# Yellow Fever Update

Maurício Lacerda Nogueira, MD, PhD

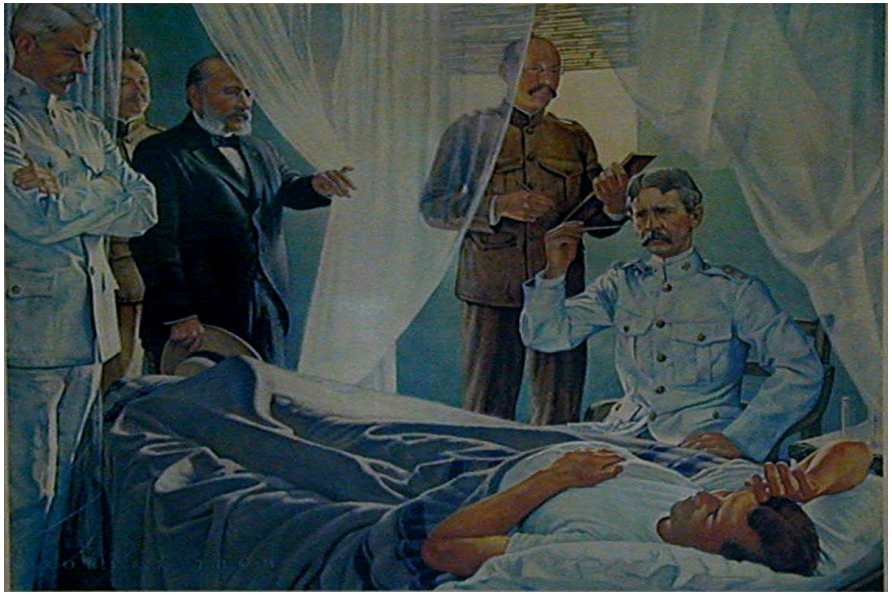
Associate Professor
Department of Microbiology and Infectious Diseases
Faculdade de Medicina de São José do Rio Preto

mnogueira@famerp.br

# Disclosure

- No conflict regarding YF Vaccine
- Hold a patent regarding pharmacological treatment of YF using MAP Kinases inhibitors
- Honorary payments or grant support regarding dengue vaccine from: Sanofi-Pasteur and/or Butantā-NIH Vaccine
- Thanks to Dr. Marcos Freire - FIOCRUZ and Prof. Betania Drumon -UFMG for sharing some data used in this presentation

# BASIC YF DATA COMES FROM EARLY XX CENTURY



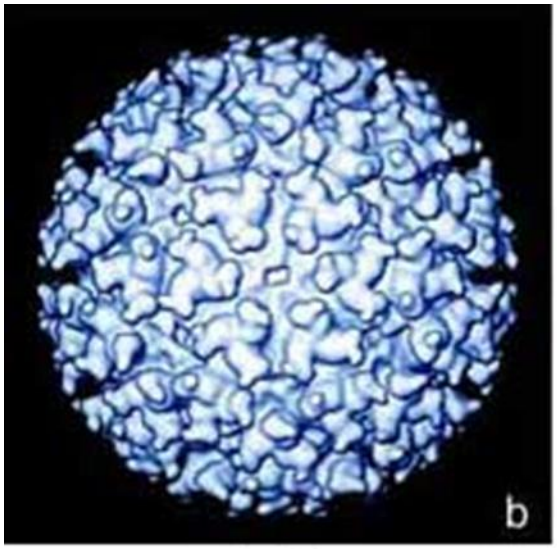
“I THANK GOD that I did not accept anybody’s opinion on this subject, but determined to put it to a through test with human beings in order to see what would happen… actual trial proven that I was right…” - Walter Reed

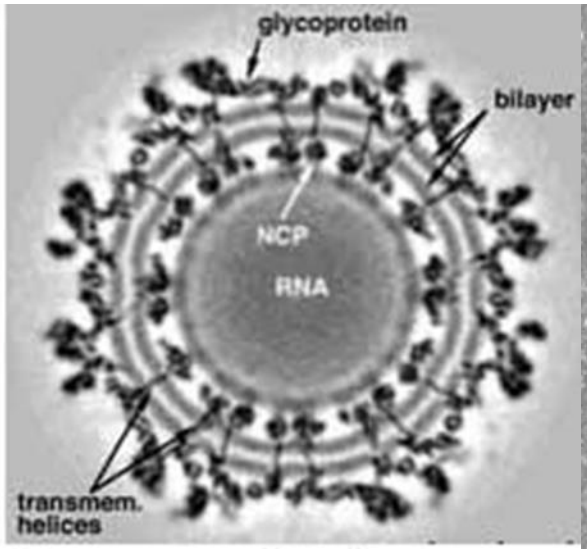
“The Etiology of Yellow Fever an Additional Note,” read before the Pan-American Medical Congress at Havana, in February, 1901

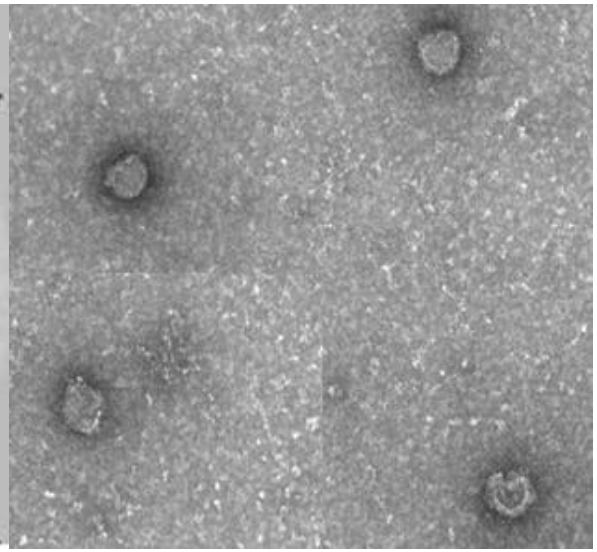
“1. The mosquito – C. facciatus – serves at the intermediate host for the parasite of yellow fever.“2. Yellow fever is transmitted to the nonimmune individual by means of the bite of the mosquito that has previously fed on the blood of those sick with this disease.“5. Yellow fever can also be experimentally produced by the subcutaneous injection of blood taken from the general circulation during the first and second days of this disease.“8. Yellow fever is not conveyed by fomites, and hence disinfection of articles of clothing, bedding, or merchandise, supposedly contaminated by contact with those sick with this disease, is unnecessary.“10. The spread of yellow fever can be most effectually controlled by measures directed to the destruction of mosquitoes and the protection of the sick against the bites of these insects.”

# YELLOW FEVER VIRUS

Etiological agent:



# Transmission Cycles of yellow fever

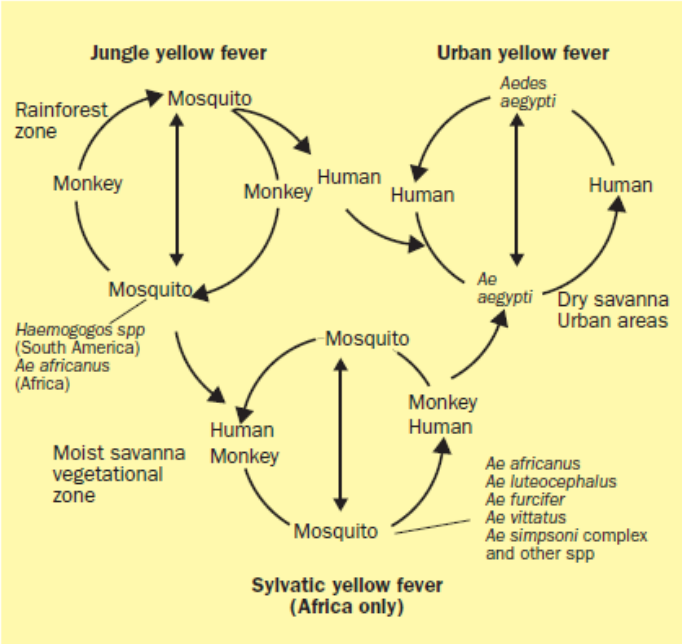
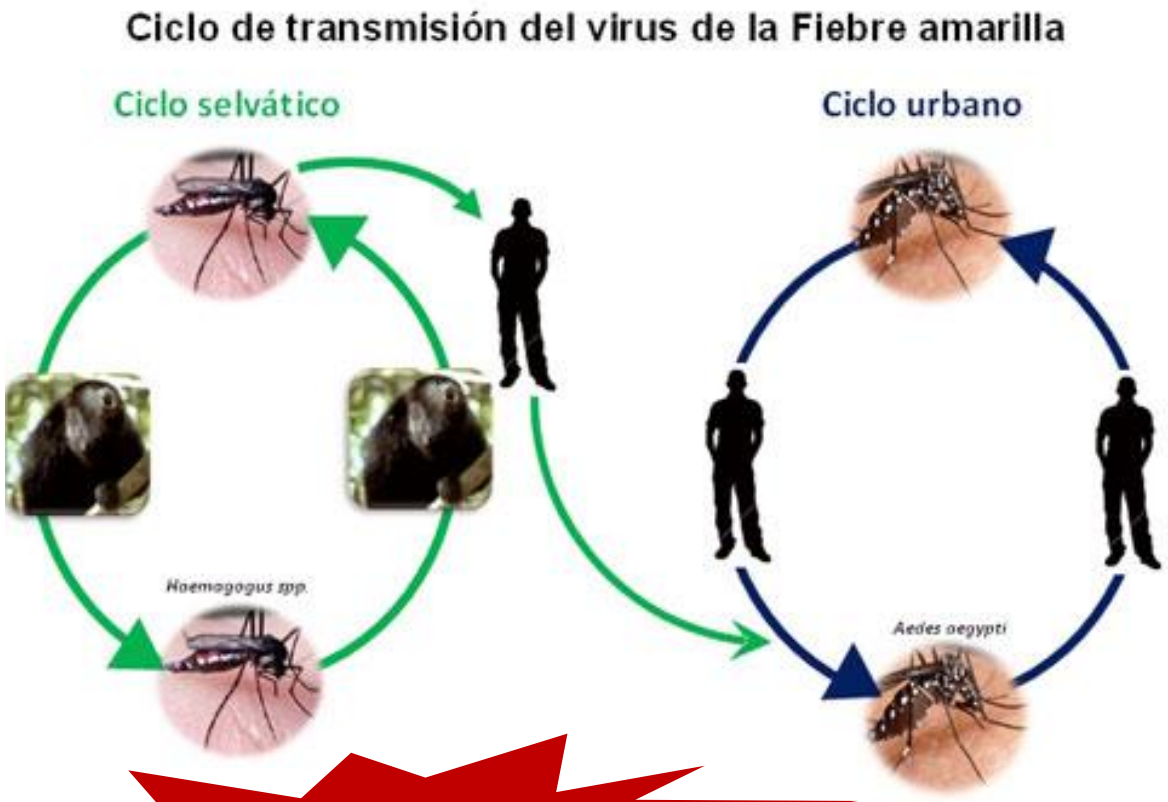



*Figure 3. The transmission cycles of yellow fever. The virus is maintained by transmission between monkeys and tree-hole breeding mosquitoes. Human beings acquire "jungle yellow fever" when exposed to the bite of mosquitoes that have previously fed on an infected monkey. The vectors and ecology differ in Africa and South America. In Africa, tree-hole breeding Aedes spp reach high densities in the moist savanna vegetational zone and transmit the virus between people. In both continents, Ae aegypti, which breeds in and around houses in man-made containers, is responsible for interhuman transmission of "urban" yellow fever virus.*




**YF: impossible to erradicate**

Fonte: SVS/MS

# Hosts

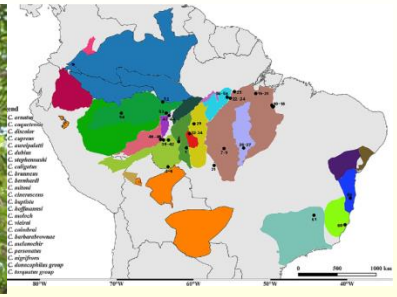

*Cebus sp*
(macaco prego)

*Alouatta sp*
(guariba, bugio)

*Callithrix sp*
(mico, soim)

Host — Amplify — Disseminate

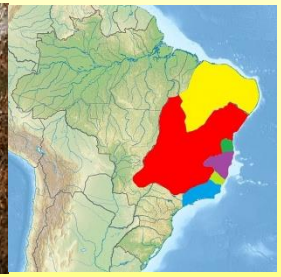
# Brazil: non-human primates (NHP): hosts for YFV



*Callicebus* spp. *(widow monkey)*


*Sapajus* spp. (tufted capuchins)

Genera: less susceptible to YFV



*Callithrix* spp. *(*marmosets) urban centers

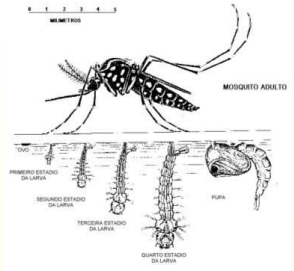
*Allouata* spp. (howler monkeys)

Genera: more susceptible to YFV

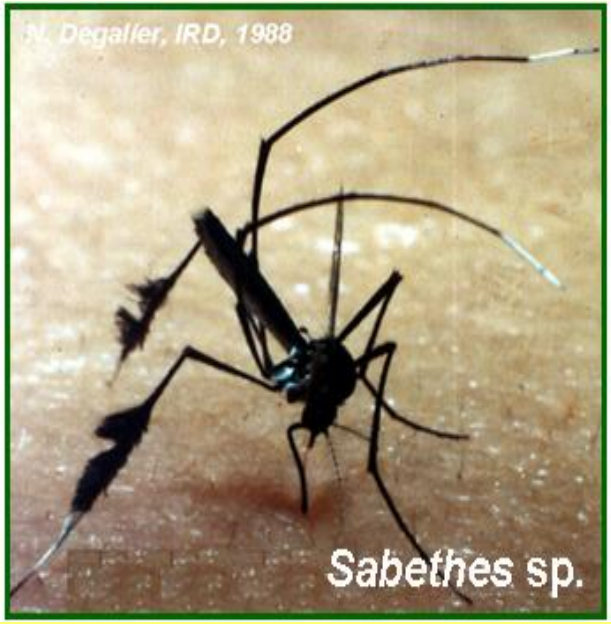
Susceptibility and wide occurrence: NHP - sentinels for YF


Hunt et al, 1978; Vasconcelos, 2003; MS-BR, 2014

# Vectors









Vectors — Reservoirs — Dissemination

# THE DISEASE

*Figure 5. Yellow fever patient during the period of infection. The patient febrile and acutely ill, with prominent conjunctival congestion. During th pre-icteric phase, the illness is difficult to differentiate from many other infectious diseases. Virus is present in the blood and the patient is infectious for blood-feeding mosquitoes.*

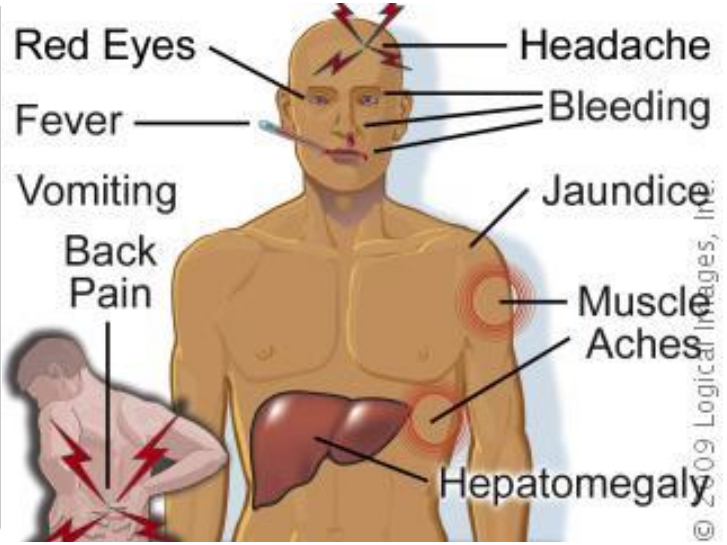




*Figura 6 - O "iceberg" da febre amarela. Distribuição das formas clínicas.*

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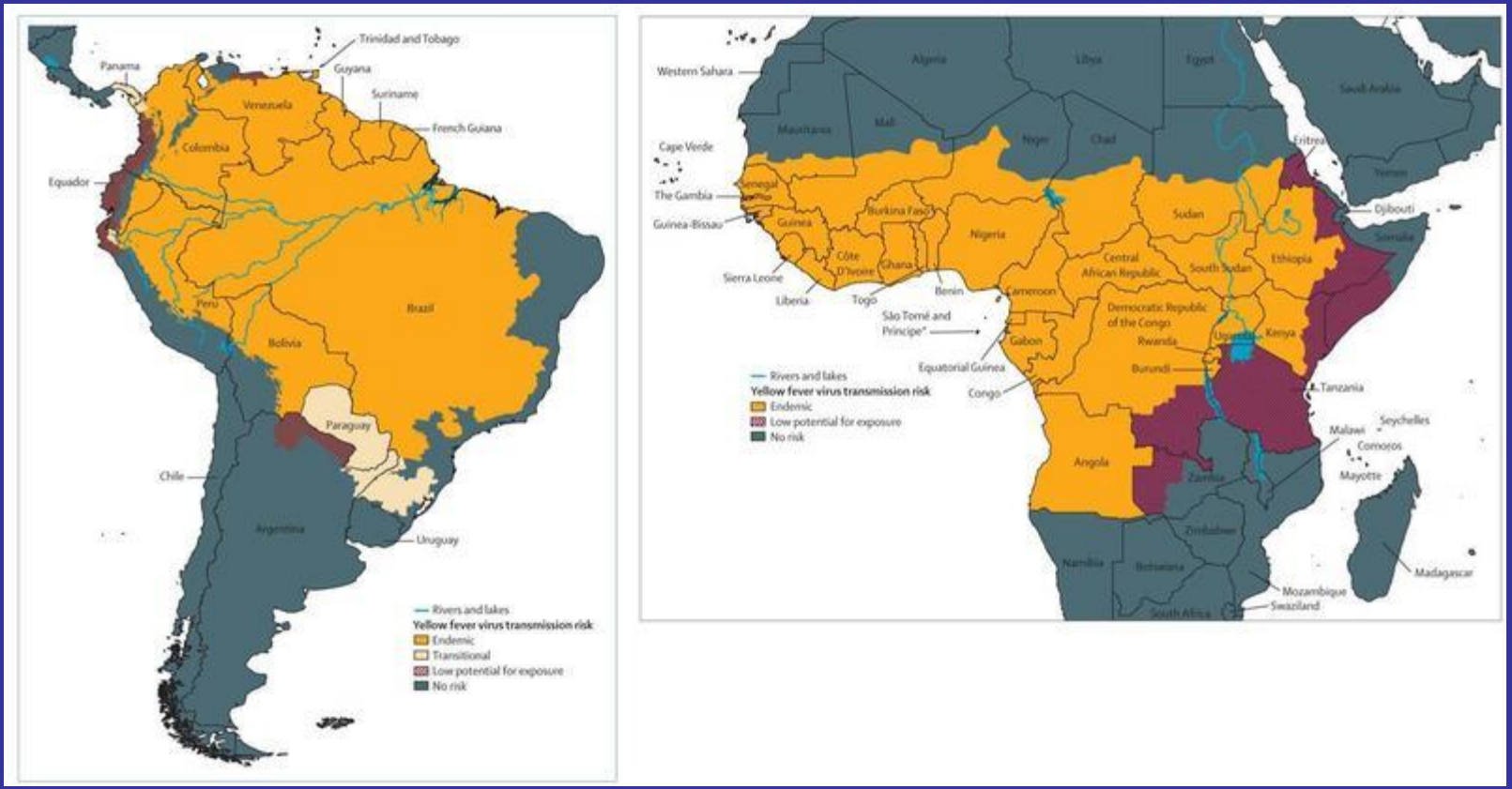
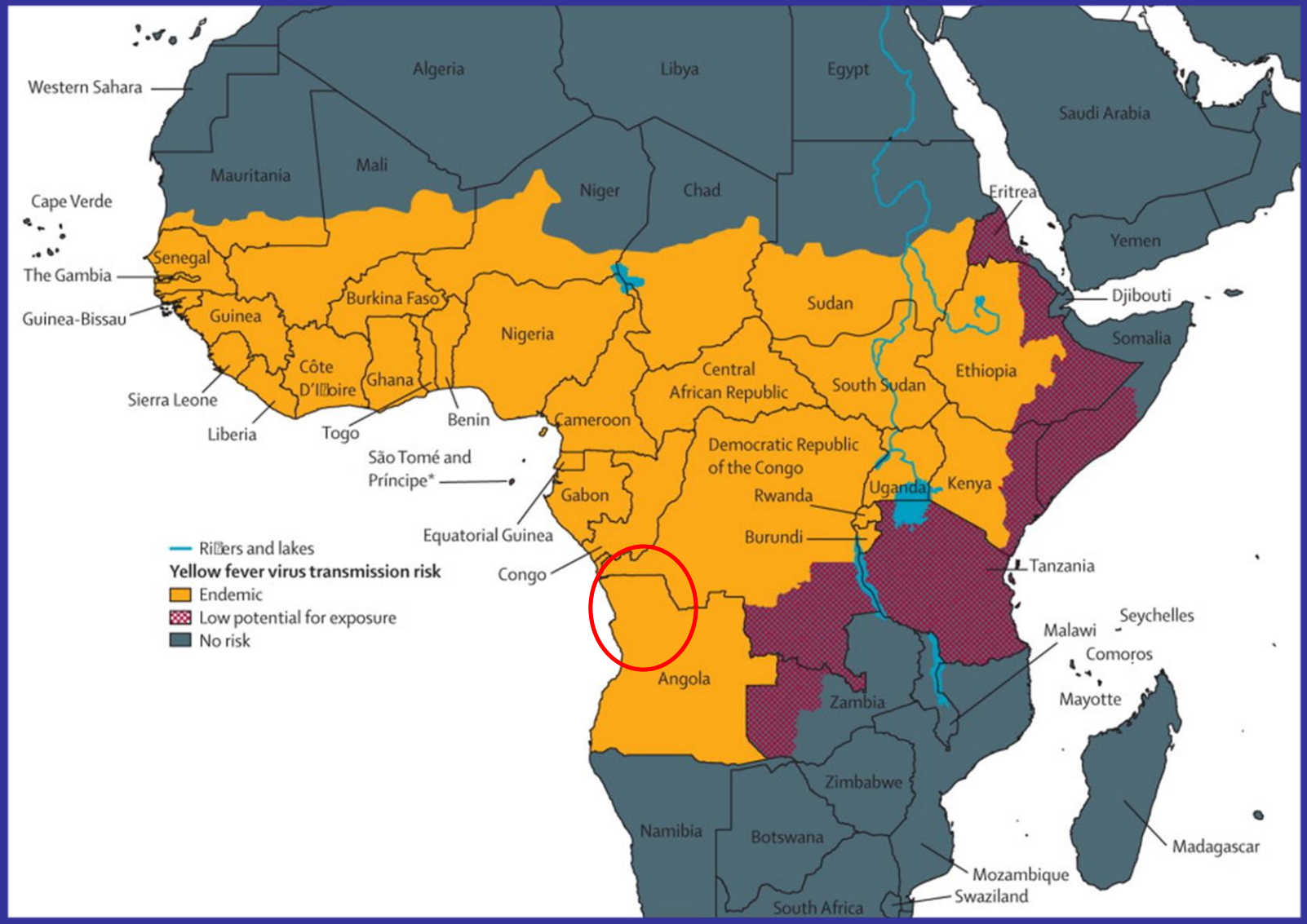
em Imunobiológ
Bio-Mar

# CURRENT SITUATION OF YFV

# YELLOW FEVER VIRUS TRANSMISSION RISK



# EPIDEMIC IN AFRICA
# 2016



# EPIDEMIC IN AFRICA (DEMOCRATIC REPUBLIC OF THE CONGO AND ANGOLA) 2016

**Angola**
884 confirmed cases
121 deaths among confirmed cases (case fatality rate, 13.7%)
4347 suspected cases
377 deaths among suspected cases (case fatality rate, 8.7%)

**Kenya**
2 confirmed cases

**China**
11 confirmed cases

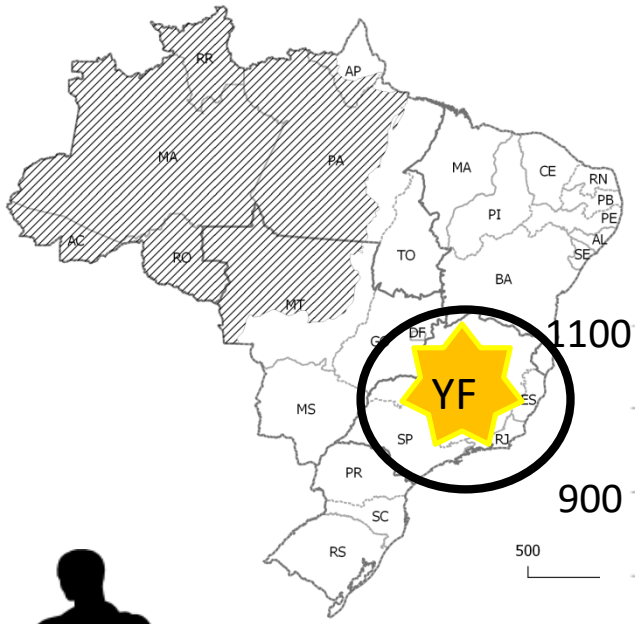
**DR Congo**
78 confirmed cases (57 imported from Angola, 8 sylvatic, 13 autochthonous)
16 deaths among confirmed cases (case fatality rate, 21.1%)
2987 suspected cases
121 deaths among suspected cases (case fatality rate, 4.0%)

Approximately 30 million people were vaccinated in the two countries.

This depleted the WHO/UNICEF and Brazilian stocks

# YELLOW FEVER IN THE AMERICAS

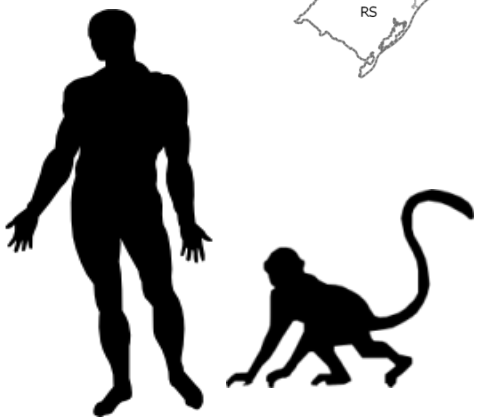
# YF in Brazil



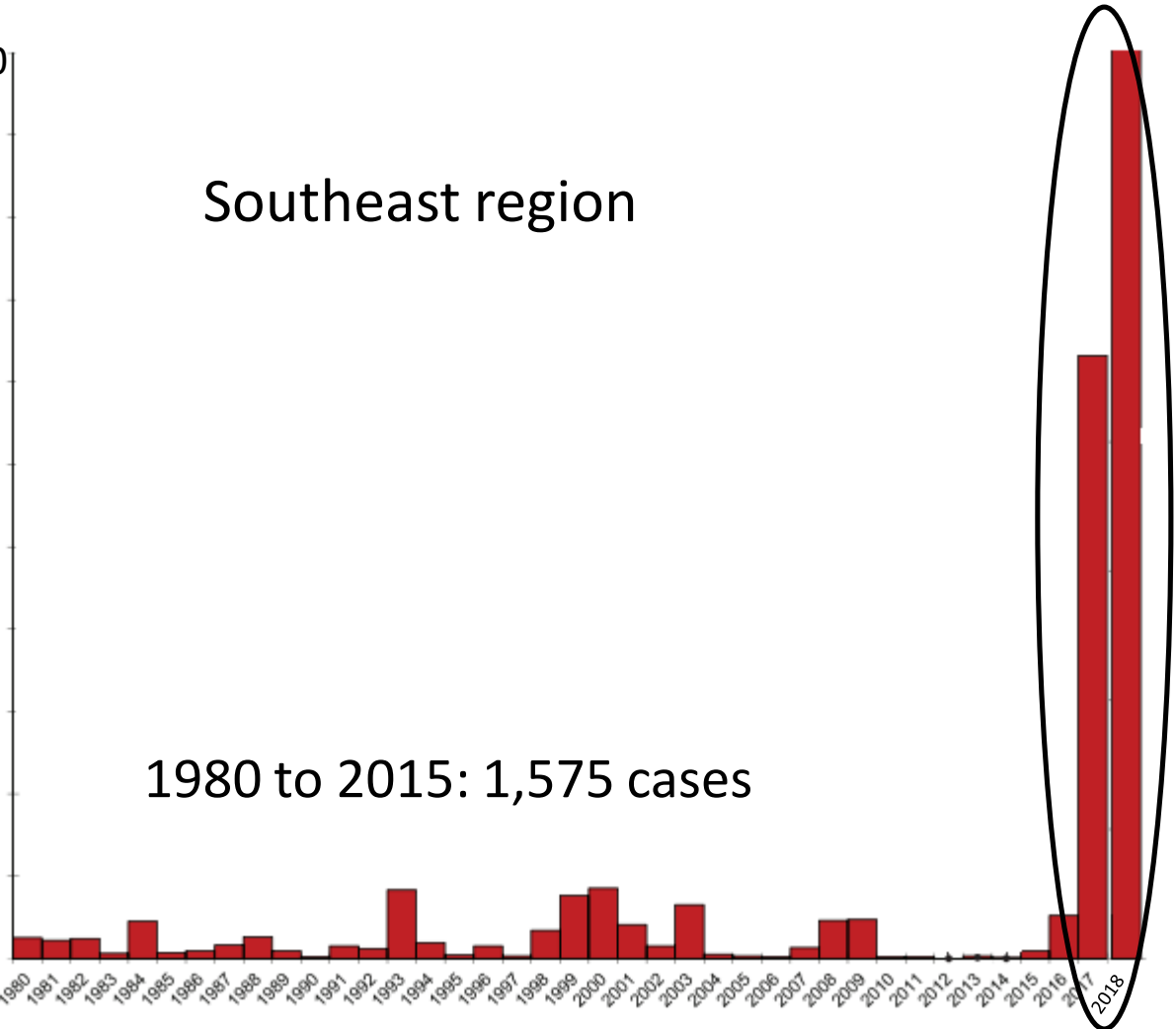

2016 to 2018: 2,153 confirmed cases

744 deaths

Southeast region



+ 5,000 deaths NHP



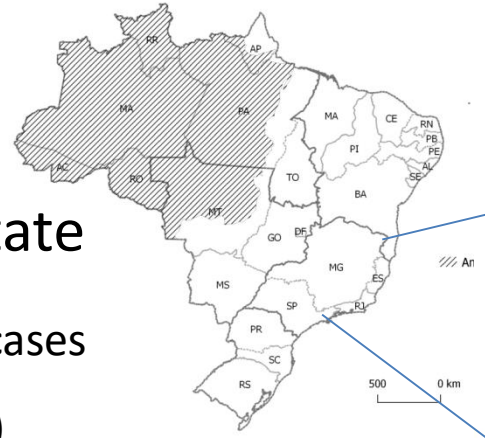

1980 to 2015: 1,575 cases

MS-BR, 2017, 2018

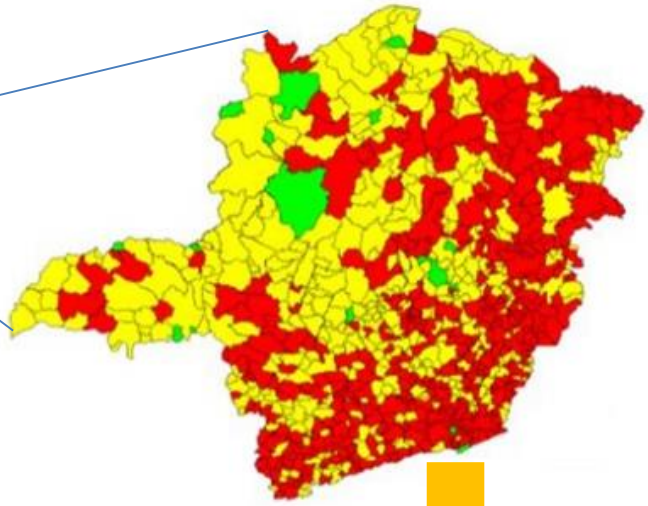
# YF: Brazil
# Minas Gerais state

2017-2018: 1,003 cases

339 deaths (33.9%)




## 2016 – vaccination coverage



≤ 50%

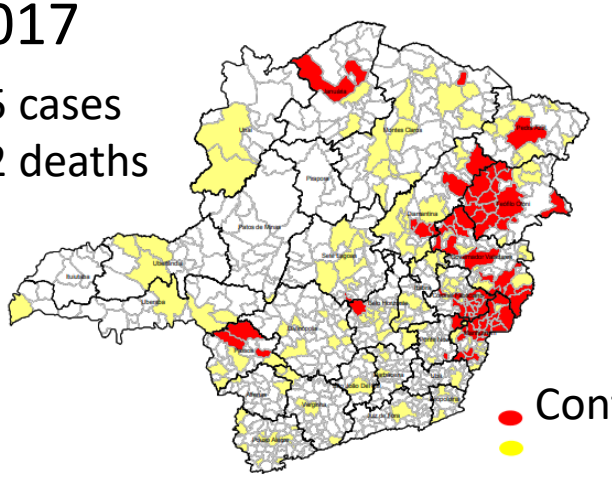
51-94%

>95%

## 2017

475 cases

162 deaths

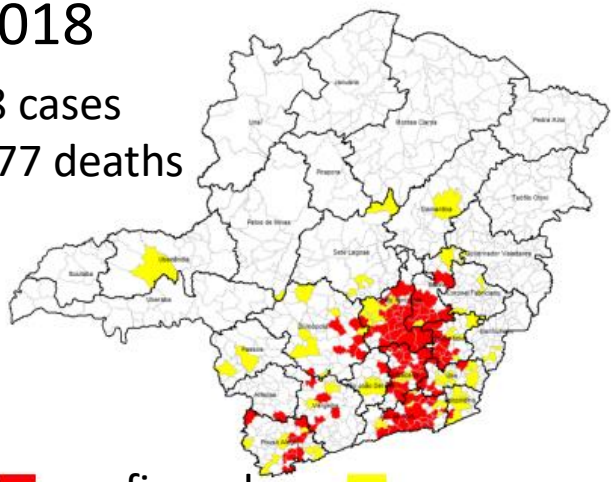


Confirmed

## 2018

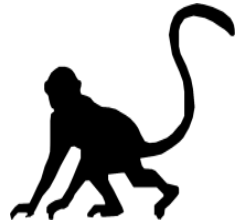
528 cases

– 177 deaths



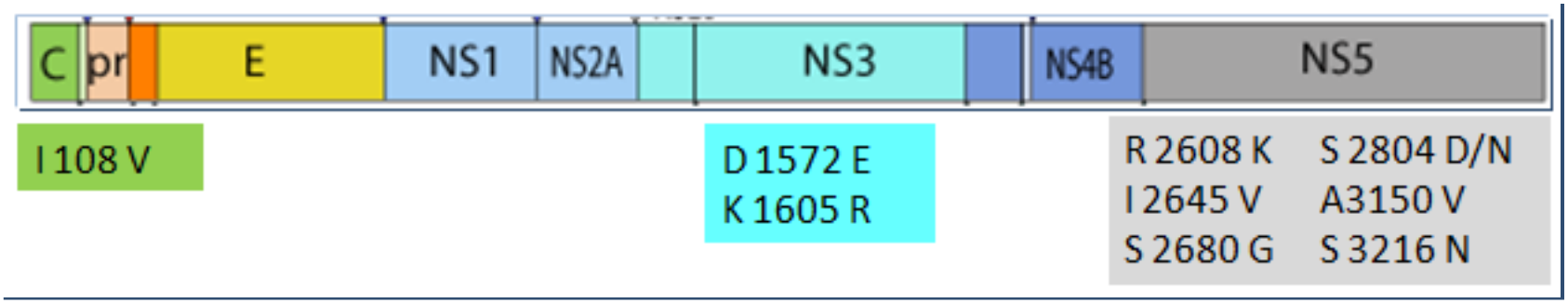
confirmed

MS-BR, 2017, 2018



A new lineage of YFV (South American GI) was responsible for outbreaks in Southeast - 2017

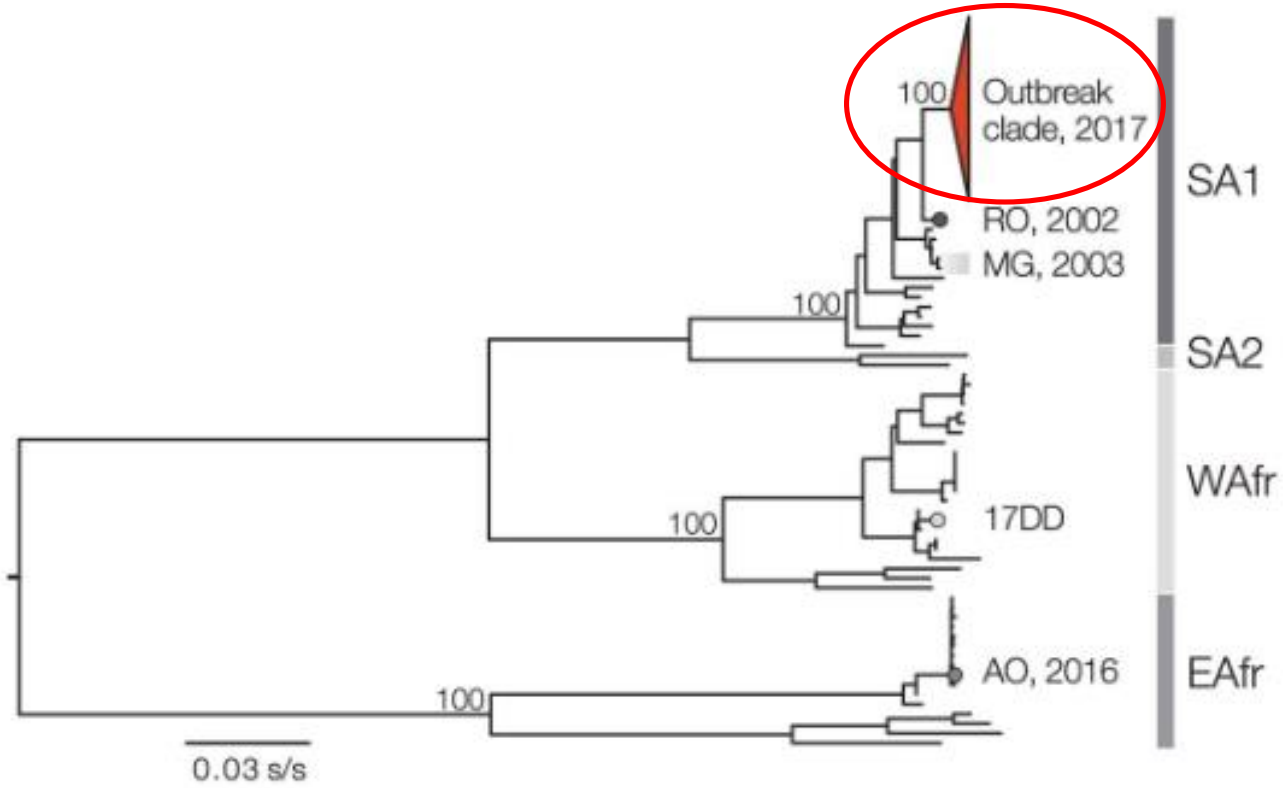
9 unique aa substitutions in the polyprotein sequence




Bonaldo et al, 2017, Moreira-Soto et al, 2018, Goméz et al, 2018, Faria et al, 2018

# Genomic and epidemiological monitoring of yellow fever virus transmission potential

N. R. Faria[1,*,†], M. U. G. Kraemer[1,2,3,*], S. C. Hill[1,*], J. Goes de Jesus[4,*], R. S. Aguiar[5,*], F. C. M. Iani[6,7,*], J. Xavier[4], J. Quick...

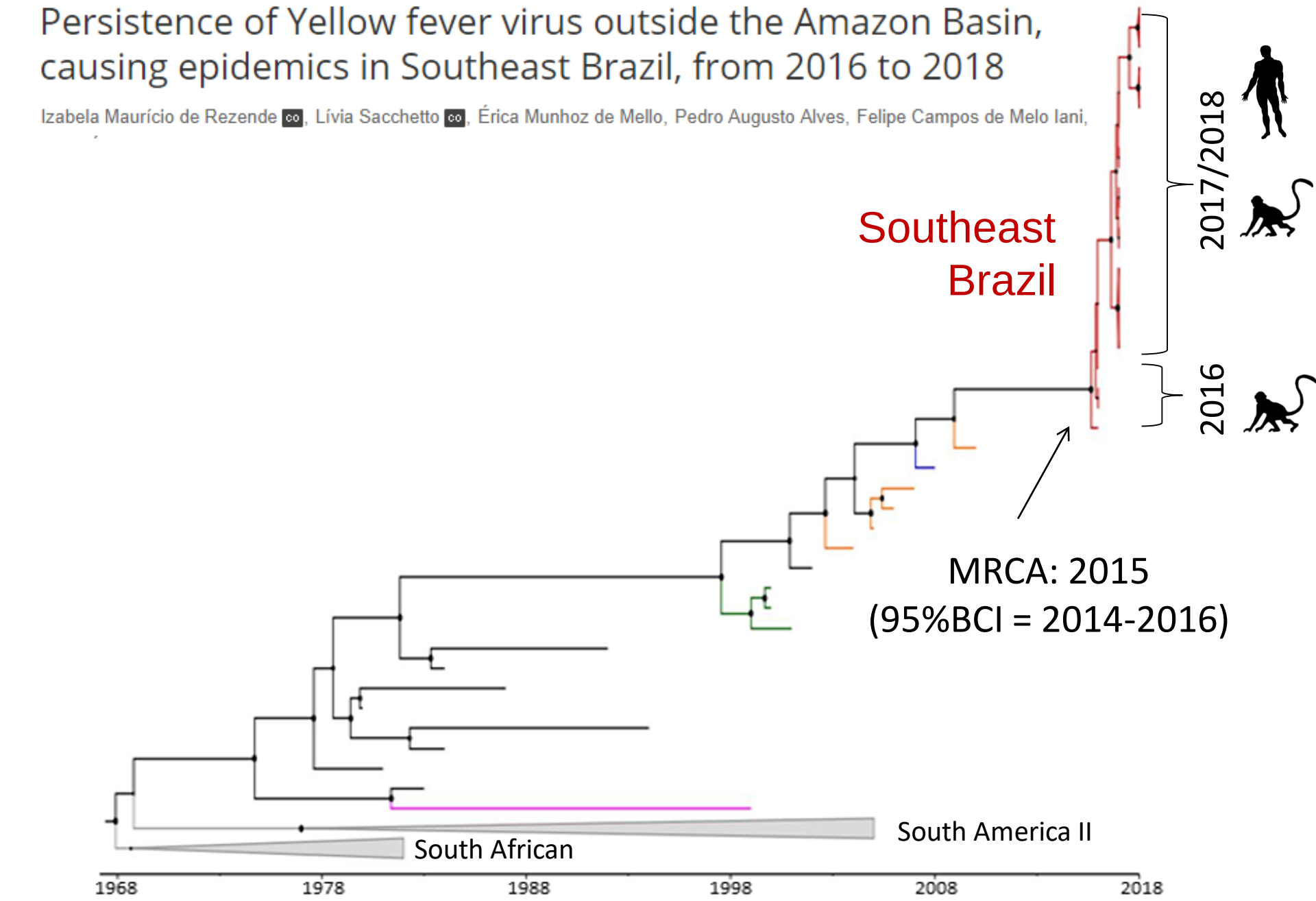



Most recent common ancestor:
July 2016
[95% BCI: Mar - Nov 2016]

Faria et al, 2018

# Persistence of Yellow fever virus outside the Amazon Basin, causing epidemics in Southeast Brazil, from 2016 to 2018

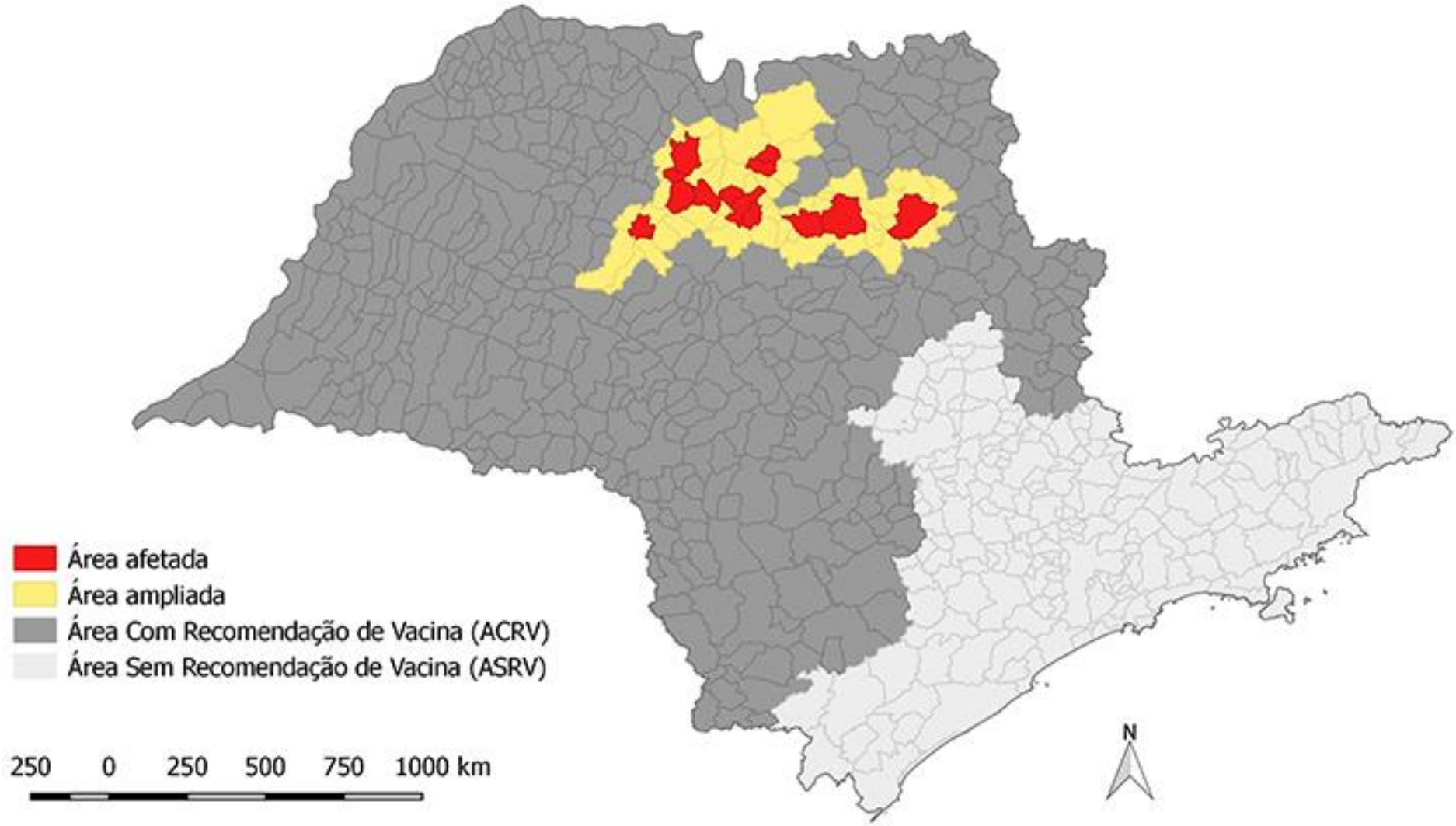
Izabela Maurício de Rezende, Lívia Sacchetto, Érica Munhoz de Mello, Pedro Augusto Alves, Felipe Campos de Melo Iani,

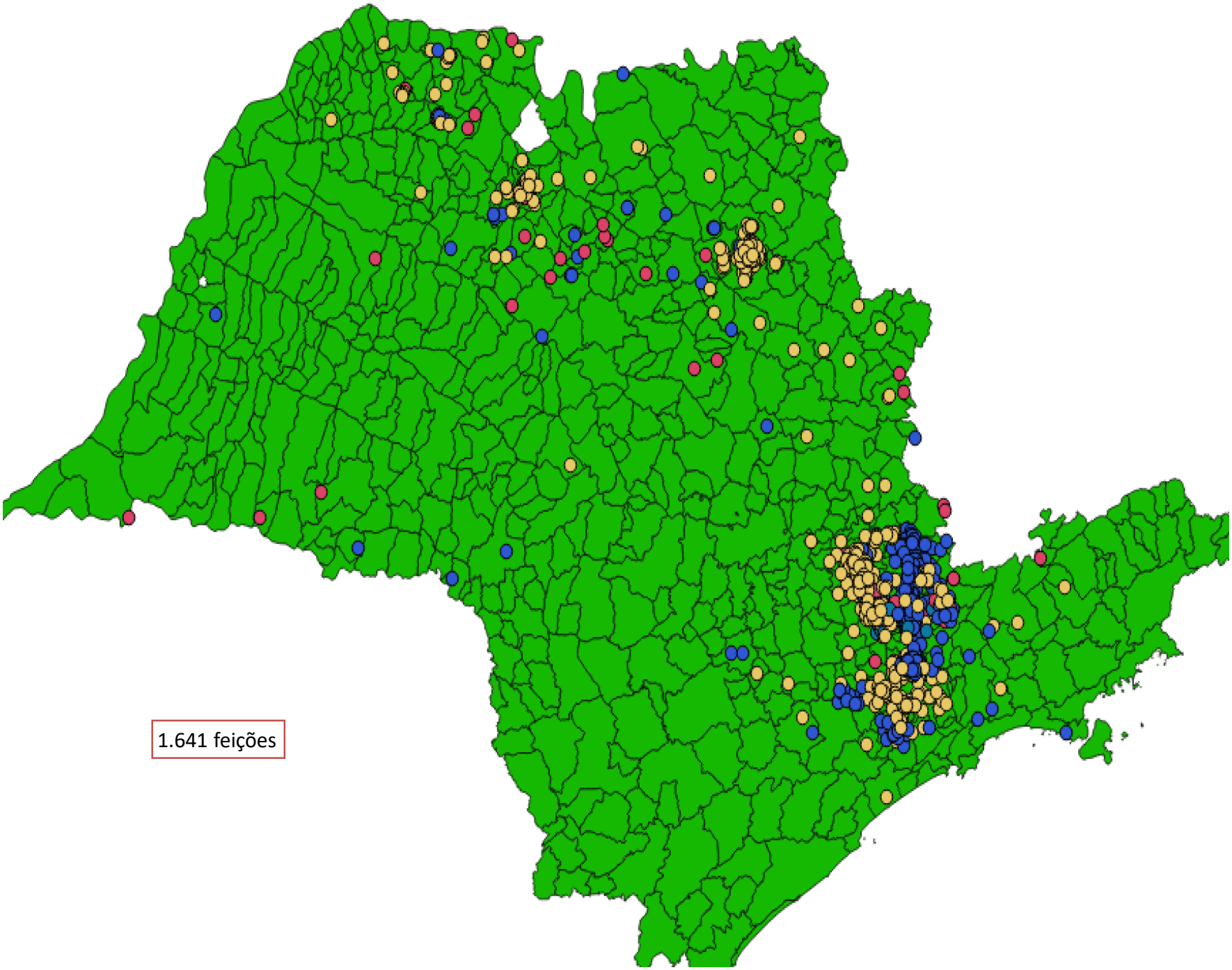



Rezende & Sacchetto et al, 2018

Beast1.8.3

# YF Sao Paulo 2016




1.641 feições


States with confirmed locally–acquired cases since July 2017

Area at risk for yellow fever transmission

Area considered at no risk for yellow fever transmission

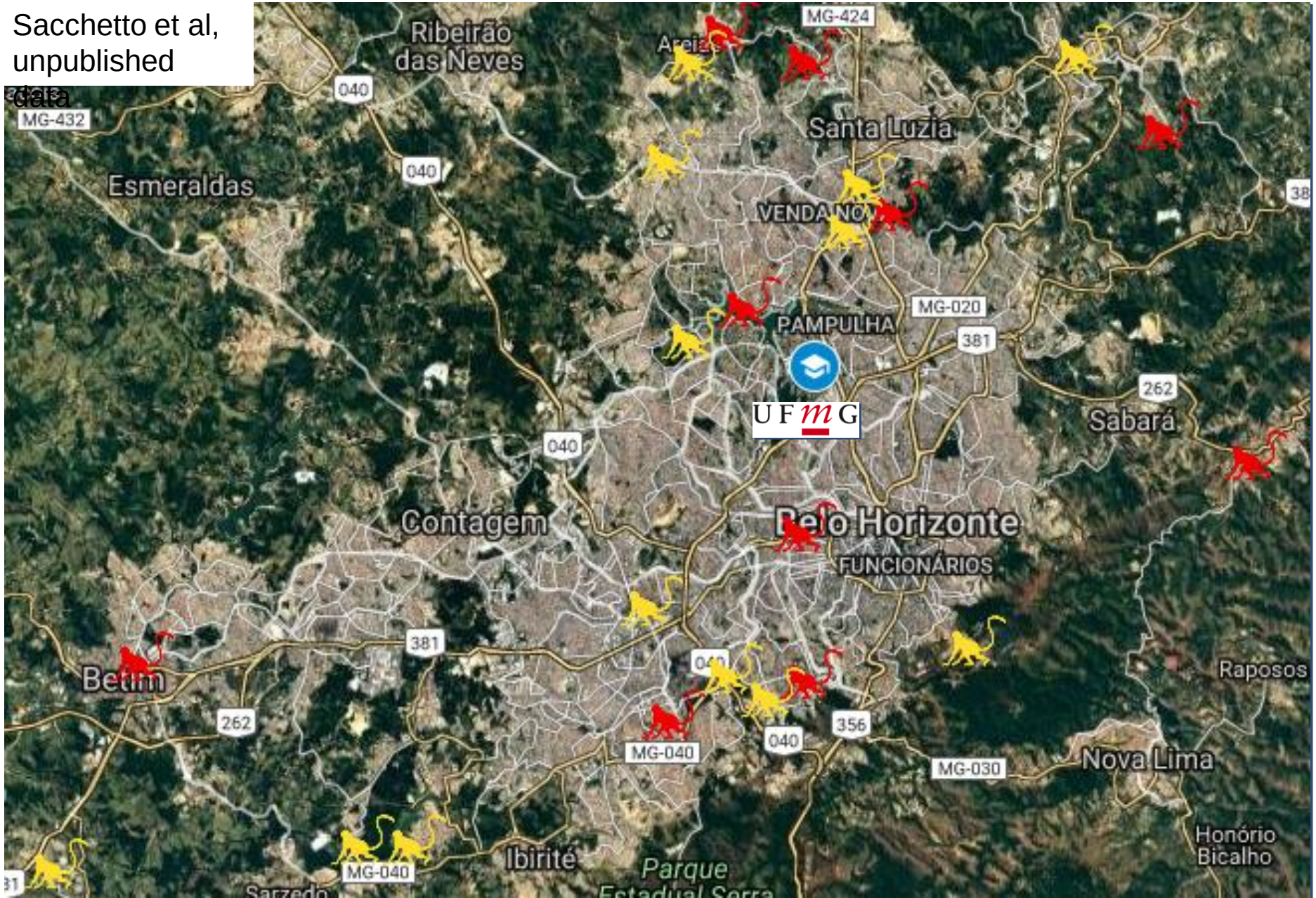
Federal state

● Probable place of infection of the European travel-related yellow fever cases

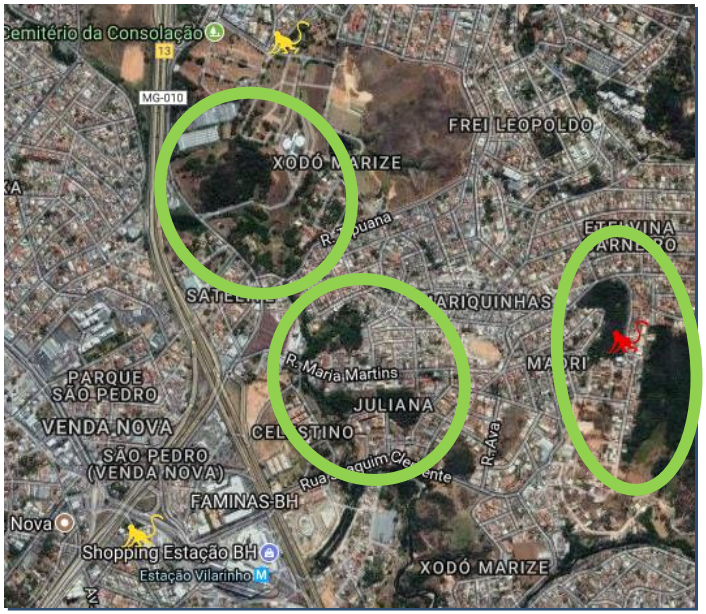
# YFV positive *Callithrix sp.* – 2017 and 2018

## Metropolitan region – Belo Horizonte 5.8 million inhabitants

Sacchetto et al, unpublished data



# YFV: *Callithrix sp.* – 2017 and 2018
# Metropolitan region – Belo Horizonte 5.8 million inhabitants

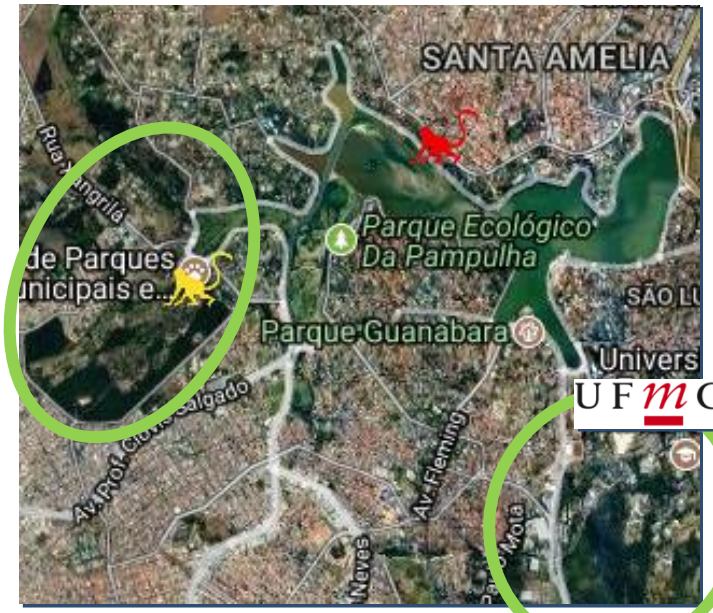



Forest pockets – commonly observed inside/boundaries of Brazilian cities

Marmosets are very common in urban areas – Brazil

Vector surveillance - needed

Sacchetto et al, unpublished data

# YELLOW FEVER VACCINE

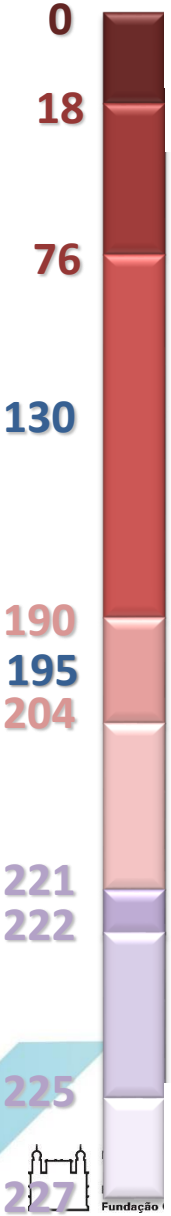
# History of Virus Attenuation of Wild Yellow Fever Asibi Strain

30/06/1927

ASIBI virus,

Mahaffi & Bauer

54 passages in rhesus monkeys




December 1933. Minced tissue were inoculated with monkey sera infected with Asibi strain

18 passages in minced mouse embryo tissue

58 passages in minced whole chick embryo

**designated 17D strain**

114 passages using chick embryo without nervous tissues

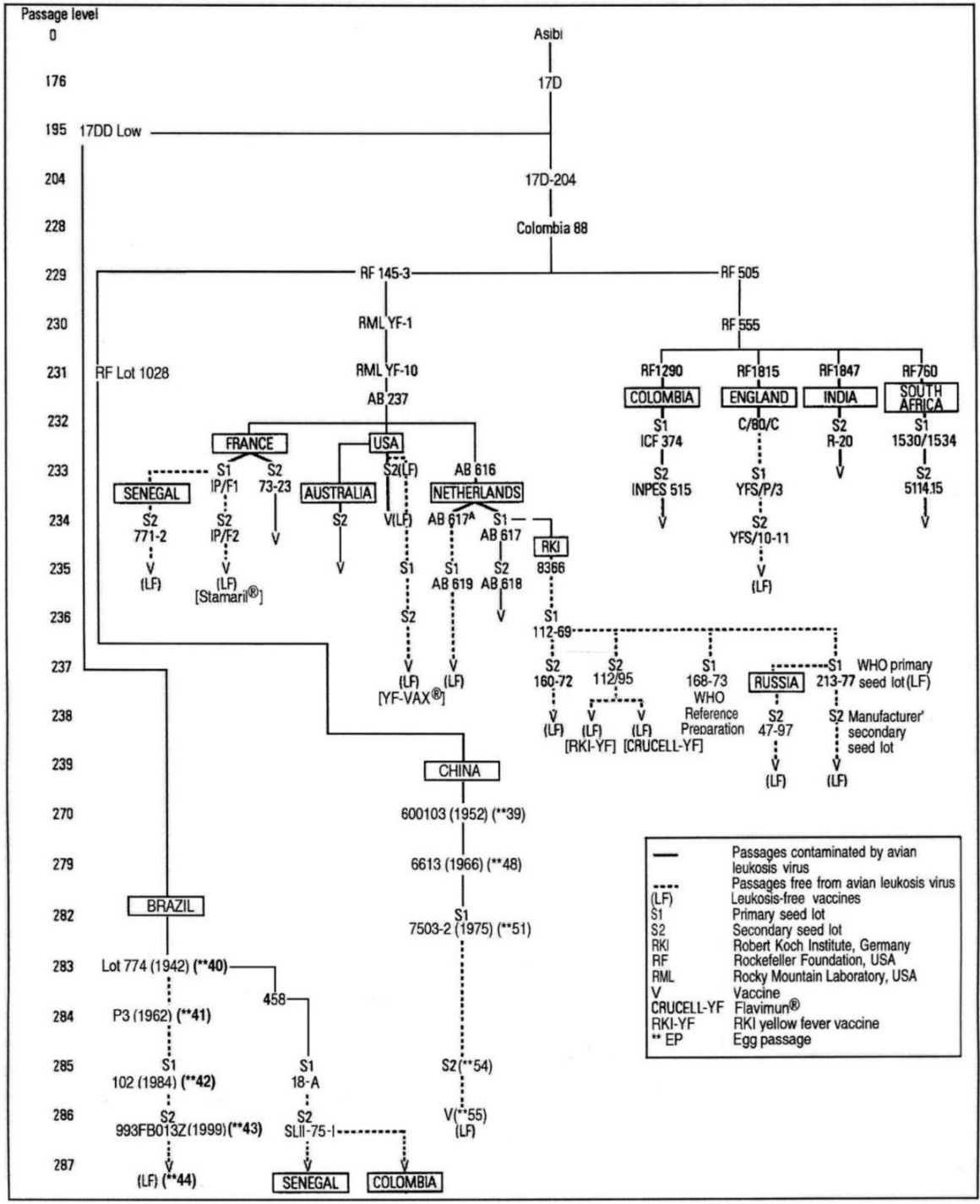
**begin the 17DD strain**

passages using chick embryo tissues without head and spinal cord and normal monkey or human serum

1936 - At 227th and 229th passages – experimental vaccination in Rockefeller Foundation employees. *Acceptable tolerability, development of neutralizing antibodies*

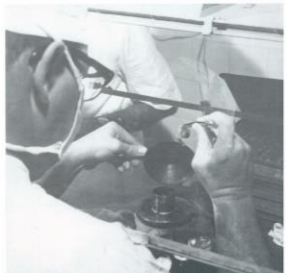
# Yellow Fever` seed virus passage in different Manufacturers

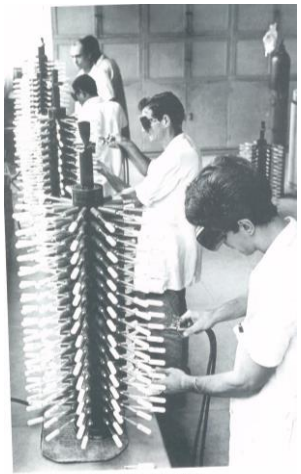
Genealogy of yellow fever vaccine strains. All strains are derived from the Asibi strain and the 176 strain derived from it by passage. The divergence of the different seed strains is shown.




https://doi.org/10.1016/j.virol.2015.03.032

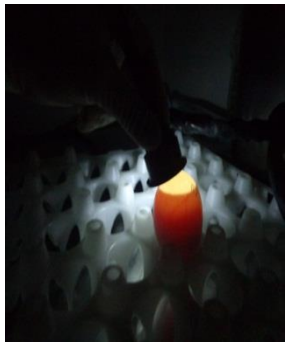
# VACCINE PRODUCTION PROCESS (1942)

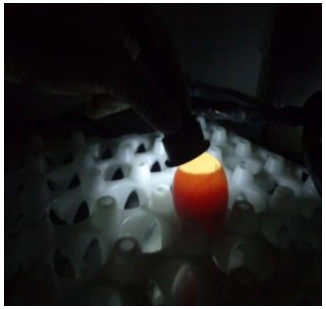




Fechamento das ampolas nas "arvores" ou "cachos".
Rio de Janeiro, 1950.
Album: LAFA/Bio-Manguinhos/Fiocruz

# VACCINE PRODUCTION PROCESS (2017).





Suspensão viral (IFA)

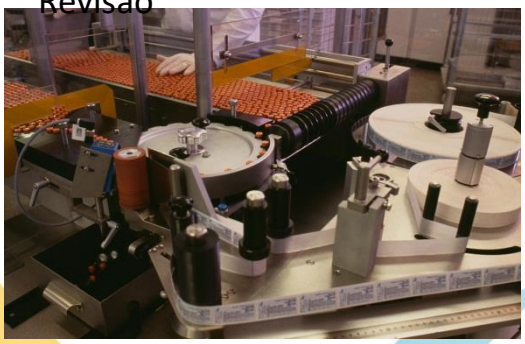
Trit

Ministério da Saúde
FIOCRUZ
Fundação Oswaldo Cruz

Instituto de Tecnologia em Imunobiológicos
Bio-Manguinhos
40 anos

# VACCINE PRODUCTION PROCESS (2018-).

Revisão






RESEARCH PAPER

Human Vaccines & Immunotherapeutics 12:2, 491–502; February 2016; Published with license by Taylor & Francis Group, LLC



## Media centre

Media centre

News

News releases

Previous years

Statements

Notes for the media

Commentaries

Events

# Yellow fever vaccination booster not needed

News release

17 MAY 2013 | GENEVA - The yellow fever 'booster' vaccination given ten years after the initial vaccination is not necessary, according to WHO. An article published in WHO's Weekly Epidemiological Record (WER) reveals that the Organization's Strategic Advisory Group of Experts on immunization (SAGE) has reviewed the latest evidence and concluded that a single dose of vaccination is sufficient to confer life-long immunity against yellow fever disease.

Since yellow fever vaccination began in the 1930s, only 12 known cases of yellow fever post-vaccination have been identified, after 600 million doses have been dispensed. Evidence showed that among this small number of "vaccine failures", all cases developed the disease within five years of vaccination. This demonstrates that

**Related links**

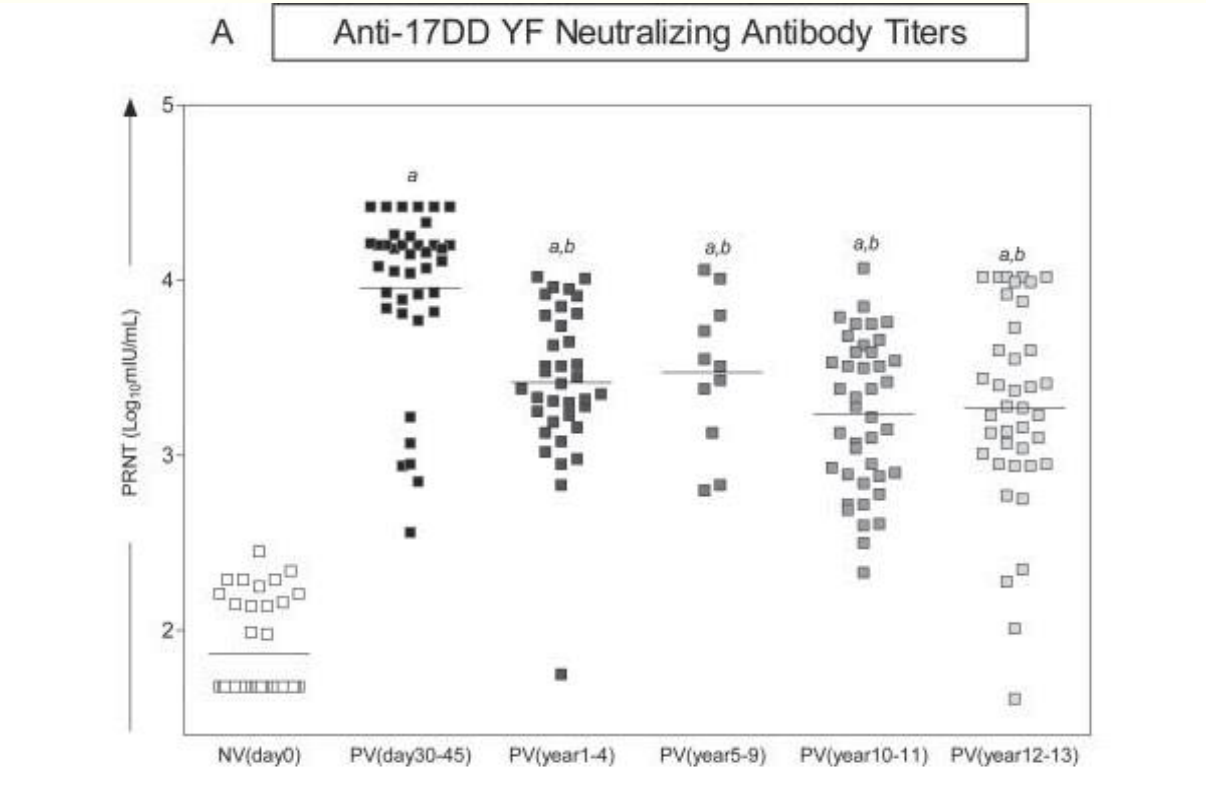
Weekly Epidemiological Record (WER)

About SAGE

Yellow fever fact sheet

More on yellow fever

# DURATION OF IMMUNITY



# Successful Use of Fractioned Doses (1/5th)

- Backed up by SAGE
- Strong political buy-in
- 2 months from decision to implementation
- Technical, Operational & Logistical challenges
  - Syringe supply, vaccine reconstitution, training of HCW, social mobilization…
- Coordinated effort among multiple partners (MoH, NGOs, National and International PH agencies, donors, community)
- INRB/CDC immunogenicity study ongoing on 742 individuals
- SAGE will meet mid-October to provide recommendations on FD

**~7, 5 m people >2y vaccinated in Kinshasa**

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

# Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak — Preliminary Report

Steve Ahuka-Mundeke, M.D., Ph.D., Rebecca M. Casey, M.B., B.S., M.P.H., Jennifer B. Harris, Ph.D., M.P.H., Meredith G. Dixon, M.D., Pierre M. Nsele, M.D., Gabriel M. Kizito, M.D., Grace Umutesi, M.P.H., Janeen Laven, B.S., Gilson Paluku, M.D., M.P.H., Abdou S. Gueye, M.D., Ph.D., Terri B. Hyde, M.D., M.P.H., Guylain K.M. Sheria, M.D., Ph.D., Jean-Jacques Muyembe-Tanfum, M.D., Ph.D., and J. Erin Staples, M.D., Ph.D.

## ABSTRACT

### BACKGROUND

In 2016, the response to a yellow fever outbreak in Angola and the Democratic Republic of Congo led to a global shortage of yellow fever vaccine. As a result, a fractional dose of the 17DD yellow fever vaccine (containing one fifth [0.1 ml] of the standard dose) was offered to 7.6 million children 2 years of age or older and nonpregnant adults in a preemptive campaign in Kinshasa. The goal of this study was to assess the immune response to the fractional dose in a large-scale campaign.

### METHODS

We recruited participants in four age strata at six vaccination sites. We assessed neutralizing antibody titers against yellow fever virus in blood samples obtained before vaccination and 28 to 35 days after vaccination, using a plaque reduction neutralization test with a 50% cutoff ($PRNT_{50}$). Participants with a $PRNT_{50}$ titer of 10 or higher at baseline were considered to be seropositive. Those with a baseline titer of less than 10 who became seropositive at follow-up were classified as having undergone seroconversion. Participants who were seropositive at baseline and who had an increase in the titer by a factor of 4 or more at follow-up were classified as having an immune response.

### RESULTS

Among 716 participants who completed follow-up, 705 (98%; 95% confidence interval [CI], 97 to 99) were seropositive after vaccination. Among 493 participants who were seronegative at baseline, 482 (98%; 95% CI, 96 to 99) underwent seroconversion. Among 223 participants who were seropositive at baseline, 148 (66%; 95% CI, 60 to 72) had an immune response. Lower baseline titers were associated with a higher probability of having an immune response ($P<0.001$).

### CONCLUSIONS

A fractional dose of the 17DD yellow fever vaccine was effective at inducing seroconversion in most of the participants who were seronegative at baseline. These findings support the use of fractional-dose vaccination for outbreak control. (Funded by the U.S. Agency for International Development and the Centers for Disease Control and Prevention.)

Annals of Internal Medicine

ORIGINAL RESEARCH

# Long-Term Protection After Fractional-Dose Yellow Fever Vaccination

## Follow-up Study of a Randomized, Controlled, Noninferiority Trial

**Anna H.E. Roukens, MD, PhD*; Karlijn van Halem, MD*; Adriëtte W. de Visser, BSc; and Leo G. Visser, MD, PhD**

**Background:** Outbreaks of yellow fever and a frequently depleted vaccine stock increase demand for a dose-sparing strategy. A fractional dose of 17D yellow fever virus (17D-YFV) vaccine has been shown to be noninferior to the standard dose in inducing seroprotection.

**Objective:** To evaluate whether fractional-dose vaccination can confer long-term immunity.

**Design:** Ten-year follow-up of a subgroup of a randomized, controlled, noninferiority trial. (Dutch Trial Register: NTR7094 [current study] and ISRCTN46326316 [original study])

**Setting:** The Netherlands.

**Participants:** Seventy-five of 155 participants in the original trial provided a blood sample for this study. These 75 participants had received primary vaccination with 17D-YFV vaccine 10 years before. Forty received a 0.1-mL fractional dose intradermally, and 35 received the standard 0.5-mL dose subcutaneously.

**Measurements:** Virus-neutralizing antibody responses were measured by a plaque reduction neutralization test.

**Results:** Thirty-nine of 40 (98% [95% CI, 89% to 100%]) participants had protective levels of yellow fever–neutralizing antibodies more than 10 years after receiving a fractional dose of 17D-YFV vaccine compared with 34 of 35 (97% [CI, 87% to 100%]) in the standard-dose group.

**Limitation:** Only 48% of participants from the original trial participated in this study.

**Conclusion:** Intradermal administration of a one-fifth dose of yellow fever vaccine induced a protective immune response that lasted for 10 years after vaccination. Persons receiving a fractional dose of yellow fever vaccine do not require a booster vaccination for long-term protection against yellow fever.

**Primary Funding Source:** Leiden University Medical Center and the International Society of Travel Medicine.

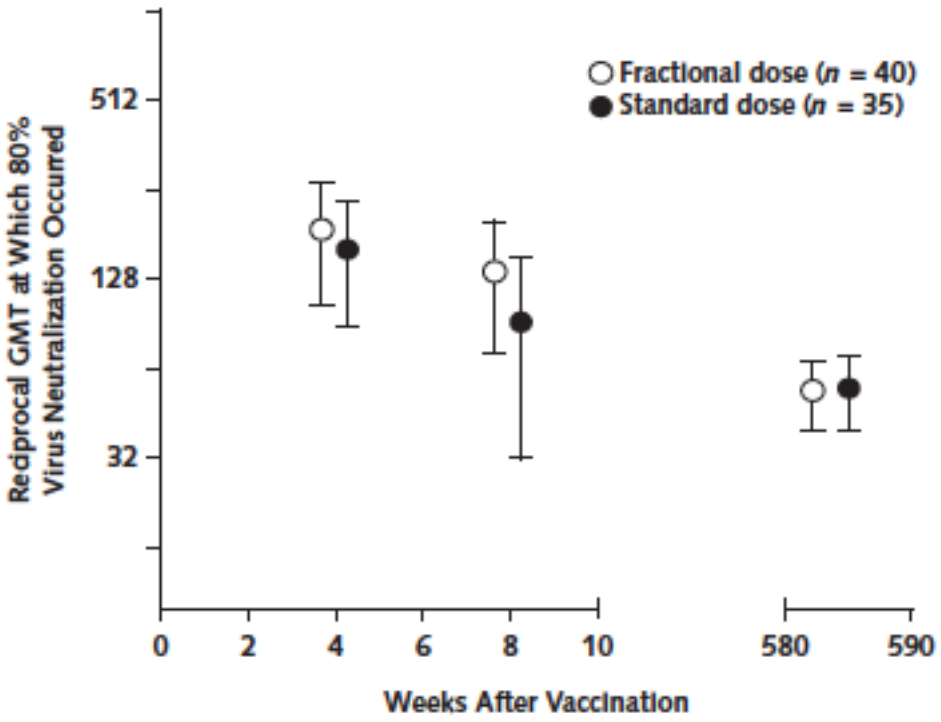
*Ann Intern Med.* doi:10.7326/M18-1529 **Annals.org**

For author affiliations, see end of text.

This article was published at Annals.org on 27 November 2018.

* Drs. Roukens and van Halem contributed equally to this work.

*Figure 2.* Protective virus neutralization after fractional- or standard-dose vaccination.




Comparison of reciprocal serum dilutions at which 80% of yellow fever virus was neutralized in constant virus-varying serum dilution tests among 75 participants 10 y after primary vaccination with the intradermal fractional dose (0.1 mL) or the subcutaneous standard dose (0.5 mL). Error bars represent 95% CIs. Virus-neutralizing capacity of serum in both groups was evaluated at similar time points, but indicators are juxtaposed for visual enhancement. GMT = geometric mean titer.

Annals of Internal Medicine ORIGINAL RESEARCH

**Long-Term Protection After Fractional-Dose Yellow Fever Vaccination**

**Follow-up Study of a Randomized, Controlled, Noninferiority Trial**

Anna H.E. Roukens, MD, PhD*; Karlijn van Halem, MD*; Adriëtte W. de Visser, BSc; and Leo G. Visser, MD, PhD

# SERIOUS ADVERSE EVENTS OF YF VACCINE

| Case | Place, year | Age, | Sex | Time after vaccin | Clinical and laboratory summary | Outcome |
|---|---|---|---|---|---|---|

Virology 290, 309–319 (2001)
doi:10.1006/viro.2001.1168, available online at http://www.idealibrary.com on IDEAL®

## Phenotypic and Molecular Analyses of Yellow Fever 17DD Vaccine Viruses Associated with Serious Adverse Events in Brazil

R. Galler,*,[1] K. V. Pugachev,† C. L. S. Santos,‡ S. W. Ocran,† A. V. Jabor,* S. G. Rodrigues,§ R. S. Marchevsky,¶ M. S. Freire,¶ L. F. C. Almeida,¶ A. C. R. Cruz,§ A. M. Y. Yamamura,¶ I. M. Rocco,‡ E. S. Travassos da Rosa,§ L. T. M. Souza,‡ P. F. C. Vasconcelos,§ F. Guirakhoo,† and T. P. Monath†

*Instituto Oswaldo Cruz and ¶Instituto de Tecnologia em Imunobiológicos, Fundação Oswaldo Cruz, 21045-900, Rio de Janeiro, RJ, Brazil; †Acambis, Inc, Cambridge, Massachusetts; ‡Instituto Adolpho Lutz, São Paulo, SP, Brazil; and §Instituto Evandro Chagas/Fundação Nacional de Saúde, Belém, PA, Brazil

Received July 6, 2001; returned to author for revision August 20, 2001; accepted August 31, 2001

| | | | | | | |
|---|---|---|---|---|---|---|
| **5** | Minas Gerais, 2001 | 19 | F | 3 | Fever, myalgia, cephalea. AST and ALT 12 e 6 x. Bilirubin 6,0. Leukopenia with left shift. Coagulation disorder. | Death 10th day |
| **6** | Rio Grande do Sul, 2001 | 4 | M | 4 | Fever, prostration, petechiae. Lymphadenopathy. AST and ALT 20 x; Bilirubin 7,01. Leukopenia with left shift. Renal failure. | Death 10th day |
| **7** | Rio de Janeiro, | 67 | M | 4 | Fever, asthenia, myalgia, cephalea and prostration. AST: 2572; TGP: 2525. Leukopenia. Respiratory failure. Yellow fever neutralizing | Recovered |

# SERIOUS ADVERSE EVENTS OF YF VACCINE

❖ International Data

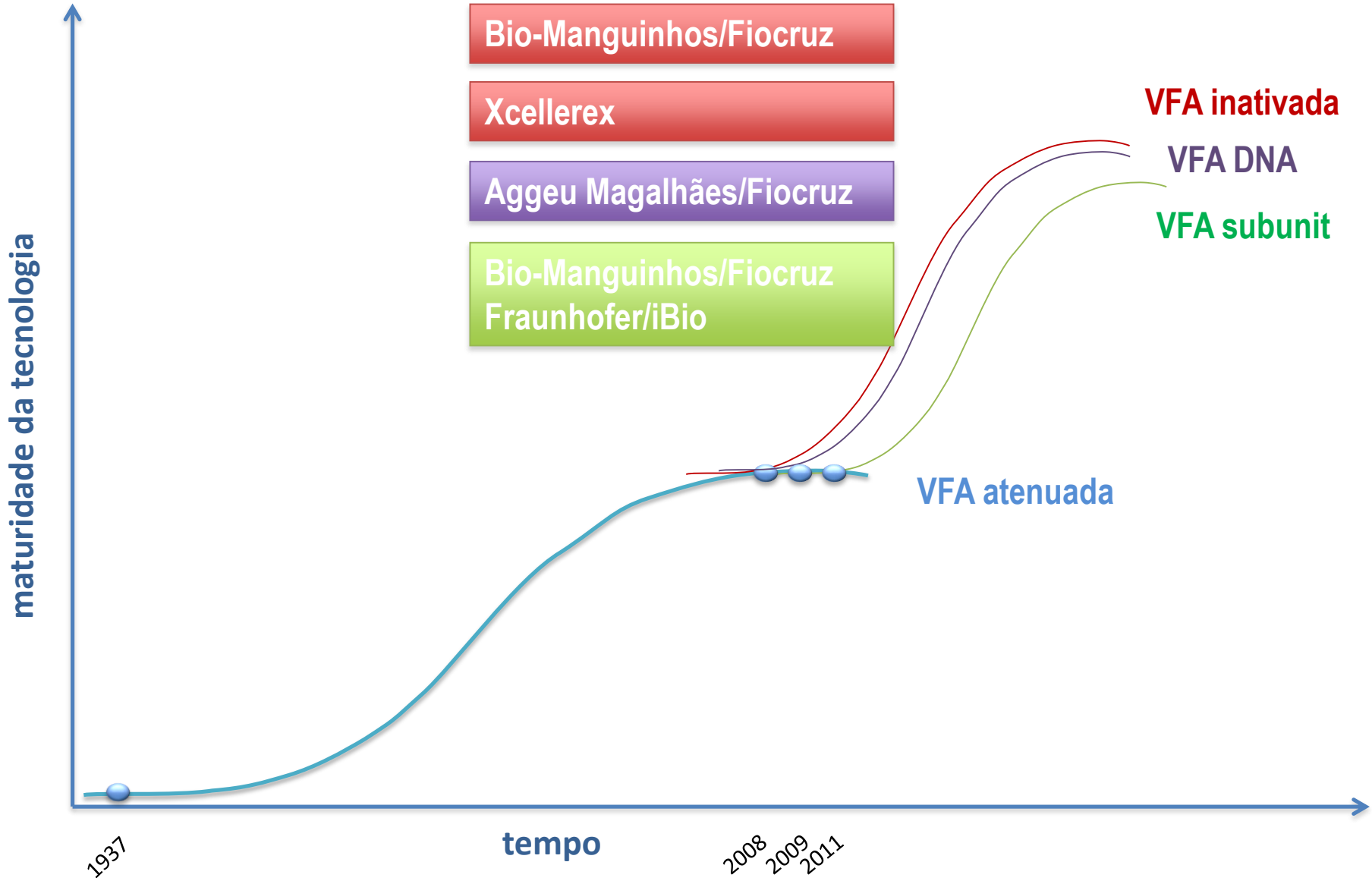
- Viscerotropic disease (0,3/100.000 doses)
- Neurologic disease (0,4/100.000 doses)
- Allergic reactions (0,8/100.000 doses)

- * death about 0,5/1.000.000. We need to vaccinate 80 Million people in Brazil

**Source: Hayes EB, 2007; Estofolete & Nogueira, 2018**

# YELLOW FEVER VACCINE: TECHNOLOGICAL IMPROVEMENT

# YELLOW FEVER VACCINE: TECHNOLOGICAL IMPROVEMENT



# AN INACTIVATE VACCINE AGAINST YF

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

## Pressure-inactivated yellow fever 17DD virus: Implications for vaccine development

Luciane P. Gaspar[a,*], Ygara S. Mendes[b], Anna M.Y. Yamamura[a], Luiz F.C. Almeida[a], Elena Caride[a], Rafael B. Gonçalves[b,1], Jerson L. Silva[b], Andréa C. Oliveira[b], Ricardo Galler[a], Marcos S. Freire[a]

[a] Programa de Vacinas Virais, Instituto de Tecnologia em Imunobiológicos, Fundação Oswaldo Cruz, Rio de Janeiro, RJ 21045-900, Brazil
[b] Programa de Biologia Estrutural, Instituto de Bioquímica Médica and Centro Nacional de Ressonância Magnética Nuclear de Macromoléculas Jiri Jonas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil

Bio-Manguinhos/Fiocruz

# AN INACTIVATE VACCINE AGAINST YF

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## An Inactivated Cell-Culture Vaccine against Yellow Fever

Thomas P. Monath, M.D., Elizabeth Fowler, Ph.D., Casey T. Johnson, D.O., John Balser, Ph.D., Merribeth J. Morin, Ph.D., Maggie Sisti, B.S., and Dennis W. Trent, Ph.D.

From Xcellerex, Marlborough, MA (T.P.M., E.F., M.J.M., M.S., D.W.T.); Johnson City Clinical Trials, Lenexa, KS (C.T.J.); and Veristat, Holliston, MA (J.B.). Address reprint requests to Dr. Monath at Kleiner, Perkins, Caufield, and Byers, 2750 Sand Hill Rd., Menlo Park, CA 94025, or at tmonath@kpcb.com.

N Engl J Med 2011;364:1326-33.



Xcellerex

# DNA VACCINE AGAINST YF

Anais da Academia Brasileira de Ciências (2009) 81(4): 663-669
(Annals of the Brazilian Academy of Sciences)
ISSN 0001-3765
www.scielo.br/aabc

## Membrane and envelope virus proteins co-expressed as lysosome associated membrane protein (LAMP) fused antigens: a potential tool to develop DNA vaccines against flaviviruses

RAFAEL DHALIA[1], MILTON MACIEL Jr.[2], FÁBIA S.P. CRUZ[1], ISABELLE F.T. VIANA[1], MARIANA L. PALMA[1], THOMAS AUGUST[2] and ERNESTO T.A. MARQUES Jr.[1,2,3]

[1]Fundação Oswaldo Cruz, Centro de Pesquisas Aggeu Magalhães, Departamento de Virologia
Laboratório de Virologia e Terapia Experimental (LaViTE), Av. Professor Moraes Rego s/n
Cidade Universitária, Caixa Postal 7472, 50670-420 Recife, PE, Brasil
[2]Johns Hopkins University, School of Medicine, Department of Pharmacology and Molecular Sciences
725 North Wolfe Street, Biophysics Building, Baltimore, Maryland 21205, USA
[3]Johns Hopkins University, School of Medicine, Department of Medicine, Division of Infectious Diseases
725 North Wolfe Street, Biophysics Building, Baltimore, Maryland 21205, USA

*Manuscript received on August 5, 2008; accepted for publication on March 3, 2009;*
*presented by* JERSON L. SILVA

**Aggeu Magalhães/Fiocruz**

# How to do a clinical trial of YF vaccine?

Cost? Market?

Markers of protection?

Population?

Ethical Considerations

# Take Home Lesson

a) YFV is re-emerging in South America and Africa with higher than usual number of cases

b) There is a good vaccine

c) There is not enough vaccine available. The stocks are in record low

d) There is technology for new vaccines. But is there interest on it?

e) No drug available

# Zika in NPH Primates, Brazil

Mauricio L Nogueira, MD,PhD

SCIENTIFIC REPORTS

OPEN

# Evidence of natural Zika virus infection in neotropical non-human primates in Brazil

Received: 19 March 2018
Accepted: 17 October 2018
Published: xx xx xxxx

Ana Carolina B. Terzian[1], Nathalia Zini[1], Lívia Sacchetto[2], Rebeca Froes Rocha[3], Maisa Carla Pereira Parra[1], Juliana Lemos Del Sarto[3], Ana Carolina Fialho Dias[3], Felipe Coutinho[3], Jéssica Rayra[3], Rafael Alves da Silva[1], Vivian Vasconcelos Costa[3], Natália Coelho Couto De Azevedo Fernandes[4], Rodrigo Réssio[4], Josué Díaz-Delgado[4], Juliana Guerra[4], Mariana S. Cunha[4], José Luiz Catão-Dias[5], Cintia Bittar[6], Andréia Francesli Negri Reis[7], Izalco Nuremberg Penha dos Santos[7], Andréia Cristina Marascalchi Ferreira[7], Lilian Elisa Arão Antônio Cruz[7], Paula Rahal[6], Leila Ullmann[8], Camila Malossi[8], João Pessoa de Araújo Junior[8], Steven Widen[9], Izabela Maurício de Rezende[2], Érica Mello[10], Carolina Colombelli Pacca[11], Erna Geessien Kroon[2], Giliane Trindade[2], Betânia Drumond[2], Francisco Chiaravalloti-Neto[12], Nikos Vasilakis[13], Mauro M. Teixeira[3] & Maurício Lacerda Nogueira[1]

| Sample ID | NHPs species | Organs (Ct value) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | KIDNEY | BRAIN | SPLEEN | LUNG | LIVER | HEART | GONADS |
| PR 17/02 | *Callithrix* sp. | n/a | n/a | 38.37 | n/a | n/a | n/a | n/a |
| PR 17/03 | *Callithrix* sp. | n/a | 37.65 | n/a | neg | neg | n/a | n/a |
| PR 17/04 | *Callithrix* sp. | 37.84 | neg | 38.37 | 38.37 | neg | 36.19 | n/a |
| PR 17/05 | *Callithrix* sp. | 38.19 | 36 | 35.14 | 36.37 | 37.88 | neg | n/a |
| PR 17/06 | *Callithrix* sp. | 37.96 | neg | 38.23 | neg | neg | n/a | n/a |
| PR 17/07 | *Callithrix* sp. | 38.18 | 37.71 | neg | 37.92 | neg | neg | n/a |
| PR 17/08 | *Sapajus* sp. | 35.98 | 34.44 | 38.91 | neg | neg | neg | n/a |
| PR 17/11 | *Callithrix* sp. | Neg | 37.36 | neg | neg | neg | neg | n/a |
| PR 17/12 | *Callithrix* sp. | 35.66 | 35.64 | neg | n/a | 37.27 | neg | n/a |
| PR 17/13 | *Callithrix* sp. | 35.57 | 32.31 | 37.13 | neg | 29.25 | neg | n/a |
| PR 17/14 | *Callithrix* sp. | Neg | 37.12 | 37 | neg | neg | neg | n/a |
| PR 17/15 | *Callithrix* sp. | Neg | neg | neg | neg | 37.51 | n/a | n/a |
| PR 17/16 | *Callithrix* sp. | 30.87 | 30.4 | 31.48 | 27.76 | 29.18 | 31.32 | n/a |
| PR 17/17 | *Callithrix* sp. | 31.66 | 31.13 | n/a | 31.75 | 32.13 | 30.36 | n/a |
| PR 17/18 | *Callithrix* sp. | 29.17 | 31.86 | 32.87 | 30.47 | 30.16 | 29.72 | n/a |
| PR 17/19 | *Callithrix* sp. | 32.65 | 32.38 | 31.4 | 31.89 | neg | 32.32 | n/a |
| PR 17/20 | *Callithrix* sp. | 34.68 | 31.11 | neg | 34.36 | neg | 34.41 | n/a |
| PR 17/21 | *Callithrix* sp. | 32.89 | 31.11 | 33.27 | 31.9 | 32.86 | 32.59 | n/a |
| PR 17/22 | *Callithrix* sp. | 29.11 | 31.86 | 30.68 | 34.06 | 31.56 | 34.47 | n/a |
| PR 17/23 | *Callithrix* sp. | Neg | neg | n/a | neg | neg | 37.85 | n/a |
| PR 17/25 | *Callithrix* sp. | 37.79 | neg | 34.75 | 37.28 | 36.44 | neg | n/a |
| PR 17/26 | *Callithrix* sp. | 37.69 | n/a | 35.94 | neg | neg | 36.97 | n/a |
| PR 17/27 | *Callithrix* sp. | 38.47 | neg | neg | neg | neg | neg | n/a |
| MG 17/01 | *Callithrix* sp. | n/a | n/a | n/a | n/a | 36.0 | n/a | n/a |
| MG 17/02 | *Callithrix* sp. | n/a | n/a | n/a | n/a | 33.9 | n/a | n/a |
| MG17/15 | *Callithrix* sp. | 27.1 | n/a | n/a | n/a | n/a | n/a | neg |
| MG 17/16 | *Callithrix* sp. | n/a | n/a | n/a | n/a | 35.3 | n/a | n/a |
| MG17/30 | *Callithrix* sp. | neg | n/a | n/a | n/a | 35.6 | n/a | 36.3 |
| MG 17/31 | *Callithrix* sp. | n/a | n/a | n/a | n/a | 35.9 | n/a | n/a |
| MG 17/32 | *Callithrix* sp. | n/a | n/a | n/a | n/a | 36.3 | n/a | n/a |
| MG 17/45 | *Callithrix* sp. | n/a | n/a | n/a | n/a | 35.6 | n/a | n/a |
| MG 17/51 | *Callithrix* sp. | n/a | n/a | n/a | n/a | 35.7 | n/a | n/a |
| Mosquitoes (Ct Value) | | | | | | | | |
| Sample ID | Mosquito species | Ct | | | | | | |
| 17/151 | *Ae. aegypti* | 35.17 | | | | | | |
| 17/160 | *Ae. aegypti* | 36.89 | | | | | | |
| 17/161 | *Ae. aegypti* | 36.48 | | | | | | |
| 17/163 | *Ae. aegypti* | 31.87 | | | | | | |
| 17/164 | *Ae. aegypti* | 36.77 | | | | | | |
| 17/169 | *Ae. aegypti* | 22.23 | | | | | | |

**Table 1.** Non-human primates positive for Zika virus, by RT-qPCR. Positive samples and mosquitoes are indicated by the Ct (cycle threshold) value. n/a: not available. neg: negative. Samples collected in São José do Rio Preto (SP), from January to March 2017 are identified by PR followed by year and sample ID. Samples collected in Minas Gerais, from January to June 2017, are identified by MG followed by year and sample ID. Mosquitoes collected in São José do Rio Preto (SP), in the first trimester of 2017 are identified by year and sample ID.

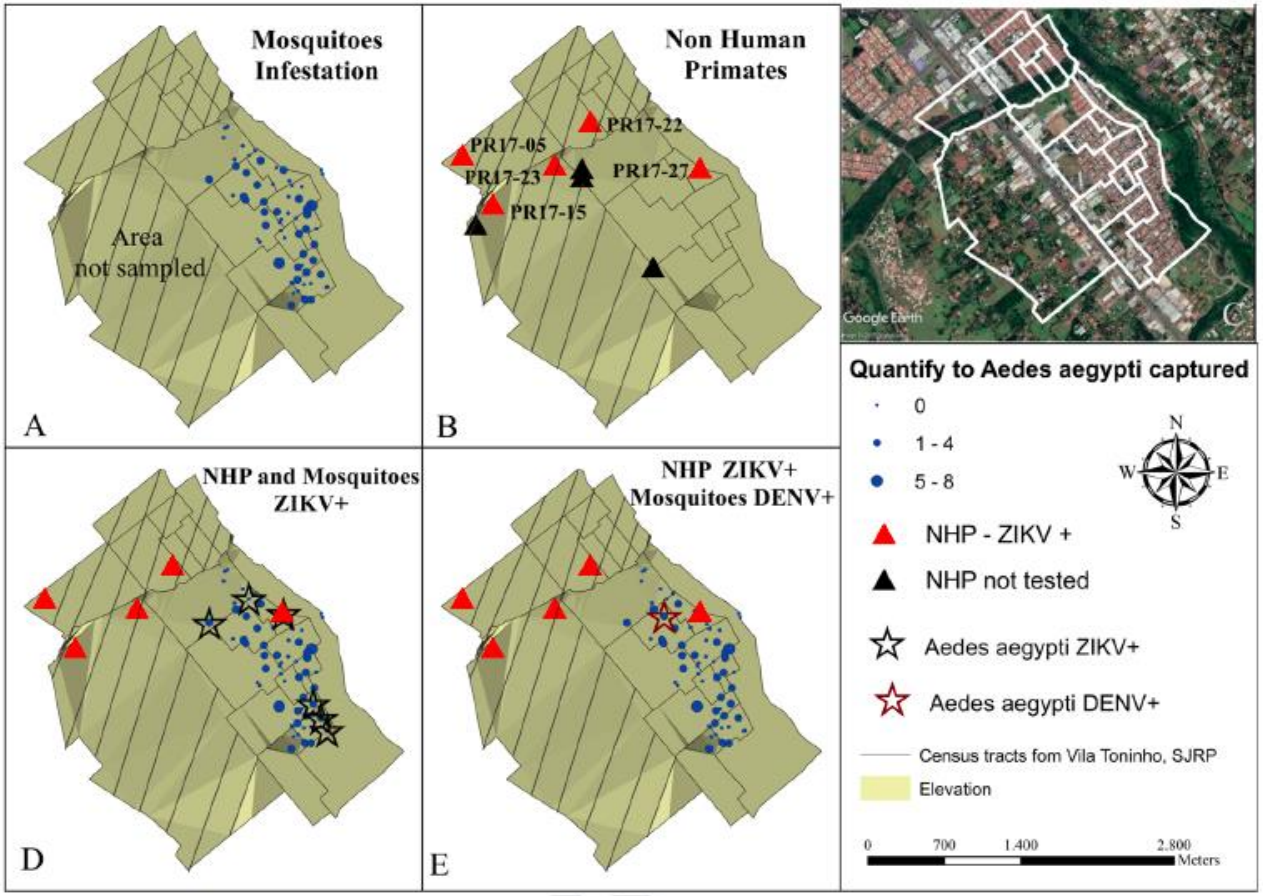



**Figure 1.** Geoprocessing map of the NHPs and mosquitoes captured in the Vila Toninho neighborhood. (**A**) Schematic representation of the area where mosquitoes are regularly collected in the Vila Toninho neighborhood. The hatched area represents the area where there is no specimen collection. The blue dots represent the collection points of the mosquitoes and the quantity of specimen collected. (**B**) Schematic representation of the collection points of the nine NHP found dead. The NHPs identified by ID PR 17-05, PR 17-15, PR 17–22, PR 17–23, PR 17–27 were analyzed and tested positive for ZIKV in one or more tissue samples and are represented by a red triangle. The black triangles represent the NHPs collected but not tested. (**C**) Satellite image of the Vila Toninho neighborhood. The boundary of the neighborhood is marked in white. Vegetation cover area can be seen in green surrounding the neighborhood. (**D**) Overlap of the area of the animals and mosquitoes collection. The ZIKV-positive PR 17–27 is overlapping with a ZIKV-positive *Ae. aegypti* mosquito pool. (**E**) Overlap of the areas of animas and mosquito collections with the presence of the DENV-positive *Ae. aegypti* mosquitoes (Vila Toninho satellite image by Google Earth Pro 7.3.1.4507 (64-bit) software. URL https://www.google.com/maps/@−20.84677,−49.34063,5682 m/data=!3m1!1e3). Map data: Google, 2018 DigitalGlobe.

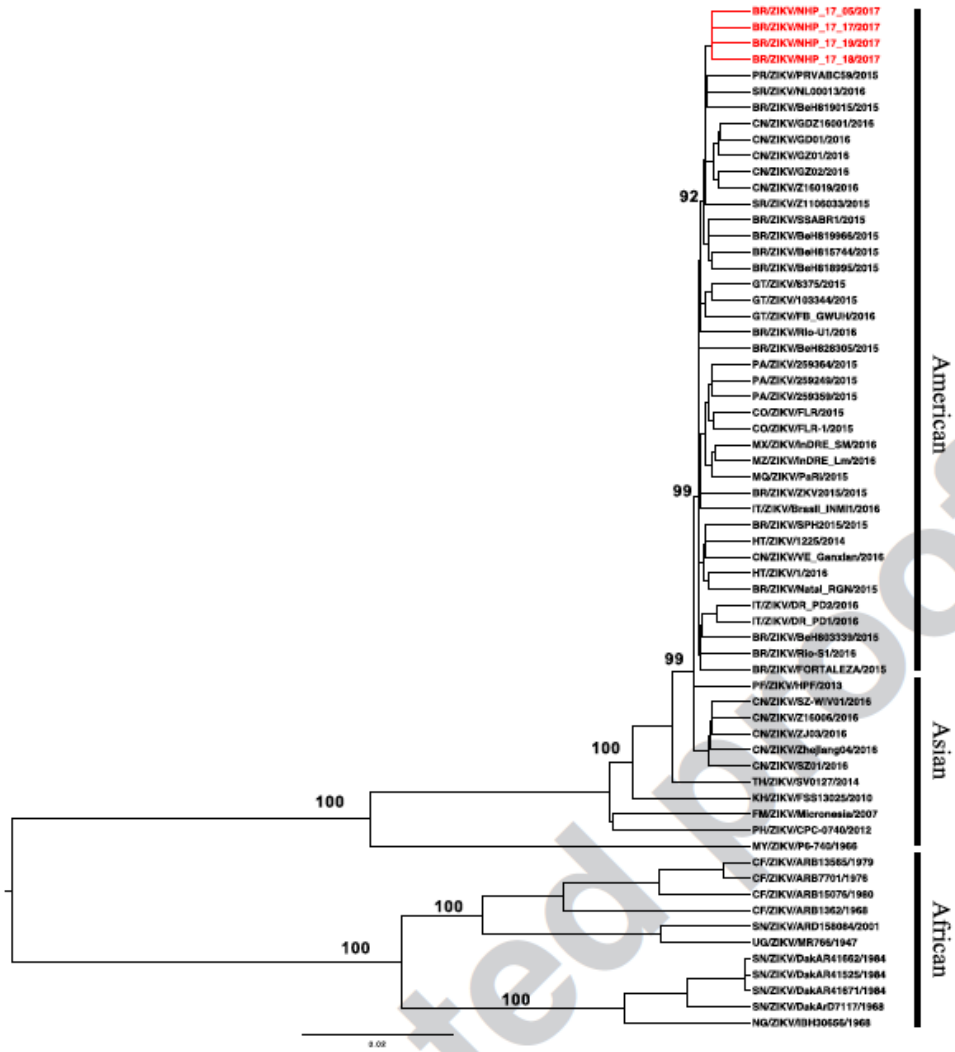



**Figure 2.** Molecular Phylogenetic analysis of Zika virus by the Maximum Likelihood method. The four strains obtained from NHPs (marmosets) are highlighted in red. Bootstrap values above 90% are shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.7699)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 52.6922% sites). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 10269 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[64].

| Days | ZIKV RNA genome copies/ml | | | |
|---|---|---|---|---|
| | NHP 1 | NHP 2 | NHP 3 | NHP 4 |
| **-1** | Negative | Negative | Negative | Negative |
| **0** | Negative | Negative | Negative | Negative |
| **2** | 200 | Negative | 1076 | 204 |
| **3** | 1150 | 214 | 238 | 2191 |
| **4** | 2848 | 626 | 786 | 3296 |
| **5** | 19958 | 1762 | 30200 | 4156 |
| **8** | Negative | 217 | Negative | Negative |
| **9** | 580 | 229 | Negative | 9840 |
| **12** | 2208 | Negative | Negative | Negative |
| **15** | Negative | Negative | Negative | Negative |
| **19** | Negative | 347 | Negative | Negative |

**Figure 3.** Viremia measurement in experimentally ZIKV-infected *Callithrix penicilata* collected from day −1 until 19 dpi. One-step qRT-PCR was used to measure semi quantitatively the ZIKV RNA loads in the serum of four animals at indicated days p.i. and represented as viral RNA copies per mL of sample standard curve. The curve was obtained from a standard sample with known titer after serial dilutions ($5 \times 10^1$ to $5 \times 10^6$ copies/mL) on the plasma of the non-infected marmosets.Values are expressed by RNA genome copies per mL for all the infected marmosets. Viremia was detected in the serum of marmosets 1, 3 and 4 on day 2 p.i. and in all infected marmosets on day 3 p.i. The figure shows that viremia increased on day 5 p.i. when compared to other evaluated days for all the infected marmosets. p.i.: post infection. NHP: non-human primates. Day −1: day before the infection.

# Conclusions - II

- Zika was detected in dead NHP in urban environment
- The sequence is similar to the human cases
- The spatial distribution was assessed
- The virus can infect experimental monkeys, with viremia and IgG seroconversion

# Thanks to ALL Collaborators

UTMB
Nikos Vasilakis
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Eduardo Massad
Luis Carlos Pereira
Edison Durigon
Dani Durigon

SJ Rio Preto Public Health
Izalco Nunes
Andreia Negri

UNESP
Joao Pessoa Araujo
Paula Rahal

FMT-Manaus
Marcus Lacerda
Maria Paula Mourao

# LPV Team


Maurício, MD, PhD

## Manager

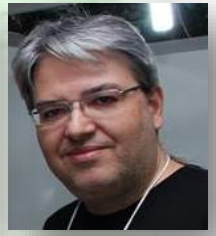
Gislaine

## PostDocs

Ana Carolina, DVM, PhD

Tatiana, PhD

Geórgia, PhD



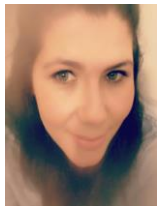
Mânlio, PhD

## Master's student

Rafael

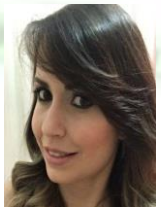
## PhD students

Maísa



Milene

Nathália



Tauyne

Cássia, MD

Karina

## Future Scientists