

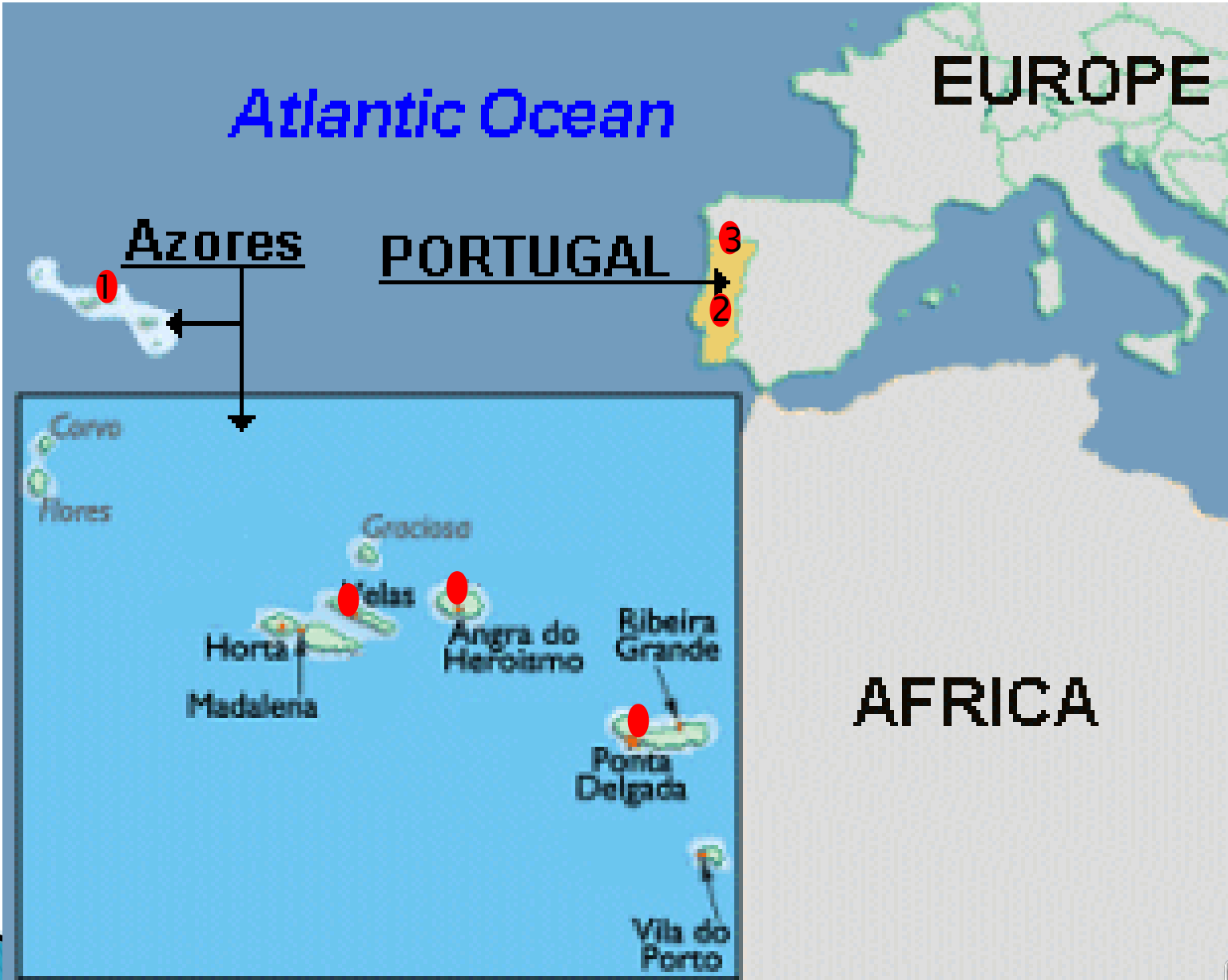


# Bovine Brucellosis in Portugal: test and slaughter along with RB51

Fernando Boinas  
*fboinas@fmv.ulisboa.pt*

Brucellosis Research Workshop  
13 – 15 May, Veterinary Faculty,  
University of Pretoria

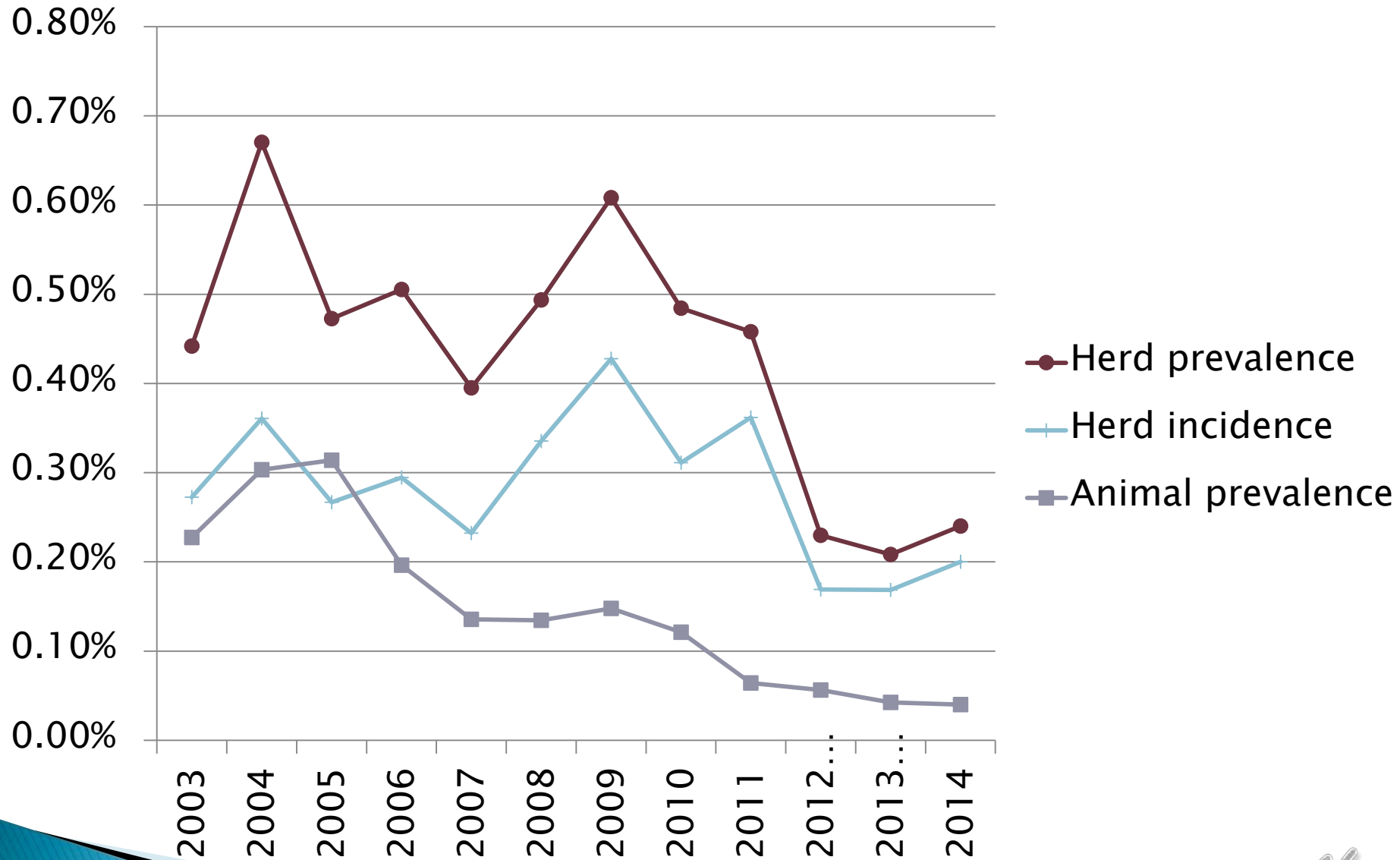




Case studies- 1,2,3



# Portugal - Bovine Brucellosis



# 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

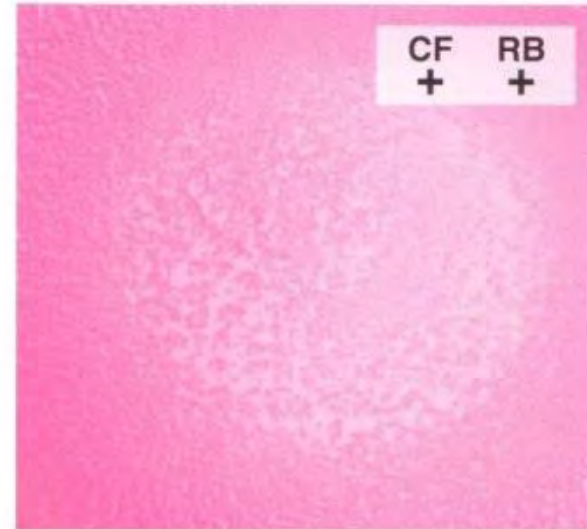
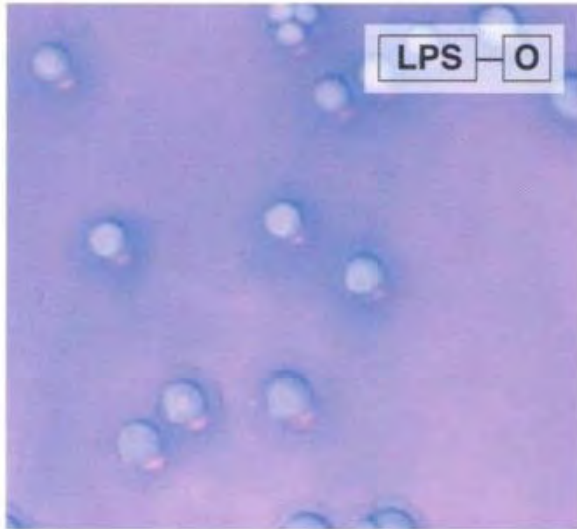


# Vaccines for BB

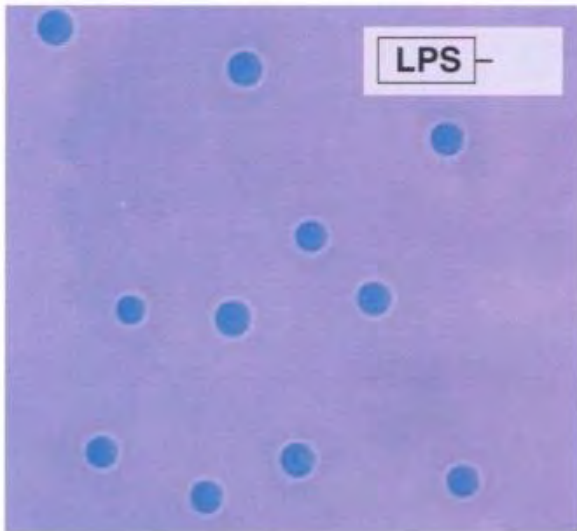
- ▶ long-term protection
- ▶ reduce clinical disease & transmission
- ▶ do not prevent seroconversion after exposure
- ▶ vaccination alone does not eradicate BB
- ▶ risks for humans and pregnant animals



*Brucella abortus* Strain 19



*Brucella abortus* Strain RB-51



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





# Lipopolysaccharide structure of virulent and vaccine strains

*Brucella abortus* field strains



*Brucella abortus* strain 19



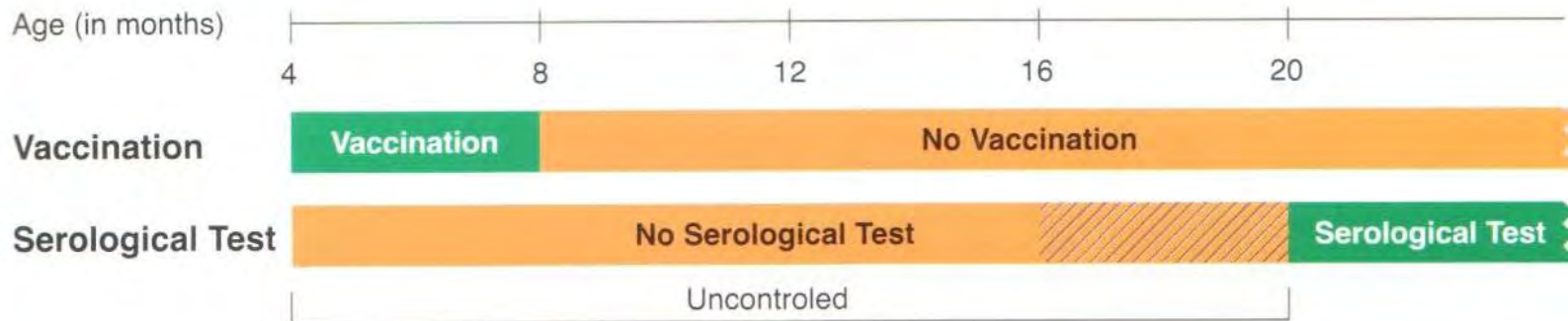
*Brucella abortus* strain RB51



The O-side chain is the immunodominant antigen of *Brucella* for antibody responses

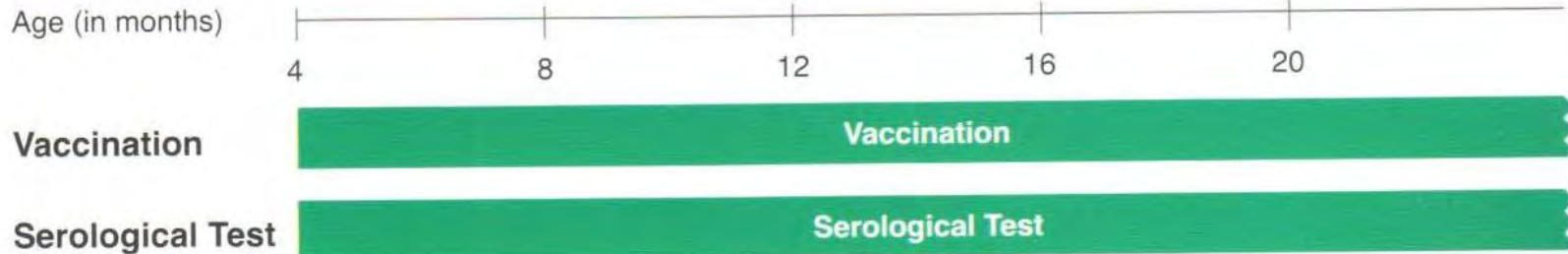


## Eradication programs based on S-19



Adults can not be vaccinated due to interference

## Eradication programs based on RB-51®



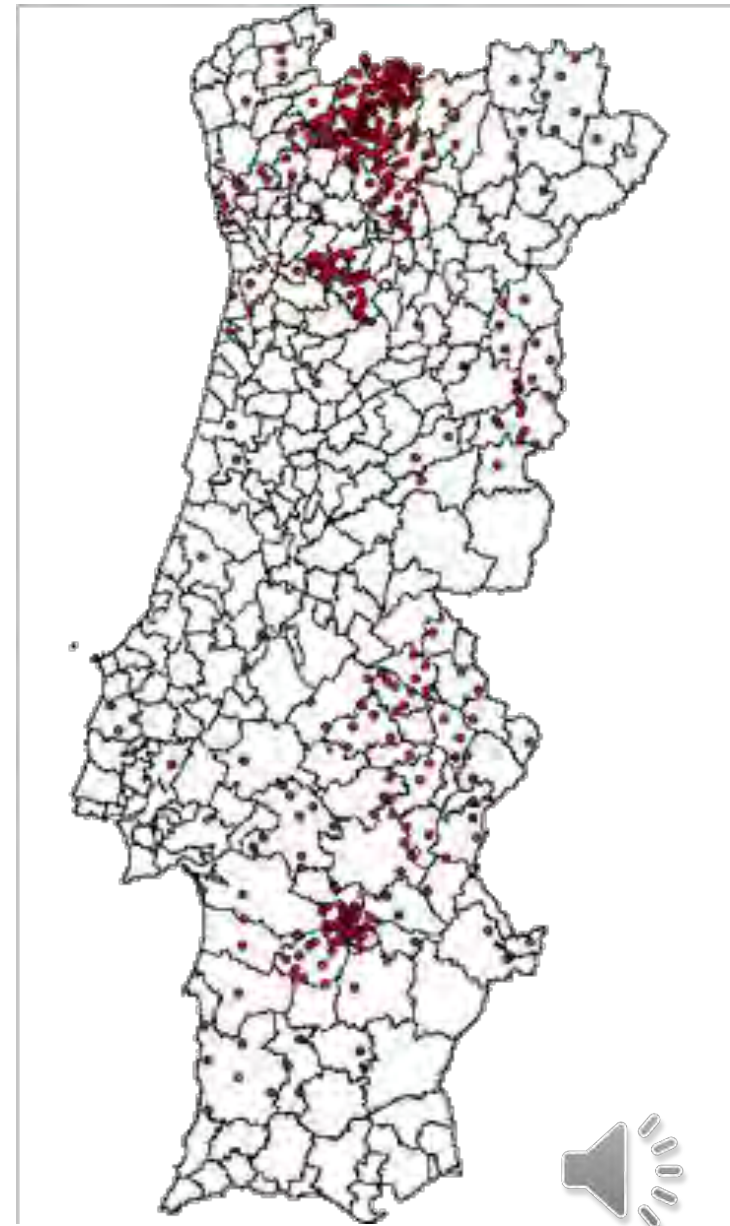
Adults can be vaccinated



# Special Vaccination Programmes (RB51)

## Justification

- ▶ High Prevalence of BB
- ▶ Increase of Incidence of BB
- ▶ Risk Factors
  - Transhumance
    - Azores Islands
    - Trás-os-Montes – Montalegre
  - High Animal Density
    - Alentejo–SAPJU
  - Depopulation ineffective
    - 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 -  $10-34 * 10^9$  CFU/dose (2 ml)
- Female animals
  - Young (+ 4 months)
  - adults
- Epidemiological follow-up
- Revaccination





**Animal Identification  
Ear tags**

**Electronic**

**Passaport & National  
Database**



9 12 2006





**Serological survey**  
**Vaccination date**  
**Epidemiological follow-up**





Contents lists available at ScienceDirect

## Preventive Veterinary Medicine

journal homepage: [www.elsevier.com/locate/prevetmed](http://www.elsevier.com/locate/prevetmed)



### 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<sup>a</sup>, B. Garin-Bastuji<sup>b</sup>, F. Lima<sup>c</sup>, L. Flor<sup>d</sup>, A. Pina Fonseca<sup>e</sup>, F. Boinas<sup>f,\*</sup>

Preventive Veterinary Medicine 94 (2010) 158–162



Contents lists available at ScienceDirect

## Preventive Veterinary Medicine

journal homepage: [www.elsevier.com/locate/prevetmed](http://www.elsevier.com/locate/prevetmed)



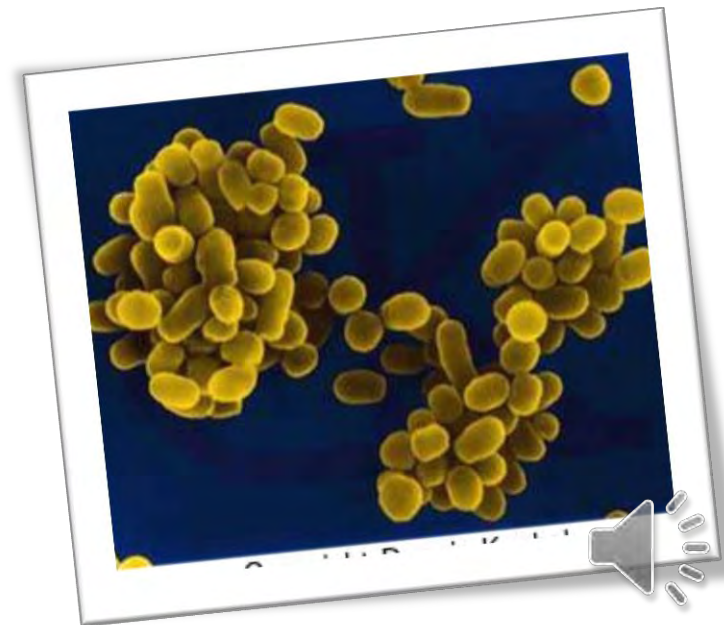
Response to Letter to the Editor

H. Martins<sup>a</sup>, B. Garin-Bastuji<sup>b</sup>, F. Lima<sup>c</sup>, L. Flor<sup>d</sup>, A. Pina Fonseca<sup>e</sup>, F. Boinas<sup>f,\*</sup>



# Bovine Brucellosis in Azores

- ▶ 1947– 1st Brucella diagnosis in Azores
- ▶ 1950– Eradication Programmes
- ▶ 1968–1984 – Vaccination S19
- ▶ 1986–1999– Vaccination M45/20
- ▶ 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

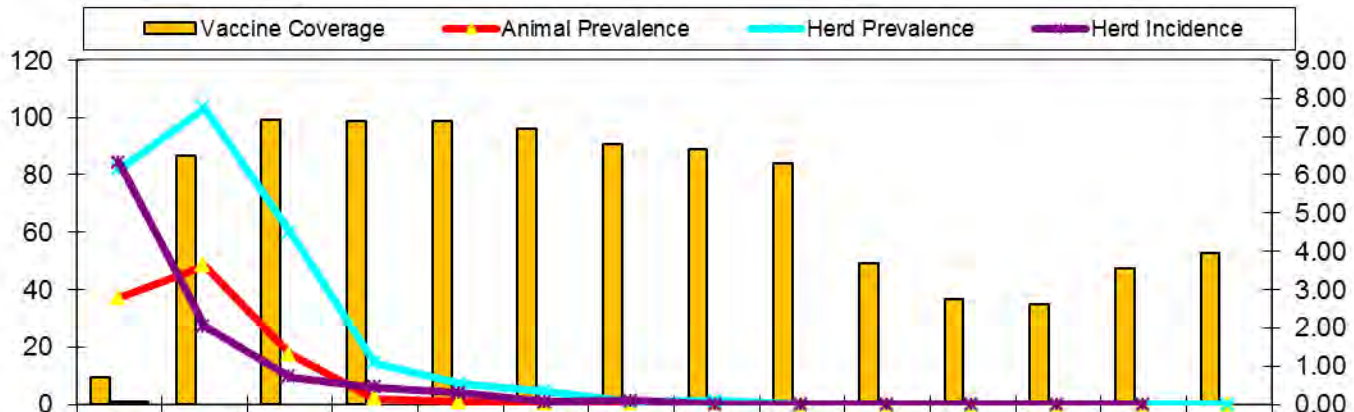


# RB51 Vaccination Programme

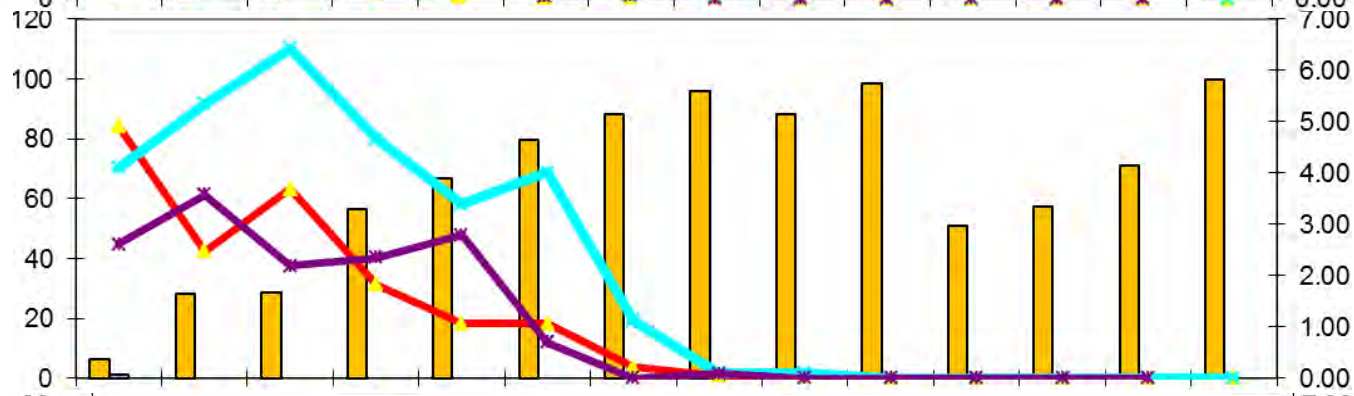
- ▶ Mass Vaccination of 3 islands
  - Terceira
  - S.Jorge
  - S.Miguel
- ▶ Pilot study
  - 2001
- ▶ Programme
  - 2002–2007



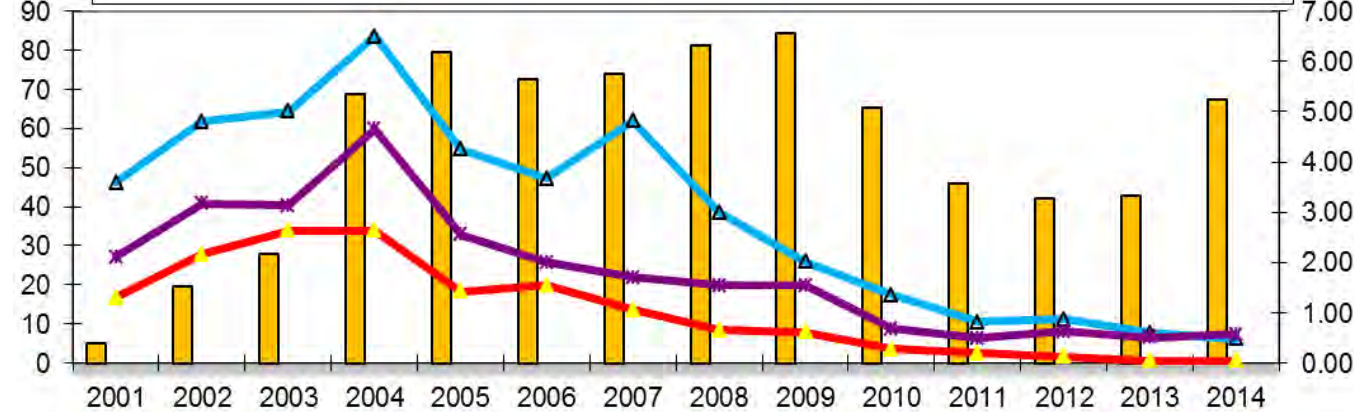
Terceira



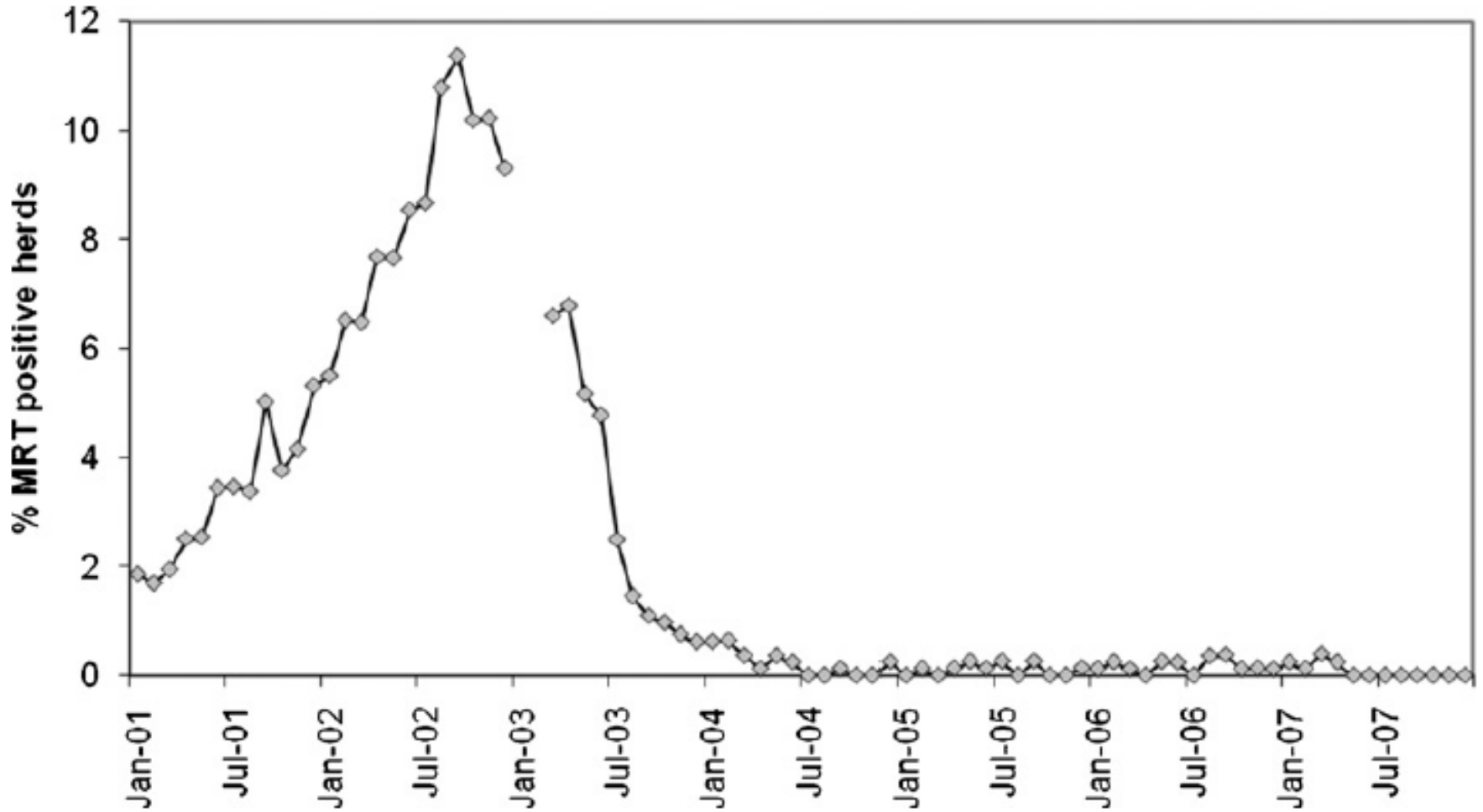
S.Jorge



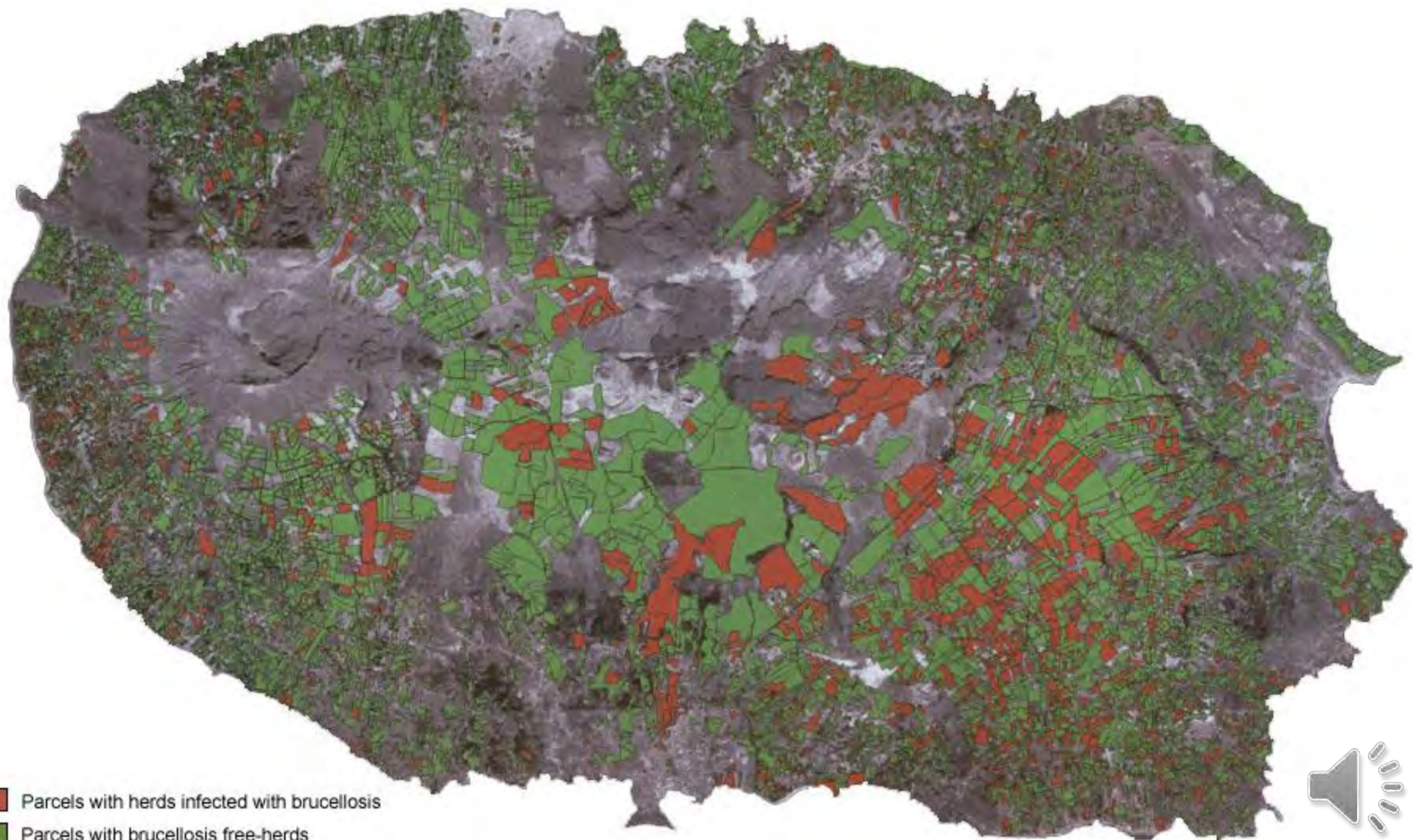
S.Miguel



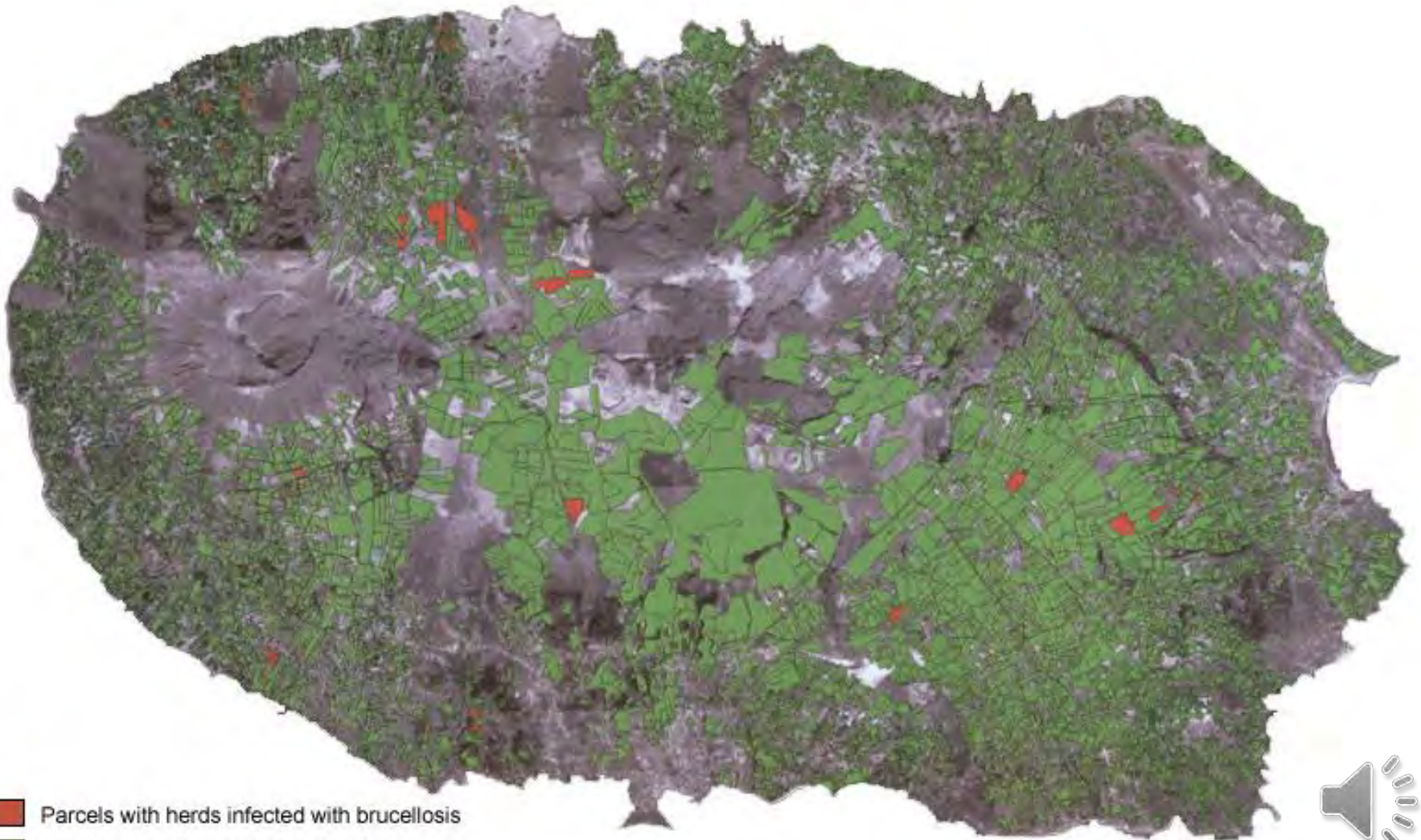
# Bulk Tank Milk Ring Test in Terceira





# Terceira, September 2002



# Terceira, June 2005



-  Parcels with herds infected with brucellosis
-  Parcels with brucellosis free-herds



# Terceira, October 2007



Parcels with brucellosis free-herds

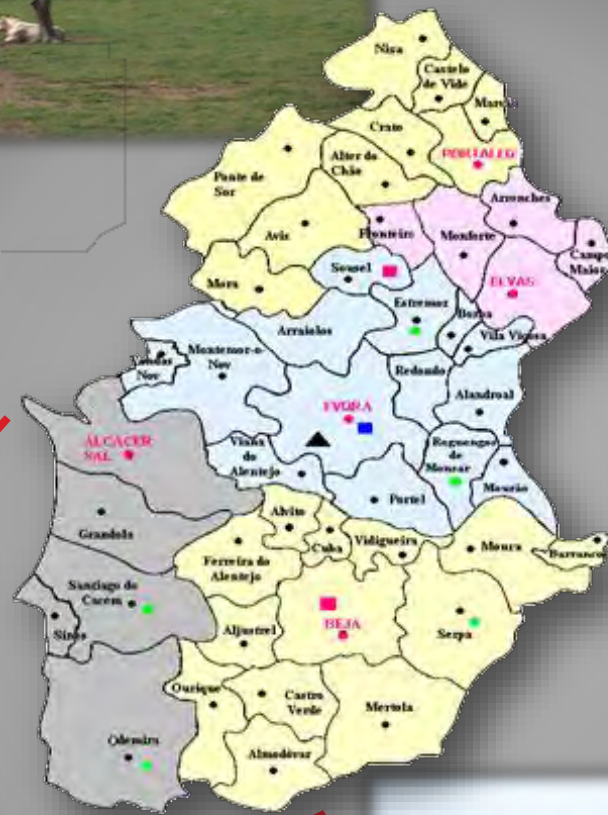
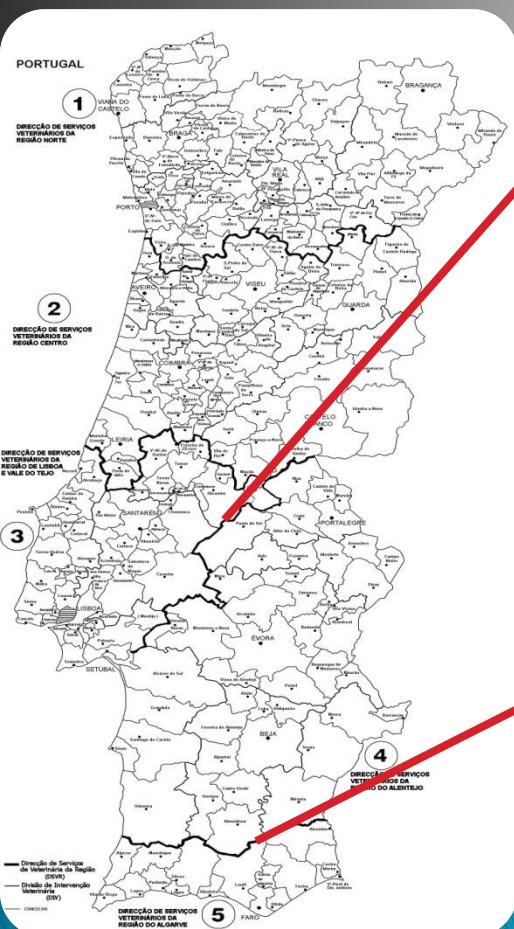


ORIGINAL ARTICLE

# **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. Caetano<sup>1</sup>, F. Afonso<sup>1</sup>, R. Ribeiro<sup>2</sup>, A. P. Fonseca<sup>1</sup>, D. A. Abernethy<sup>3</sup> and F. Boinas<sup>2</sup>

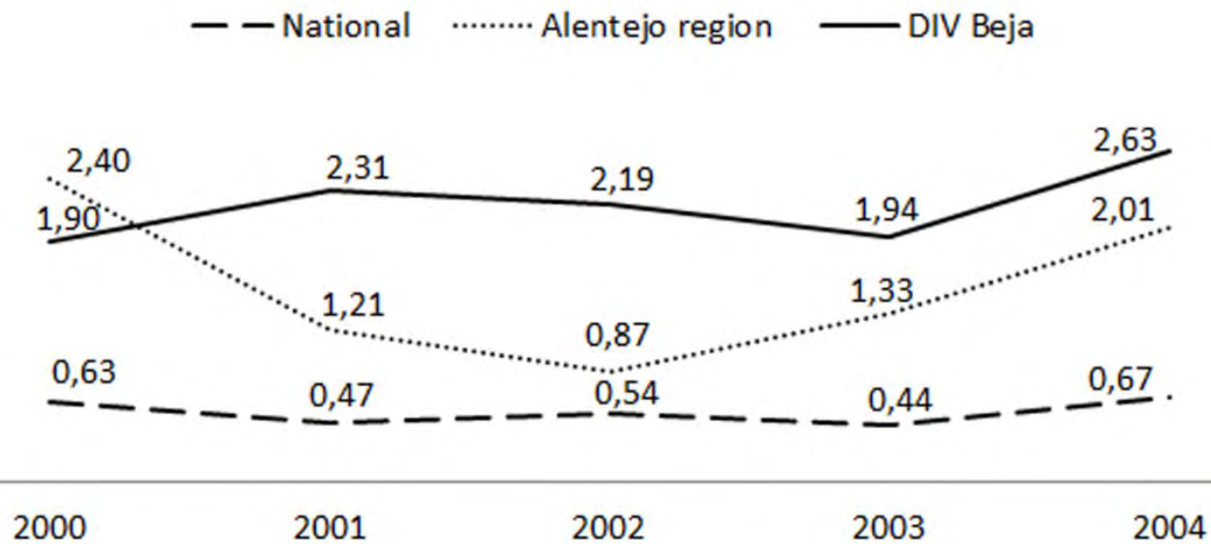




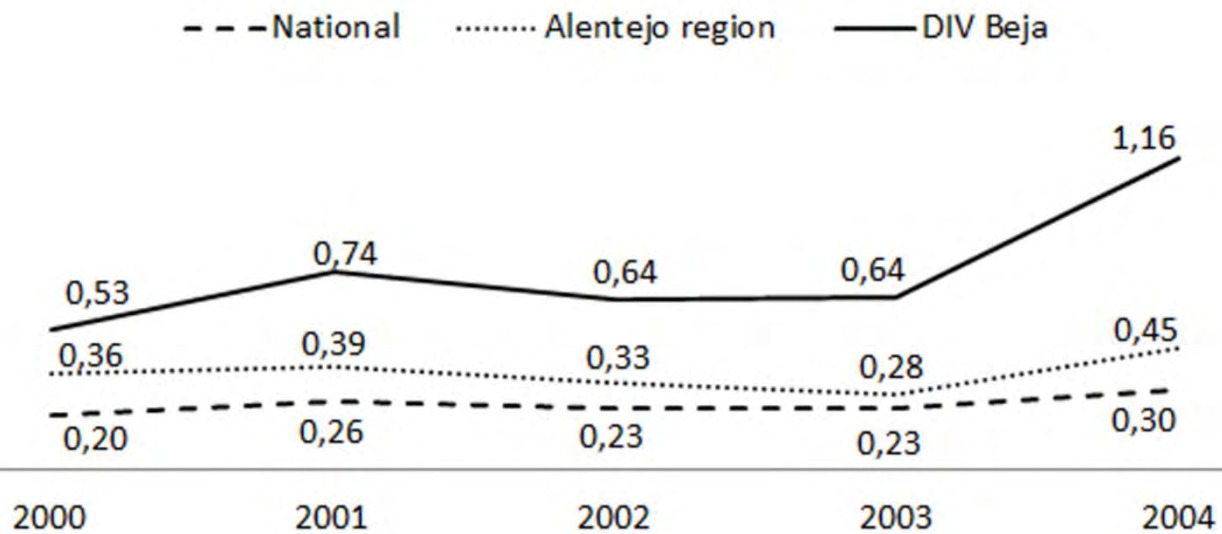
# ALENTEJO



### Percentage of positive herds (%)



### Percentage of positive animals (%)

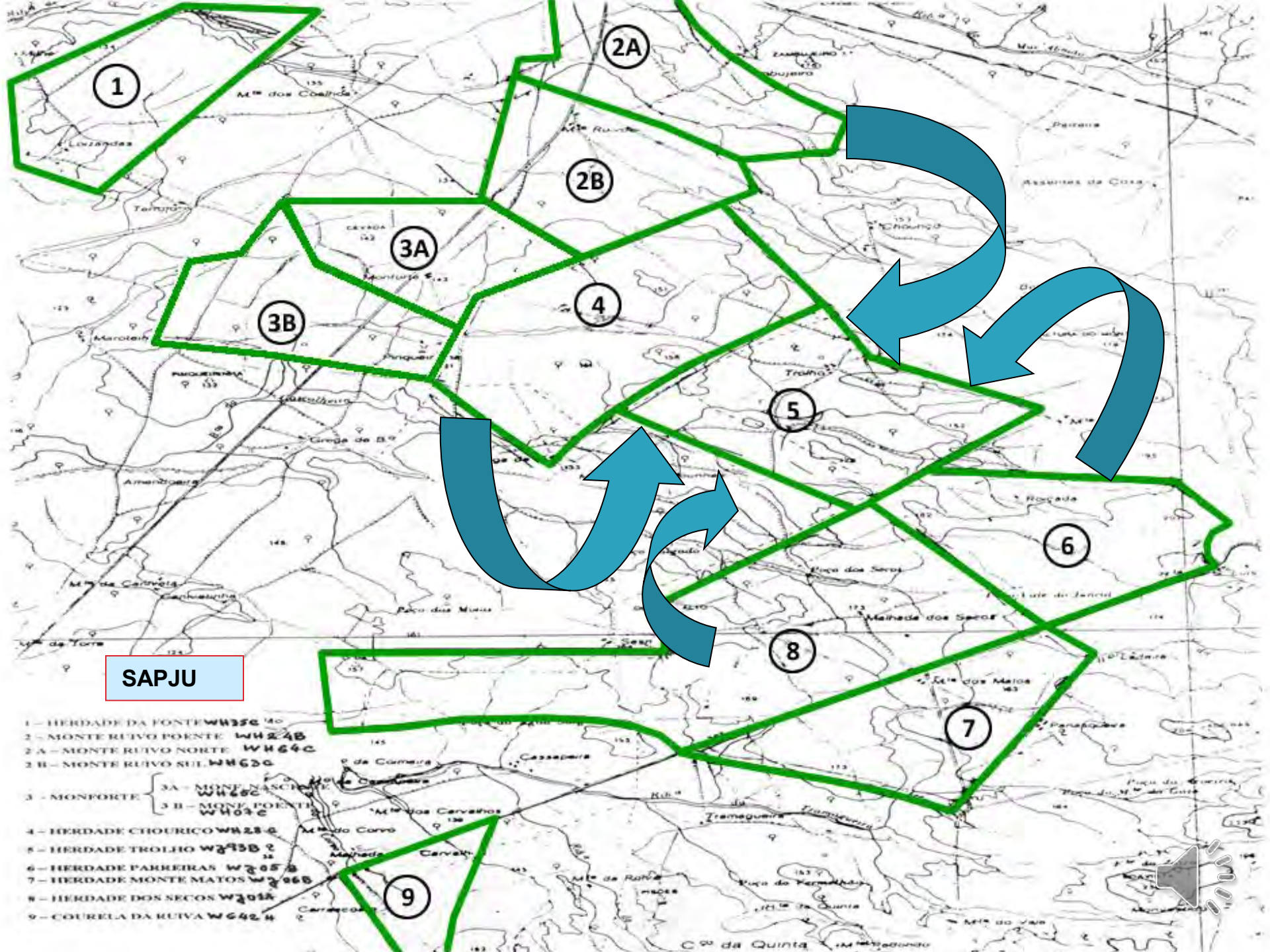


# Alentejo- SAPJU

## RB51 Special Vaccination Programme

- ▶ Programme
  - Beginning: 7-2004
  - Duration: 5 Years
  
- ▶ N° of Vaccinated Herds: 10
  
- ▶ N° of Breeding Animals:
  - Existing: 4277
    - heifers: 1485
    - cows: 2792





1

2A

2B

3A

3B

4

5

6

8

7

9

SAPJU

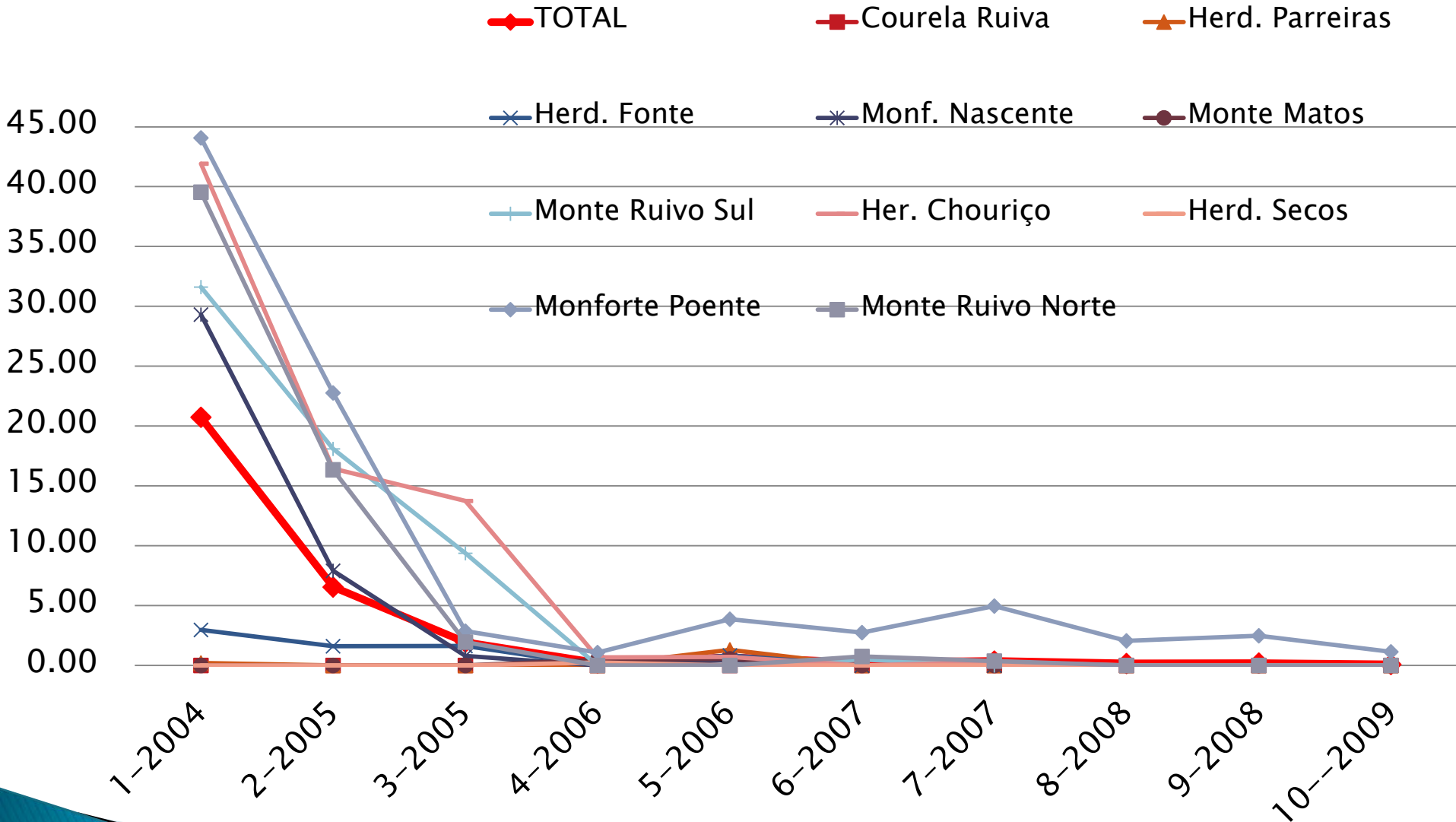
- 1 - HERDADE DA FONTE WH25C 140
- 2 - MONTE RUIVO PONTE WH24B
- 2 A - MONTE RUIVO NORTE WH64C
- 2 B - MONTE RUIVO SUL WH63C
- 3 - MONFORTE
  - 3 A - MONTE NASCIMENTO WH66C
  - 3 B - MONTE PONTE WH03C
- 4 - HERDADE CHOURICO WH23C
- 5 - HERDADE TROLHO W215B 2
- 6 - HERDADE PARREIRAS W205B
- 7 - HERDADE MONTE MATOS W206B
- 8 - HERDADE DOS SECOS W201A
- 9 - COURELA DA RUIVA W642 4

## Regulatory actions, seroprevalence and birth rate in ten *Brucella*-infected herds in the Alentejo before and after initiation of RB51 vaccination in 2004 .

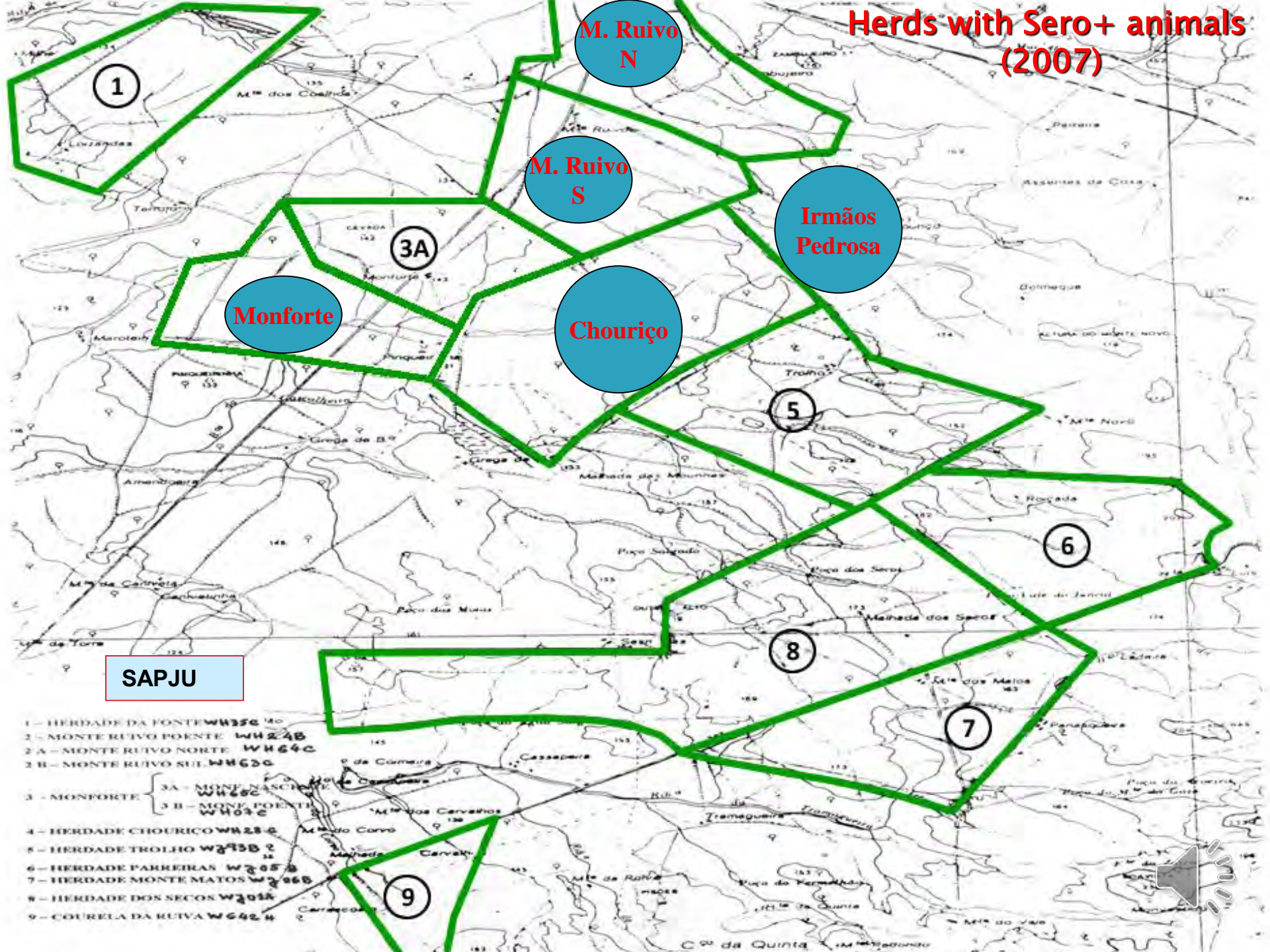
Year	Herds					Cattle	Prev. (%)	Birth rate (%)
	Total	Positive	Depopulated	Restocking	Purchase	Vaccinated		
1999	8	8	3	0	0	0	26.4	NA
2000	5	5	2	0	0	0	18.1	NA
2001	7	7	0	3	1	0	7.6	NA
2002	9	9	0	2	0	0	4.3	NA
2003	10	8	0	0	1	0	12.6	NA
2004 <sup>1</sup>	10	7	0	0	0	3370	19	60.1
2005	10	6	0	0	0	846	5.7	44.3
2006	10	6	0	0	0	709	0.5	66.0
2007	10	3	0	0	0	755	0.5	68.6
2008	10	1	0	0	0	331	0.2	71.9
2009 <sup>1</sup>	10	1	0	0	0	0	0.1	73.5



# Animal Prevalence Rates SAPJU



# Herds with Sero+ animals (2007)



1

M. Ruivo N

M. Ruivo S

Irmãos Pedrosa

Monforte

Chouriço

5

6

8

7

SAPJU

9

- 1 - HERDADE DA FONTE WH24C
- 2 - MONTE RUIVO PONTE WH24B
- 2 A - MONTE RUIVO NORTE WH64C
- 2 B - MONTE RUIVO SUL WH63C
- 3 - MONFORTE
  - 3A - MONTE NASCIMENTO WH66C
  - 3B - MONTE PONTE WH03C
- 4 - HERDADE CHOURIÇO WH23C
- 5 - HERDADE TROLHO W235B
- 6 - HERDADE PARREIRAS W205B
- 7 - HERDADE MONTE MATOS W206B
- 8 - HERDADE DOS SECOS W205A
- 9 - COURELA DA RUIVA W642H

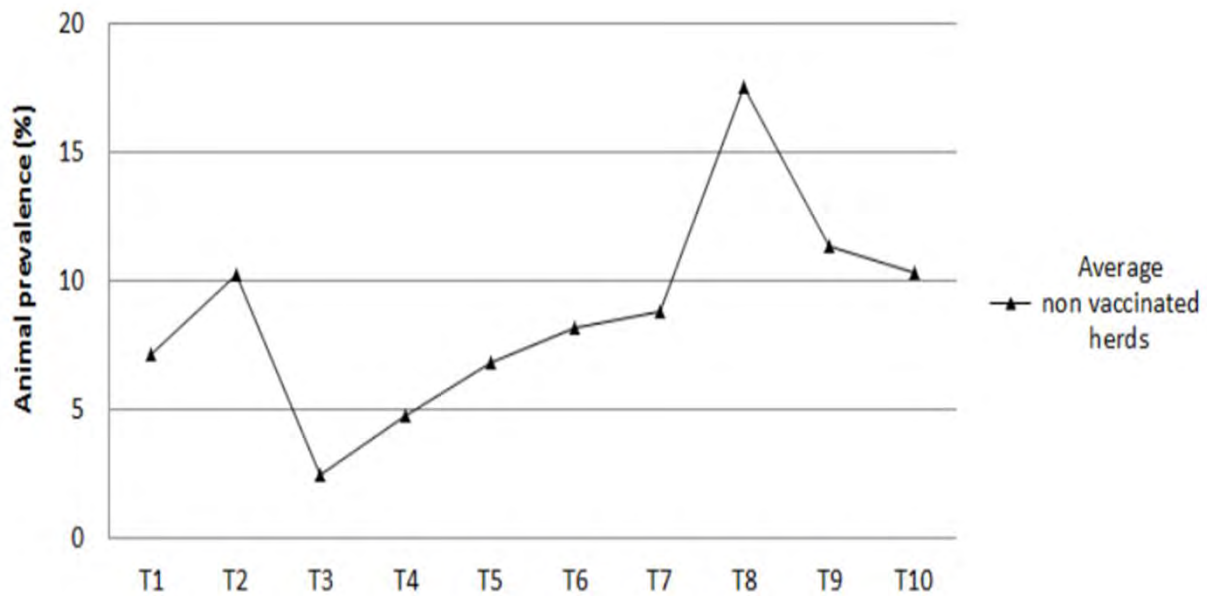
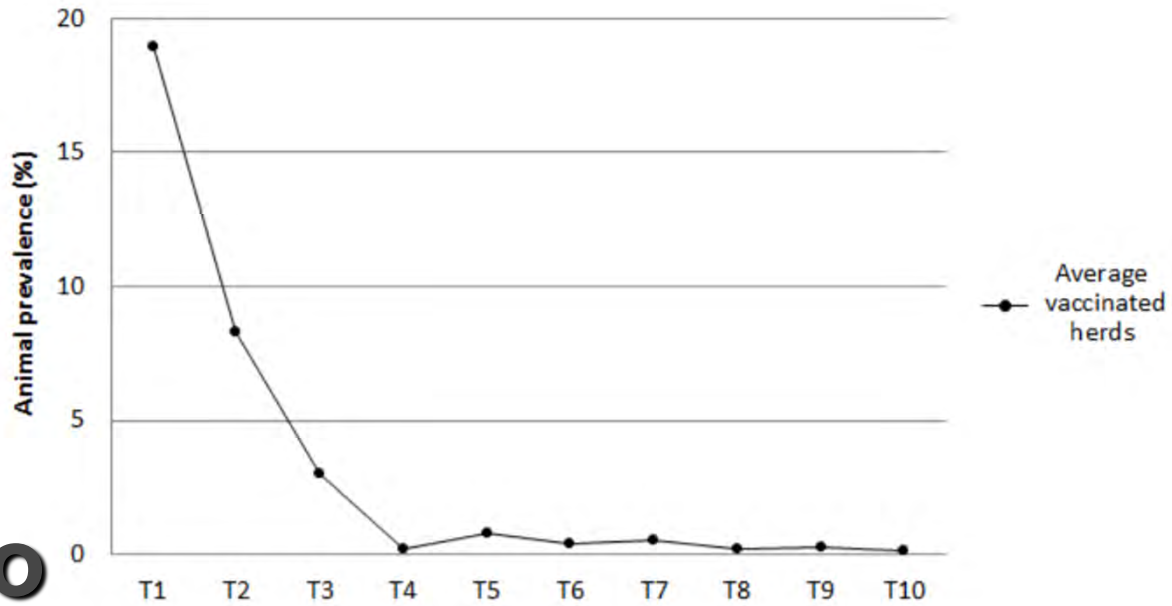


## Follow-up of the ten Brucella-infected herds in the Alentejo region not subjected to RB51 vaccination .

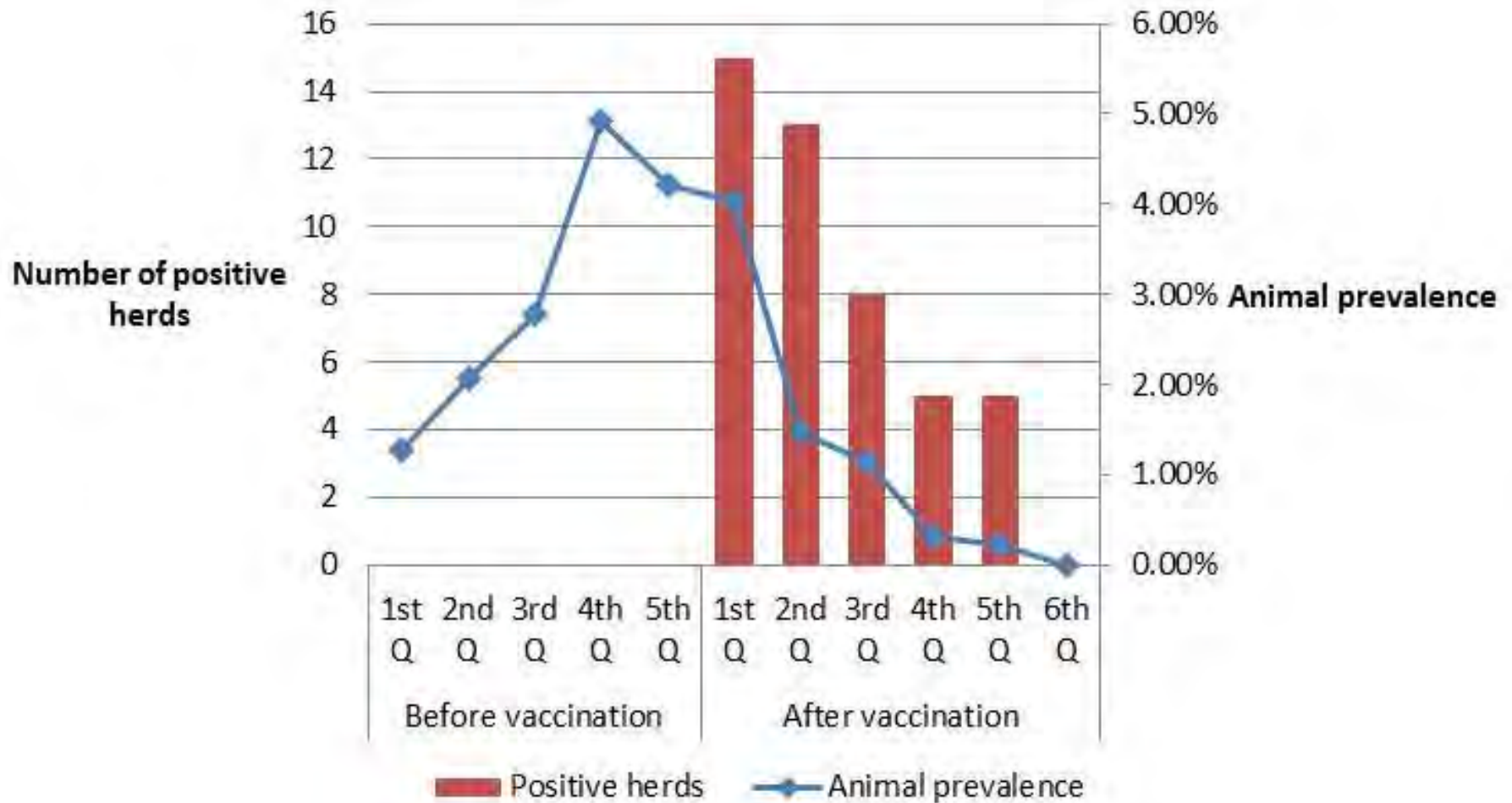
ID herd	1 <sup>st</sup> intervention with positives	Depopulation (year)	Repopulation (year)	Year of first seropositivity after repopulation	Follow-up
1	2001	2003	Yes (2004)		Negative
2	2001	2003	Yes (2004)	2004	Positive until 2006 and negative thereafter
3	1999	2003	Yes (2004)	2004	Positive until 2005 and negative thereafter
4	2003	2004	No		Ceased activity
5	2001	2004	Yes (2009)		Negative
6	2004	2005	No		Ceased activity
7	2003	2006	Yes (2007)	2009	Positive after 2009 thereafter
8	2005	2006	Yes (2007)		Negative
9	2006	2007	No		Ceased activity
10	2007	2008	No		Ceased activity



# Alentejo SAPJU



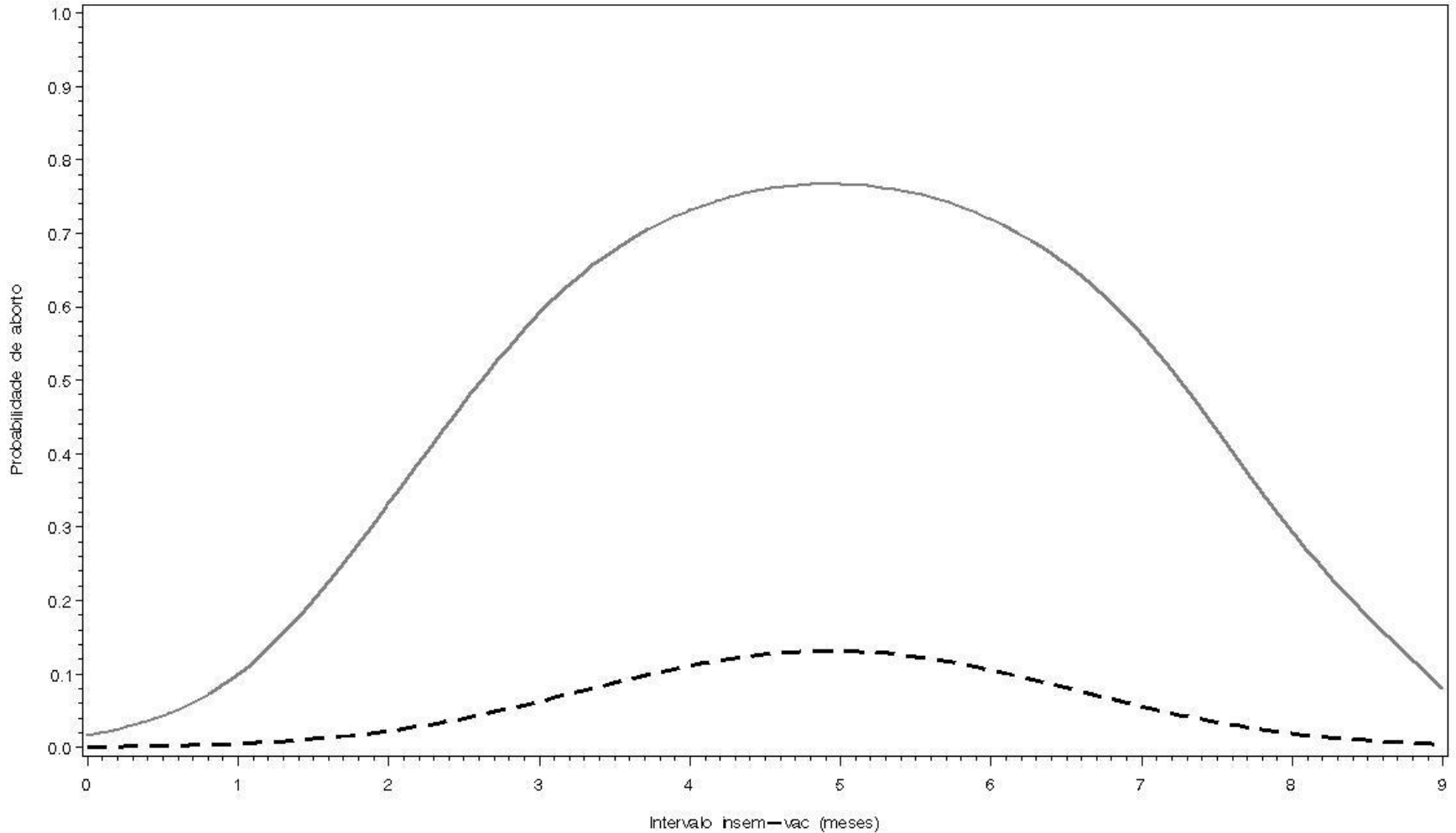
# Alentejo – other special vaccination programmes



*Q- Quarter (4 months)*

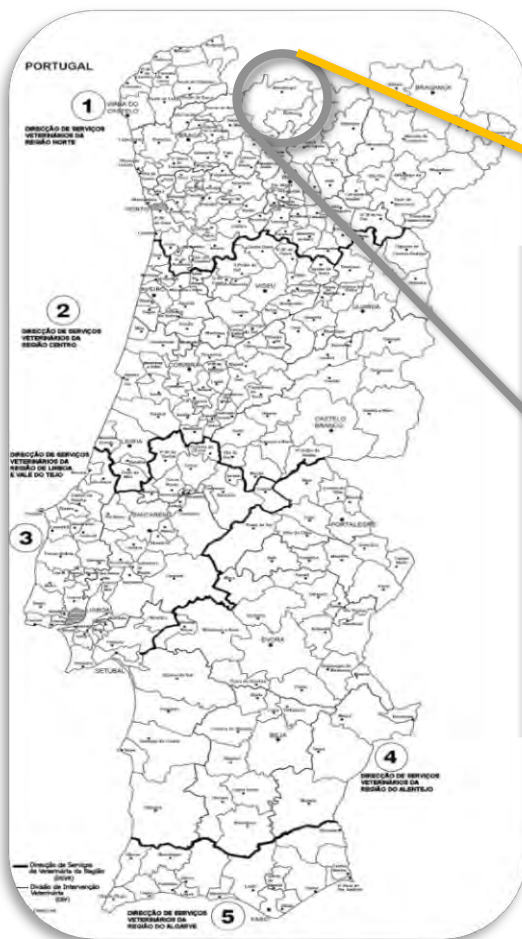


# RB51 Vaccination & probability of abortion in 1 EU



# Montalegre

**TRÁS-OS-MONTES (ToM)**



Small holdings (villages), genetic pool (DOP), communal grazing



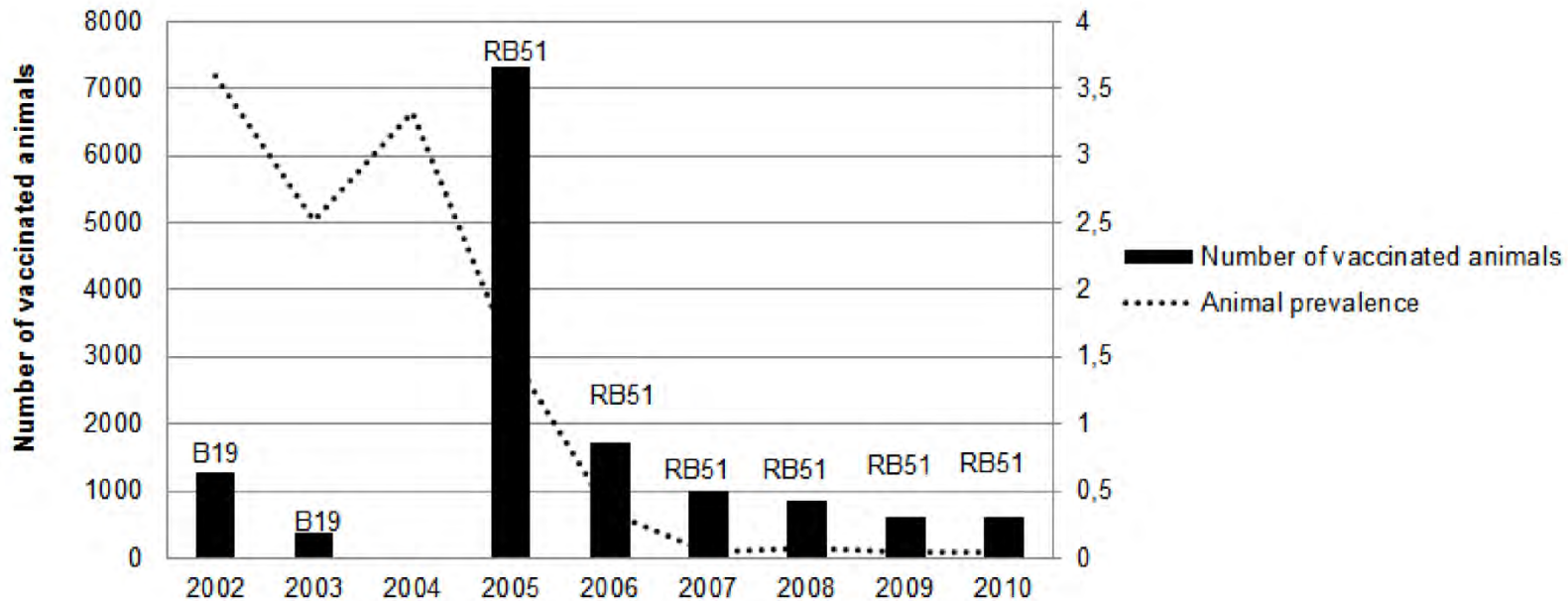
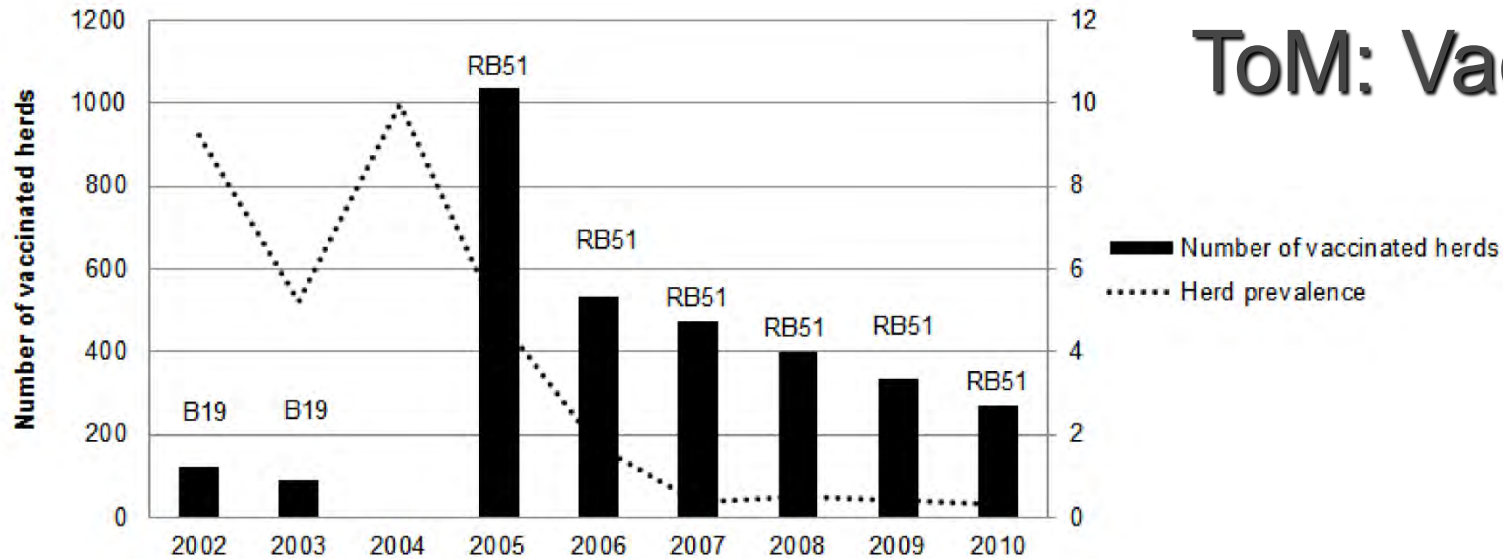
# Trás-os-Montes (ToM)

## S19 -> RB51 Vaccination Programme

- ▶ Period:
  - S19 – RB51
    - Beginning: 10/2002–3/2005
    - Duration: 7/2003 – 5 Years
- ▶ N° of villages: 7/35
- ▶ N° of herds: 127/1103
- ▶ N° of animals: 1584/
  - 8640



# ToM: Vaccination



# ToM: RB51 Vaccination

	Region	2005	2006	2007	2008	2009	2010	Median value	Difference between regions ( <i>Wilcoxon rank sum test</i> )
Herd prevalence (%)	Montalegre	4,82	1,71	0,38	0,50	0,42	0,34	0.46	<i>p-value</i> = 0.24
	Rest of DIV of Vila Real	0,58	1,21	0,86	1,12	4,54	2,16	1.16	
Animal prevalence (%)	Montalegre	1,53	0,35	0,05	0,08	0,05	0,05	0.07	<i>p-value</i> = 0.04
	Rest of DIV of Vila Real	0,79	1,02	0,71	0,87	3,02	1,33	0.94	





# 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

Age abortion (m.)	Time vaccination to abortion	
	days	months
7	90	3.0
6	93	3.1
7	93	3.1
7	109	3.6
7	113	3.8
7	123	4.1
7	125	4.2
8	132	4.4
7	139	4.6
8	147	4.9
8	152	5.1

**Average**

**7,09**

**119,64**

**3,99**



# 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 3.0–5.1 m



# 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 = 6055; 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



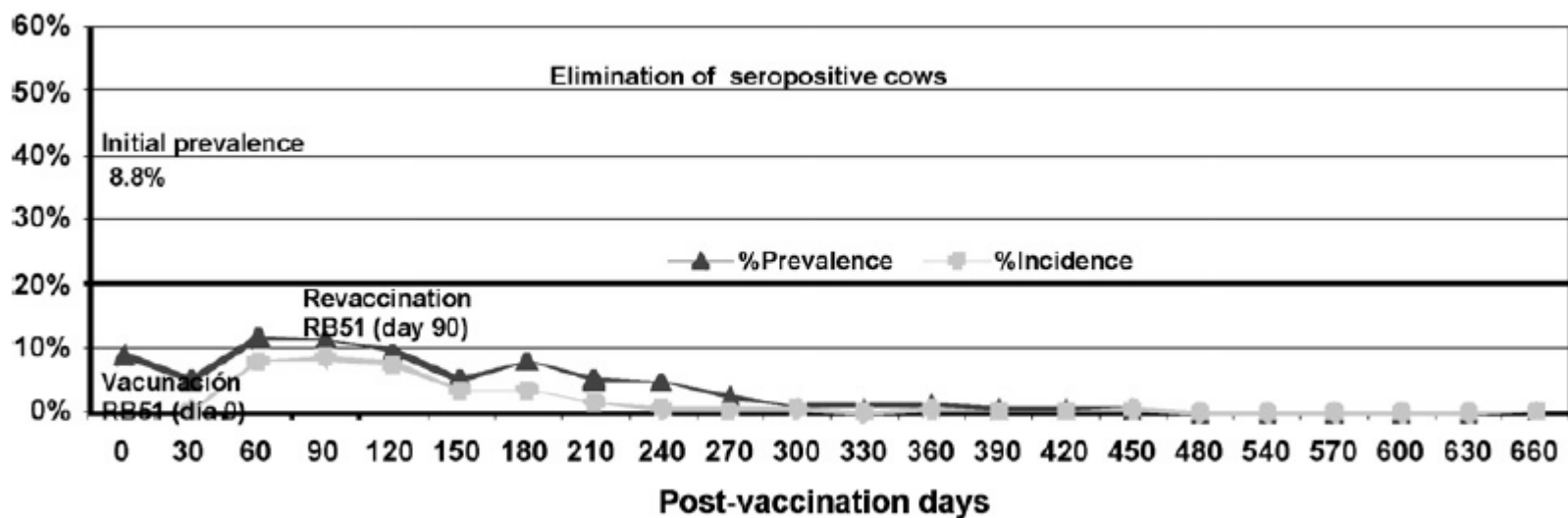


Fig. 1. Prevalence and incidence in dairy herd with low brucellosis prevalence, post-vaccination with *B.*

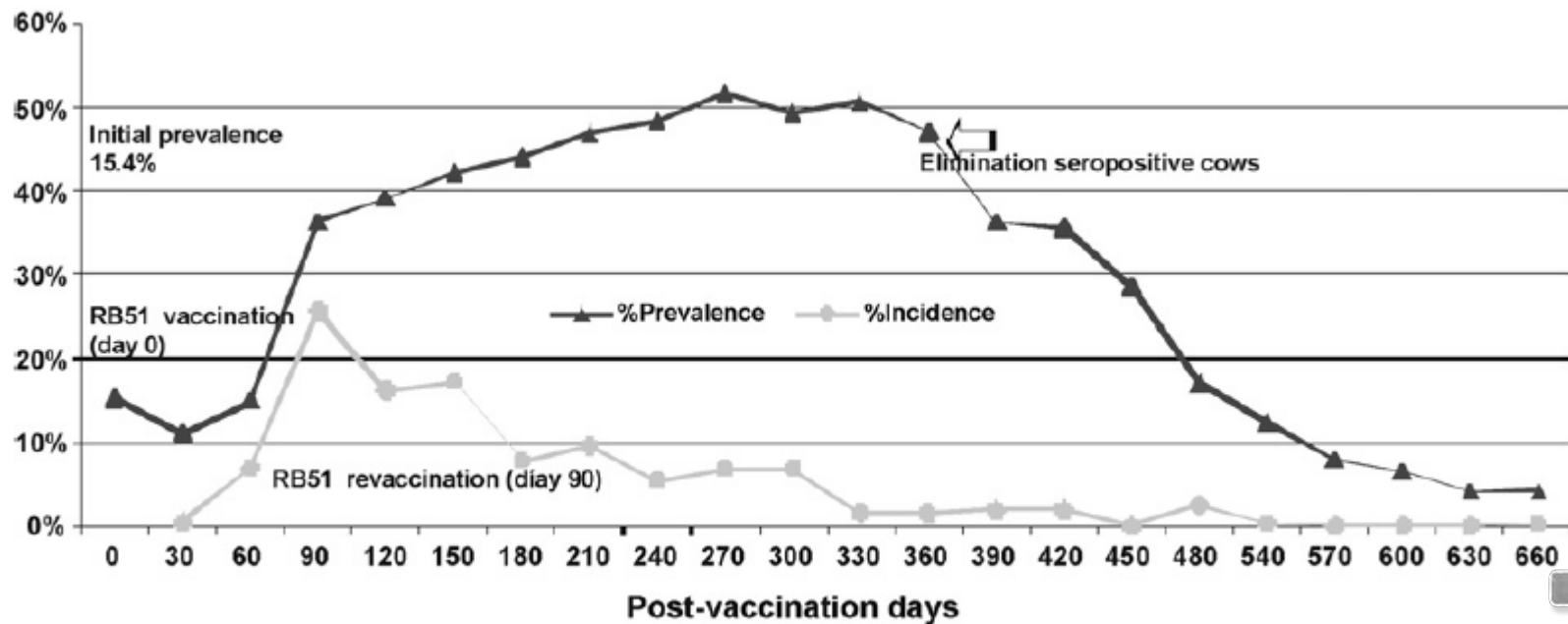


Fig. 3. Monthly prevalence and incidence in dairy herd with high brucellosis prevalence, post-vaccination with *B. abortus* RB51.

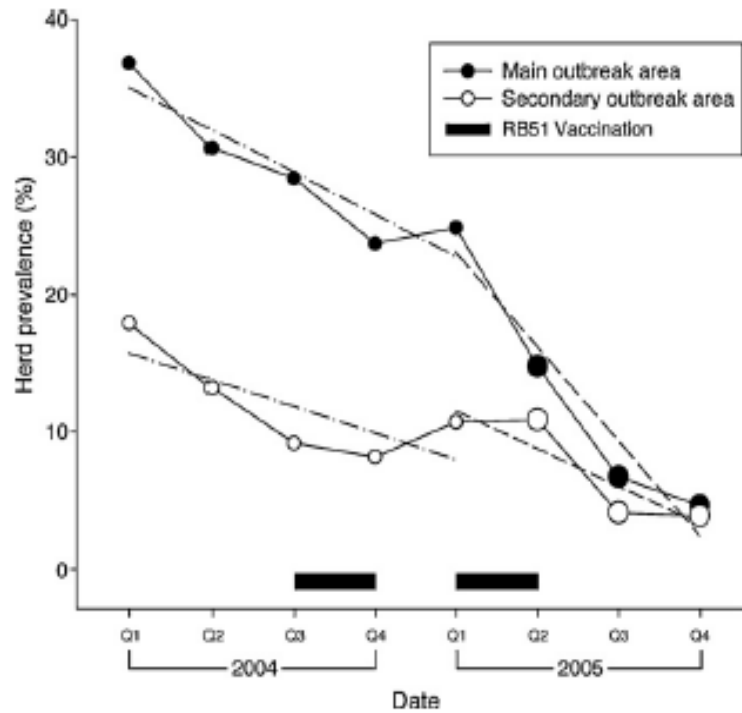
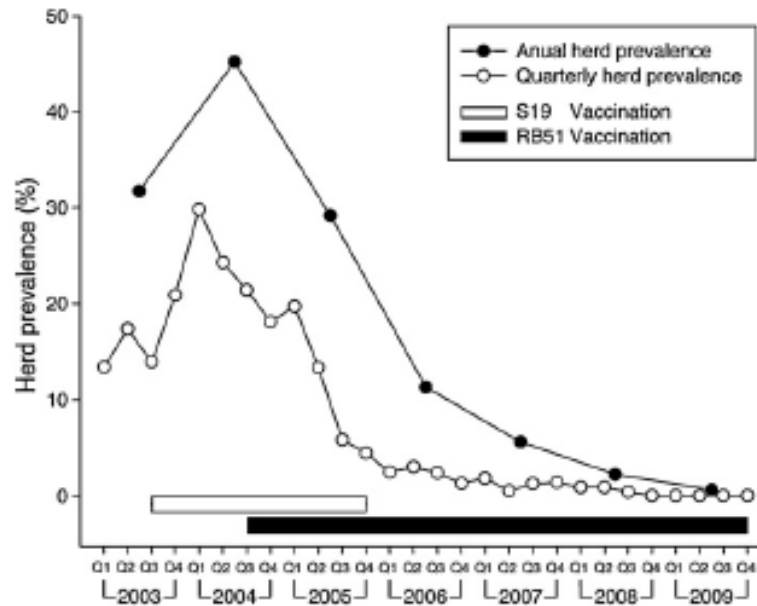




Table 1

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.

Year	Semester	Animal incidence (%)	Reactors vaccinated with S19 <sup>a</sup>	Proportion of S19 Vaccinated reactors (%) <sup>b</sup>
2004	First	7.77	45	1.39
	Second	6.56	19	0.72
2005	First	2.03	57	7.95
	Second	1.11	64	14.58
2006	First	0.23	28	34.15
	Second	0.27	40	35.40
2007	First	0.08	17	54.84
	Second	0.11	13	27.08
2008	First	0.02	5	33.33
	Second	0.01	2	22.22
2009	First	0.01	3	100
	Second	0.00	0	0

<sup>a</sup> Number of reactors that had been previously vaccinated with S19.

<sup>b</sup> 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**

General measures included in the national eradication program

- Individual identification of all animals and herds
- Periodic testing of all animals >12 months of age and removal of reactors
- Movement restrictions in case of suspicious or positive\* herds
- Compulsory reporting and aetiological investigation of abortions
- Epidemiological investigation in positive herds (trace-back and trace-forward of breakdowns)

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

\*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 / n° of Sero+ animals
  - Depopulation – : 2475€ (95% CI: 1418–3532€)
  - Vaccination – 967€ (CI 572–1368€)
- ▶ Other costs
  - vaccine
  - loosing the OBF status
  - etc.



**Thank you**  
**Obrigado**



Alentejana  
bull  
Portugal



Africander  
bull  
South Africa

