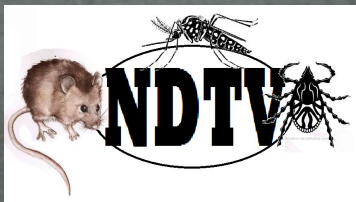


“Vigilância Laboratorial do Zika Vírus no Estado de São Paulo”

Renato Pereira de Souza, PhD

Pesquisador Científico – NDTV/CV/IAL



Zika vírus

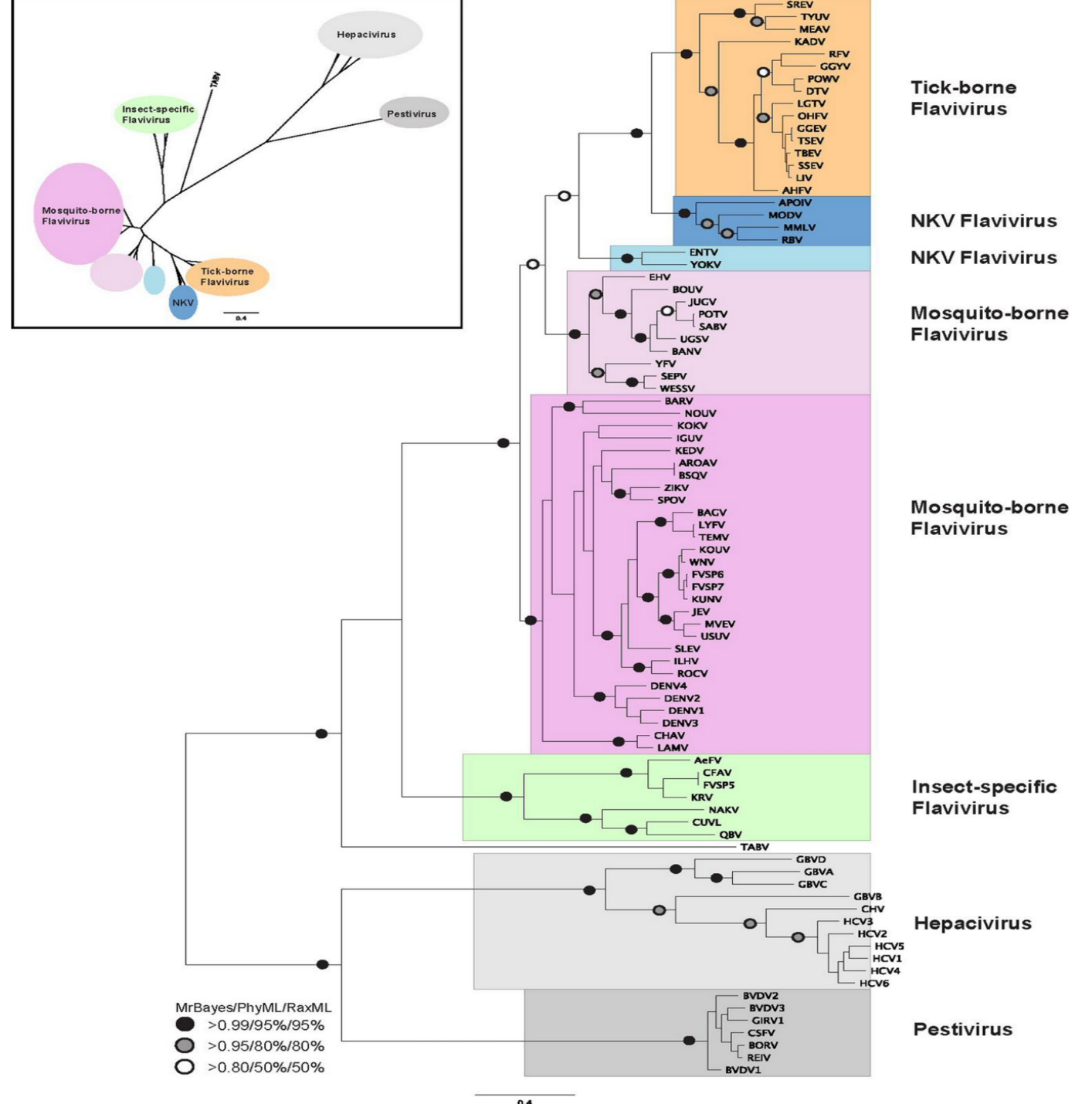
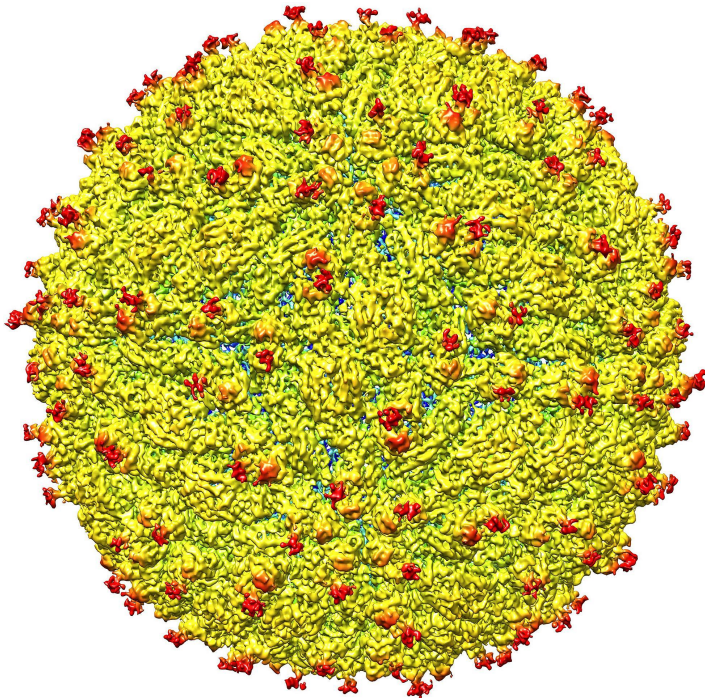
Flavivírus – Flaviviridae

Grande Diversidade

Impacto Considerável à Saúde Pública

Ramo das Encefalites– WNV, JEV, ROCV.

Clado do Spondweni



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

Oumar Faye¹, Caio C. M. Freire², Atila Iamarino², Ousmane Faye¹, Juliana Velasco C. de Oliveira², Mawlouth Diallo¹, Paolo M. A. Zanotto², Amadou Alpha Sall^{1*}

1 Institut Pasteur de Dakar, Dakar, Senegal, **2** Laboratory of Molecular Evolution and Bioinformatics, Department of Microbiology, Biomedical Sciences Institute, University of Sao Paulo, Sao Paulo, Brazil

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.

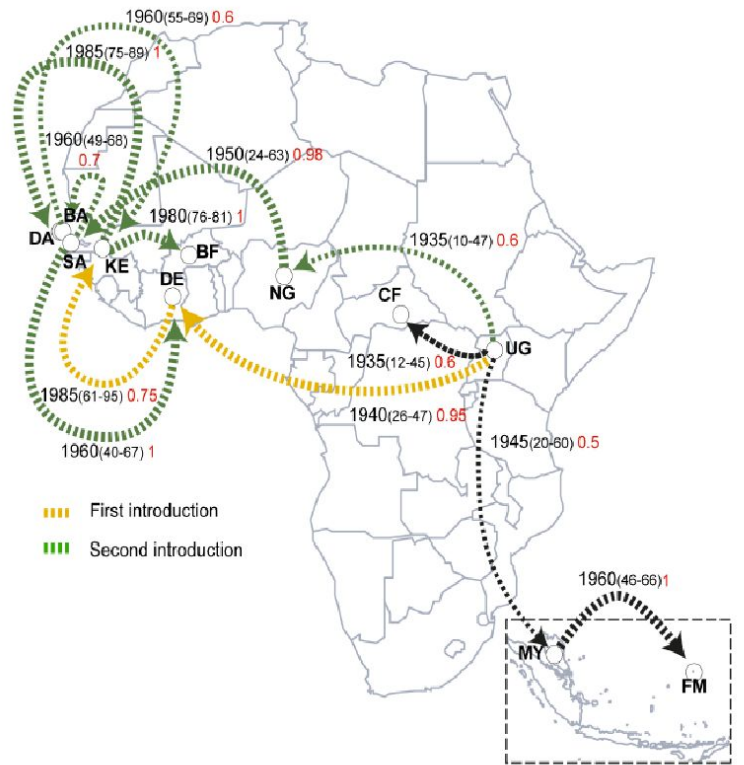
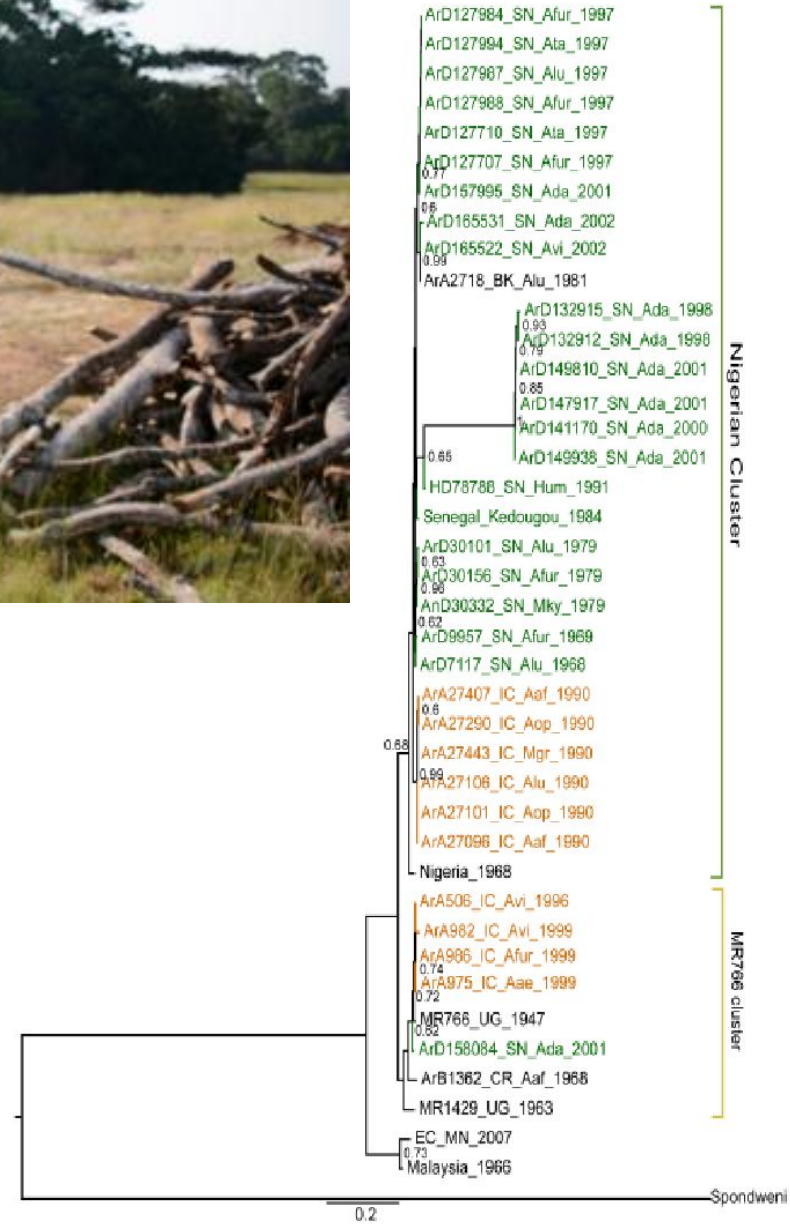
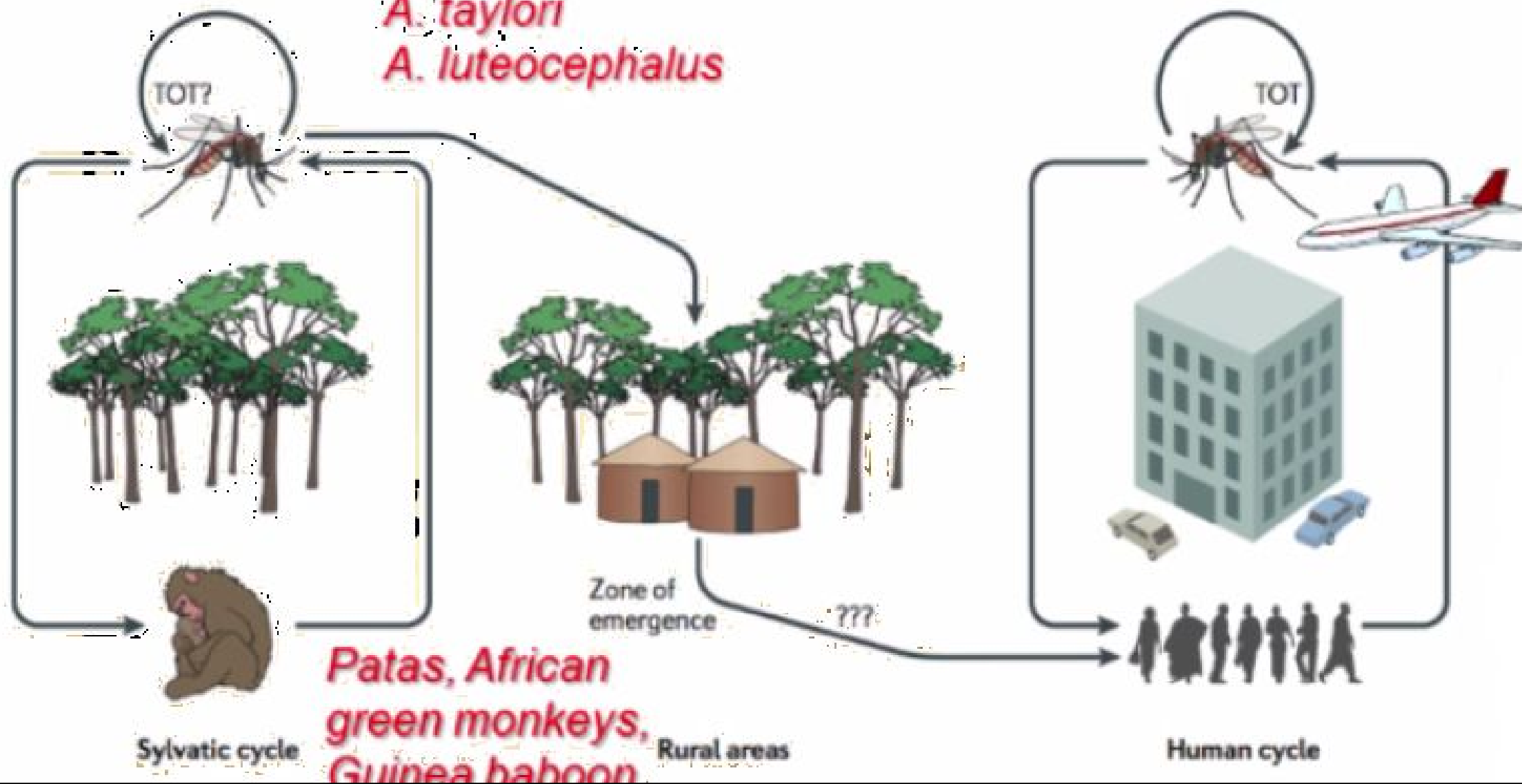


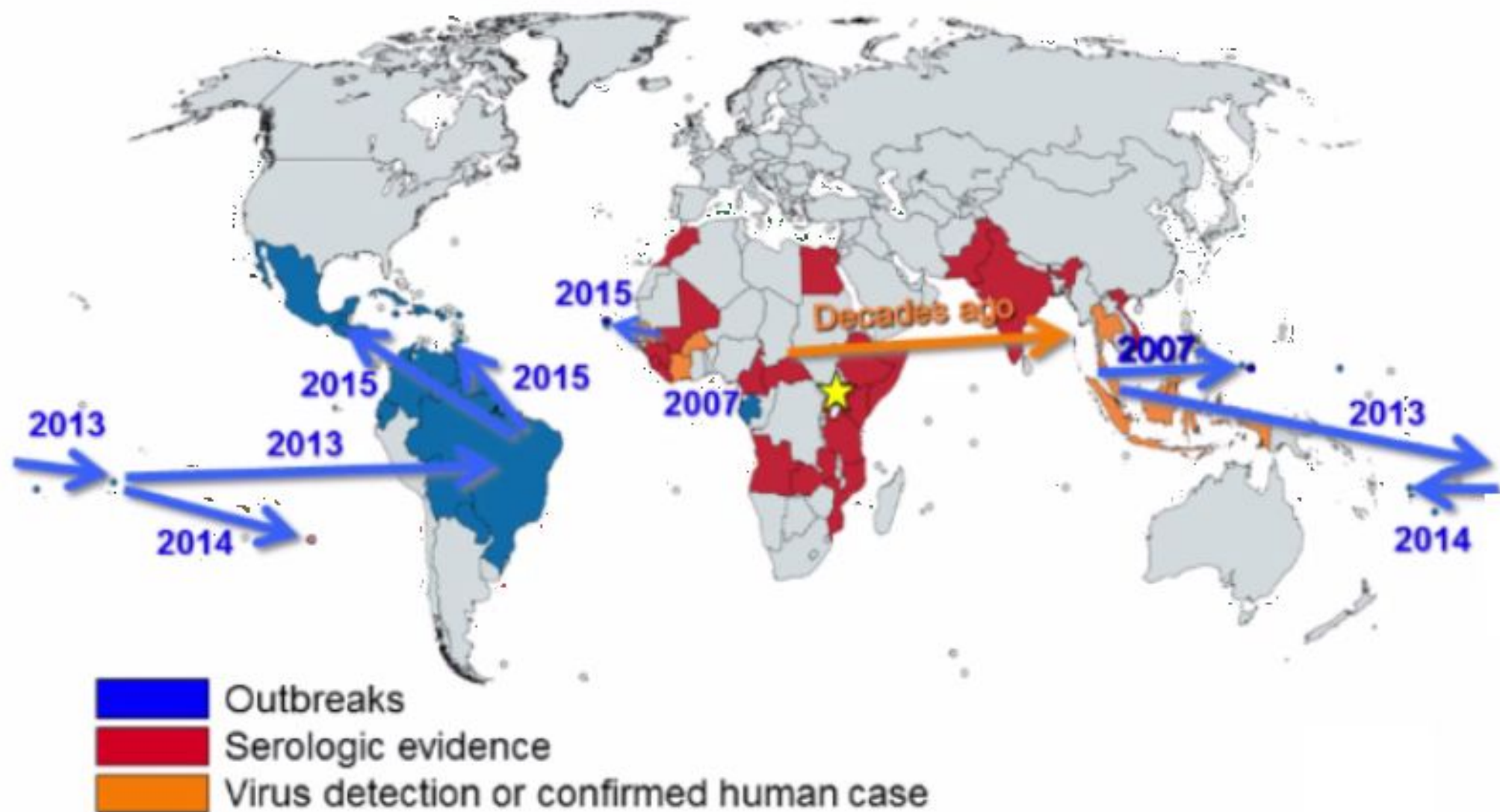
Figure 2. Geographic spread of ZIKV in Africa and Asia. The directed lines connect the most probable sources and target localities of viral lineages (shown by arrows), with widths proportional to the posterior probabilities and values shown in red. Only plausible routes with probabilities above 50% are shown. The distinct introductions into Senegal and Cote 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

A. furcifer
A. taylori
A. luteocephalus

A. aegypti
A. albopictus (and
other *Stegomyia*)



Historic Spread of Zika Virus





Encefalite
Japonesa
Nilo Ocidental
Rift Valley
Zika
Ebola
Marburg
Lassa



ZIKA

SINTOMAS

Febre baixa
Olhos vermelhos
Dores nas articulações
Dor de cabeça
Manchas na pele



A metodologia empregada é de alta sensibilidade e especificidade.

Nominalmente o protocolo Lanciotti et al, 2008 tem a capacidade de detecção a partir de 25 cópias de RNA/Reação, sendo altamente específico mesmo quando desafiado com outros flavivírus.

O protocolo Faye et al, 2013 relata uma sensibilidade de 0,05 PFU/reação e também apresentou alta especificidade, comprovada por sua não reatividade à outros 31 flavivírus.

RESEARCH

Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007

Robert S. Lanciotti,* Olga L. Kosoy,* Janeen J. Laven,* Jason O. Velez,* Amy J. Lambert,*
Alison J. Johnson,* Stephanie M. Stanfield,* and Mark R. Duffy*

Zika virus (ZIKV) is a mosquito-borne flavivirus first isolated in Uganda from a sentinel monkey in 1947. Mosquito and sentinel animal surveillance studies have demonstrated that ZIKV is endemic to Africa and Southeast

studies in the absence of epidemics (6–8). Additional serologic studies in the 1950s and 1960s detected ZIKV infections among humans in Egypt, Nigeria, Uganda, India, Malaysia, Indonesia, Pakistan, Thailand, North Vietnam,

Faye et al. *Virology Journal* 2013, **10**:311
<http://www.virologyj.com/content/10/1/311>



VIROLOGY JOURNAL

RESEARCH

Open Access

Quantitative real-time PCR detection of Zika virus and evaluation with field-caught Mosquitoes

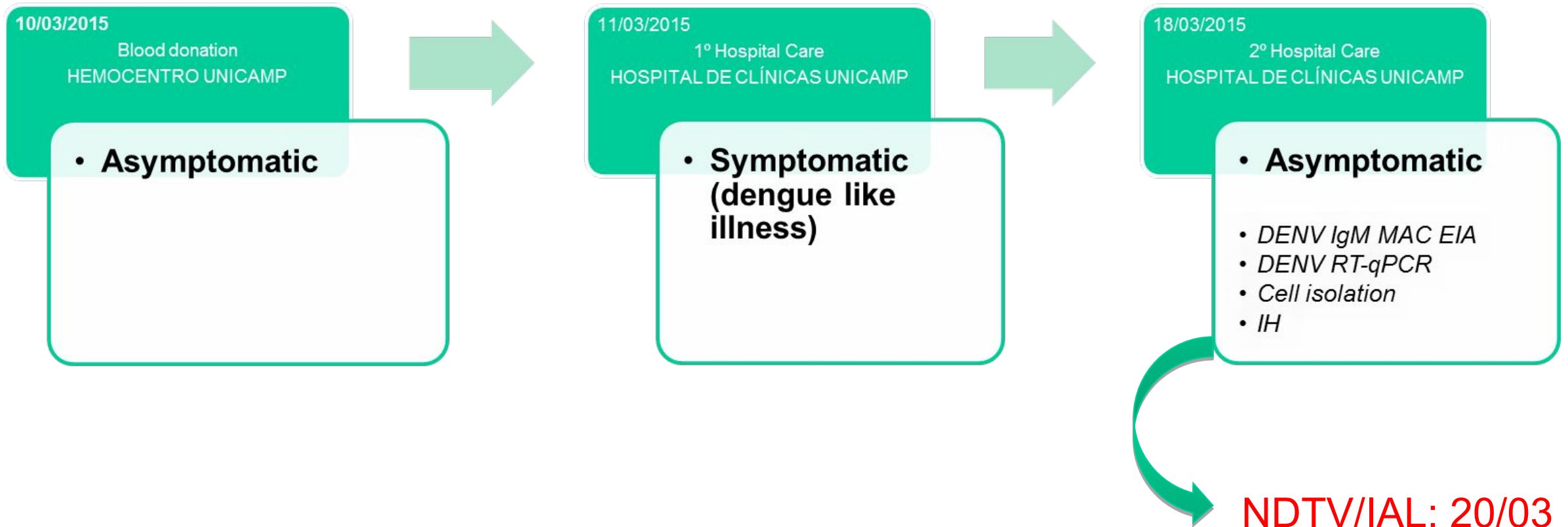
Oumar Faye¹, Ousmane Faye¹, Diawo Diallo², Mawlouth Diallo², Manfred Weidmann³ and Amadou Alpha Sall^{1*}

Abstract

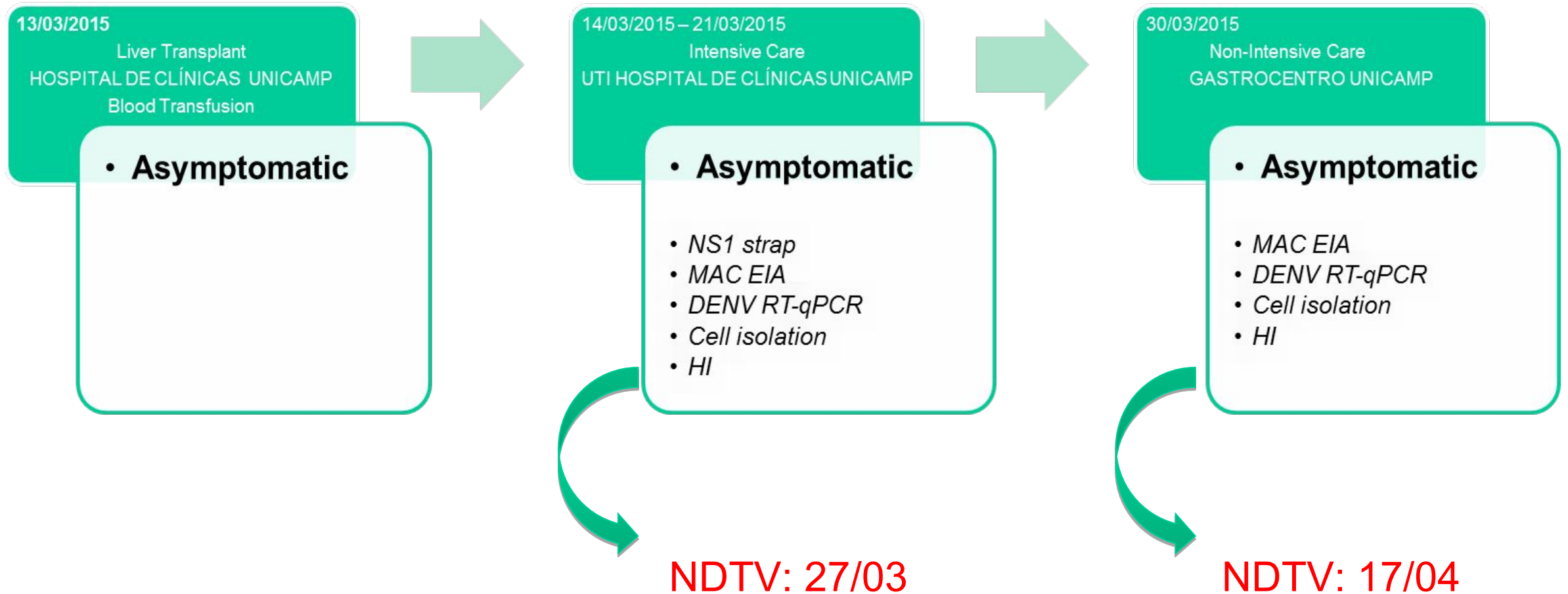
Background: Zika virus (ZIKV), a mosquito borne flavivirus is a pathogen affecting humans in Asia and Africa. ZIKV infection diagnosis relies on serology—which is challenging due to cross-reactions with other flaviviruses and/or absence or low titer of IgM and IgG antibodies at early phase of infection- virus isolation, which is labor intensive, time consuming and requires appropriate containment. Therefore, real-time RT-PCR (rRT-PCR) is an appealing option as a rapid, sensitive and specific method for detection of ZIKV in the early stage of infection. So far, only one rRT-PCR assay has been described in the context of the outbreak in Micronesia in 2007. In this study, we described a one step rRT-PCR for ZIKV which can detect a wider genetic diversity of ZIKV isolates from Asia and Africa.

Results: The NSS protein coding regions of African ZIKV isolates were sequenced and aligned with representative flaviviruses sequences from GenBank to design primers and probe from conserved regions. The analytical sensitivity of the assay was evaluated to be 32 genome-equivalents and 0.05 plaque forming unit (pfu). The assay was shown to detect 37 ZIKV isolates covering a wide geographic in Africa and Asia over 36 years but none of the 31 other flaviviruses tested showing high analytical specificity. The rRT-PCR could be performed in less than 3 hours. This method was used successfully to detect ZIKV strains from field-caught mosquitoes.

Doador de Sangue, 52 anos, Sexo masculino - Sumaré (SP) Sem Histórico de Viagem



Receptor, Sexo masculino, 55 anos



No Laboratório...

NS1 ELISA Biorad® - IAL Campinas

DENV fourplex real-time RT-PCR (Johnson et al., 2005)

MAC-ELISA IgM (in-house)

Tentativa de Isolamento em células C6/36 seguida por
Ensaio de Imunofluorescência Indireta(IFA) usando
anticorpo policlonal para Flavivírus e Anticorpo
monoclonal para DENV.

Resultados



This sample was
retrieved from
HC/UNICAMP and
was sent later to
NDTV

Blood Donor	D ₋₁	D ₆
DENV IgM MAC EIA	Negative	Negative
DENV RT-qPCR	Negative	Negative
IFA/Zika RT-PCR	POSITIVE	Negative
HI	-	Negative

Receptor	D ₀ (transfusion)	D ₁₇
IgM MAC EIA	Negative DENV, ROCV, SLEV, YFV	DENV POSITIVE
DENV RT-qPCR	Negative	Negative
IFA	POSITIVE	Negative
HI	Anti-Flavi atb	Anti-Flavi atb
NS1	Negative	-

Receptor

IFA Anti-flavivírus
Positive
IFA Anti-DENV
Negative

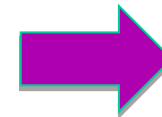
RT-PCR
SLEV

RT-PCR
WNV

RT-PCR YFV

Zika Virus RT-PCR
Positive

(Lanciotti et al., 2008)



Envelope sequenced
Applied Biosystems
377 DNA sequencer

CASE REPORT

Probable transfusion-transmitted Zika virus in Brazil

*Maria L. Barjas-Castro,¹ Rodrigo N. Angerami,² Mariana S. Cunha,³ Akemi Suzuki,³
Juliana S. Nogueira,³ Iray M. Rocco,³ Adriana Y. Maeda,³ Fernanda G.S. Vasami,³ Gizelda Katz,⁴
Ilka F.S.F. Boin,⁵ Raquel S.B. Stucchi,⁵ Mariângela R. Resende,^{2,5} Danillo L.A. Esposito,⁶
Renato P. de Souza,³ Benedito A. da Fonseca,⁶ and Marcelo Addas-Carvalho¹*

BACKGROUND: Zika virus (ZIKV) is an emerging arthropod-borne flavivirus transmitted by *Aedes* mosquitoes. Recent commentaries regarding ZIKV routes of transmission describe a potential transmission by transfusion. Herein, we report a probable case of transfusion-transmitted ZIKV infection through a platelet transfusion that was detected from postdonation information.

CASE REPORT: A blood donor made a voluntary telephone report to the blood donor facility 3 days after donation and informed the facility of a febrile illness (fever,

Zika virus (ZIKV) is an emerging arthropod-borne virus transmitted by *Aedes* mosquitoes that was first identified in Uganda in 1947.¹ The virus belongs to the genus *Flavivirus*, family *Flaviviridae*, and is closely related to other viruses from this family, such as dengue, yellow fever, and West Nile viruses. Until recently, only sporadic human ZIKV infections had been reported, mainly in Africa. The virus emerged in 2007 for the first time in Micronesia, causing an important outbreak on Yap Island. In 2013 and 2014, ZIKV rapidly spread to Oceania^{2,3}

TRANSFUSION-TRANSMITTED ZIKA VIRUS

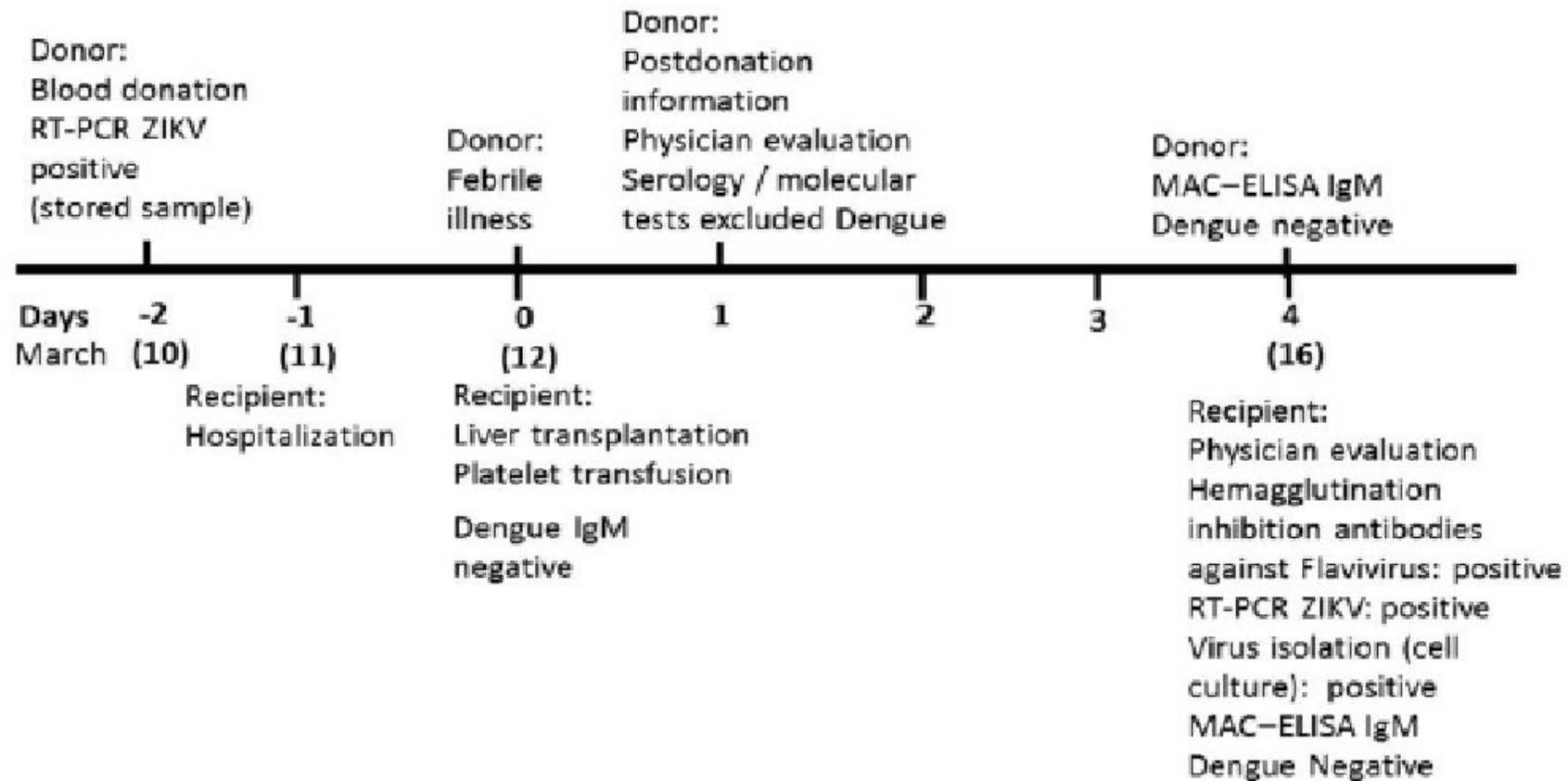


Fig. 1. Timeline of major events related to the case of Zika virus transfusional transmission, 2015. RT-PCR, reverse transcriptase polymerase chain reaction; ZIKV, Zika virus; MAC-ELISA IgM, immunoglobulin M antibody capture-enzyme-linked immunosorbent assay.

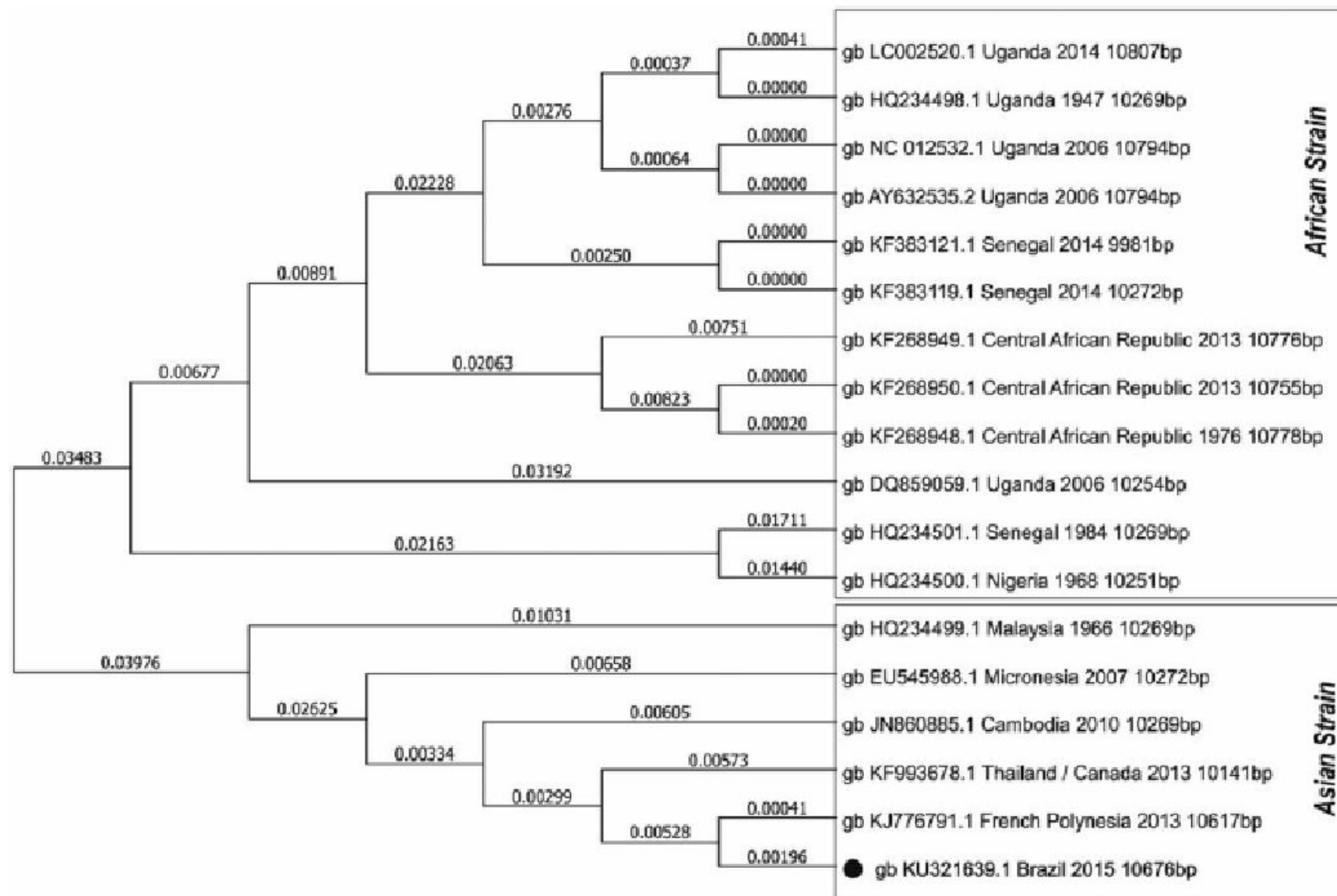


Fig. 2. Molecular phylogenetic analysis of Zika viruses using the maximum likelihood method. The analysis involved 18 nucleotide complete sequences available at the National Center for Biotechnology Information (NCBI) website. All positions containing gaps and missing data were eliminated. In total, there were 9825 positions in the final data set. The numbers above the branches indicate the distance between sequences, ranging from 0 (no difference) to 1. Evolutionary analyses were conducted in MEGA6 software.



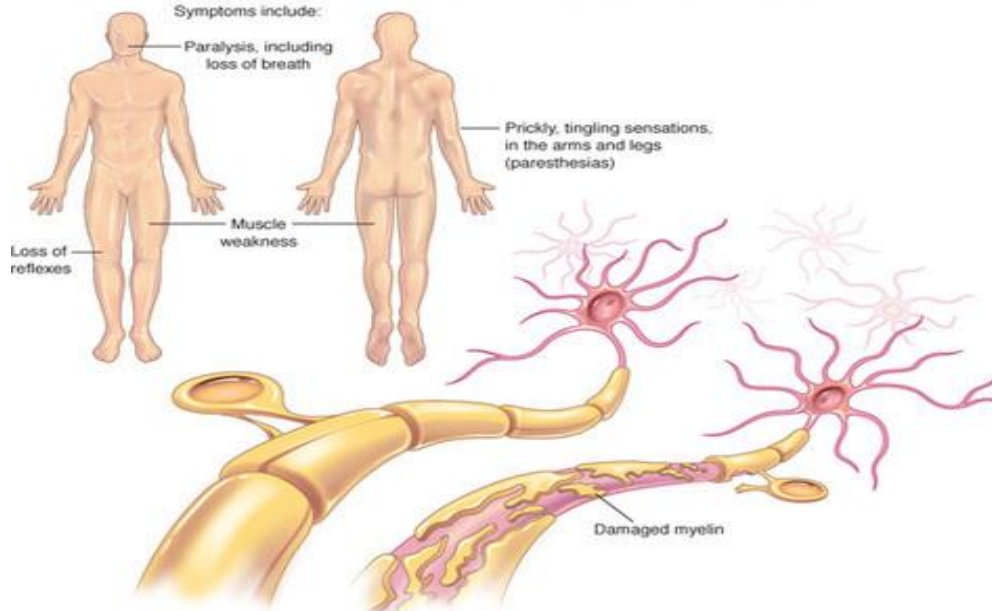
Ministério da
Saúde

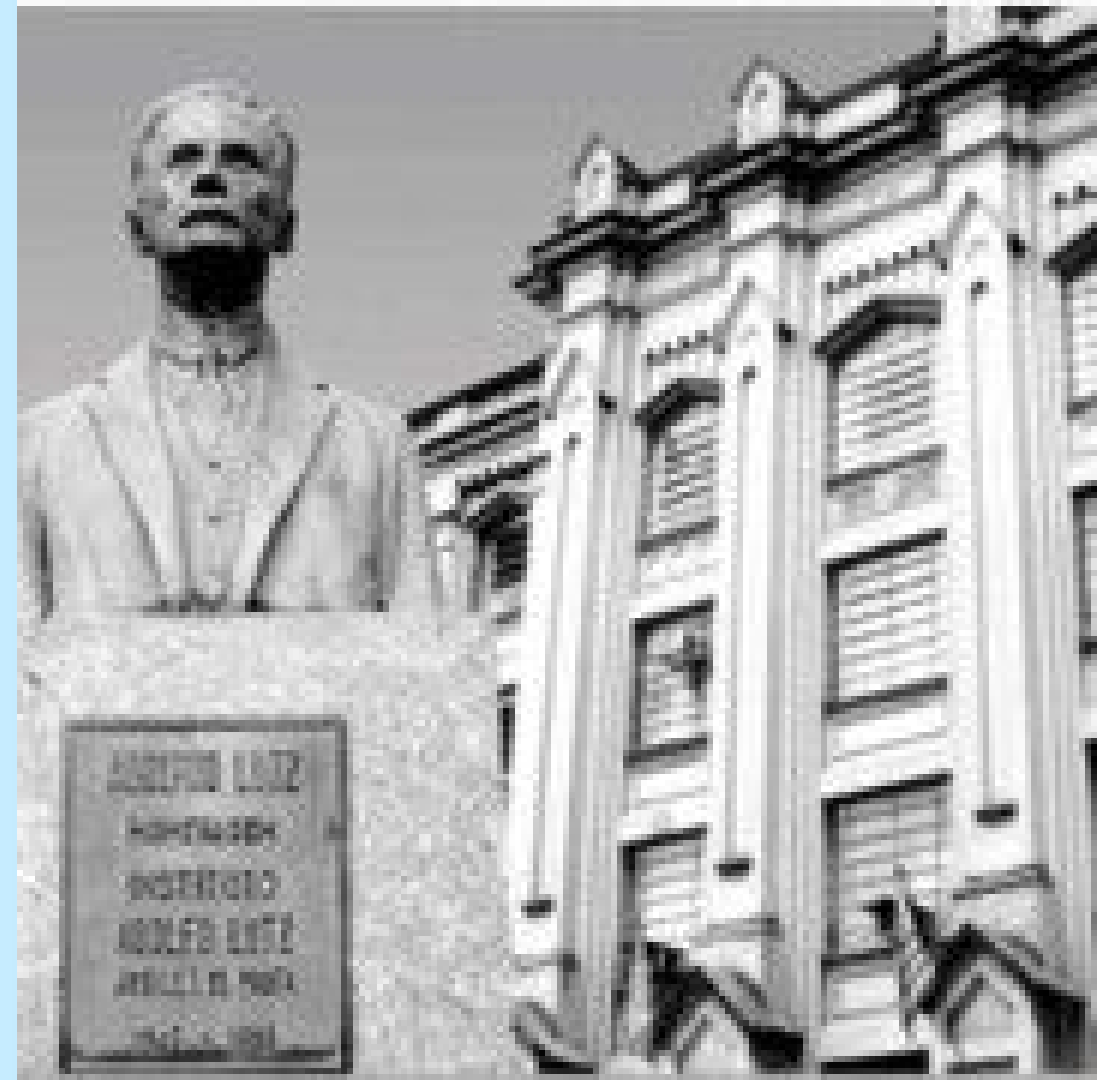


Cause and symptoms of Guillian-Barré syndrome

Guillain-Barré syndrome is caused by the destruction of myelin, known as demyelination.

Symptoms include:







**GOVERNO DO ESTADO DE SÃO PAULO
SECRETARIA DE ESTADO SAÚDE
COORDENADORIA DE CONTROLE DE DOENÇAS
CENTRO DE VIGILÂNCIA EPIDEMIOLÓGICA "PROF. ALEXANDRE VRANJAC"
INSTITUTO ADOLFO LUTZ**

NOTA INFORMATIVA Nº 02 – NOVEMBRO DE 2015

VÍRUS ZIKA – ORIENTAÇÕES TÉCNICAS

Com a finalidade de organizar o sistema de vigilância e as estratégias de organização da rede assistencial diante da introdução do ZIKA vírus (ZIKV) no Brasil, e em razão das características da doença cujas medidas de controle prescindem da caracterização dos casos individuais,



GOVERNO DO ESTADO DE SÃO PAULO
SECRETARIA DE ESTADO SAÚDE
COORDENADORIA DE CONTROLE DE DOENÇAS
CENTRO DE VIGILÂNCIA EPIDEMIOLÓGICA "PROF. ALEXANDRE VRANJAC"
INSTITUTO ADOLFO LUTZ

Serão disponibilizados, semanalmente, 30 exames RT-PCR em Tempo Real para ZIKV, para o estado de São Paulo. Serão analisadas 10 amostras/semana no CLR-São José do Rio Preto e 20 amostras/semana no Instituto Adolfo Lutz Central (IAL).

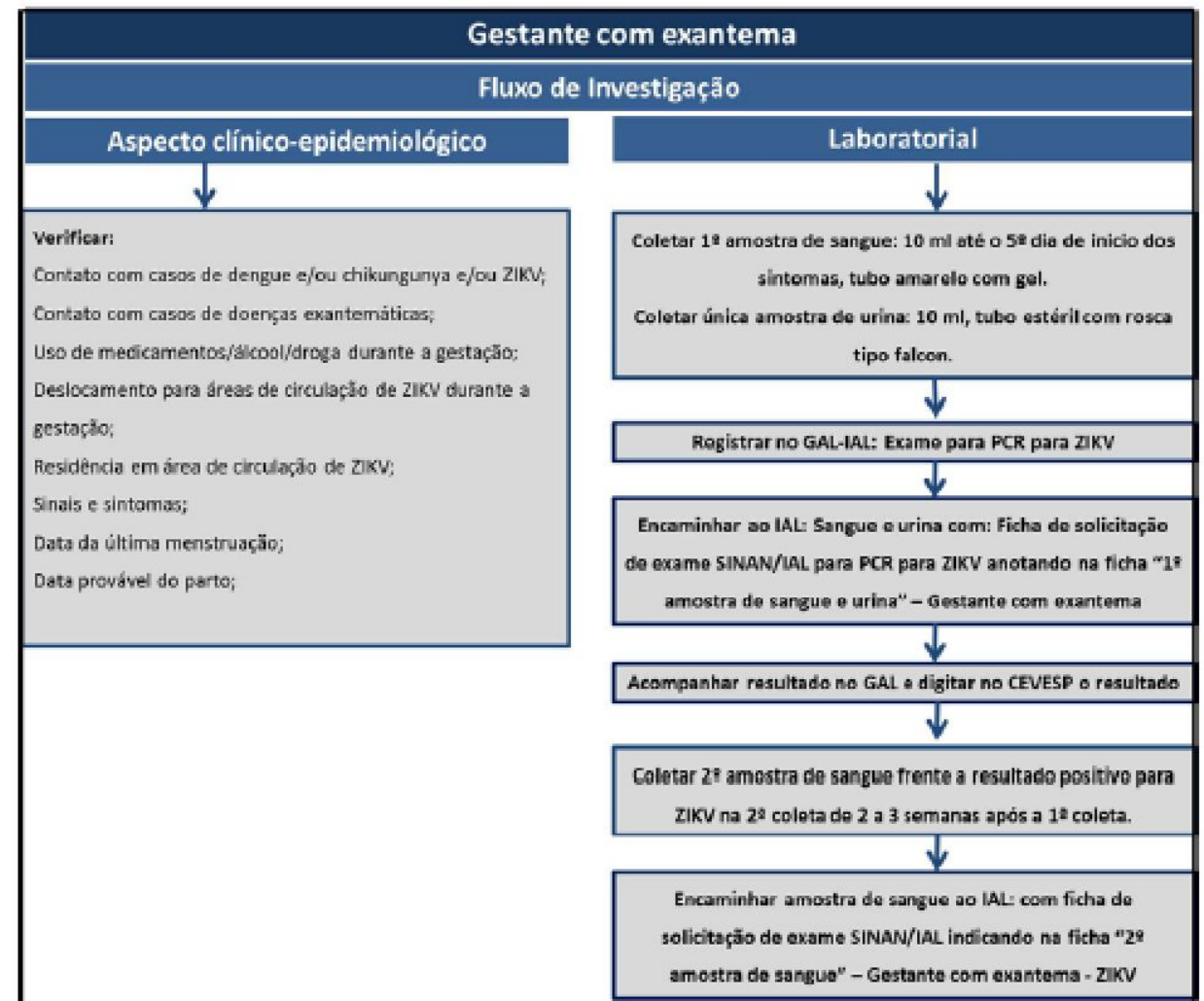
O Laboratório não receberá amostras oriundas de demanda/notificação espontânea, para diagnóstico de Zika. A **Vigilância Laboratorial de Zika**, no estado de São Paulo, se dará por meio da pesquisa de vírus Zika (ZIKV) em amostras **Dengue-NS1 negativas**, de pacientes que atendam à definição de caso de Zika, especialmente em municípios com baixa positividade para Dengue-NS1.

Protocolo de Vigilância para Gestantes com Exantema



4.3 Fluxo de Investigação

Fluxo de investigação para gestantes relacionadas à infecção pelo ZIKV. Central/CIEVS-SP. Março de 2016



Relatório de Resultado Quantitativo Mensal (Exame / Metodologia)

Todos os Laboratórios

Data Início:

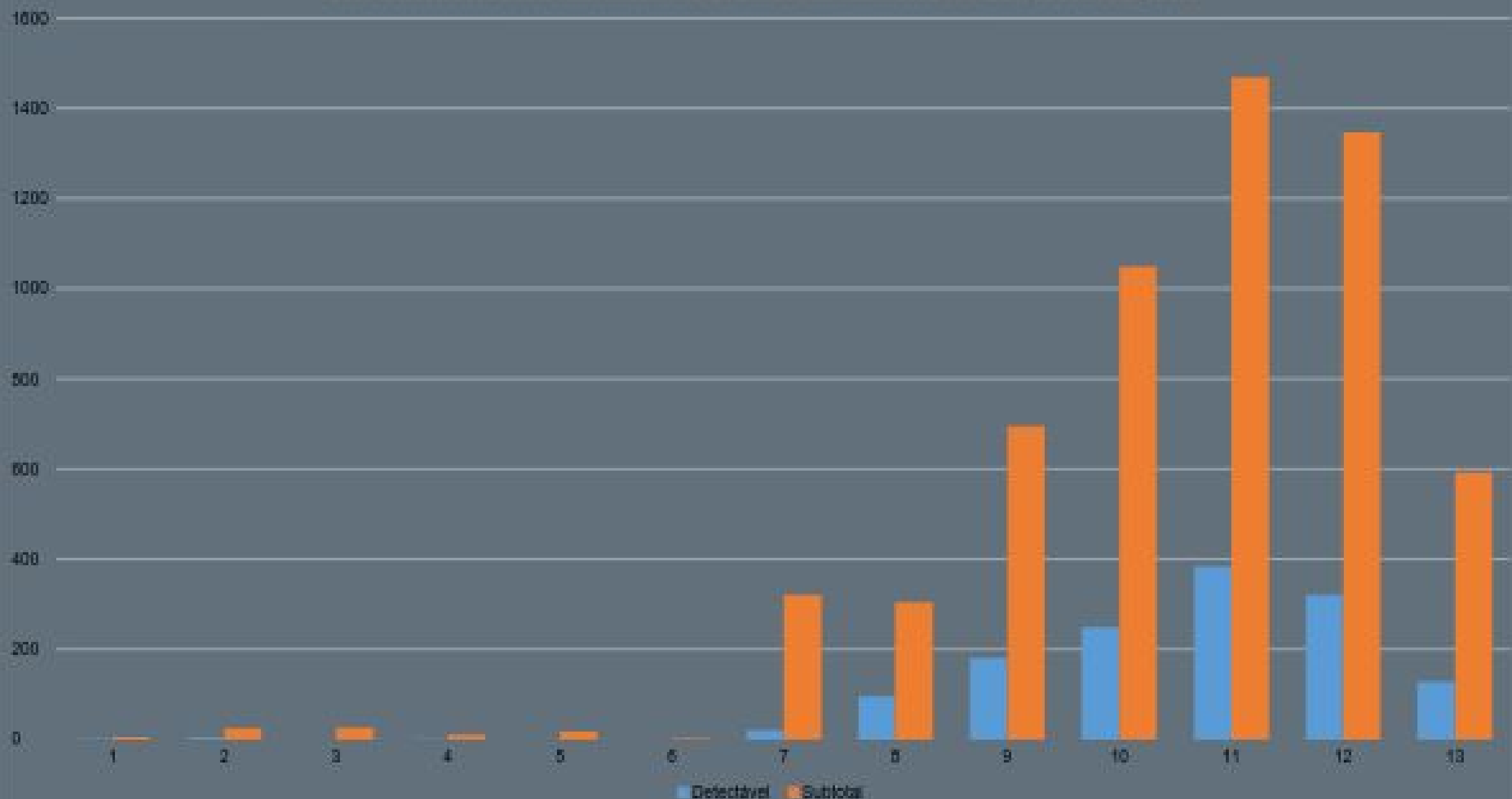
01/06/2015

Data Fim:

30/06/2016

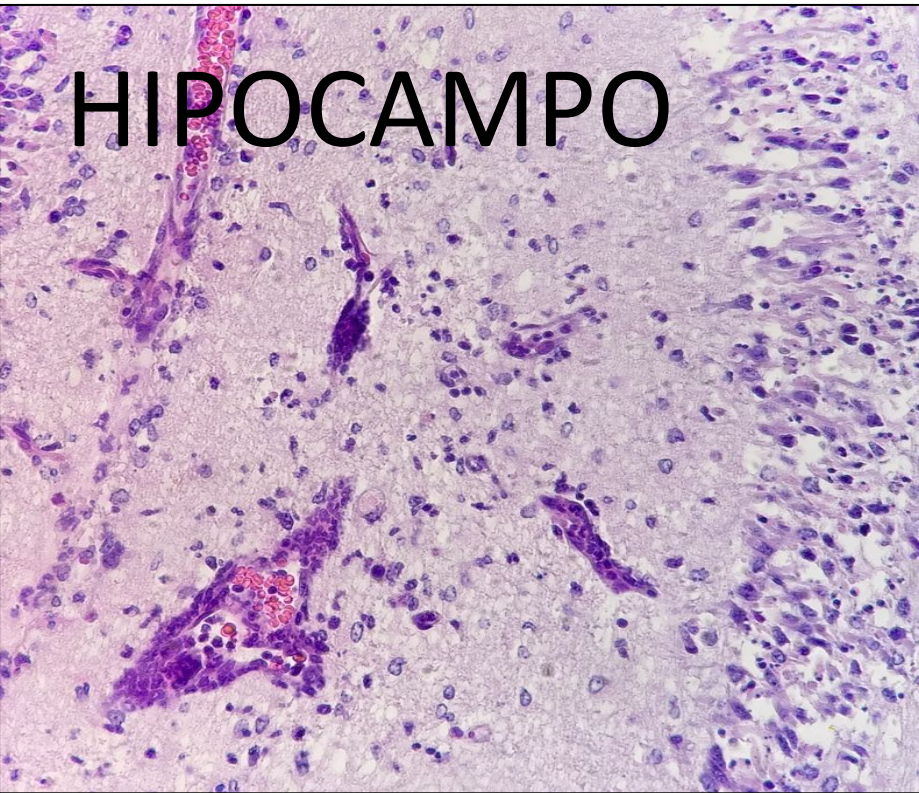
Exame/Metodologia	Jun/2015	Jul/2015	Ago/2015	Set/2015	Out/2015	Nov/2015	Dez/2015	Jan/2016	Fev/2016	Mar/2016	Abr/2016	Mai/2016	Jun/2016	Total Exame
Zika, Biologia Molecular/RTTR														
<i>Detectável</i>	1	3	0	1	0	0	20	94	180	248	383	320	127	1377
<i>Não Detectável</i>	4	21	25	9	16	2	301	210	514	796	1076	1012	456	4442
<i>Inconclusivo</i>	0	0	0	0	0	0	0	0	0	4	10	15	8	37
<i>Indeterminado</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Subtotal	5	24	25	10	16	2	321	304	694	1048	1469	1347	592	5857
Total Geral	5	24	25	10	16	2	321	304	694	1048	1469	1347	592	5857

Casos detectados laboratorialmente e Suspeitos encaminhados ao IAL/Un/2015 a Jun/2016

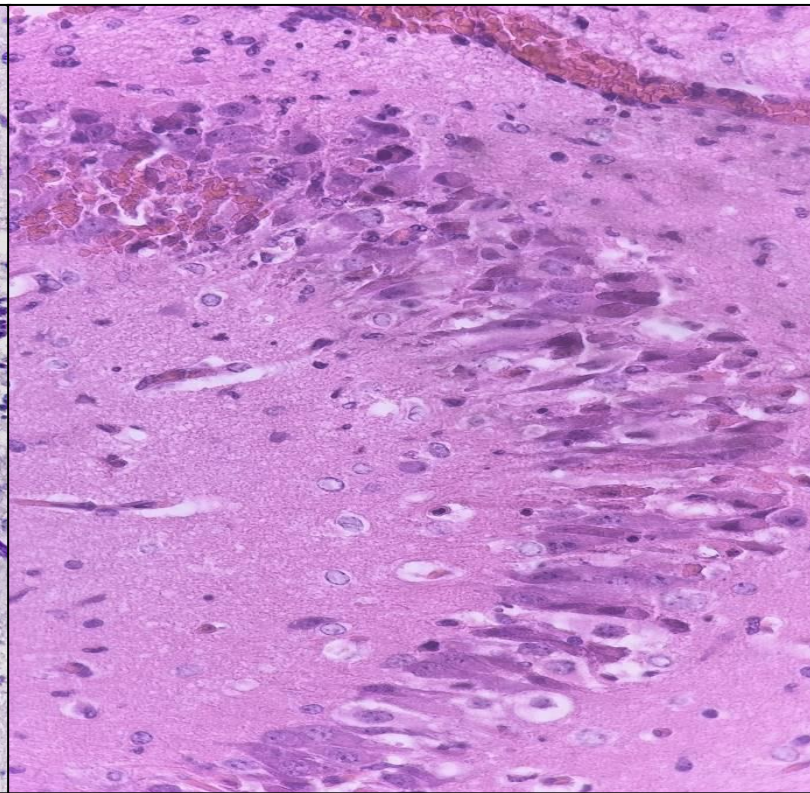


PATOLOGIA IAL

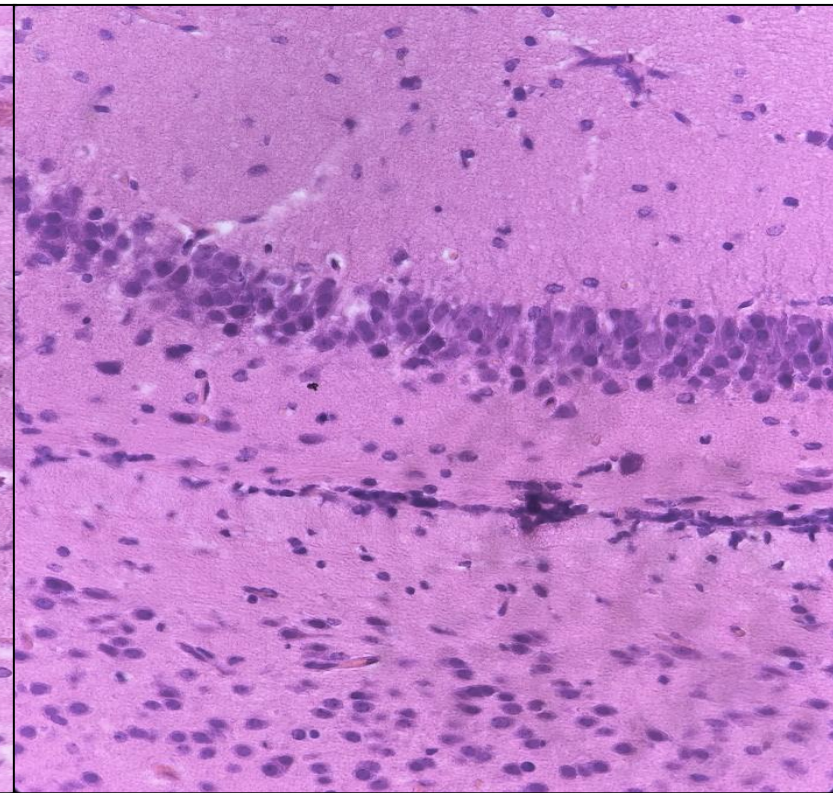
Modelo Animal – Efeitos Neurológicos



Inoculação IC. 6dias



Inoculação SC. 13 dias



Controle negativo. 13 dias

Instituto Adolfo Lutz

Validação de ensaio imunoenzimático para detecção de anticorpos
anti-NS1 do Zika vírus em amostras de indivíduos com ou sem
suspeita da infecção por Zika vírus

Dra. Paula Ordonhez Rigato
Centro de Imunologia



First Complete Genome Sequence of Zika Virus (*Flaviviridae*, *Flavivirus*) from an autochthonous transmission in the Americas

Mariana Sequetin Cunha¹, Danillo Lucas Alves Esposito², Iray Maria Rocco¹, Adriana Yurika Maeda¹, Fernanda Gisele da Silva Vasami¹, Juliana Silva Nogueira¹, Renato Pereira de Souza¹, Akemi Suzuki¹, Marcelo Addas-Carvalho³, Maria de Lourdes Barjas-Castro³, Rodrigo Nogueira Angerami⁴, Mariângela Ribeiro Resende^{4,5}, Raquel Silveira Bello Stucchi⁵, Ilka de Fátima Santana Ferreira Boin⁵, Gizelda Katz⁶, and Benedito Antonio Lopes da Fonseca²

- Division of Vector-borne Diseases, Adolfo Lutz Institute, São Paulo, SP, Brazil
- Department of Internal Medicine, Division of Infectious Diseases, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
- Blood Center, State University of Campinas/UNICAMP, Campinas, SP, Brazil
- Section of Hospital Epidemiology, Hospital of Clinics, State University of Campinas/UNICAMP, Campinas, SP, Brazil
- Clinical Medical Department, Faculty of Medical Sciences, State University of Campinas/UNICAMP, Campinas, SP, Brazil
- Infectious Diseases Surveillance Center “Prof. Alexandre Vranjac”/CCD, São Paulo, SP, Brazil

We report here the genome sequence of Zika virus, ZikaSPH2015 strain, containing all structural and nonstructural proteins flanked by the 5' and 3' UTR. It was isolated in São Paulo state, Brazil, 2015, from a patient who received a blood transfusion from an asymptomatic donor at the time of donation.

Zika em expansão

Sequenciamento confirma que variedade em circulação no país veio da Polinésia e projeção estima que deve se espalhar por outros países

Ricardo Zorzetto

Abaixo, o genoma completo da variedade do vírus zika que circulou no surto de 2013 e 2014 na Polinésia Francesa

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Das equipes brasileiras completaram nas últimas semanas o sequenciamento do material genético do vírus zika isolado nos estados de São Paulo e da Paraíba. Os resultados sugerem que a variedade do zika em circulação em diferentes regiões brasileiras é mesmo originária da Polinésia Francesa, onde houve um surto em 2013 e 2014. Também indicam que o vírus possivelmente foi introduzido no Brasil em um único evento.

No Instituto Adolfo Lutz, em São Paulo, o virologista Renato Pereira de Souza e sua equipe sequenciaram o material genético do zika extraído de uma pessoa que desenvolveu a doença em Campinas. Esse indivíduo contraiu o vírus ao receber uma transfusão de sangue. O doador adoeceu dias mais tarde e avisou ao hemocentro da Universidade Estadual de Campinas (Unicamp) que estava com suspeita de dengue. Análises feitas no Adolfo Lutz descartaram a dengue e confirmaram a infecção por zika. "Os hemocentros terão de prestar atenção também a esse vírus, uma vez que muitos casos são assintomáticos", afirma Souza. "No caso em questão, o vírus permaneceu viável e infectou outra pessoa", conta o virologista, um dos coordenadores da análise,

se, realizada em parceria com pesquisadores da Unicamp e da Universidade de São Paulo (USP), em Ribeirão Preto, e aceito para publicação na revista *Genome Announcements*.

O sequenciamento do material genético do vírus revelou um genoma enxuto. São cerca de 10,6 mil unidades (nucleotídeos) compondo uma fita simples de ácido ribonucleico (RNA). Essa fita abriga ao todo apenas seis genes, capazes de produzir 10 diferentes proteínas – alguns genes são polivalentes. "A análise do genoma indica que o vírus é de uma linhagem muito próxima à que circulou na Polinésia Francesa e na Ilha de Páscoa", conta Souza.

Na Universidade Federal do Rio de Janeiro, o virologista Amílcar Tanuri e sua equipe também sequenciaram o genoma do zika extraído do líquido amniótico de duas gestantes que tiveram bebês com microcefalia na Paraíba. As conclusões são as mesmas. "Não tenho dúvida de que é a mesma linhagem da Polinésia", afirma Tanuri. Segundo o virologista carioca, a diferença entre o material genético do vírus em circulação aqui e o de lá é pequena, cerca de 20 nucleotídeos e apenas 1 dos 3.500 aminoácidos (unidades formadoras das proteínas). Tamanha semelhança, segundo ele, indica que o vírus está se espalhando

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Zika virus in the Americas: Early epidemiological and genetic findings

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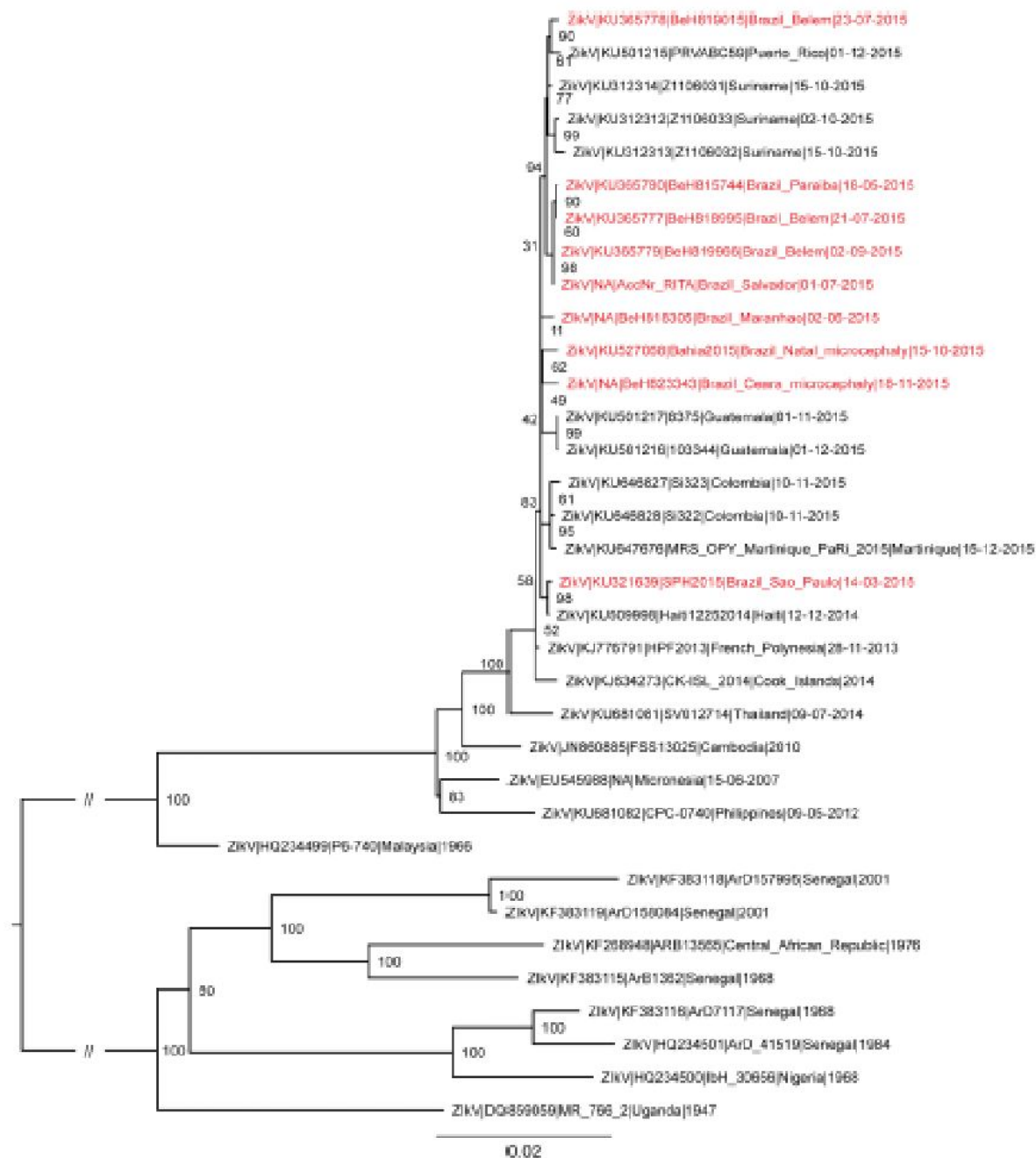
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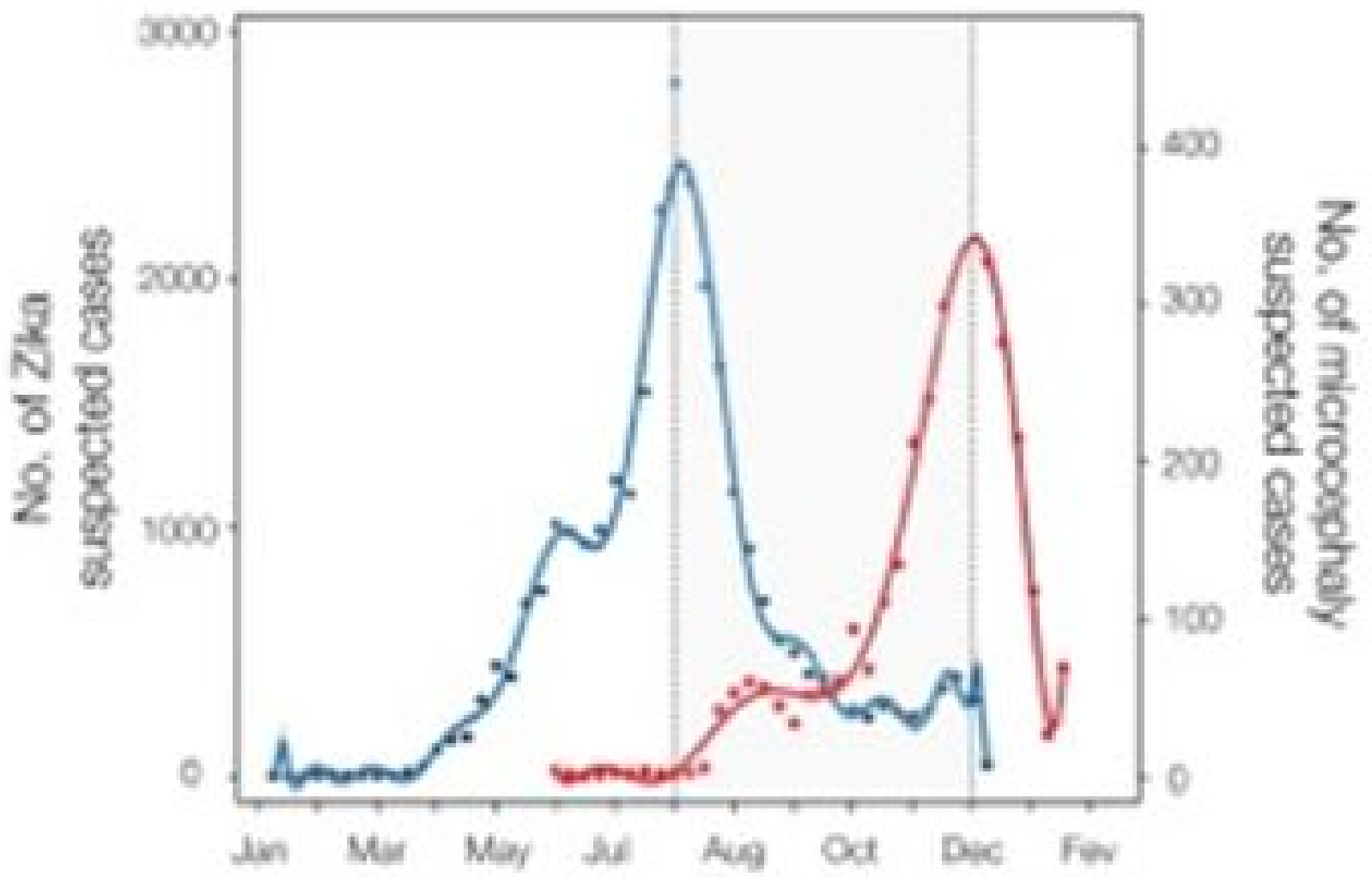
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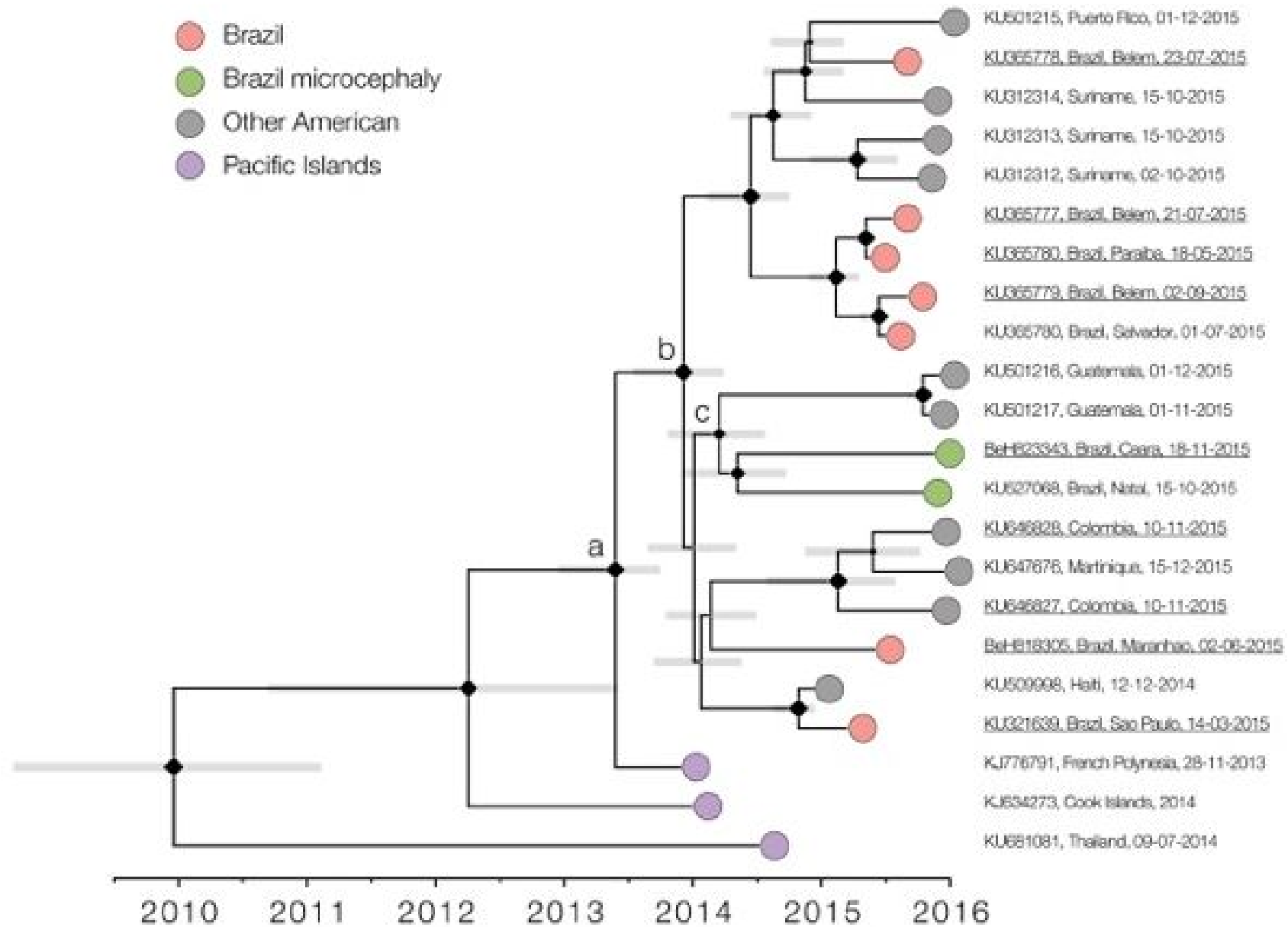
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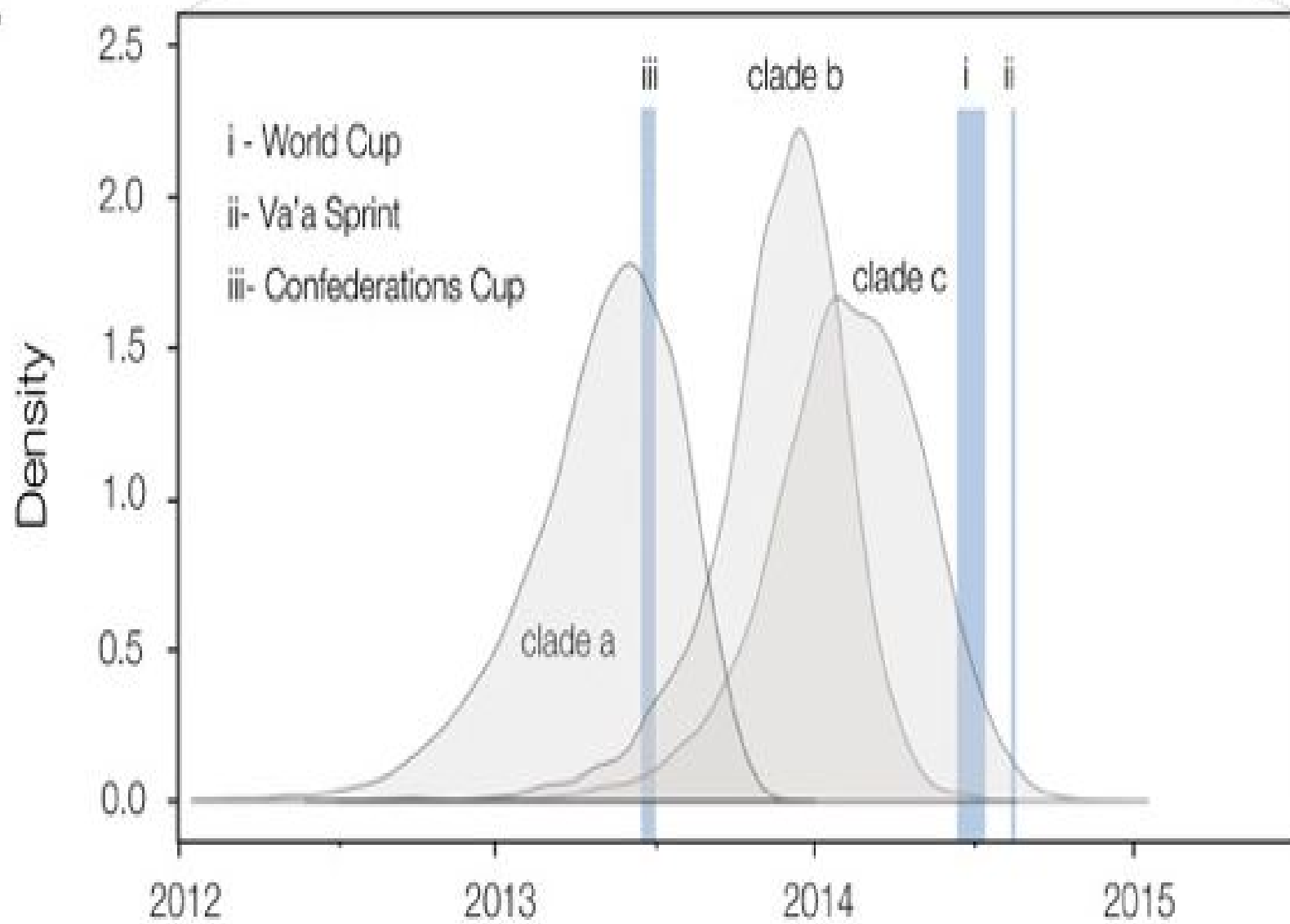




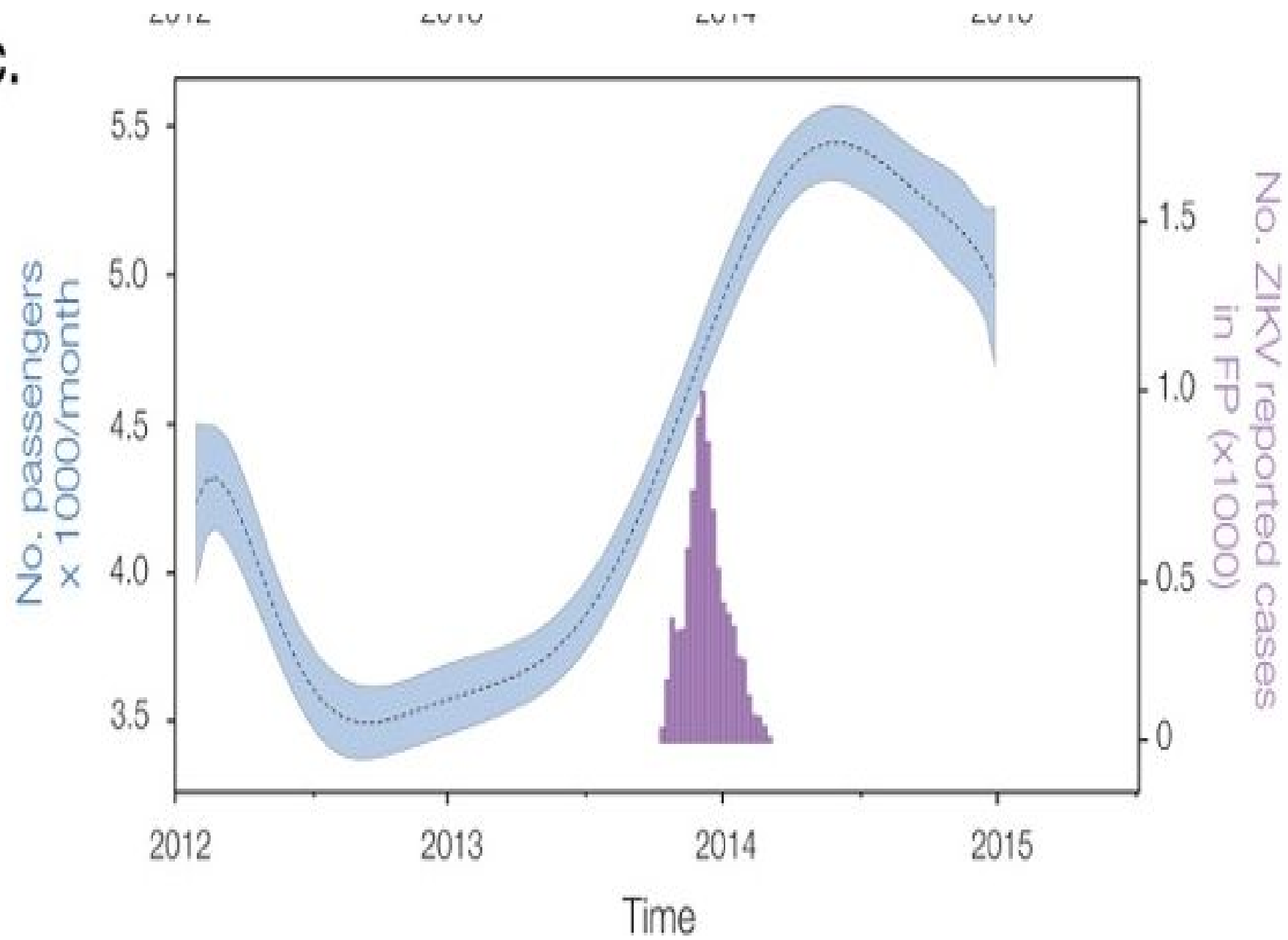
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Vírus zika provavelmente chegou ao Brasil em 2013

 revistapesquisa.fapesp.br/2016/03/24/virus-zika-provavelmente-chegou-ao-brasil-em-2013/

Agente infeccioso deve ter entrado no país em um único evento, 18 meses antes de os primeiros casos serem registrados



Imprimir

O vírus zika pode ter entrado no Brasil em meados de 2013, quase um ano e meio antes de os primeiros casos começarem a ser registrados no país. Até então, o Ministério da Saúde estimava que o vírus havia aportado em território nacional entre 2014 e 2015, o mesmo período em que se começou a detectar um aumento nos casos de microcefalia — um tipo de malformação congênita em que os bebês nascem com a cabeça menor que o esperado para o tempo de gestação —, sobretudo na região Nordeste. Em um estudo publicado nesta quinta-feira (24/3) na revista *Science*, um grupo internacional de pesquisadores, do qual participam brasileiros dos institutos Evandro Chagas e Adolfo Lutz e da Fundação Oswaldo



O fato de a maioria das infecções não provocar sintomas pode ter

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The 2013 Confederations Cup soccer tournament may have brought people infected with the Zika virus to Brazil, a new study of viral genomes suggests.

AP Photo/Eugene Hoshiko

Don't blame sports for Zika's spread

By [Gretchen Vogel](#) | Mar. 24, 2016, 2:30 PM

World Cup fans are probably not to blame for bringing Zika virus to Brazil in June and July 2014. And, contrary to other speculation, neither are the teams that attended a championship canoe race in September 2014. According to a new genome analysis, the virus, first detected in northeastern Brazil in March 2015, had likely been spreading there long before either event, having arrived sometime between May and December 2013. The researchers say it most likely slipped in on one of thousands of airline flights from French Polynesia or Southeast Asia.

That is an important insight, says Matthew Ferrari, an epidemiologist and modeler at Pennsylvania State University, University Park, who wasn't involved in the study. "Starting with [one-time events] as hypotheses can be distracting," he says. "The genome data

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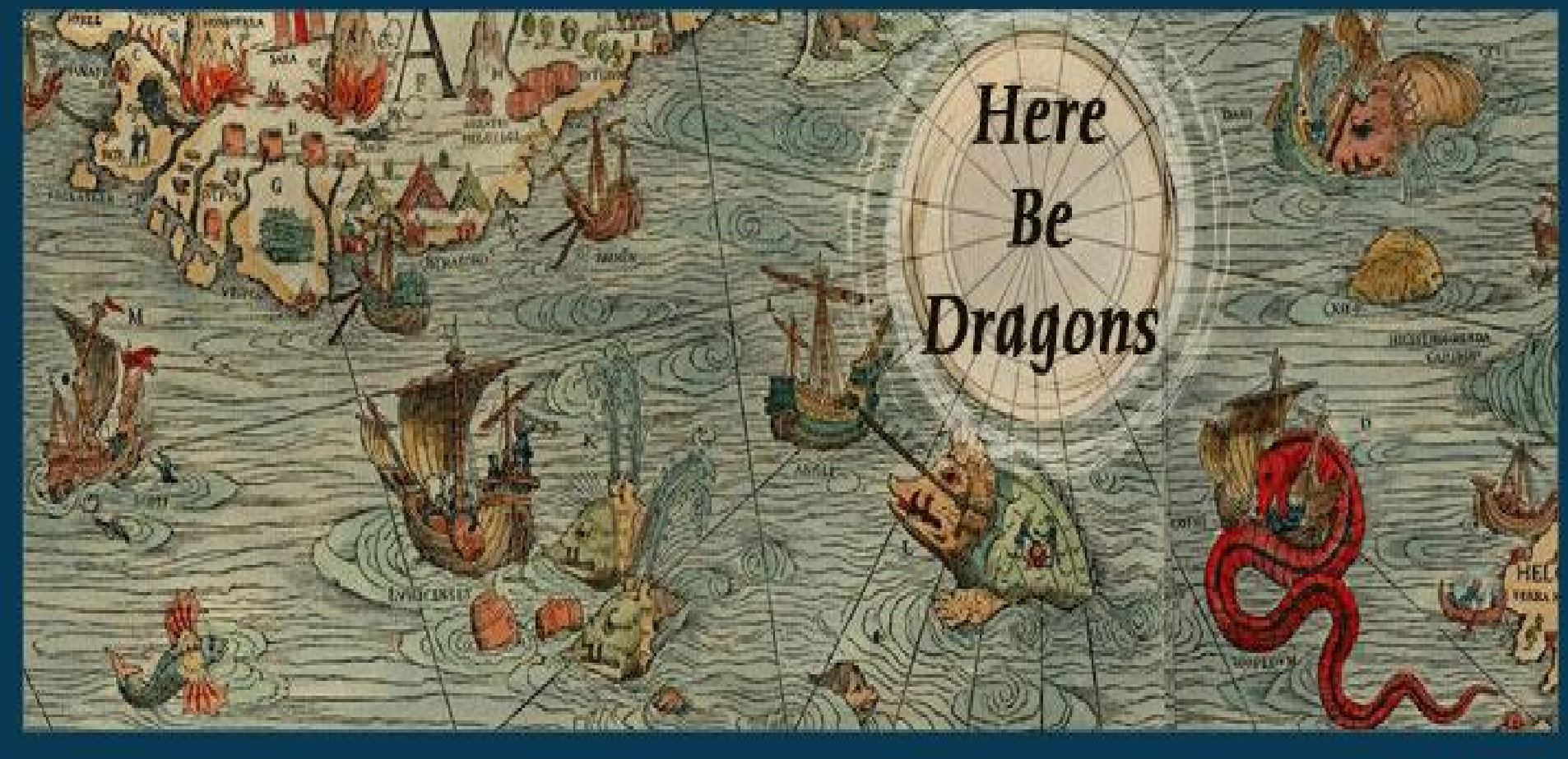
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Is it going to adapt
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