17DD Yellow Fever Vaccine: na updatee

New Cells, New Vaccines VII: Protein to Product
17-20 March 2013, Wilmington, DE

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Ministry of Health
OUTLINE

• Yellow Fever disease
  – World situation
  – YF in Brazil

• Technological innovation in YFV vaccine
  – 17DD vaccine improvements
  – Future vaccines
200,000 cases of yellow fever annually with 30,000 deaths

Risk areas of yellow fever in Brazil and immunization recommendation, 1997 to 2008

Source: SVS/MS.
Cases of Yellow Fever in Brazil, 1990-2010

Total: 587 cases; 41.8% death
Yellow Fever is not an eradicable disease !!!

- Last Urban YF case in Sena Madureira, 1942
- Existance of Silvester YF cycle
  - 1898 – Adolfo Lutz, Emilio Ribas in SP state
  - 1930 – Canan valley in ES state – F. Soper, Arnaldo de Andrade, Américo de Oliveira
- Monkeys: Cebus (prego); Alouatta (guariba); Aleles (aranha); Callitrix (sagui)
- Mosquitos: Genus Haemagogus - H. janthinopmys > important in virus maintenance; H. albomaculatus > fly authonomy
  - Genus Sabethes – Latin American countries

- Vector Aedes albopictus – its role not well established

✓ The Silvester YF will remain forever, therefore we must develop a better vaccine which does protect for life and does not cause Serious Adverse Events
✓ The infestation by the vector A. aegypti in the majority of urban regions is a threat for re-introduction of YF virus in urban areas.
Transmission Cycles: primates reservoir and vectors

Fig. 5. Yellow fever transmission cycles. The yellow fever virus is transmitted between human and nonhuman primate hosts by mosquitoes in 3 cycles: the sylvatic (jungle) cycle in which mosquitoes of the forest canopy transmit virus to monkeys and secondarily to humans entering the jungle; the intermediate cycle (or zone of emergence) in which virus enters rural towns and villages bordering jungle areas; and the urban cycle in which humans serve as the viremic host and virus is transmitted from human to human by the domesticated *Aedes aegypti* mosquito.
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

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.
Figure 36–15 Derivation of early vaccine lots from the original 17D virus, prior to the institution of a seed lot system (see text). (From Fox and Penna\textsuperscript{491} and Brès P, Koch M,\textsuperscript{482} with permission.)

Monath, T. In Vaccine, Plotkin S. Orenstein W & Offit Paul, 2008
Yellow Fever Vaccine in Brazil

- 1937 March - H. Smith brought 17D strain and started the vaccine production in Brazil.

✓ One year later with more than 60,000 people vaccinated Smith, Penna e Paoliello, concluded that “exists a safe and possible method for large scale immunization against Yellow Fever”.

- Bica, Alfredo, Anais Simpósio 50 aniversário da introdução da cepa 17 D no Brasil, 1988

Vaccination at Fazenda Pedra Preta, on Três Pontas, MG, August 15, 1937
Technological innovation in YFV: 17DD attenuated vaccine
Technological innovation in YFV: 17DD attenuated vaccine

1937, start of vaccination in Brazil
Technological innovation in YFV: 17DD attenuated vaccine

1961, Dr. Alberto Nicolau, change of inoculation route of embryonated eggs (vitellin cavity instead amniotic cavity) reducing mortality to 8% of embryos

1980, Dr. Oscar Sebastião de Souza Lopez, facility upgrade and standardization of methodology to estimate vaccine potency using Vero cell

1982, use of Specific Pathogen-Free (SPF) eggs

1983, facility upgrade
Technological innovation in YFV: 17DD attenuated vaccine

1999, New Vaccine Processing Plant
- New vaccine presentation: 5 dose vial
- 2 new industrial freeze dryers increased the capacity of production > 32 million doses in 5 doses presentation/year

1984, Dr. Oscar Sebastião de Souza Lopez, thermal stabilizer was incorporated for the first time into the vaccine, significantly improving their quality
Technological innovation in YFV: 17DD attenuated vaccine

- **2006**: New vaccine presentation
- **2009**: Dose response clinical study in adults
- **2001**: WHO pre-qualification

Time line:
- 1937
- 1944
- 1961
- 1980
- 1982
- 1983
- 1984
- 1999
- 2000
- 2001
- 2006
- 2009
- 2011
## Dose Response Study

<table>
<thead>
<tr>
<th>Group in IU (PFU)/dose</th>
<th>Number</th>
<th>SC %</th>
<th>PRNT&lt;sub&gt;50&lt;/sub&gt; GMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 (246)</td>
<td>118</td>
<td>66.9</td>
<td>1970.2</td>
</tr>
<tr>
<td>158 (740)</td>
<td>122</td>
<td>88.5</td>
<td>6837.3</td>
</tr>
<tr>
<td>587 (2,200)</td>
<td>131</td>
<td>96.9</td>
<td>12,145</td>
</tr>
<tr>
<td>3013 (6,600)</td>
<td>131</td>
<td>97.7</td>
<td>13,479.2</td>
</tr>
<tr>
<td>10,447 (20,000)</td>
<td>115</td>
<td>99.1</td>
<td>12,190.6</td>
</tr>
<tr>
<td>27,476 (60,000)</td>
<td>131</td>
<td>97.7</td>
<td>13,479.2</td>
</tr>
</tbody>
</table>

SC - % of seroconversion
GMT – geometric mean titer

1,000 LD<sub>50</sub> - dose recommended by WHO 11,607.5
Production and use of 17DD vaccine in Brazil

• From 1937 to 1986 – 154,383,814 persons vaccinated

• From 1987 to 2010 – 500,000,000 doses supplied to the National Immunization Program
  • Derem/Bio-Manguinhos, 2011
YF vaccine – Exportation after WHO pre-qualification in 2001

Total: 72 countries

✓ 2001-2010 > 115 milhões doses exported

Source: Derem/Bio-Manguinhos, 2011
International data

✓ Serious Adverse Events are rare: viscerotropic events (0.3/100,000 doses), neurological events (0.4/100,000 doses) and anaphylaxis (0.8/100,000 doses)

Source: Hayes EB, 2007
<table>
<thead>
<tr>
<th>Case</th>
<th>Place, year</th>
<th>Age, years</th>
<th>Sex</th>
<th>Time after vaccination days</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>4</td>
<td>Minas Gerais, 1999</td>
<td>12</td>
<td>F</td>
<td>3</td>
<td>Fever, asthenia, myalgia. 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, cepahlea. 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, cepahlea and prostration. AST: 2572; TGP: 2525. Leukopenia. Respiratory failure. Yellow fever neutralizing antibodies: 3533 mUI/mL (10 days after vaccination); 43875 mUI/mL (23 days after vaccination).</td>
<td>Recovered 48th day</td>
</tr>
</tbody>
</table>
### Eventos Adversos Graves associados à VFA

<table>
<thead>
<tr>
<th>Ano</th>
<th>Número de EAPV Graves</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DVA-VFA*</td>
<td>DNA-VFA**</td>
</tr>
<tr>
<td>2007</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2008</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>2009</td>
<td>11</td>
<td>79</td>
</tr>
<tr>
<td>2010</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>2011</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2012</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>116</td>
</tr>
</tbody>
</table>

Fonte: SIEAPV/SIPNI/CGPN/DEVEP/SVS/MS.
* Doença Viscerotrópica associada à vacina febre amarela (DVA-VFA)
** Doença Neurológica associada à vacina febre amarela (DNA-VFA)
Technological innovation in YFV: trends

- 2008 -- Bio-Manguinhos/Fiocruz
  YFV inactivated

- 2010 -- Xcellerex/GE
  YFV inactivated

- 2009 -- Aggeu Magalhães/Fiocruz
  YFV DNA

- 2011 ---Bio-Manguinhos/Fiocruz
  Fraunhofer/iBio
  YFV subunit
Upstream/Dowstream Development
Flow of current steps in the project of inactivated vaccine for yellow fever

Virus production in bioreactor

Clarification

Chromatographic step

Viral inactivation
B-propiolactone

Hold step (-70C)

Process control
- Virus titration;
- Total protein;
- HCP;
- Residual DNA;
- Endotoxin.

Preclinical studies

Mice;
Nonhuman primates

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

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Yellow Fever DNA Vaccine
FhCMB’s approach to target protein production in plants is based on three key components:

- **Launch vectors for target expression**: flexible tool for easy target engineering, delivery into biomass and expression.

- **Vacuum infiltration for delivery of launch vectors into plant biomass**: scalable, easy to control methodology that delivers vector to nearly 100% of biomass, therefore, eliminating the dependence on pathogenicity of plant virus.

- **Hydroponic system for contained plant biomass generation**: controlled growth conditions that eliminate the need for soil.
The design of immunogenicity studies and the results obtained are showed in the poster presentations, as follows:

1- Immunization with recombinant, plant produced Yellow Fever virus envelop (E) Protein Vaccine Candidate in Rhesus macaques. Guimarães, RC et al.

2- Kinects of antibody response induced by recombinant E Protein of Yellow Fever virus. Shoji, Y. et al.
Obrigado!
Thank you!

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