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 Butantã-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, Aedes aegypti, which breeds in and around houses in man-made containers, is responsible for interhuman transmission of "urban" yellow fever virus.

YF: impossible to eradicate
**Hosts**

*Alouatta sp*  
(guariba, bugio)

*Callithrix sp*  
(mico, soim)

*Cebus sp*  
(macaco prego)
Brazil: non-human primates (NHP): hosts for YFV

*Callicebus* spp. (*widow monkey*)

*Sapajus* spp. (*tufted capuchins*)

*Callithrix* spp. (*marmosets*)

*Allouata* spp. (*howler monkeys*)

**Genera: less susceptible to YFV**

**Genera: more susceptible to YFV**

Susceptibility and wide occurrence: NHP - sentinels for YF

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

Figure 5. Yellow fever patient during the period of infection. The patient is febrile and acutely ill, with prominent conjunctival congestion. During the 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.
CURRENT SITUATION OF YFV
YELLOW FEVER VIRUS TRANSMISSION RISK
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%)

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%)

Kenya
2 confirmed cases

China
11 confirmed cases

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

1980 to 2015: 1,575 cases

+ 5,000 deaths
NHP

MS-BR, 2017, 2018
YF: Brazil
Minas Gerais state

2017-2018: 1,003 cases
339 deaths (33.9%)

2018
528 cases
– 177 deaths

2017
475 cases
162 deaths

2016 – vaccination coverage

≤ 50%
51-94%
>95%
A new lineage of YFV (South American GI) was responsible for outbreaks in Southeast - 2017

9 unique aa substitutions in the polyprotein sequence

Most recent common ancestor: 
July 2016 
[95% BCI: Mar - Nov 2016]
Persistence of Yellow fever virus outside the Amazon Basin, causing epidemics in Southeast Brazil, from 2016 to 2018

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

MRCA: 2015 (95%BCI = 2014-2016)

Southeast Brazil


South African

South America II

Rezende & Sacchetto et al, 2018

Beast1.8.3
YF São Paulo 2016
1.641 feições
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
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

classify as 17DD strain

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

30/06/1927
ASIBI virus, Mahaffi & Bauer
54 passages in rhesus monkeys

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)
VACCINE PRODUCTION PROCESS (2017).
VACCINE PRODUCTION PROCESS (2018-).
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 lifelong 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
DURATION OF IMMUNITY

Anti-17DD YF Neutralizing Antibody Titers

PRNT (Log_{10}IU/mL)

NV(day0)  PV(day30-45)  PV(year1-4)  PV(year5-9)  PV(year10-11)  PV(year12-13)
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
Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak — Preliminary Report


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.)
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 (97% [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.
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.
## SERIOUS ADVERSE EVENTS OF YF VACCINE

<table>
<thead>
<tr>
<th>Case</th>
<th>Place, year</th>
<th>Age</th>
<th>Sex</th>
<th>Time after vaccin</th>
<th>Clinical and laboratory summary</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Goiânia 1999</td>
<td>5</td>
<td>F</td>
<td>3</td>
<td>Fever, dyspnea, hyperemia of oropharynx. AST and ALT: 4 x. Bilirubin 1,1 mg. Leukocytosis, anemia. R-X: Diffuse interstitial infiltration at left.</td>
<td>Death 5th day</td>
</tr>
<tr>
<td>2</td>
<td>São Paulo 1999</td>
<td>11</td>
<td>M</td>
<td>3</td>
<td>Fever, malaise, diarrhea, jaundice, petechiae and epistaxis. AST: 162; ALT: 150; Total Bilirubin 13,5; Cr 3,2.</td>
<td>Death 5th day</td>
</tr>
<tr>
<td>3</td>
<td>São Paulo 2000</td>
<td>22</td>
<td>F</td>
<td>4</td>
<td>Fever, myalgia, hyperemia of oropharynx. Jaundice. AST and ALT: 10,5 x. Leukopenia; thrombocytopenia and increase of urea and creatinine.</td>
<td>Death 6th day</td>
</tr>
<tr>
<td>4</td>
<td>Minas Gerais, 1999</td>
<td>12</td>
<td>F</td>
<td>3</td>
<td>Fever, asthenia, myalgia, cephalea. Oral hyperemia, jaundice, hypotension and bleeding. Leukopenia; thrombocytopenia and increase of urea and creatinine.</td>
<td>Recovered 9th day</td>
</tr>
<tr>
<td>5</td>
<td>Minas Gerais, 2001</td>
<td>19</td>
<td>F</td>
<td>3</td>
<td>Fever, myalgia, cephalea. AST and ALT 12 e 6 x. Bilirubin 6,0. Leukopenia with left shift. Coagulation disorder.</td>
<td>Death 10th day</td>
</tr>
<tr>
<td>6</td>
<td>Rio Grande do Sul, 2001</td>
<td>4</td>
<td>M</td>
<td>4</td>
<td>Fever, prostration, petechiae. Lymphadenopathy. AST and ALT 20 x; Bilirubin 7,01. Leukopenia with left shift. Renal failure.</td>
<td>Death 10th day</td>
</tr>
<tr>
<td>7</td>
<td>Rio de Janeiro, 2003</td>
<td>67</td>
<td>M</td>
<td>4</td>
<td>Fever, asthenia, myalgia, cephalea and prostration. AST: 2572; TGP: 2525. Leukopenia. Respiratory failure. Yellow fever neutralizing antibodies: 3533 mUI/mL (10 days after).</td>
<td>Recovered 48th day</td>
</tr>
</tbody>
</table>
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

- Bio-Manguinhos/Fiocruz
- Xcellerex
- Aggeu Magalhães/Fiocruz
- Bio-Manguinhos/Fiocruz Fraunhofer/iBio

VFA inativada
VFA DNA
VFA subunit
VFA atenuada

1937
2008 2009 2011

maturidade da tecnologia
tempo
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, 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
AN INACTIVATE VACCINE AGAINST YF

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.
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 DHALIA¹, MILTON MACIEL Jr.², FÁBIA S.P. CRUZ¹, ISABELLE F.T. VIANA¹, MARIANA L. PALMA¹, THOMAS AUGUST² and ERNESTO T.A. MARQUES Jr.¹,²,³

¹Fundação 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
²Johns Hopkins University, School of Medicine, Department of Pharmacology and Molecular Sciences 725 North Wolfe Street, Biophysics Building, Baltimore, Maryland 21205, USA
³Johns 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
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
Evidence of natural Zika virus infection in neotropical non-human primates in Brazil

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>NHPs species</th>
<th>Organ(s) (Ct value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KIDNEY</td>
</tr>
<tr>
<td>PR 17/02</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>PR 17/03</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>PR 17/04</td>
<td>Callithrix sp.</td>
<td>37.84</td>
</tr>
<tr>
<td>PR 17/05</td>
<td>Callithrix sp.</td>
<td>38.19</td>
</tr>
<tr>
<td>PR 17/06</td>
<td>Callithrix sp.</td>
<td>37.96</td>
</tr>
<tr>
<td>PR 17/07</td>
<td>Callithrix sp.</td>
<td>38.18</td>
</tr>
<tr>
<td>PR 17/08</td>
<td>Cebus sp.</td>
<td>35.98</td>
</tr>
<tr>
<td>PR 17/11</td>
<td>Callithrix sp.</td>
<td>Neg</td>
</tr>
<tr>
<td>PR 17/12</td>
<td>Callithrix sp.</td>
<td>35.66</td>
</tr>
<tr>
<td>PR 17/13</td>
<td>Callithrix sp.</td>
<td>35.57</td>
</tr>
<tr>
<td>PR 17/14</td>
<td>Callithrix sp.</td>
<td>Neg</td>
</tr>
<tr>
<td>PR 17/15</td>
<td>Callithrix sp.</td>
<td>Neg</td>
</tr>
<tr>
<td>PR 17/16</td>
<td>Callithrix sp.</td>
<td>30.87</td>
</tr>
<tr>
<td>PR 17/17</td>
<td>Callithrix sp.</td>
<td>31.66</td>
</tr>
<tr>
<td>PR 17/18</td>
<td>Callithrix sp.</td>
<td>29.17</td>
</tr>
<tr>
<td>PR 17/19</td>
<td>Callithrix sp.</td>
<td>32.65</td>
</tr>
<tr>
<td>PR 17/20</td>
<td>Callithrix sp.</td>
<td>34.68</td>
</tr>
<tr>
<td>PR 17/21</td>
<td>Callithrix sp.</td>
<td>32.89</td>
</tr>
<tr>
<td>PR 17/22</td>
<td>Callithrix sp.</td>
<td>29.11</td>
</tr>
<tr>
<td>PR 17/23</td>
<td>Callithrix sp.</td>
<td>Neg</td>
</tr>
<tr>
<td>PR 17/25</td>
<td>Callithrix sp.</td>
<td>37.79</td>
</tr>
<tr>
<td>PR 17/26</td>
<td>Callithrix sp.</td>
<td>37.49</td>
</tr>
<tr>
<td>PR 17/27</td>
<td>Callithrix sp.</td>
<td>38.47</td>
</tr>
<tr>
<td>MG 17/01</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>MG 17/02</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>MG 17/15</td>
<td>Callithrix sp.</td>
<td>27.1</td>
</tr>
<tr>
<td>MG 17/16</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>MG 17/30</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>MG 17/31</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>MG 17/32</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>MG 17/45</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
<tr>
<td>MG 17/51</td>
<td>Callithrix sp.</td>
<td>n/a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mosquito species</th>
<th>Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/151</td>
<td><em>Ae. aegypti</em></td>
<td>35.17</td>
</tr>
<tr>
<td>17/160</td>
<td><em>Ae. aegypti</em></td>
<td>36.89</td>
</tr>
<tr>
<td>17/161</td>
<td><em>Ae. aegypti</em></td>
<td>36.48</td>
</tr>
<tr>
<td>17/163</td>
<td><em>Ae. aegypti</em></td>
<td>31.87</td>
</tr>
<tr>
<td>17/164</td>
<td><em>Ae. aegypti</em></td>
<td>36.77</td>
</tr>
<tr>
<td>17/169</td>
<td><em>Ae. aegypti</em></td>
<td>22.23</td>
</tr>
</tbody>
</table>

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 animals 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,–49.34063,5682m/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.
<table>
<thead>
<tr>
<th>Days</th>
<th>ZIKV RNA genome copies/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NHP 1</td>
</tr>
<tr>
<td>-1</td>
<td>Negative</td>
</tr>
<tr>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>1150</td>
</tr>
<tr>
<td>4</td>
<td>2848</td>
</tr>
<tr>
<td>5</td>
<td>19958</td>
</tr>
<tr>
<td>8</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>580</td>
</tr>
<tr>
<td>12</td>
<td>2208</td>
</tr>
<tr>
<td>15</td>
<td>Negative</td>
</tr>
<tr>
<td>19</td>
<td>Negative</td>
</tr>
</tbody>
</table>

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 x 10^1 to 5 x 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
Sasha Azar
Scott Weaver

UFMG
Betania Drumond
Mauro Teixeira

Fiocruz
Patricia Brasil

USP
Franscisco Chiaravalotti
Neto
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