynecol 2016; **47**: 6–7
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Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?

In Case 2, fetal ultrasound examination was performed at 29.2 weeks' gestation. HC was 229 mm (3.1 SD below expected value) and estimated fetal weight was 1018 g (19th percentile). AC was below the 3rd percentile but FL was normal. The cerebral hemispheres were markedly asymmetric with severe unilateral ventriculomegaly, displacement of the midline, thinning of the parenchyma on the dilated side, failure to visualize the corpus callosum and almost complete disappearance or failure to develop the thalami. The pons and brainstem were thin and continuous with a non-homogeneous small mass at the position of the basal ganglia. Brain calcifications were more subtle than in Case 1 and located around the lateral ventricles and fourth ventricle. Both eyes had cataracts and intraocular calcifications, and one eye was smaller than the other (Figure 2).

Ultrasound Obstet Gynecol 2016; **47**: 6–7
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Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?

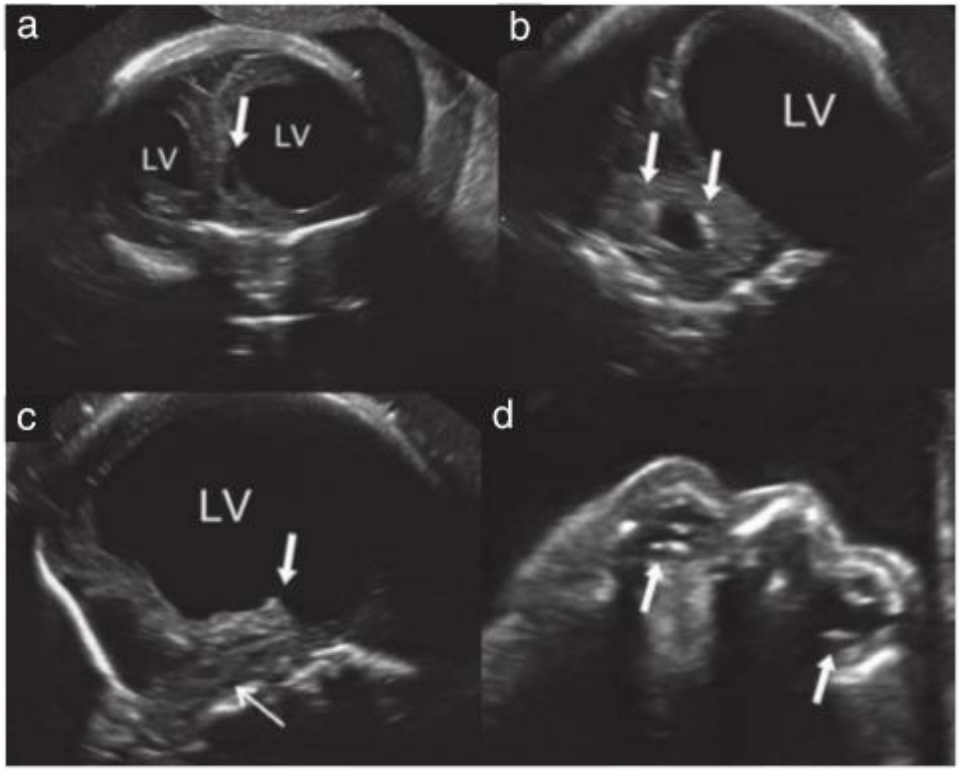

Figure 2 Case 2: (a) Anterior coronal view shows severe asymmetric ventriculomegaly with cystic formation (arrow). (b) Posterior horn of the lateral ventricle (LV) in coronal view is dilated. Note calcifications in the fourth ventricle (arrows). (c) The thalamus is absent (arrow) and the brainstem and pons are thin and difficult to visualize (sagittal view). (d) Axial view shows calcifications in both eyes (arrows). Note that the proximal eye is very small and lacks normal anatomic landmarks.

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FIGURE. Interim guidance: testing algorithm*,†,§ for a pregnant woman with history of travel to an area¶ with Zika virus transmission, with or without clinical illness consistent with Zika virus disease**

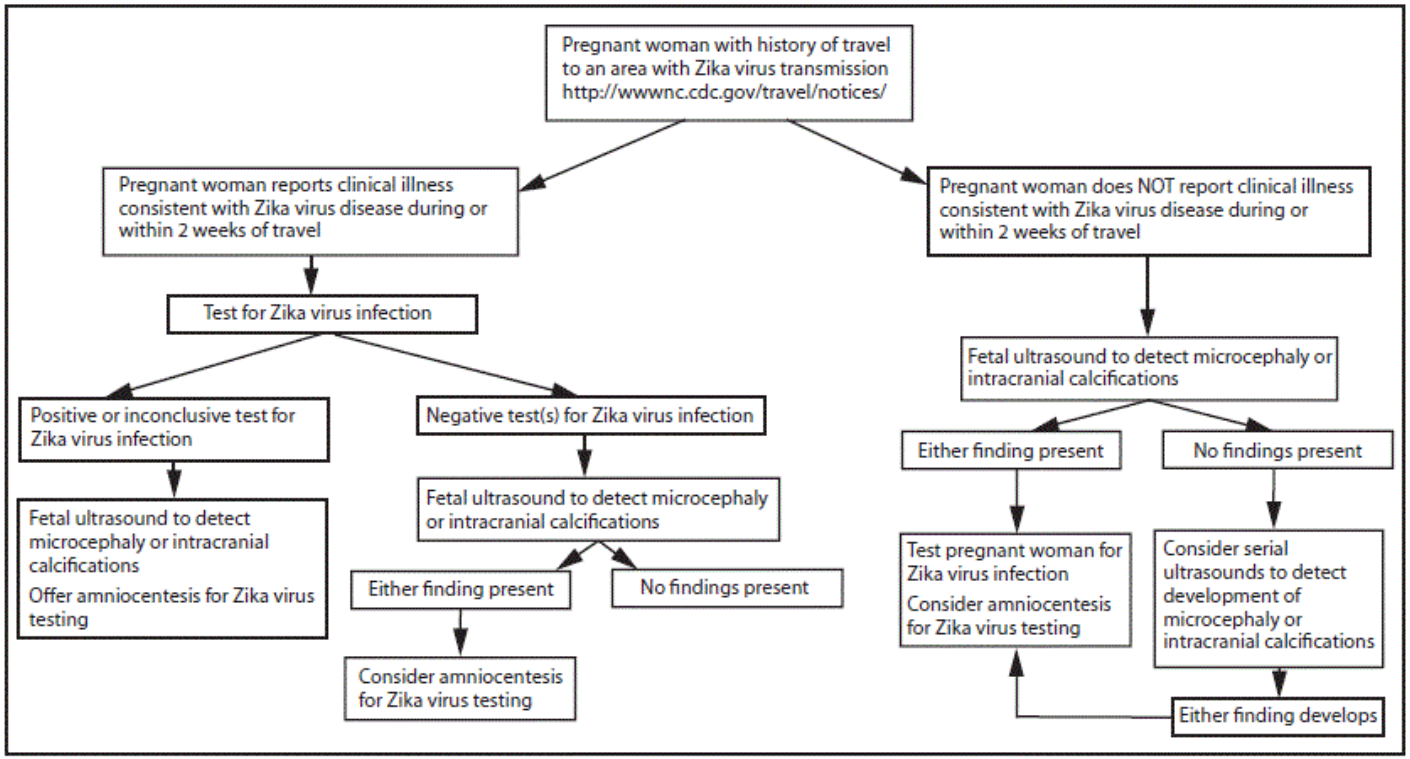

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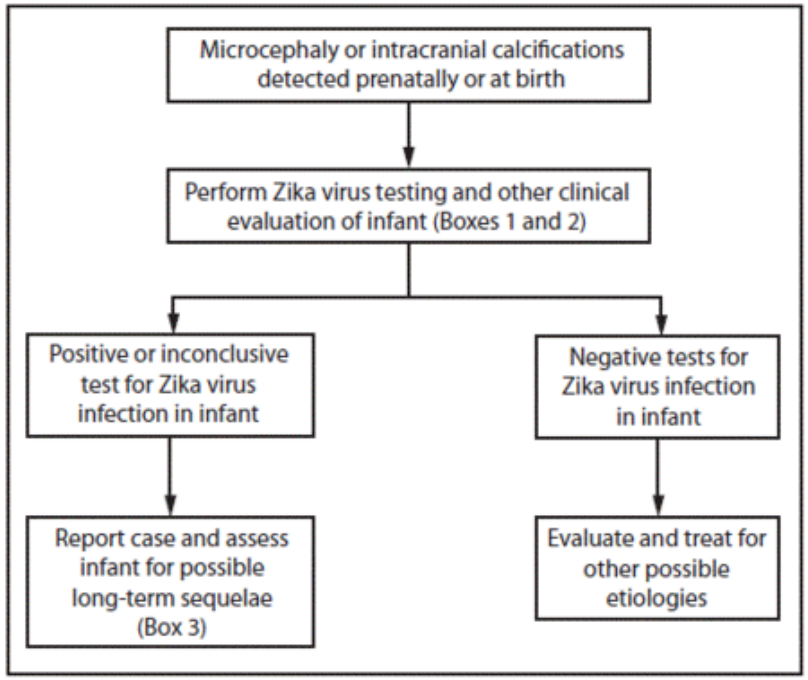
FIGURE 1. Interim guidelines for the evaluation and testing of infants with microcephaly* or intracranial calcifications whose mothers traveled to or resided in an area with Zika virus transmission† during pregnancy§



Neurological Complications

- Guillain-Barré Syndrome:
  - 42 from 74 patients with neurological syndromes were GBS (2013-2014)
  - July 2015 Brazil: From 76 patients with neurological syndromes, 42 were GBS
  - Jan 2016 El Salvador: unusual increase in GBS cases. Usually 14 cases/month, last month 46 cases all suspected to have ZIKV
- Meningitis (French Polynesia Outmbreak 2013-2014)
- Meningoencephalitis (French Polynesia Outmbreak 2013-2014)
- Myelitis (French Polynesia Outmbreak 2013-2014)

Prevention

- **Low tech**
  - House screens, air-conditioning, mosquito repellant
  - Removal of debris and containers that provide mosquito-breeding sites around the home
- **High tech**
  - No Zika vaccines in advanced development
  - Could existing flavivirus vaccine platforms presumably be adapted?
  - Barriers to Zika vaccine development: Same issues for chikungunya, WNV, St. Louis encephalitis, and other arboviruses
    - Preemptively vaccinating large populations in anticipation of sporadic, unpredictable outbreaks may be prohibitively expensive / not cost-effective
    - Vaccine stockpiling & rapid deployment may be too slow to counter sudden explosive epidemics

Avoid This!!!!

How to Contact CDC?

- For all states and territories, questions about laboratory testing or sending specimens to CDC should be directed to the **Arboviral Diseases Branch on-call epidemiologist at 970-221- 6400**.
- More information about submitting specimens to CDC is at:
  - http://www.cdc.gov/ncezid/dvbd/specimensub/arboviral-shipping.html.

Thank you!