Viral metagenomics as a tool to identify new pathogens directly in the human samples: Zika outbreak

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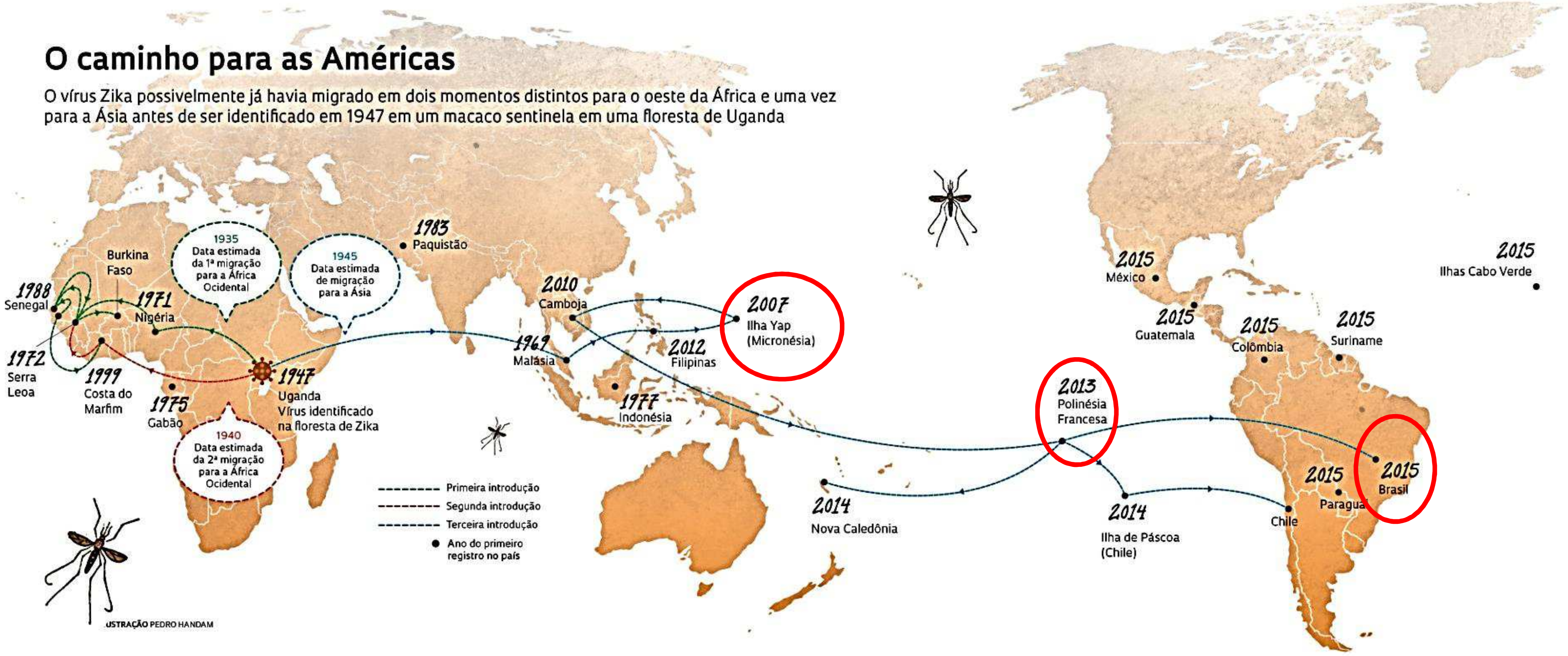
Today Talk

1. Zika outbreak
2. Viral Metagenomics
3. Applications and future perspectives

Zika outbreak

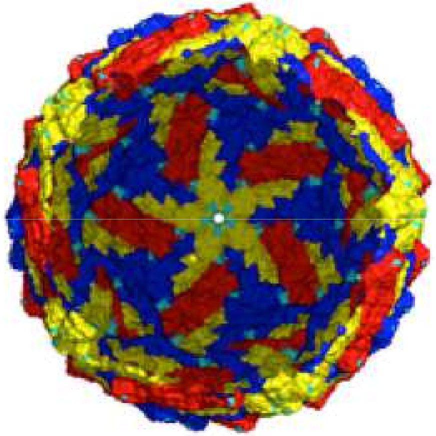


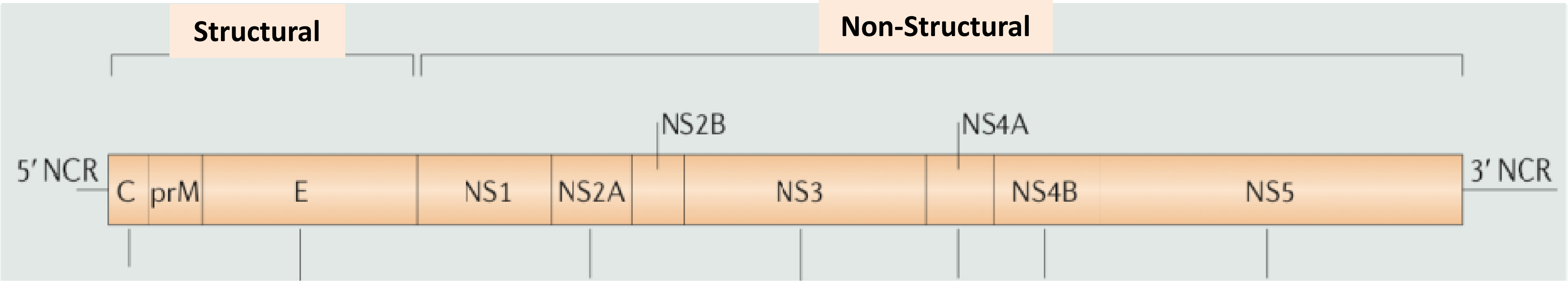
O caminho para as Américas

O vírus Zika possivelmente já havia migrado em dois momentos distintos para o oeste da África e uma vez para a Ásia antes de ser identificado em 1947 em um macaco sentinela em uma floresta de Uganda

Zika genome

Genus: *Flavivirus*



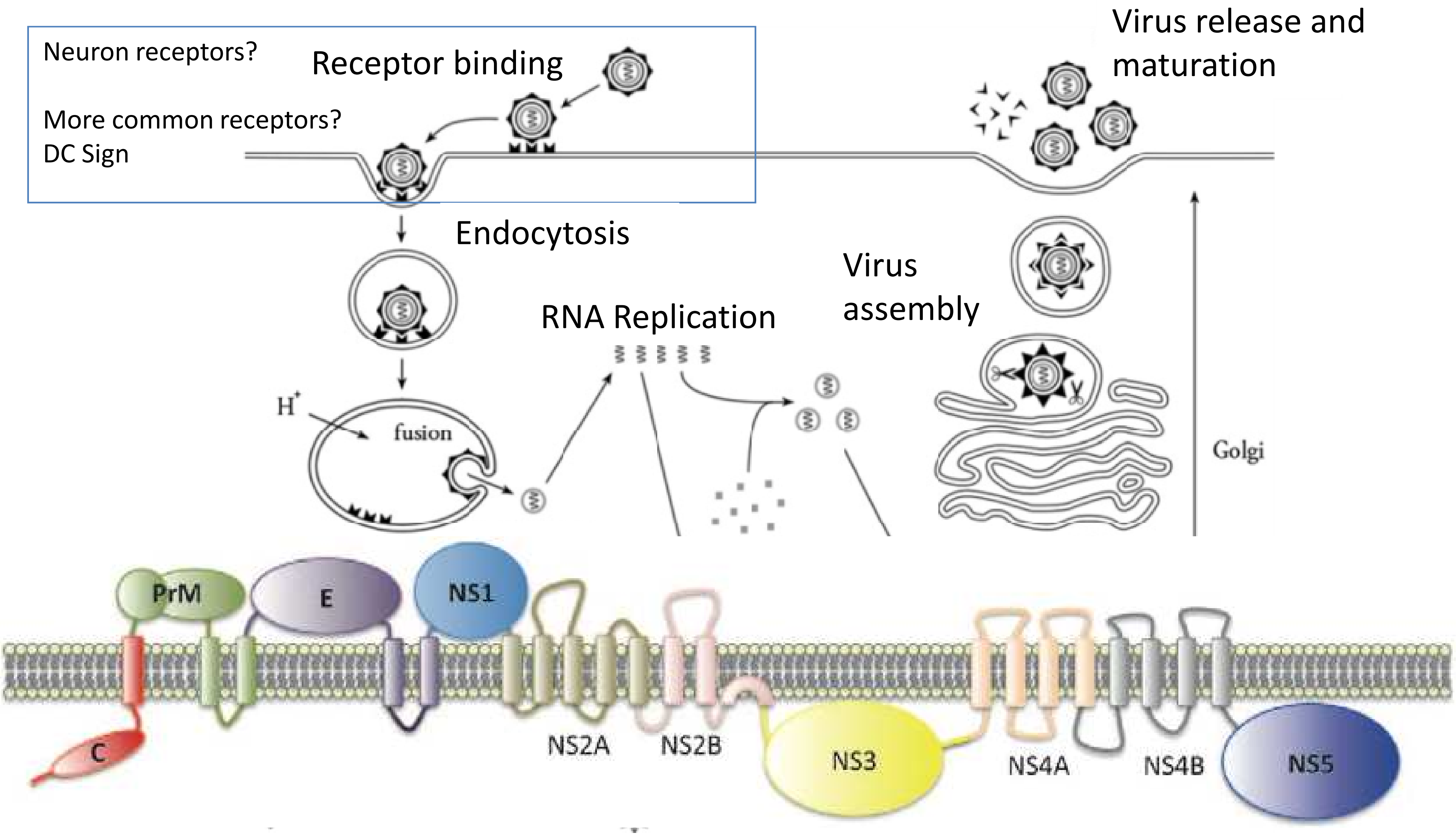


Positive RNA strand virus – about 11kb

Structural proteins: C, prM and Envelope
Non structural proteins: regulatory and replication.

VASILAKIS, *et al.*, 2011

Zika: Replicative Cycle ?



Neuron receptors?

More common receptors?
DC Sign

Receptor binding

Endocytosis

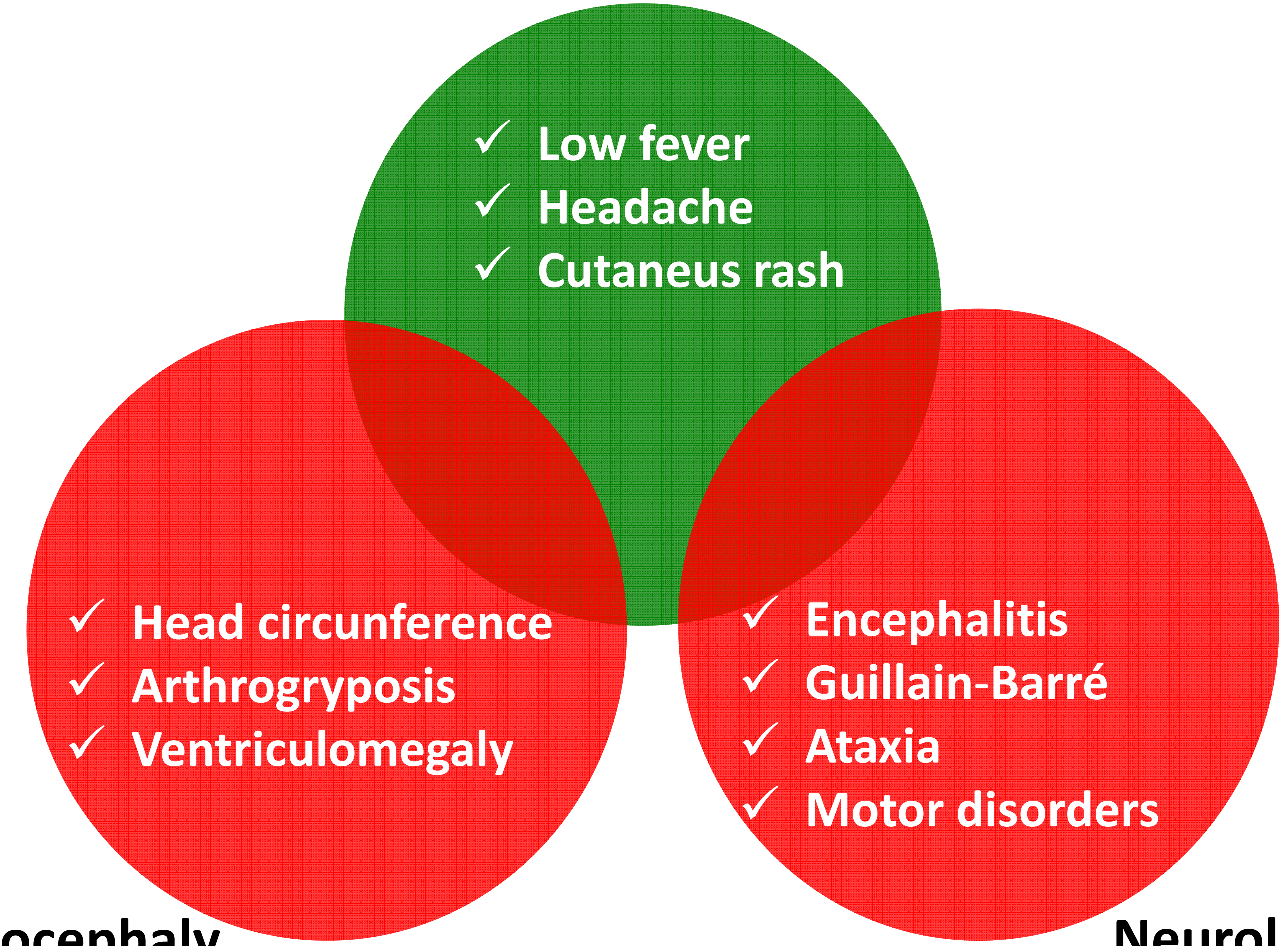
RNA Replication

Virus assembly

Virus release and maturation

Weaver and Vasilakis, 2009

Zika: Symptoms



Classic symptoms

- ✓ Low fever
- ✓ Headache
- ✓ Cutaneus rash

Neonate microcephaly

- ✓ Head circunference
- ✓ Arthrogryposis
- ✓ Ventriculomegaly

Neurologic disorders

- ✓ Encephalitis
- ✓ Guillain-Barré
- ✓ Ataxia
- ✓ Motor disorders

Possible causes of microcephaly

- ✓ **Genetic disorders** (autosomal recessive microcephaly, Aicardi–Goutières syndrome, chromosomal trisomy, Rett syndrome, and X-chromosomal microcephaly among several others).
- ✓ **Drug and chemical intoxication** (alcohol, cocaine, antiepileptic drugs, lead/mercury intoxication and radiation).
- ✓ **Maternal malnutrition**
- ✓ **Bacteria and viral infection** (syphilis, rubella, cytomegalovirus, herpes simplex, varicella zoster virus, HIV-1, Chikungunya **and possible Zika????**

WHO: occipital frontal circumference of the head of the newborn child or fetus is **2 standard deviations (SD) smaller**.

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Research

Protocol for Metagenomic Virus Detection in Clinical Specimens[1]

Claudia Kohl✉ , Annika Brinkmann, Piotr W. Dabrowski, Aleksandar Radonić, Andreas Nitsche, and Andreas Kurth

Author affiliations: Robert Koch Institute, Berlin, Germany

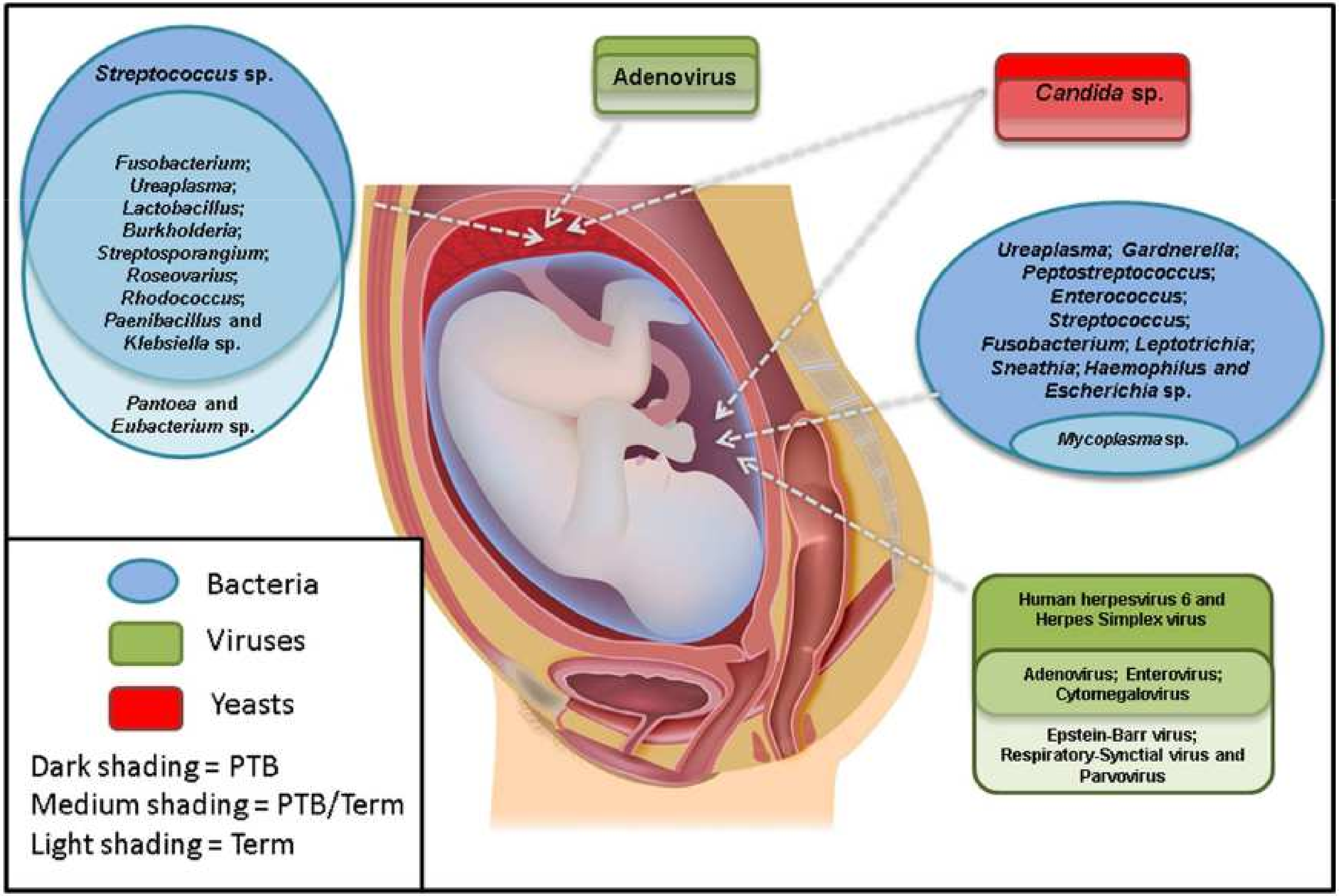
Suggested citation for this article

Abstract

Sixty percent of emerging viruses have a zoonotic origin, making transmission from animals a major threat to public health. Prompt identification and analysis of these pathogens are indispensable to taking action toward prevention and protection of the affected population. We quantifiably compared classical and modern approaches of virus purification and enrichment in theory and experiments. Eventually, we established an unbiased protocol for detection of known and novel emerging viruses from organ tissues

No necessity of isolation and cultive

Aminiotic fluid metagenomics



Streptococcus sp.

Fusobacterium; *Ureaplasma*; *Lactobacillus*; *Burkholderia*; *Streptosporangium*; *Roseovarius*; *Rhodococcus*; *Paenibacillus* and *Klebsiella* sp.

Pantoea and *Eubacterium* sp.

Adenovirus

Candida sp.

Ureaplasma; *Gardnerella*; *Peptostreptococcus*; *Enterococcus*; *Streptococcus*; *Fusobacterium*; *Leptotrichia*; *Sneathia*; *Haemophilus* and *Escherichia* sp.

Mycoplasma sp.

Human herpesvirus 6 and Herpes Simplex virus

Adenovirus; Enterovirus; Cytomegalovirus

Epstein-Barr virus; Respiratory-Synctial virus and Parvovirus

🔵 Bacteria

🟩 Viruses

🟥 Yeasts

Dark shading = PTB
Medium shading = PTB/Term
Light shading = Term

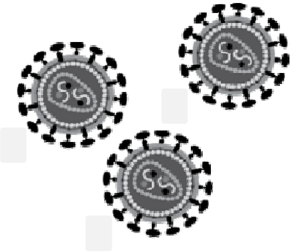
Matthew S. Payne and Sara Bayatibojakhi , 2014*

Viral Metagenomics

Depend on NGS and unbiased sequencing

Method	Advantage	Disadvantages
Sanguer sequencing	• Sequencing can be focused on any taxon of interest, regardless of prevalence in community • Good for microbial communities with high diversity • Cheaper	• Do not reconstruct entire genome • Cannot identify novel types • Sequence data focused on single group, not entire community
Unbiased sequecing	• All genomes in the sample are sequenced • Can identify novel genotypes • Can assemble full genomes of dominant types • Good for subpopulations	• Only dominant genomes are well-represented • Automated assembly of genome is problematic, requires manual checking for some assemblies • Expensive

Viral Metagenomics – Work Flow



Filtrate (0,45 uM) and ultracentrifugate (concentrate virus particles)

↓

RNase and Dnase treatment (remove human RNA and DNA)

↓

RNA and DNA extraction and double strand DNA synthesis

↓

DNA fragmentation and tagmentation

↓

Sequencing in Miseq Plataform

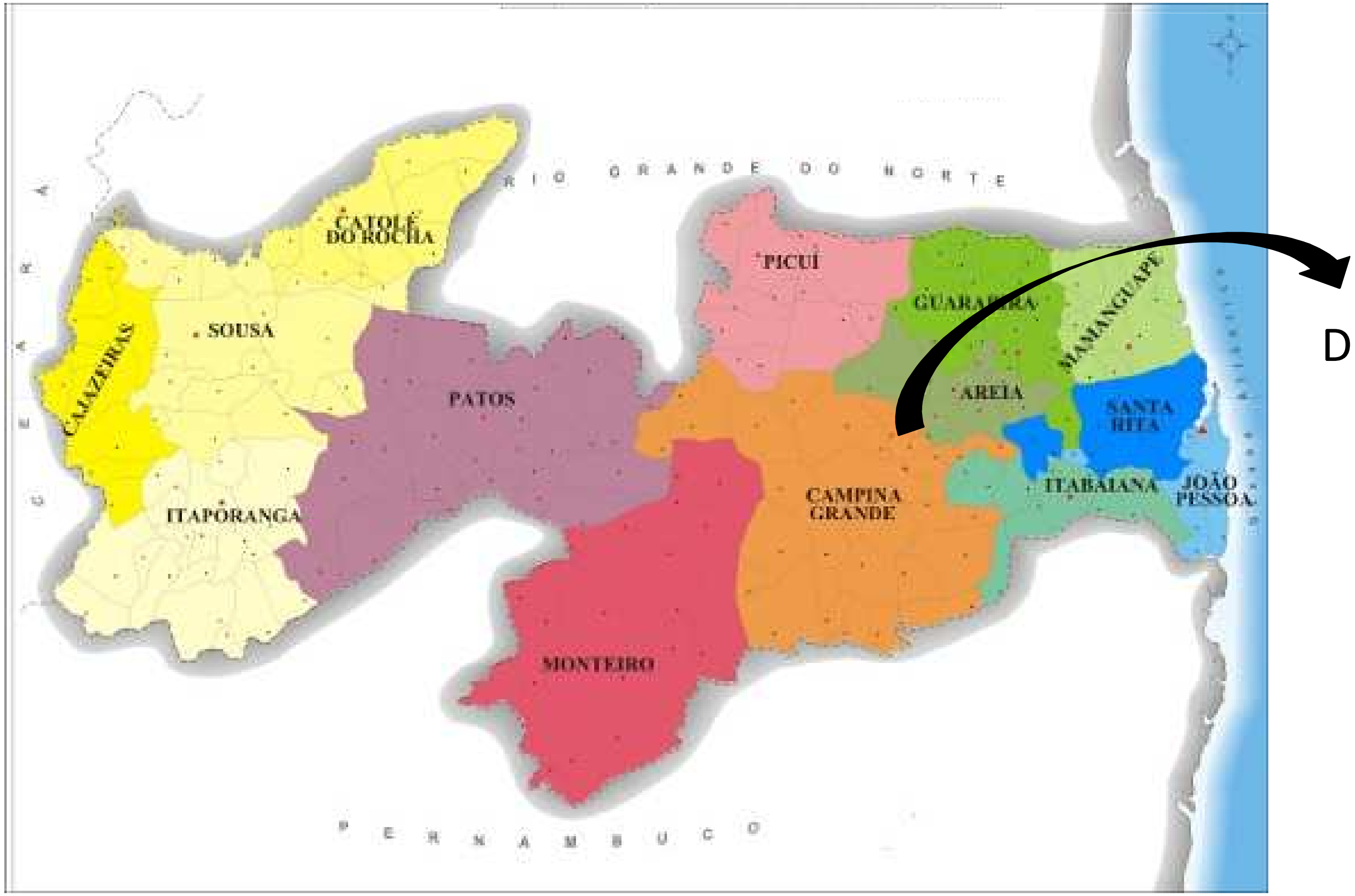
↓

Analyzing the data (clean up human reads, blast sequences, align in reference genome).

The Lancet ID, 2016

Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study

Guilherme Calvet, Renato S Aguiar*, Adriana S O Melo, Simone A Sampaio, Ivano de Filippis, Allison Fabri, Eliane S M Araujo, Patricia C de Sequeira, Marcos C L de Mendonça, Louisi de Oliveira, Diogo A Tschoeke, Carlos G Schrago, Fabiano L Thompson, Patricia Brasil, Flavia B dos Santos, Rita M R Nogueira, Amilcar Tanuri†, Ana M B de Filippis†*



Dra. Adriana Melo
IPESQ

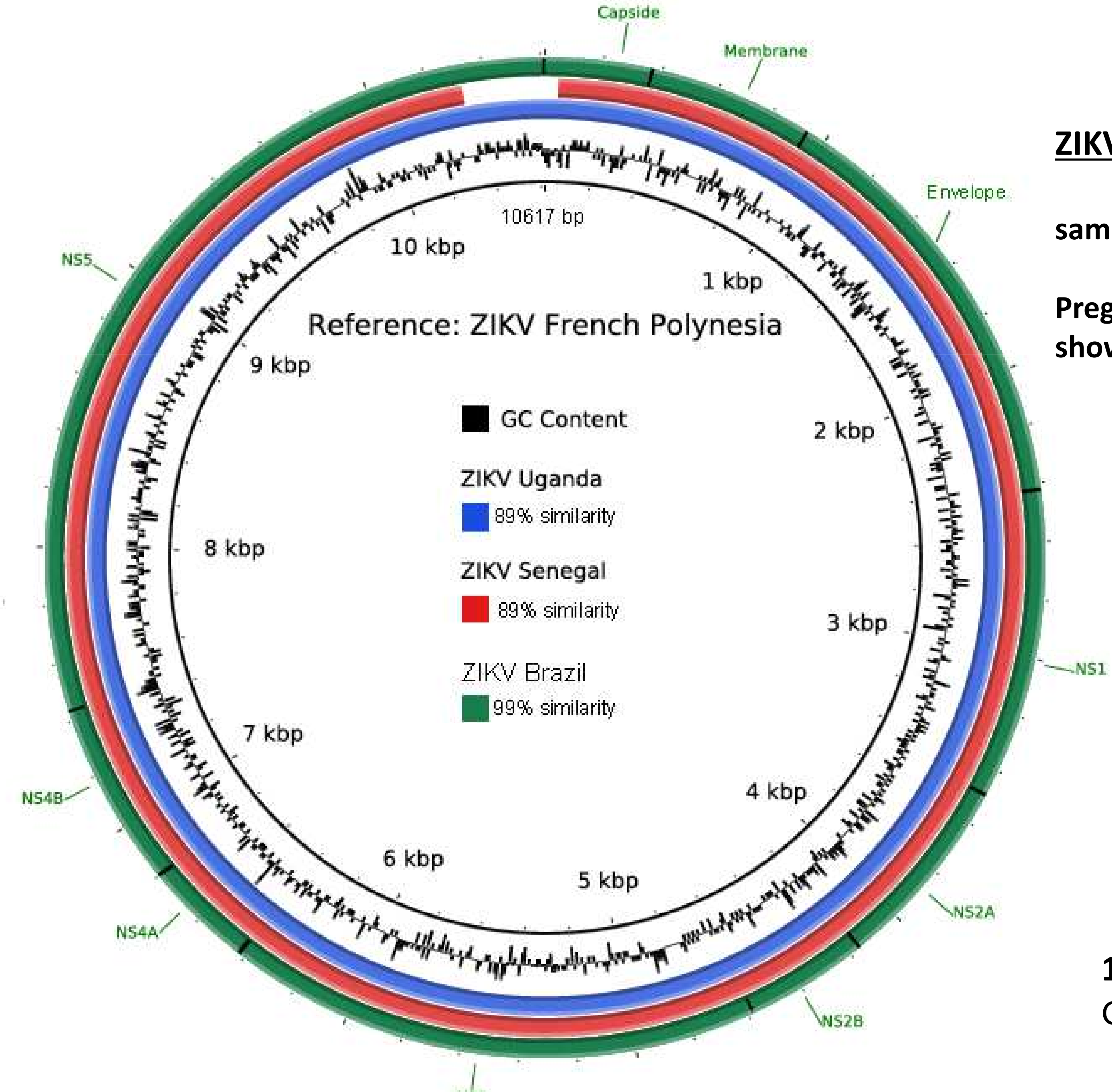
FIOCRUZ

UFRJ

Patients, diagnostic and sampling

Case	Age	Zika symtoms	Amniocentesis (amniotic fluid)	Last ultrasson	Sorology	RT-PCR Zika	Metagenomic
#1	27	18th week cutaneous rash with itching of the hands and back	28th week	• Microcephaly • dilation of ventricles • asymmetry of hemispheres • hypoplastic cerebellum with absence of cerebellar vermis.	Anti-DENV • IgG Neg • IgM Neg Anti-CHIK • IgG Neg • IgM Neg Anti-Zika • IgM Pos	positive	683 sequences ZIKV positive 5 contigs 10,793 bases whole genome
#2	35	10th week cutaneous rash with fever	28th week	• Microcephaly • severe hypoplasia of the cerebellar vermis • enlargement of the posterior fossa • parenchyma was normal.	Anti-DENV • IgG Neg • IgM Neg Anti-CHIK • IgG Neg • IgM Neg Anti-Zika • IgM Pos	positive	Only 2 fragments 1641-1763 6466-6566

All samples TORCH negative, HIV, Syphilis, Cytomegalovirus (CMV) and Parvovirus B19 negative

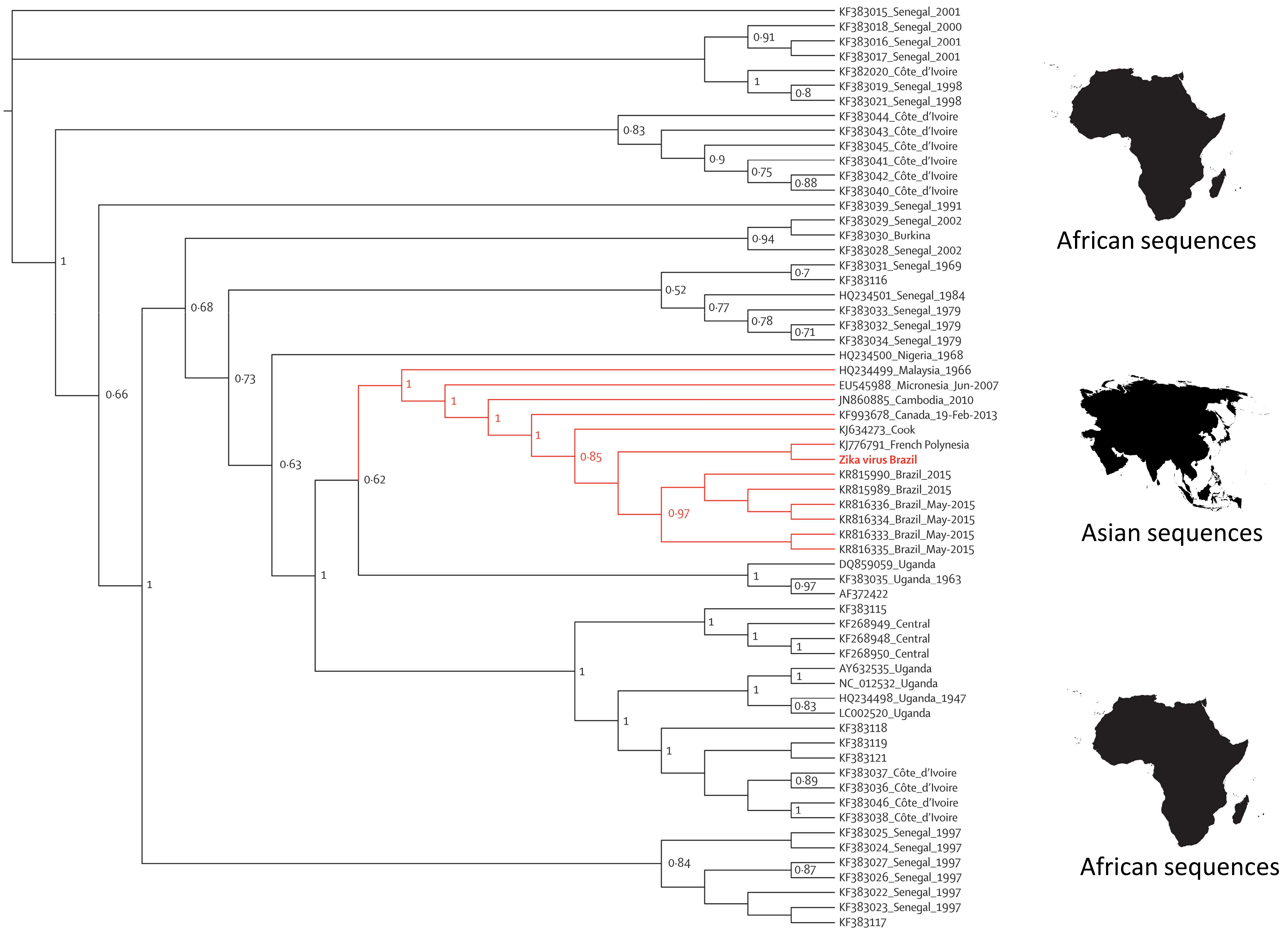


ZIKV BR genome

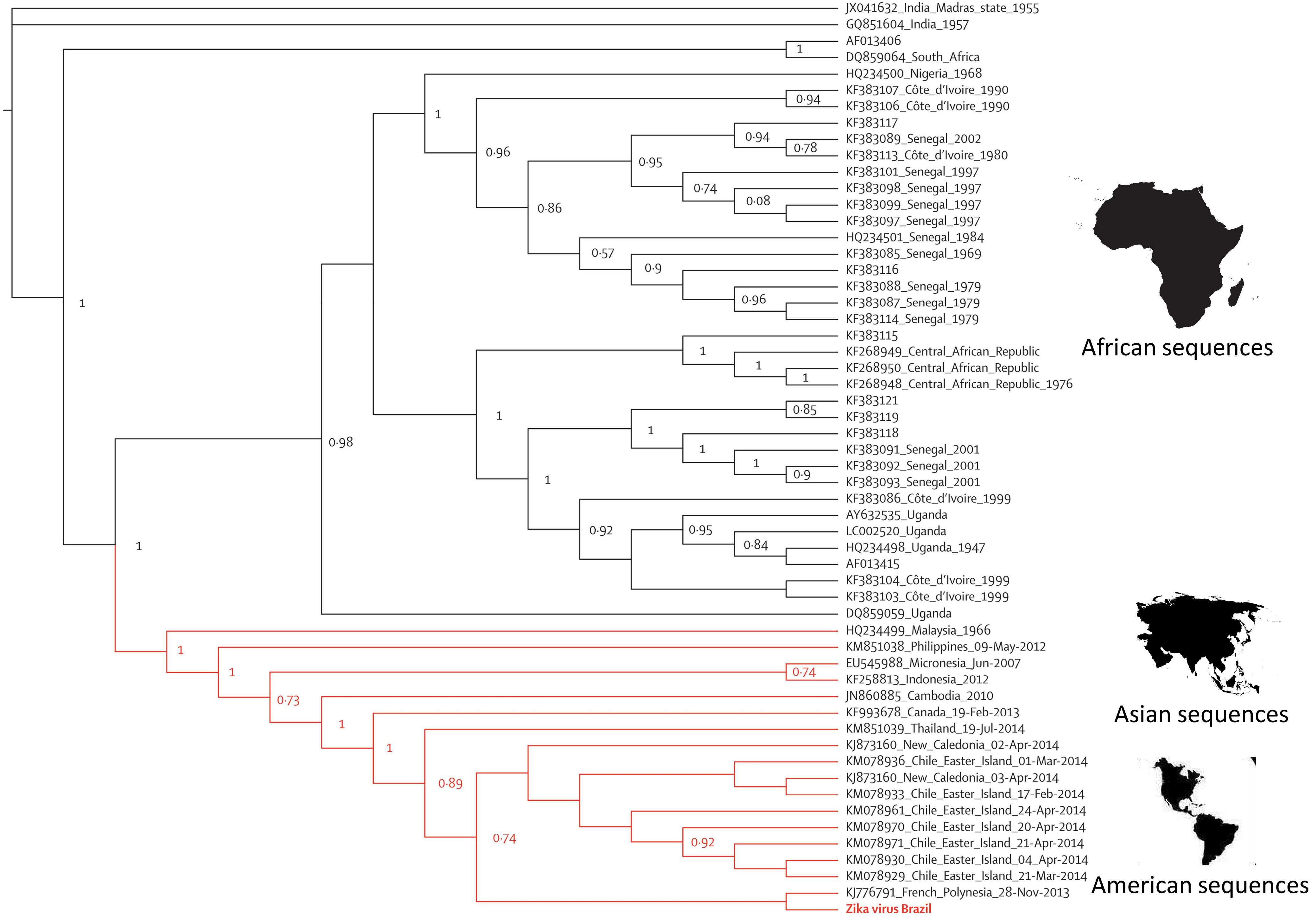
sample: amniotic fluid

Pregnant woman with fetus showing microcephaly

10,793 bases
GenBank :



Envelope phylogeny: maximum likelihood



NS5 phylogeny: maximum likelihood

African versus Asian strains

Open questions ???

- ✓Is there any difference in the pathology ?
- ✓ Viral fitness and replication?
- ✓ Vector transmission?
- ✓Diagnosis and antiviral development?

In vivo model of Zika infection

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

David O'Connor visiting UFRJ

NATURE | NEWS

Zika researchers release real-time data on viral infection study in monkeys

Raw data from macaque experiment published daily online.

Declan Butler

23 February 2016

Gabrielle Lehrer-Brey, part of a team that is experimentally infecting macaques with Zika virus, works with blood-plasma samples at the University of Wisconsin-Madison.

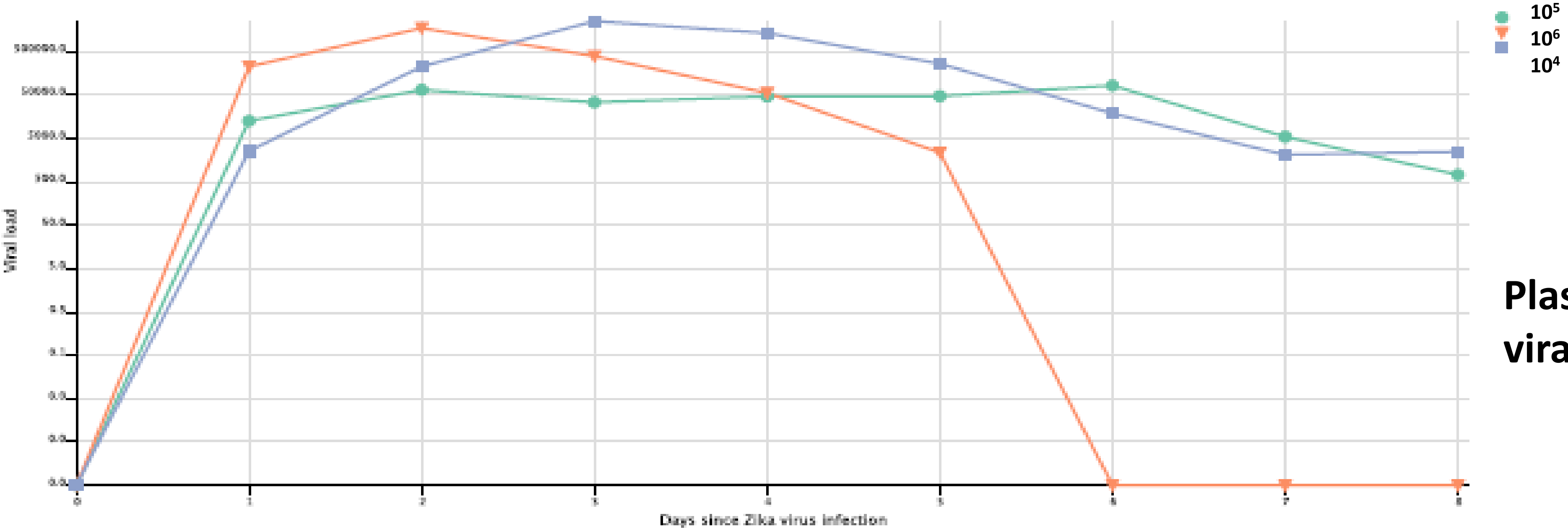
Zika Experiemental Team - UWMD

Dr David O'Connor, whose 'ZEST' team at the University of Wisconsin-Madison are releasing Zika data in real time.

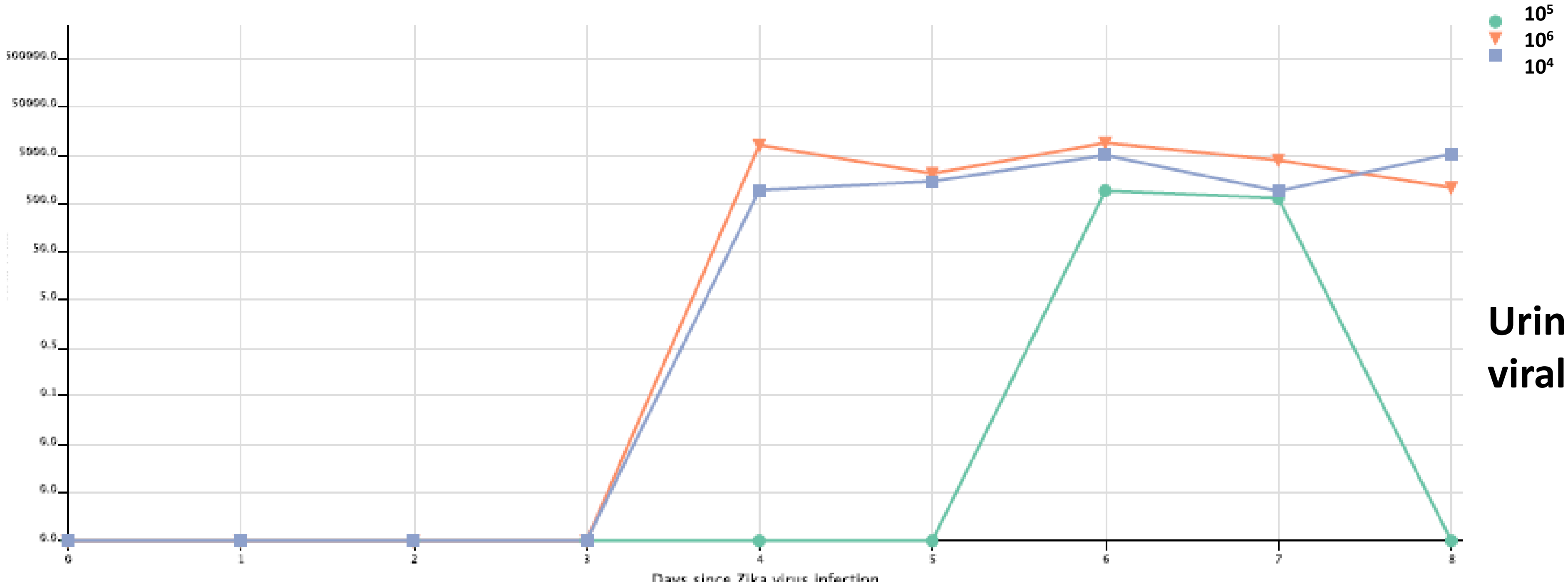
https://dholk.primate.wisc.edu/project/dho/public/Zika/public/ZIKV-001-public/begin.view

On February 15, we challenged 3 male Indian rhesus macaques with French Polynesian Zika virus.
Each animal was challenged with a different dose:

- 826226 = 10^6 PFU
- 393422 = 10^5 PFU
- 912116 = 10^4 PFU

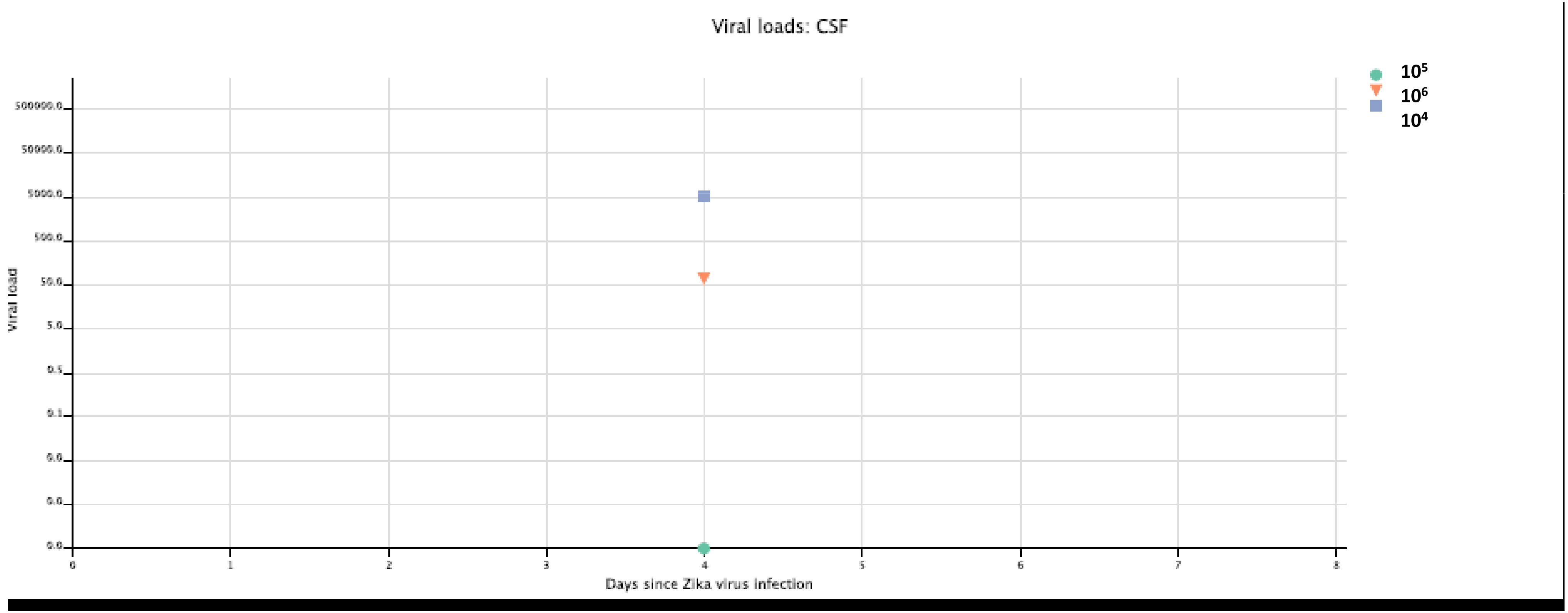


Plasma viral load



Urine viral load

Neutropism of Zika (CSF)



To view the data:
https://dholk.primate.wisc.edu/project/dho/public/Zika/public/ZIKV-001-public/begin.view

- monkeys health (weight, biochemistry and metabolic tests)
- Immunophenotyping (CD4%, CD8%, NK%, cytokine production).
- Tissues necropsy

New round of infection in pregnant monkeys.

Future Perspectives of microcephaly cases

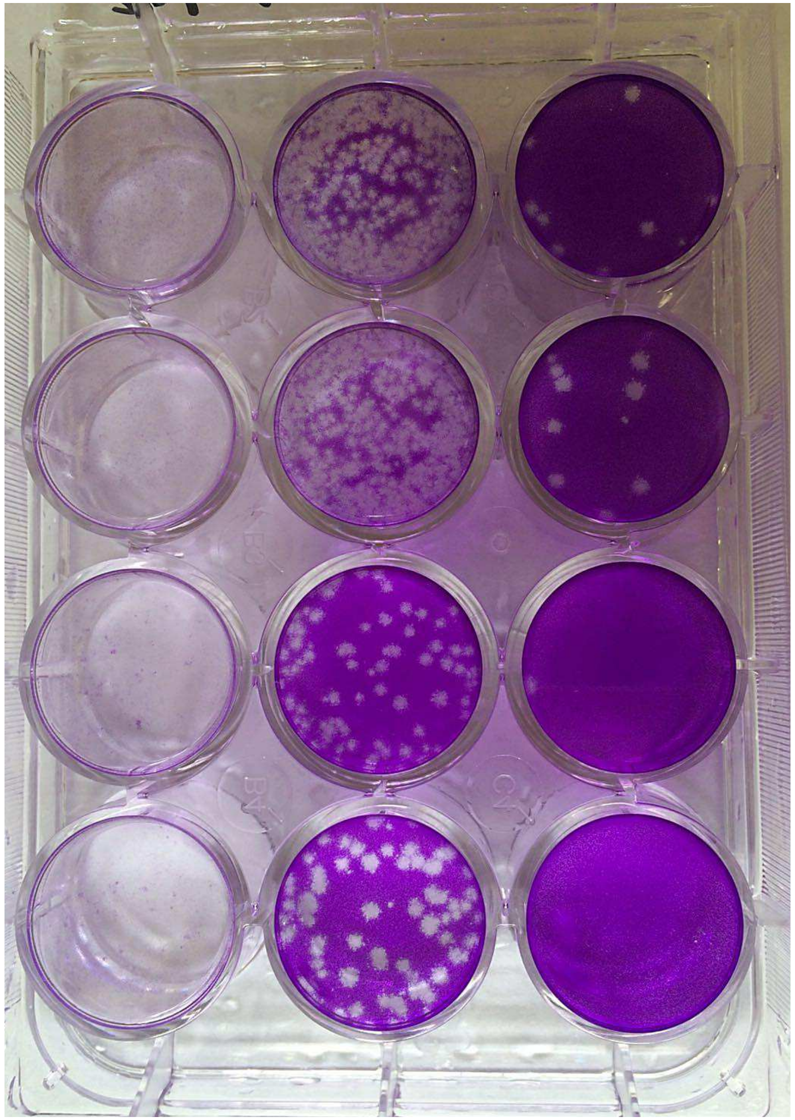
Neonate with Arthrogryposis

- ✓ **Incresaing the numbers of cases to be analysed (14 cases described by Adriana Melo)**
- ✓ **Virus identification in the samples (RT-PCR, metagenomics and immunohistochemistry)**
- ✓ **Inflammation status (cytokine storm).**
- ✓ **Cellular Pathaways modulated by Zika infection (transcrpitome of infected tissues)**

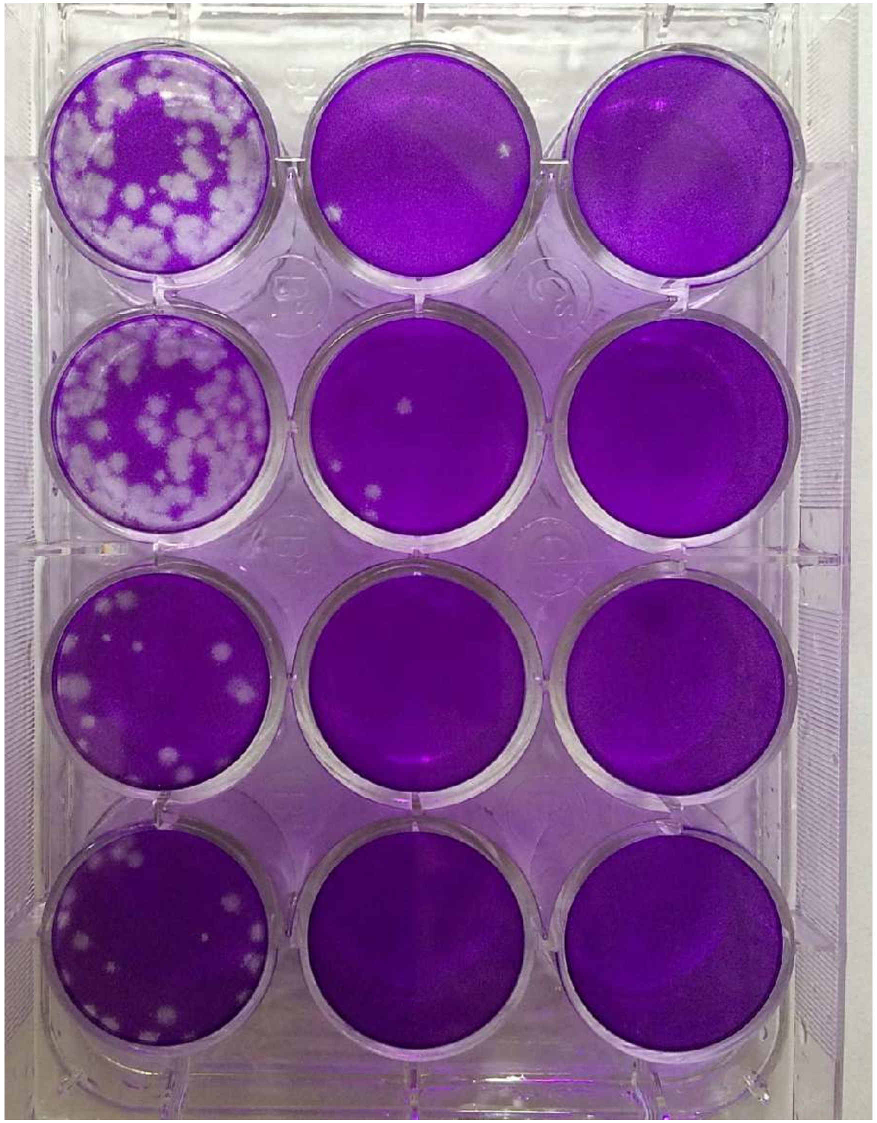
Zika identification in neonates

# Paciente	Material																	
	Líquidos						Sólidos											
	Liquido Amniotico [LA]	Urina da mãe [UM]	Urina Bebê [UI]	Sangue materno [SM]	Sangue de cordão [SC]	Líquor (LCR) [LC]	Placenta [PL]	Cordão (tecido) [CU]	Cérebro [CE]	Cerebelo (CB)	Nervo Periférico [NP]	Medula Espinhal [ME]	Intestino [IN]	Pulmão [PU]	Rim [RI]	Baço [BA]	Fígado [FI]	Coração [CO]
S.0001	Pos	espera		espera	Neg		Neg	Neg	Pos				espera	Neg	Neg		Neg	Neg
S.0002	Pos				Neg	Neg	Neg	Neg										
S.0003																		
S.0004	espera			espera			Pos											
S.0005	Neg		espera	espera	Neg		Pos	Neg										
S.0006	espera	espera	espera	espera			Neg											
S.0007	Pos	espera		espera	Neg	Neg	Neg	Neg	Pos	Pos	Neg	Pos	espera	Pos	Neg	Neg	Neg	Neg
S.0008				espera														
S.0009	Neg			espera	Neg		Neg	Neg	Neg				espera	Neg	Neg	Neg	Neg	Neg
S.0010	Pos																	
S.0011	Neg			espera														
S.0012	Pos			espera														
S.0013	Pos																	
S.0014		espera			Neg		Neg											

African reference 766



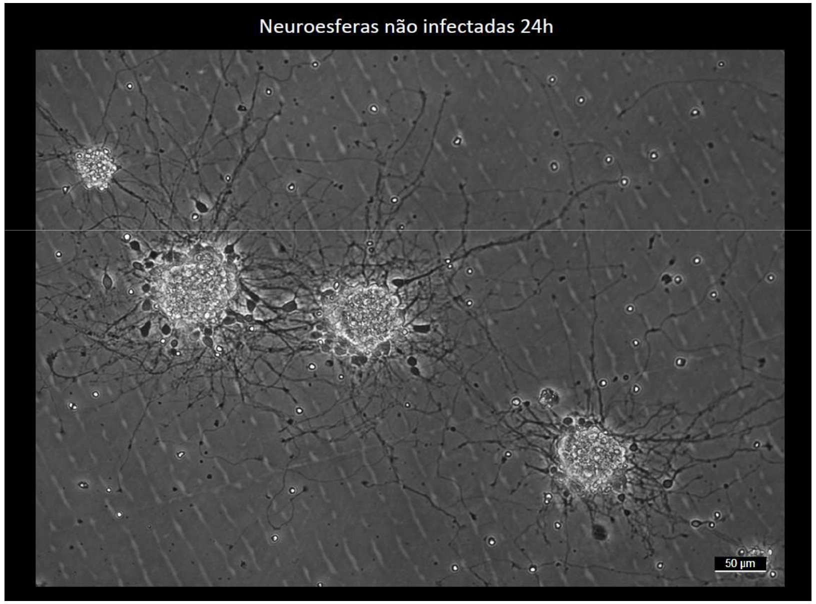
Zika Br

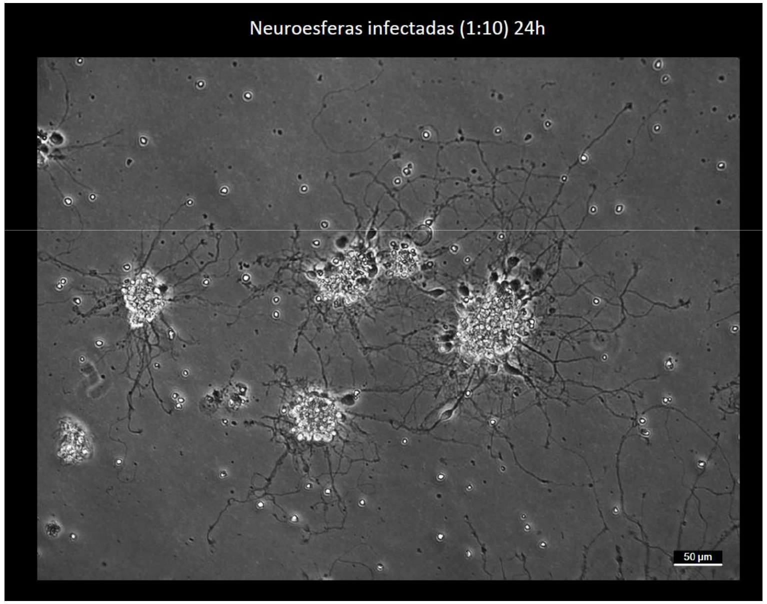


Zika infected neurospheres

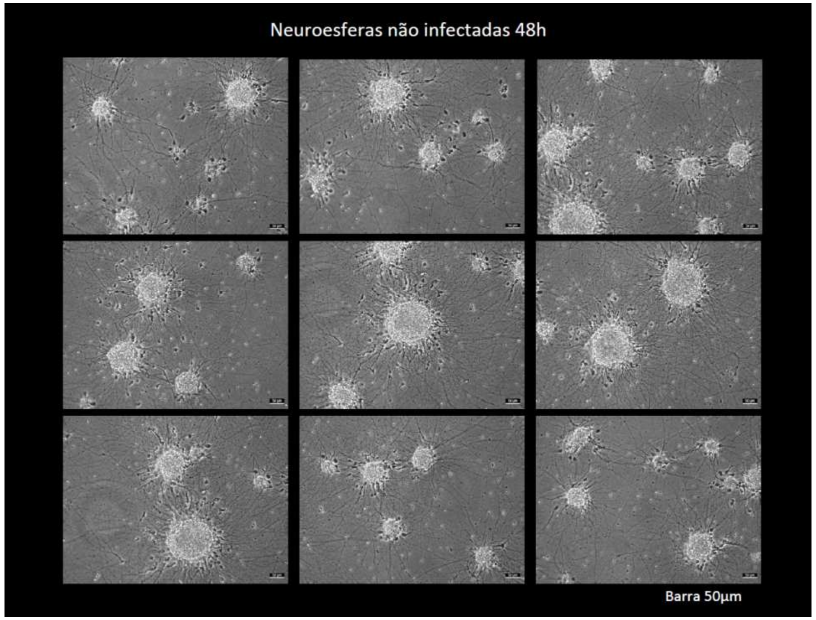
Non infected

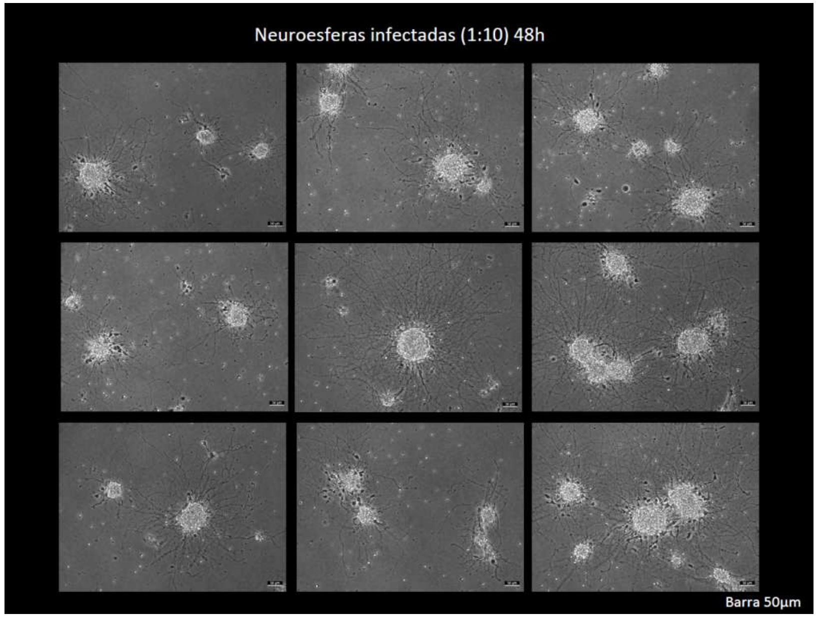
Zika Br





24 h





48 h

Conclusions

- The only virus identified in the liquid amniotic fluid of microcephaly cases by viral metagenomics was Zika.
- Zika-BR more related to French Polynesia sequences.
- In vivo monkeys models recapitulate the human infection with viremia peaks.
- Virus-cell interactios studies should be performed camparing African and Brazilian Zika viruses to elucidate virus neurotropism and pathology.
- All the raw sequence data was deposited as SVA file in the viromica.org to general public.
- Increasing the Encephalitis cases non Guillain-Barré

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- Rodrigo Delvechio (UFRJ)
- Loraine Campanati (UFRJ)
- Patricia Garcez (UFRJ)
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