Zika research in Brazil and the Latin American and Caribbean region

Paolo Zanotto, D.PHIL., Institute of Biomedical Sciences, USP

GloPID-R Zika virus research workshop
University of São Paulo, Brazil, 30 Nov – 2 Dec 2016
Life Cycle of arboviruses and ‘emergence’

Sylvatic
- Mild headaches
- Maculopapular rash
- Fever

- Malaise
- Conjunctivitis
- Arthralgia

Endemic/Epidemic

Ae. furcifer (West Africa)
Ae. albopictus (Southeast Asia)

Transovarial transmission

Ae. aegypti
Ae. albopictus
Ae. polynesiensis

25°C (15.0 days)
30°C (5.9 days)

Intrinsic incubation period (6.5 days)

Zone of Emergence

Humans

Primates

Ae. luteocephalus (West Africa)
Ae. furcifer (West Africa)
Ae. niveus (Southeast Asia)

Transovarial transmission
MODES OF TRANSMISSION

- Vectors (*Aedes* spp – *Aedes aegpty*)
- Sexual (Semen?)
- Perinatal
- Congenital
- Blood transfusion and organ transplants
- Saliva and urine (?)
The Flaviviruses

Phylogeny of 100 nearly complete polyprotein coding regions
The Spondweni serocomplex viruses
Geographic Spread of ZIKV in Africa, Asia and beyond

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

Oumar Faye*, Caio C. M. Freire**, Atila Iamarino*, Ousmane Faye1, Juliana Velasco C. de Oliveira2, Mawouth Diallo1, Paolo M. A. Zanotto1, Amadou Alpha Sall1*

1 Tropical Pathology Unit, Dakar, Senegal; 2 Laboratory of Molecular Evolution and Biodiversity, Department of Microbiology, Biomolecular Sciences Institute, University of São Paulo, São Paulo, Brazil
Figure 1. Cumulative number of countries, territories and areas by WHO region reporting mosquito-borne Zika virus transmission in years, 2007-2014, and monthly from 1 January 2015 to 1 June 2016

Source: WHO, 2 June, 2016

* By 1st SEP – 72 countries/territories
51 Nations with ZIKV transmission in the Americas

- Anguilla
- Antigua and Barbuda
- Argentina
- Aruba
- The Bahamas
- Barbados
- Belize
- Bolivia
- Bonaire
- Brazil
- British Virgin Islands
- Cayman Islands
- Colombia
- Commonwealth of Puerto Rico, US territory
- Costa Rica
- Cuba
- Curacao
- Dominica
- Dominican Republic
- Ecuador
- El Salvador
- French Guiana
- Grenada
- Guadeloupe
- Guatemala
- Guyana
- Haiti
- Honduras
- Jamaica
- Martinique
- Mexico
- Montserrat
- Nicaragua
- Panama
- Paraguay
- Peru
- Saba
- Saint Barthélemy
- Saint Lucia
- Saint Martin
- Saint Vincent and the Grenadines
- Sint Eustatius
- Sint Maarten
- St. Kitts and Nevis
- Suriname
- Trinidad and Tobago
- Turks and Caicos
- United States
- U.S. Virgin Islands
- Venezuela

Source: CDC Atlanta
ZIKA: Epidemiological situation, Brazil Epi Week 1-32/2016*

Zika cases have already dropped by 99.9% in 2016

Peak was in February, with 19,837 notified cases. In the second week of August, there were 5 cases.

CASES
- Probable: 196,976 (2,277 counties)

DEATHS
- 2015: 3 confirmed
- 2016: 3 confirmed

PREGNANT WOMEN
- Probable: 16,264

Source: Sinan-NET
Timeline

Perception: increase of live births with microcephaly

Aug - Sep

SES-PE¹ receive notification from clinicians

Oct

Clinical picture suggestive of congenital infection

Start of investigation SES-PE¹, SVS/MS² and PAHO³

11/Nov

National Public Health Emergency Declaration

28/Nov

Diagnostic of first case connecting ZIKV and Microcephaly by the IEC⁴

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¹ Secretaria de Saúde do Estado de Pernambuco
² Secretaria de Vigilância em Saúde do Ministério da Saúde
³ Pan American Health Organization
⁴ Instituto Evandro Chagas
Zika virus and microcephaly: why is this situation a PHEIC?  
(Feb. 1\textsuperscript{st}, 2016)

Public Health Emergency of International Concern (PHEIC) must:

• (1) Constitute a health risk to other countries through international spread.

• (2) Potentially require a coordinated response because it is unexpected, serious, or unusual.

• (3) Have implications beyond the affected country that could require immediate action.

Heymann et al., 2016 The Lancet DOI: [http://dx.doi.org/10.1016/S0140-6736(16)00320-2](http://dx.doi.org/10.1016/S0140-6736(16)00320-2)
Notified cases of microcephaly in Brazil, 2010-2014 and 2015 for 14 states (under investigation)
## Microcephaly birth rates per 100,000 live births.
### Brazil 2010-2014 and 2015

<table>
<thead>
<tr>
<th>States</th>
<th>Average microcephaly birth rate per 100,000 live births. Brazil, 2010-2014 (*)</th>
<th>Microcephaly birth rate per 100,000 live births. Brazil, 2015(**)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Central West Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distrito Federal</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Goiás</td>
<td>3.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Mato Grosso do Sul</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td><strong>Northeast Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alagoas</td>
<td>6.5</td>
<td>112.4</td>
</tr>
<tr>
<td>Bahia</td>
<td>5.2</td>
<td>18.2</td>
</tr>
<tr>
<td>Ceará</td>
<td>5.3</td>
<td>20.0</td>
</tr>
<tr>
<td>Maranhao</td>
<td>2.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Paraíba</td>
<td>7.4</td>
<td>436.2</td>
</tr>
<tr>
<td>Pernambuco</td>
<td>6.1</td>
<td>456.7</td>
</tr>
<tr>
<td>Piauí</td>
<td>6.5</td>
<td>77.6</td>
</tr>
<tr>
<td>Rio Grande do Norte</td>
<td>3.8</td>
<td>168.8</td>
</tr>
<tr>
<td>Sergipe</td>
<td>4.7</td>
<td>225.0</td>
</tr>
<tr>
<td><strong>Northern Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tocantins</td>
<td>5.0</td>
<td>49.8</td>
</tr>
<tr>
<td><strong>Southeast Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rio de Janeiro</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>4.9</td>
<td>99.7</td>
</tr>
</tbody>
</table>

(*) Calculated using the average number of Microcephaly cases that occurred between 2010 and 2014 in the numerator, and the number of live births per mother’s state of residency from 2013 in the denominator.

(**) Calculated using the number of Microcephaly cases that occurred up until EW 47, 2015, and the number of live births per state of the mother’s residency from 2013 in the denominator.
### Key early findings: contribution to the pathogenesis of ZIKV congenital disease

<table>
<thead>
<tr>
<th>Date</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOV 17</td>
<td>Detection of ZIKV genome in amniotic fluid. Osvaldo Cruz Institute. RT-PCR in amniotic fluid samples from two pregnant women from Paraíba, whose fetuses have been diagnosed with microcephaly by ultrasound exams.</td>
</tr>
<tr>
<td>NOV 28</td>
<td>Detection of ZIKV in a neonate fatal case with microcephaly and other congenital malformations (Nov 18, Ceara State, Brazil). Mother infection with 8 weeks of pregnancy (presented anasarca) Evandro Chagas Institute</td>
</tr>
</tbody>
</table>
Initial connection linking Zika Virus (ZIKV), microcephaly and other malformations

- Baby whose mother had ZIKV in the 8th week of pregnancy and died 5 minutes after born.

- Clinically, newborn had microcephaly and several congenital malformations including arthrogryposis, atresia of (incipient) esophagus and trachea, etc.

- Blood sample all viscera fragments obtained (brain, heart, lung, liver, kidney and spleen) were positive by RT-qPCR to ZIKV.

- ZIKV strains were also isolated from the blood, brain and pooled viscera's in C6/36 cells, which were sequenced showing to be related to the Asian genotype of ZIKV.

- By immunohistochemical assay, ZIKV antigens were found in all analyzed viscera fragments (brain, liver, heath, lung and kidney), but a semi-quantitative exam showed the brain expressing much more ZIKV antigens.

Source: IEC, 2015
Ongoing research in South America & Caribbean
1) Data from airports within 50 km of areas conducive to year-round Zika virus transmission from September, 2014, to August, 2015.

2) Used LandScan, a gridded global population dataset, to estimate numbers of people living in geographies at risk for autochthonous Zika virus transmission.

3) 9.9 million travellers departed from the aforementioned Brazilian airports for international destinations, with 65% to the Americas (figure), 27% to Europe, and 5% to Asia. Traveller volumes were greatest to the USA (2 767 337), Argentina (1 314 694), Chile (614 687), Italy (419 955), Portugal (411 407), and France (404 525). China and Angola received the highest volume of travellers in Asia (84 332) and Africa (82 838), respectively.

4) Argentina, Italy, and the USA have more than 60% of their populations residing in areas conducive to seasonal Zika virus transmission, whereas Mexico, Colombia, and the USA have an estimated 30.5, 23.2, and 22.7 million people, respectively, living in areas conducive to year-round transmission.
Argentina: Haplotype frequencies for ND5 gene in *Aedes* populations


http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004839
Venezuela: ONI & ZIKV Spread

Figure 1. The index of Google Trends in Colombia (blue color) from October 2014 until March 2016, and the temperature of the Pacific Ocean using ENSO in red color.

Figure 2. Number of suspected cases of Zika in Colombia from September 2015 to March 2016 in bars, and the red line the Oceanic Niño Index temperature (see text for details).

Phylogenetic relations between the envelope gene sequences of Suriname ZIKV and other ZIKV

Patients with Symptoms of Zika Virus Disease (ZVD), Including Laboratory-Confirmed Cases, in Colombia (August 9, 2015–April 2, 2016).

From 582 pregnant women with symptoms “similar” to Zika virus disease, 326 (56%) were positive for Zika virus by (RT-PCR) assay.

Is there a difference in microcephaly numbers in Brazil and Colombia?

Pacheco et al., 2016 NEJM June 15, 2016 DOI: 10.1056/NEJMoa1604037
Zika, Dengue, and Chikungunya co-infection in a pregnant woman from Colombia

Villamil-Gómez et al.. 2016 International Journal of Infectious Diseases Volume 51, October 2016, Pages 135–138
http://dx.doi.org/10.1016/j.ijid.2016.07.017
Fatal Zika Virus Infection in Patient with Sickle Cell Disease, Colombia


✔ October/2015, Female, 15 years-old
✔ High fever, arthralgia, retro-orbital pain, mialgia, ictericia
✔ Falciform anemia, genotypo SC
✔ Abdominal pain, no bleeding - suspected dengue
✔ Worsening of jaundice, respiratory failure, intense abdominal pain, hepatoesplenomegaly.
✔ Anemia e thrombocytopenia
✔ DENV negative RT-PCR
✔ ZIKV positive RT-PCR para

Severe vaso-occlusive crisis & splenic sequestration

Technical Appendix Figure. Autopsy findings for liver and spleen of a 15-year-old girl with sickle cell disease who died of Zika virus infection, Colombia. A) Liver showing paracanal necrosis. B) Liver showing erythropagocytosis of Kupffer cells. C) Spleen showing severe decrease of white pulp (functional asplenia). D) Spleen showing multiple splenic drepanocytes (splenic sequestration). (Hematoxylin and eosin stained) (Original magnification, ×10 in A, ×40 in B and C, ×60 in D.)
Ongoing research in Brazil

• Causal relation of ZIKV & congenital ZIKV syndrome (CZS)
• ZIKV infection in 2-, 3-D systems, animal models, following “persistent” infections.
• Anti-ZIKV molecules & MAbs.
• Serological & molecular diagnostics tools (different platforms).
• Vaccine development (different platforms).
• Assemble & manage cohorts of pregnant women in consort with research groups.
• Entomology: control, vector genetics, physiology & ecology


“Brazilian storm” - Steven Hehen
Funding research in Brazil

Main funding agencies:

- **CNPq** - Conselho Nacional de Desenvolvimento Científico e Tecnológico - "National Counsel of Technological and Scientific Development" ([cnpq.br](http://cnpq.br/)).


- **FAPESP** - Fundação de Amparo à Pesquisa do Estado de São Paulo - “São Paulo Research Foundation” ([www.fapesp.br/en/](http://www.fapesp.br/en/)).
Viral Genetic Diversity Network (VGDN): the precursor of the FAPESP ZIKV network

[Graph showing the growth of publications related to Zika virus in São Paulo and Rio de Janeiro over time.]
Freitas et al., JAMA Ophthalmol.
Published online February 9, 2016.

Table. Ocular Findings in Infants with Microcephaly and Presumed Zika Virus Congenital Infection

<table>
<thead>
<tr>
<th>Ocular Finding</th>
<th>Patient No.</th>
<th>Affected Eyebird No. (Total 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal pigment mottling</td>
<td>1 U</td>
<td>U</td>
</tr>
<tr>
<td>Chorioretinal atrophy</td>
<td>2 U</td>
<td>U</td>
</tr>
<tr>
<td>Optic nerve abnormalities</td>
<td>3 U</td>
<td>U</td>
</tr>
<tr>
<td>Iris coloboma</td>
<td>4 U</td>
<td>U</td>
</tr>
<tr>
<td>Lens subluxation</td>
<td>5 U</td>
<td>U</td>
</tr>
<tr>
<td>Scleral scleeral ossicles</td>
<td>6 U</td>
<td>U</td>
</tr>
<tr>
<td>Vasculature</td>
<td>7 U</td>
<td>U</td>
</tr>
</tbody>
</table>

Abbreviations: U, right eye; O, left eye; C, each eye.

Conclusions

In summary, 10 of 29 infants with microcephaly (34.5%) had severe ocular abnormalities; these infants were born after a ZIKV outbreak in the state of Bahia in Brazil. The posterior ocular findings were focal pigment mottling and chorioretinal atrophy with a predilection for the macular area, as well as optic disc abnormalities. These findings can contribute to the diagnosis of ZIKV congenital infection in children with congenital microcephaly, although the retinal lesions found in this sample may resemble West Nile virus or toxoplasmosis retinochoroiditis.35 Advances in serologic ZIKV tests and additional studies are necessary to confirm such findings.

This study can help guide clinical management and practice, as we observed that a high proportion of the infants with microcephaly had ophthalmologic lesions. Infants with microcephaly should undergo routine ophthalmologic evaluations to identify such lesions. In high-transmission settings, such as South America, Central America, and the Caribbean, ophthalmologists should be aware of the risk of congenital ZIKV-associated ophthalmologic sequelae.
Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg?

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).

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.
Zika virus in the Americas: Early epidemiological and genetic findings


*Equal contribution to the work

1Center for Technological Innovation, London Chagas Institute, Ministry of Health, Brazil, 2Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PJ, UK, 3Department of Zoology and Genetics, Faculty of Sciences, University of Brasilia, Brasilia, Brazil, 4Department of Epidemiology, Faculty of Medicine, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil, 5Department of Zoology and Genetics, Faculty of Medicine, University of Uberlândia, Uberlândia, Minas Gerais, Brazil, 6Department of Epidemiology, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil, 7Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PJ, UK, 8Laboratório de Virologia e Biologia Molecular, Instituto Evandro Chagas, FUNDUNE, Manaus, Brazil, 9Federative University of Rio de Janeiro, Brazil, 10Federative University of Rio de Janeiro, Brazil, 11Federative University of Rio de Janeiro, Brazil, 12Department of Public Health, Universidade do Porto, Porto, Portugal, 13Department of Public Health, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, 14Department of Public Health, University of Texas Medical Branch, Galveston, TX, USA.

Brazil has experienced an unprecedented epidemic of Zika virus (ZIKV), with approximately 30,000 cases reported to date. ZIKV was first detected in Brazil in May 2015 and cases of microcephaly potentially associated with ZIKV infection were identified in November 2015. Using next-generation sequencing we generated seven Brazilian ZIKV genomes, sampled from four self-limiting cases, one blood donor, one fatal adult case, and one newborn with microcephaly and congenital malformations. Phylogenetic and molecular clock analyses show a simple introduction of ZIKV into the Americas, estimated to have occurred between May-Dec 2013, more than 12 months prior to the detection of ZIKV in Brazil. The estimated date of origin coincides with an increase in air passengers to Brazil from ZIKV endemic areas, and with reported outbreaks in Pacific Islands. ZIKV genomes from Brazil are phylogenetically interspersed with those from other South American and Caribbean countries. Mapping mutations onto existing structural models revealed the context of viral amino acid changes present in the outbreak lineage; however no shared amino acid changes were found among the three currently available virus genomes from microcephaly cases. A genomic-level analysis reveals an anomalously high rate of microsatellite drift. Further analysis of ZIKV in Brazil provides a baseline for future studies of the evolution and molecular epidemiology of ZIKV in the Americas.

Fig. 2. Maximum likelihood phylogeny of ZIKV complete coding region sequences. Bootstrap scores are shown next to well-supported nodes and the phylogeny was midpoint rooted. A fully annotated tree is provided in fig. S2. The American ZIKV clade is drawn as a white triangle and a star in detail in Fig. 3. Asterisks highlight the four internal branches that are ancestral to the American ZIKV lineage (see main text and fig. S3). Correlation between the sampling date of each sequence and the genetic distance of that sequence from the root of a maximum likelihood phylogeny of the Asian genotype ($R^2 = 0.997$). A molecular clock phylogeny of this data is shown in Fig. 3. The Malaysian strain (HQ234499) sampled in 1966 is the oldest representative of that genotype and falls on the regression line, indicating that it does not appear to be unusually divergent for its age. A similar analysis with the HQ234499 strain excluded is shown in fig. S5C.
Fig. 3. Timescale of the introduction of ZIKV to the Americas. (A) Molecular clock phylogeny of the ZIKV outbreak lineage estimated from complete coding region sequences, plus 6 sequences (KJ634273, KU312315, KU312314, KU212313, KU646828, and KU646827) longer than 1500nt (available data as of 7th March 2016). For visual clarity, three basal sequences, HQ23499 (Malaysia, 1966), EU549888 (Micronesia, 2007) and JN860885 (Cambodia, 2010) are not displayed here (see fig. S3). Gray horizontal bars represent 95% Bayesian credible intervals for divergence dates. A and B denote clades discussed in main text and numbers next to them denote posterior probabilities. Diamond sizes represent, at each node, the posterior probability support of that node. Taxa are labeled with accession number, sampling location, and sampling date. Names of sequences generated in this study are underlined. (B) Posterior distributions of the estimated ages (TMRCA) of clades A and B, estimated in BEAST using under the best fitting evolutionary model (table S2). The time and duration of the three events (i-iii) discussed in the main text are shown. (C) The blue curve (left hand axis) shows a polynomial fitting of the number of travelers (blue points) from countries with recorded ZIKV outbreaks between 2012 and 2015 (French Polynesia, Thailand, Indonesia, Malaysia, Cambodia, New Caledonia, supplementary materials section 6), aggregated across 20 Brazilian national airports. The purple bars represent weekly numbers of suspected ZIKV cases (right hand side) in French Polynesia from the 30 Oct 2013 to 14 Feb 2014 (4).
Emerging Problems in Infectious Diseases

Zika virus infection, associated microcephaly, and low yellow fever vaccination coverage in Brazil: Is there any causal link?

Luciano Pamplona de Góes Cavalcanti¹, Pedro Luiz Tauli¹, Carlos Henrique Alencar¹, Wanderson Oliveira², Mauro Martins Teixeira³, Jorg Heukeleibach⁴

¹ Department of Community Health, School of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil
² Coordenação Geral de Vigilância e Respostas às Emergências em Saúde Pública do Ministério da Saúde do Brasil, Brasília, Brazil
³ Department of Biochemistry and Immunology, Biological Science Institute, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
⁴ Antônio Brasil Centre for Public Health and College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, Queensland, Australia
⁵ Faculty of Medicine, University of Brasília (UnB), Distrito Federal, DF, Brazil

Abstract

Introduction: Since the end of 2014, Zika virus (ZIKV) infection has been rapidly spreading in Brazil. Methodology: To analyze the possible association of yellow fever vaccine with a protective effect against ZIKV-related microcephaly, the following spatial analyses were performed, using Brazilian municipalities as units: i) yellow fever vaccination coverage in Brazilian municipalities in individuals aged 15-49; ii) reported cases of microcephaly by municipality; and iii) confirmed cases of microcephaly related to ZIKV, by municipality. SaTScan software was used to identify clusters of municipalities for high risk of microcephaly. Results: There were seven significant high risk clusters of confirmed microcephaly cases, with four of them located in the Northeast where yellow fever vaccination rates were the lowest. The clusters harbored only 2.9% of the total population of Brazil, but 15.2% of confirmed cases of microcephaly. Conclusion: We hypothesize that pregnant women in regions with high yellow fever vaccination coverage may pose their offspring to lower risk for development of microcephaly. There is an urgent need for systematic studies to confirm the possible link between low yellow fever vaccination coverage, Zika virus infection and microcephaly.
Zika virus damages the human placental barrier and presents marked fetal neurotropism

Lucia de Noronha¹, Camila Zanluca², Marina Luize Viola Azevedo¹, Kleber Giovanni Luz², Claudia Nunes Duarte dos Santos²/

¹Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brasil; ²Fundação Oswaldo Cruz, Instituto Carlos Chagas, Laboratório de Virologia Molecular, Curitiba, PR, Brasil; ³Universidade Federal do Rio Grande do Norte, Instituto de Medicina Tropical, Natal, RN, Brasil

Fig. 1: pathological findings and immunohistochemistry reactions in placental tissues. (A) Histological section of case 1 immunostained by the conventional immunohistochemistry technique, omitting the primary antibody, which was used as a negative control. We observed chorionic placenta (TORCH type) with chorionic villus inflammation (histiocytic-predominant villi - arrow), edema and trophoblastic epithelial lesions (arrow head) as compared to normal villus tissue (dashed arrow). There was an increase in villous Hofbauer cells and villous stromal lymphohistiocytic cells. (B) Histological section of case 1 immunostained with a non-related anti-Chikungunya virus monoclonal antibody as the primary antibody, which was used as a negative control. We observed the same features observed in A (arrow shows histiocytic-predominant villi, arrow head shows non lesioned trophoblastic epithelial cells and dashed arrow shows normal villi). (C-D) Histological section of case 1 immunostained with the anti-flavivirus monoclonal antibody 402. Chorionic placenta (TORCH type) was observed with immunopositivity in Hofbauer cells (arrow) and some histiocytes in the intervillous spaces (dashed arrow). There was no immunopositivity in the trophoblastic epithelium (arrow head).

Fig. 2: anatomopathological findings on brain tissues samples stained with H&E. (A) Histological section of brain tissue samples of case 2 with a mildly affected white matter region revealing diffuse microglial hyperplasia, gliosis with reactive astrocytes and microglia nodules (arrow). (B) Histological section of a brain tissue sample from case 3 with a mildly affected white matter region containing microlglia nodules (arrow) and demyelination (dashed arrow). (C) Histological section of a brain tissue sample from case 4 with a more severely affected white matter region, revealing extensive destruction and infiltration by mononuclear inflammatory cells. Diffuse microglial hyperplasia was also present. In addition, severe gliosis with reactive microglial astrocytes were diffusely distributed. (D) Histological section of a brain tissue sample from case 4 with a more severely affected white matter region showing extensive perivascular cuffing by lymphocytes (arrow). In addition, moderate gliosis with reactive microglial astrocytes (dashed arrow) were diffusely distributed.

Fig. 3: pathological findings and immunohistochemical reactions in brain tissues. (A-D) Histological sections of brain tissue samples of cases 2 (E-G) and 3 (C-D) immunostained with 402 (B and D) or with a non-related anti-Chikungunya virus monoclonal antibody (A and C). We observed some positive glial cells (arrow) and scattered nodular microglial formations (dashed arrow). (E-G) Histological sections of brain tissue samples of case 4 immunostained with 402 (F-G) or with a non-related anti-Chikungunya virus monoclonal antibody (B) as the primary antibody. We observed some positive germinal glial cells (G - arrow). We also observed scattered positive endothelial cells (F - arrow).
Is CZS caused by ZIKV?

The Brazilian Zika virus strain causes birth defects in experimental models

size reduction of brains
MOI calculation for ZIKV infection in 3D-systems
Correspondence

Prolonged Shedding of Zika Virus Associated with Congenital Infection

Toxoplasma/rubella/CMV: IgM− and IgG+
Normal USG Apr. 28, 2015
HIV−/VDRL−

Symptoms of ZIKV infection at 23 wk of gestation
Normal USG Dec. 28, 2015

Symptoms of ZIKV infection at 26 wk of gestation

Day 0
Newborn delivery

Day 1

Day 4
CSF: normal results and VDRL−

Day 45
Symptoms of ZIKV− on qRT-PCR; IgM− and IgG+

Day 54
Toxoplasma/rubella/CMV: IgM− and IgG+

Day 59
CSF: normal results with CMV−, EBV−, herpes (1-2)−, and toxoplasma− on PCR; serum: toxoplasma− on PCR

Day 67
Serum: ZIKV− on qRT-PCR; IgM− and IgG+

Day 216
Serum and semen: ZIKV− on qRT-PCR; IgG+
Serum: ZIKV− on qRT-PCR; IgM− and IgG+ (low titer)
Serum: ZIKV− on qRT-PCR; IgG+ (titer, >320)

2015
January 2016
February 2016
March 2016
August 2016

Mother
Father
Newborn
Vaccine protection against Zika virus from Brazil

Rafael A. Larocca, Peter Abbink, Jean Pierre S. Peron, Paolo M. de A. Zanotto, M. Justin Lampietro, Alexander Badamchi-Zadeh, Michael Boyd, David Ng'ang'a, Marina KiriLOva, Ramya Nityanandam, Noe B. Mercado, Zhenfeng Li, Edward T. Moseley, Christine A. Bricault, Erica N. Bordinchi, Patricia B. Giglio, David Jetton, George Neubauer, Joseph P. NkoloLa, Lori F. Maxfield, Rafael A. De La Barrera, Richard G. Jarman, Kenneth H. Eckels, Nelson L. Michael, Stephen J. Thomas, Dan H. Barouch

Affiliations | Contributions | Corresponding author

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


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Conclusions

• Despite the difficulties associated with a fast-spreading emerging disease, the scientific community in the Americas has shown the capacity to respond.

• Smart & fast articulation of research groups has been paramount.

• FAPESP as an interesting paradigm for fast response: *fund ongoing projects at once*!

• Follow up children born from mothers that were infected by ZIKV is paramount: The disease has not been fully described yet. We expect more surprises in the future.

• Importance of sexual transmission & viral persistence is a focal research point now.

• Funding is at the essence, but a comprehensive plan on potential emerging pathogens (not only ZIKV) is the right proactive policy.

• Crucially, we have to *think, plan & act beyond ZIKV*. 
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