Bovine Brucellosis in Portugal: test and slaughter along with RB51

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PT Bovine Brucellosis Eradication Programmes Measures

Sanitary

- Serological surveys
- Sanitary Slaughter of Sero+ animals & bacteriological examination
- Herd quarantine
- Control of Animal Movement

Medical

- Live vaccination with RB51
- Electronic Id

Abortion
- Compulsory notification of abortion
- Sample collection for bacteriology
  - abortion products
  - vaginal swabs
Control and Eradication Programme

- Test and Slaughter Policy
- Annual serosurveillance
  - RBT & CFT – Series / Parallel
  - MRT
- Abortion notification
- Bacteriology of abortions & Sero+ animals
  - Isolation
  - Tipification
Control and Eradication Programme

- Animal Movement
  - Live – restriction
  - Slaughter

- Awareness campaigns
  - Producers and Staff
  - Human Doctors
Vaccines for BB

- long-term protection
- reduce clinical disease & transmission
- do not prevent seroconversion after exposure
- vaccination alone does not eradicate BB
- risks for humans and pregnant animals
**Brucella abortus Strain 19**

LPS = Lipopolysaccharide
O = O-side chain
CF = Complement fixation
RB = Rose Bengal

**Brucella abortus Strain RB-51**

LPS = Lipopolysaccharide
O = O-side chain
CF = Complement fixation
RB = Rose Bengal
Lipopolysaccharide structure of virulent and vaccine strains

\[ \text{Brucella abortus} \quad \text{field strains} \]
\[ \text{Lipid A} \quad \text{KDO} \quad \text{Sugars} \quad \text{O-side chain} \]

\[ \text{Brucella abortus} \quad \text{strain 19} \]
\[ \text{Lipid A} \quad \text{KDO} \quad \text{Sugars} \quad \text{O-side chain} \]

\[ \text{Brucella abortus} \quad \text{strain RB51} \]
\[ \text{Lipid A} \quad \text{KDO} \quad \text{Sugars} \]

The O-side chain is the immunodominant antigen of \textit{Brucella} for antibody responses.
Eradication programs based on S-19

Age (in months)  4  8  12  16  20

Vaccination  | Vaccination  | No Vaccination
Serological Test  | No Serological Test  | Serological Test

Uncontrolled

Adults can not be vaccinated due to interference

Eradication programs based on RB-51®

Age (in months)  4  8  12  16  20

Vaccination  | Vaccination
Serological Test  | Serological Test

Adults can be vaccinated
Special Vaccination Programmes (RB51) Justification

- High Prevalence of BB
- Increase of Incidence of BB
- Risk Factors
  - Transumance
    - Azores Islands
    - Trás-os-Montes – Montalegre
  - High Animal Density
    - Alentejo–SAPJU
  - Depopulation ineffectively
    - Alentejo – SAPJU
- Limited supply of S19
- Socio-economic
  - Subsistence
  - Genetic Poll
Special Vaccination Programmes
Vaccination Protocol - RB51

– Epidemiological Units

– Breeding Herds

– RB51 – (CZV)
  – S/C – $10^{-34} \times 10^9$ CFU/dose (2 ml)

– Female animals
  Young (+ 4 months)
  adults

– Epidemiological follow-up
  Revaccination
Animal Identification
Ear tags
Electronic
Passaport & National Database
Serological survey
Vaccination date
Epidemiological follow-up
Eradication of bovine brucellosis in the Azores, Portugal—Outcome of a 5-year programme (2002–2007) based on test-and-slaughter and RB51 vaccination

H. Martins\textsuperscript{a}, B. Garin-Bastuji\textsuperscript{b}, F. Lima\textsuperscript{c}, L. Flor\textsuperscript{d}, A. Pina Fonseca\textsuperscript{e}, F. Boinas\textsuperscript{f,*}

Response to Letter to the Editor

H. Martins\textsuperscript{a}, B. Garin-Bastuji\textsuperscript{b}, F. Lima\textsuperscript{c}, L. Flor\textsuperscript{d}, A. Pina Fonseca\textsuperscript{e}, F. Boinas\textsuperscript{f,*}
Bovine Brucellosis in Azores

- 1947–1st Brucella diagnosis in Azores
- 1950–Eradication Programmes
- 2000 –
  ◦ Eradication
    • Corvo, Flores, Graciosa e Pico
  ◦ Low Prevalence
    • Faial & Sta Maria
  ◦ Infection
    • S.Miguel, Terceira, S. Jorge
Bovine Brucellosis in Azores Problems in the Control

- Climate
- Large Bovine Population
  - 250,000 Bovine / 12,000 Herds
    - 20.8 animals/ herd
  - S. Miguel, Terceira and S. Jorge – 80% of bovine
- Extensive Production system
  - 1.3 animal/ ha
- Large number of parcels
  - Avge farm size: 5.3ha
  - Avge 6.3 parcels /farm
Bovine Brucellosis in Azores Problems in the Control

- Animal movement between pastures
  - Communal paths
  - Common watering points
Mass Vaccination of 3 islands
- Terceira
- S.Jorge
- S.Miguel

Pilot study
- 2001

Programme
- 2002–2007
Bulk Tank Milk Ring Test in Terceira
Terceira, September 2002
Terceira, June 2005
Terceira, October 2007

![Map of Terceira Island](image)

- **Parcels with brucellosis free-herds**
ORIGINA L AR TICLE

Control of Bovine Brucellosis from Persistently Infected Holdings Using RB51 Vaccination with Test-and-Slaughter: A Comparative Case Report from a High Incidence Area in Portugal

M. C. Caetano¹, F. Afonso¹, R. Ribeiro², A. P. Fonseca¹, D. A. Abernethy³ and F. Boinas²
Programme
- Beginning: 7–2004
- Duration: 5 Years

Nº of Vaccinated Herds: 10

Nº of Breeding Animals:
- Existing: 4277
  - heifers: 1485
  - cows: 2792
Regulatory actions, seroprevalence and birth rate in ten Brucella-infected herds in the Alentejo before and after initiation of RB51 vaccination in 2004.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Positive</th>
<th>Depopulated</th>
<th>Restocking</th>
<th>Purchase</th>
<th>Vaccinated</th>
<th>Prev. (%)</th>
<th>Birth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26.4</td>
<td>NA</td>
</tr>
<tr>
<td>2000</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18.1</td>
<td>NA</td>
</tr>
<tr>
<td>2001</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>7.6</td>
<td>NA</td>
</tr>
<tr>
<td>2002</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4.3</td>
<td>NA</td>
</tr>
<tr>
<td>2003</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>12.6</td>
<td>NA</td>
</tr>
<tr>
<td>2004</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3370</td>
<td>19</td>
<td>60.1</td>
</tr>
<tr>
<td>2005</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>846</td>
<td>5.7</td>
<td>44.3</td>
</tr>
<tr>
<td>2006</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>709</td>
<td>0.5</td>
<td>66.0</td>
</tr>
<tr>
<td>2007</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>755</td>
<td>0.5</td>
<td>68.6</td>
</tr>
<tr>
<td>2008</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>331</td>
<td>0.2</td>
<td>71.9</td>
</tr>
<tr>
<td>2009</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>73.5</td>
</tr>
</tbody>
</table>
Follow-up of the ten Brucella–infected herds in the Alentejo region not subjected to RB51 vaccination.

<table>
<thead>
<tr>
<th>ID</th>
<th>1st intervention with positives</th>
<th>Depopulation (year)</th>
<th>Repopulation (year)</th>
<th>Year of first seropositivity after repopulation</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2003</td>
<td>2004</td>
<td>No</td>
<td></td>
<td>Ceased activity</td>
</tr>
<tr>
<td>6</td>
<td>2004</td>
<td>2005</td>
<td>No</td>
<td></td>
<td>Ceased activity</td>
</tr>
<tr>
<td>8</td>
<td>2005</td>
<td>2006</td>
<td>Yes (2007)</td>
<td>2009</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>2006</td>
<td>2007</td>
<td>No</td>
<td></td>
<td>Ceased activity</td>
</tr>
<tr>
<td>10</td>
<td>2007</td>
<td>2008</td>
<td>No</td>
<td></td>
<td>Ceased activity</td>
</tr>
</tbody>
</table>
Alentejo – other special vaccination programmes

Number of positive herds

Before vaccination

<table>
<thead>
<tr>
<th>1st Q</th>
<th>2nd Q</th>
<th>3rd Q</th>
<th>4th Q</th>
<th>5th Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

After vaccination

<table>
<thead>
<tr>
<th>1st Q</th>
<th>2nd Q</th>
<th>3rd Q</th>
<th>4th Q</th>
<th>5th Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Positive herds
Animal prevalence

Q – Quarter (4 months)
RB51 Vaccination & probability of abortion in 1 EU
Montalegre

Small holdings (villages), genetic pool (DOP), communal grazing
Trás-os-Montes (ToM)
S19 -> RB51 Vaccination Programme

- Period:
  - S19 – RB51
    - Duration: 7/2003 – 5 Years

- № of villages: 7/35

- № of herds: 127/1103

- № of animals: 1584/8640
ToM: Vaccination

Number of vaccinated herds

Number of vaccinated animals

B19
RB51

- Number of vaccinated herds
- Herd prevalence
- Number of vaccinated animals
- Animal prevalence
ToM: RB51 Vaccination

<table>
<thead>
<tr>
<th>Region</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Median value</th>
<th>Difference between regions (Wilcoxon rank sum test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd prevalence (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-value = 0.24</td>
</tr>
<tr>
<td>Montalegre</td>
<td>4.82</td>
<td>1.71</td>
<td>0.38</td>
<td>0.50</td>
<td>0.42</td>
<td>0.34</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Rest of DIV of Vila</td>
<td>0.58</td>
<td>1.21</td>
<td>0.86</td>
<td>1.12</td>
<td>4.54</td>
<td>2.16</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Real</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal prevalence (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-value = 0.04</td>
</tr>
<tr>
<td>Montalegre</td>
<td>1.53</td>
<td>0.35</td>
<td>0.05</td>
<td>0.08</td>
<td>0.05</td>
<td>0.05</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Rest of DIV of Vila</td>
<td>0.79</td>
<td>1.02</td>
<td>0.71</td>
<td>0.87</td>
<td>3.02</td>
<td>1.33</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Real</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Epidemiological evaluation of abortions in herds vaccinated with RB51

- Regional epidemiological evaluation
- Epidemiological questionnaire
- Risk factors
- Rate of notification of:
  - births
  - abortions
  - perinatal mortality
### Abortions with *B. abortus* RB51 strain isolation

<table>
<thead>
<tr>
<th>Age abortion (m.)</th>
<th>Time vaccination to abortion</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>months</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>90</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>93</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>93</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>109</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>113</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>123</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>125</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>132</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>139</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>147</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>152</td>
<td>5.1</td>
<td></td>
</tr>
</tbody>
</table>

Average: 7.09 months, 119.64 days, 3.99 months
RB51 vaccination & abortions

- Bacteriology of abortion products and vaginal swabs
  - 61 samples
  - Herds with BB free status: 11 samples with isolation of RB51
  - Rate of isolation: 18%

- Bovine – Critical Period:
  - Bibliography: 5 – 6 m. gestation
  - ToM: Median 7 m. – range 6-8m.

- Time from vaccination to abortion
  - Bibliography: 1.5 – 2 m.
  - ToM: Median 4.1 – range 3.0–5.1m
RB51 vaccination & abortions

Field Studies

- Rate of abortion after vaccination of pregnant cows:
  - Bibliography: 0–2%
  - ToM: 0.18% (75% of 8,073 adults vaccinated = 6,055; 11 RB51+)
- No abortions in 348 revaccinated females
Case studies: discussion & conclusions

- Implementation of mass vaccination with RB51
  - High vaccination coverage – Excellent results in 1–1.5 years
  - Low vaccination coverage – Delayed results

- Association with a Test and Slaughter Programme

- Continuous surveillance

- Minimal side-effects
  - No human cases
  - Reduced number of abortions
Case studies: discussion & conclusions

Complementary measures
- Improvement of the awareness
  - Veterinarians
  - Producers
- Investigation of abortions
  - Notification
  - Epidemiological
  - Laboratory
- Implementation of epidemiological units
  - Herds
  - Areas
Fig. 1. Prevalence and incidence in dairy herd with low brucellosis prevalence, post-vaccination with B. abortus RB51.

Fig. 3. Monthly prevalence and incidence in dairy herd with high brucellosis prevalence, post-vaccination with B. abortus RB51.
Table 1
Animal incidence (%), number of positive animals previously vaccinated with S19 and proportion of S19 vaccinated reactors from all positive animals in the period of 2004–2009 (bi-annual data) during an outbreak of bovine brucellosis (Brucella abortus) in the Extremadura region of West Spain.

<table>
<thead>
<tr>
<th>Year</th>
<th>Semester</th>
<th>Animal incidence (%)</th>
<th>Reactors vaccinated with S19</th>
<th>Proportion of S19 Vaccinated reactors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>First</td>
<td>7.77</td>
<td>45</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>6.56</td>
<td>19</td>
<td>0.72</td>
</tr>
<tr>
<td>2005</td>
<td>First</td>
<td>2.03</td>
<td>57</td>
<td>7.95</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>1.11</td>
<td>64</td>
<td>14.58</td>
</tr>
<tr>
<td>2006</td>
<td>First</td>
<td>0.23</td>
<td>28</td>
<td>34.15</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>0.27</td>
<td>40</td>
<td>35.40</td>
</tr>
<tr>
<td>2007</td>
<td>First</td>
<td>0.08</td>
<td>17</td>
<td>54.84</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>0.11</td>
<td>13</td>
<td>27.06</td>
</tr>
<tr>
<td>2008</td>
<td>First</td>
<td>0.02</td>
<td>5</td>
<td>33.33</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>0.01</td>
<td>2</td>
<td>22.22</td>
</tr>
<tr>
<td>2009</td>
<td>First</td>
<td>0.01</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\* Number of reactors that had been previously vaccinated with S19.
\[ Number of S19 vaccinated reactors divided by all positive animals detected every 6 months.\]
Table 2: Eradication measures implemented in each special incidence area (SIA) depending on the main control strategy applied

<table>
<thead>
<tr>
<th>General measures included in the national eradication program</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual identification of all animals and herds</td>
</tr>
<tr>
<td>Periodic testing of all animals &gt;12 months of age and removal of reactors</td>
</tr>
<tr>
<td>Movement restrictions in case of suspicious or positive* herds</td>
</tr>
<tr>
<td>Compulsory reporting and aetiological investigation of abortions</td>
</tr>
<tr>
<td>Epidemiological investigation in positive herds (trace-back and trace-forward of breakdowns)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measures implemented in SIAs</th>
<th>SIA I-III (stamping out)</th>
<th>SIA IV-V (vaccination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased routine testing (serology)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Segregation and rapid compulsory culling of positive reactors</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Total depopulation of herd</td>
<td>Yes</td>
<td>No†</td>
</tr>
<tr>
<td>Disinfection under official supervision and quarantine of facilities and pastures (90 days)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S19 vaccination of replacement heifers</td>
<td>No</td>
<td>Yes‡</td>
</tr>
<tr>
<td>Mass RB51 vaccination+annual revaccinations</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Herds in which one or more reactors were found
†Occasionally performed in municipalities not subjected to vaccination (surveillance municipalities)
‡Performed in the first 3-5 years of the study (see text)

Higher annual rate of decrease in SIA I–III (46.9%) than in SIA IV&V (14.9%)
Cost of depopulation vs vaccination

- Annual compensations cost per Sero+ animal / nº of Sero+ animals
  - Depopulation – 2475€ (95% CI: 1418–3532€)
  - Vaccination – 967€ (CI 572–1368€)

- Other costs
  - vaccine
  - loosing the OBF status
  - etc.

Saez et al., 2014
Thank you
Obrigado

Africander
bull
South Africa