Eradication of bovine brucellosis in the Azores, Portugal—Outcome of a 5-year programme (2002–2007) based on test-and-slaughter and RB51 vaccination

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ABSTRACT

Bovine brucellosis is an important contagious disease that can cause abortions and infertility in cattle, and can be transmitted to humans. Despite having an eradication programme in place since 1994, in 2000 the situation of bovine brucellosis due to Brucella abortus was not significantly improving in 3 of the 9 islands (Terceira, S. Miguel and S. Jorge) of the archipelago of Azores, an autonomous region of Portugal. Farming on these islands, particularly dairy, is extensive. Therefore, the use of RB51 vaccine, which does not induce antibodies detectable with routine brucellosis diagnostic tests, was implemented. This article reports the results of an eradication programme based on RB51 mass vaccination combined with test-and-slaughter, which was implemented in the Azores during the 2002–2007 period. During the first round of vaccination, both adult cows and heifers were vaccinated. Subsequently, only replacement stock aged 4–12 months, were immunized with RB51. The test-and-slaughter policy based on bulk milk ring test (MRT) and serological surveillance was maintained. During this period, the average brucellosis herd incidence, herd prevalence and individual prevalence decreased 69.26%, 39.26% and 75.41% respectively for the three above-mentioned islands. However, disease reduction approaching eradication was obtained only on the island of Terceira, where a high level of vaccine coverage was rapidly reached and regularly maintained together with strict application of a test-and-slaughter programme.

This work shows that the RB51 vaccine could be a useful tool for eradicating bovine brucellosis in well-controlled epidemiological units provided that there is mass vaccine coverage for a sufficiently long period of time and it is combined with an appropriate test-and-slaughter programme.
countries and, when necessary, vaccines have been utilized to improve control and eradication efforts.

Despite its proven effectiveness, the S19 classical vaccine can induce antibodies to the O-chain of the outer-membrane smooth lipopolysaccharide, particularly when used in adults. Such vaccine-induced antibodies may be difficult to distinguish from those resulting from natural infection and thus complicate diagnosis. The more recently developed RB51 vaccine is a rough attenuated mutant of \textit{B. abortus} strain 2308, which produces very low levels of the O-chain (Schurig et al., 1991; Stevens and Olsen, 1996; Cloeckaert et al., 2002). This vaccine has the advantage of not inducing an antibody response measurable by standard assays in both young heifers and adult cows, even when vaccination is repeated (Uzal et al., 2000). Due to this advantage, it has been increasingly used instead of S19 in the USA and in several other American countries, where good results in disease control have been reported (Lord et al., 1998; Samartino, 2002; review of Schurig et al., 2002; Poester et al., 2006). However, its effectiveness remains controversial (Moriyon et al., 2004; Leal-Hernandez et al., 2005, Blasco and Moriyon, 2005; Diaz, 2005). Most studies on the protection induced by RB51 under experimental conditions in cattle have concluded that animals are protected against moderate challenge. However, some authors have suggested that RB51 could be less effective than S19 against severe challenges (for a review see Moriyon et al., 2004). RB51 is rifampin resistant and, although considered rare, it can infect humans (Ashford et al., 2004). As a consequence, rifampin, which is one of the drugs recommended for brucellosis post-exposure chemo-prophylaxis cannot be used for treatment. As with any other live \textit{Brucella} vaccines, if administered during pregnancy in cattle, it could cause abortion and may be excreted in milk (Van Metre et al., 1999; Moriyon et al., 2004).

Azores, an archipelago composed of 9 islands with an area of 2322 km$^2$ and 246,000 inhabitants located in the North Atlantic, is an Autonomous Region of Portugal (Fig. 1). Bovine brucellosis was first reported in the Azores in 1947 and official programmes for disease control started in 1950, being reinforced in 1994 through an EU co-financed eradication programme. By 2000, bovine brucellosis had been eradicated in four islands (Corvo, Flores, Graciosa and Pico) and reached a very low prevalence in Faial and Santa Maria. However, brucellosis was still present in the three remaining islands (Terceira, Miguel and S. Jorge). In the Azores, vaccination against bovine brucellosis started in 1968. The S19 vaccine was used until 1984 and was then replaced by Strain 45/20, which was used until 1999. Due to the very low animal prevalence rate reached in each of the islands (<1\%), vaccination was then stopped. However, 3–4 years later, depending on the island, levels of infection increased dramatically and a new vaccination scheme, using the RB51 vaccine was considered.

This paper reports the results of a mass RB51 vaccination campaign of the entire cattle population implemented from 2002 to 2007 and integrated into an eradication programme based on test-and-slaughter, on the Azores islands of S. Jorge, Miguel and Terceira.

2. Materials and methods

2.1. The bovine production system in the Azores

Cattle production, especially dairy production, is the most important industry of Azores employing 30% of the
active population full time and 80% part time, and accounts for 20% of the Regional Gross Internal Product. The dairy cattle population of the Azores accounts for around 30% of the total cattle population in Portugal. Consequently, Azores milk production represents almost 30% of Portuguese milk production. There are presently approximately 12,000 herds with about 5000 fattening herds and 7000 dairy herds. Animals are reared extensively (1.3 animal/ hectare) with approximately 250,000 adult animals mostly distributed in the main islands of S. Miguel (48%), Terceira (25%) and S. Jorge (76%). Amongst these, 200,000 adult animals are dairy (Holstein-Friesian breed). These three islands account for 80% of the total cattle population and for 95% of the milk production of the archipelago.

There is a great variation in herd size. Depending on the island, 60–70% of the animals are concentrated in 25–30% of herds. These general characteristics of the cattle population in the Azores remain almost unchanged from 2002 to 2007, except that the number of herds decreased by 13% while the number of animals increased by 2.6%, raising the mean number of animals per herd from 24.8 to 29.5.

The Azores has a temperate, maritime climate with mild annual temperature oscillations (averages of 14 °C in winter and 21 °C in summer) and high levels of precipitation with relative humidity (averages of 69% in summer and up to 91% in winter). These weather conditions allow for an extensive production system based on permanent pastures, which account for 90% of the agrarian production.

Most of the farms are fragmented into parcels (average: 6.3 parcels per farm), sometimes spread along the island. Direct contact between herds is possible in contiguous parcels. Production systems are mainly extensive, with animals moving frequently between pastures through communal paths and using common watering points. Milking is almost always operated with portable machines on the parcels. All milk produced is pasteurised, except in S. Jorge, where raw milk is used for the production of a regional cheese that undergoes a 3-month maturation process.

2.2. Laboratory testing

2.2.1. Serological tests

Milk Ring Test (MRT), Rose Bengal Test (RBT) and Complement Fixation Test (CFT) were performed according to the OIE standard procedures using antigens complying with the OIE and EU requirements (World Organisation for Animal Health, 2004; Commission Regulation, 2002).

2.2.2. Bacteriology for isolation of Brucella

Cultures were performed on Farrell’s medium, according to procedures described by Alton et al. (1988), on stomach contents, lung and spleen of aborted foetuses, or on retropharyngeal and supramammary lymph nodes and spleen from slaughtered animals.

Brucella strains isolated were biotyped by the Regional Veterinary Diagnostic Laboratory and confirmed at the National Reference Laboratory (LNVH, Lisbon) according to procedures described by Alton et al. (1988).

2.3. Surveillance and eradication programme

The test and slaughter policy implemented since 1994 in the whole archipelago was continued during the vaccination campaign. It consisted of an annual sero-surveillance of all adult cattle, using RBT as a screening test and CFT as a confirmatory test.

In addition, MRT was performed on bulk milk in dairy herds. In Terceira, MRT was performed on a monthly basis during the study period. On the other 2 islands, it has been performed on a regular basis since 2005, but only 7 to 9 times a year. When a bulk milk sample was positive on the MRT, all the animals of the contributing herds were blood-tested. As in the rest of EU, abortion notification is compulsory in Azores.

Whenever possible, and if further confirmation of brucellosis was needed, Brucella isolation was attempted from samples from slaughtered animals and from abortion materials submitted to the laboratory.

Herds were considered “herds suspect of brucellosis” if at least one animal tested positive to RBT and CFT, and were considered as “infected herds” when Brucella was isolated, or on the basis of an epidemiological investigation. Animals positive to either RBT or CFT and belonging to infected herds were considered as “infected animals” and were slaughtered within 10 days of receipt of test results. After 2003, females less than 12 months of age born to positive cows were considered at-risk and slaughtered. The herds with positive animals were followed-up at 30-day intervals until a first negative herd test was obtained. According to the EU regulation and to the Official Veterinary Services of Portugal (Council Directive 64/432, 1964/2007; Direcção Geral de Veterinária, 2005), 2 negative herd tests (using RBT and CFT in parallel) of adult animals after the last positive animal was slaughtered, at 30–60 day intervals, and two additional negative herd tests at 3-month intervals were required to re-qualify the herd as free.

2.4. Vaccination campaign

The objective of the vaccination campaign was to implement mass vaccination of the cattle population of the 3 islands (Terceira, S. Miguel and S. Jorge). The target population (named “eligible” in the rest of this article) consisted of the breeding herds, excluding the fattening herds.

The RB51® vaccine was supplied by CZ Veterinaria S.A (Pontevedra, Spain) and administered only to heifers or adult female cattle by the subcutaneous route at 10–34 × 109/dose (2 ml).

A pilot study was implemented from August to December 2001, on a limited number of herds in order to evaluate potential side effects of the vaccine and increase the awareness of the people involved (breeders, veterinarians and veterinary services). Priority was given to herds previously identified as infected, as well as to their neighbouring herds. On the 3 islands, 10,300 animals from 381 herds were vaccinated (Table 1).

After April 2002, the mass vaccination plan was extended to all the herds on the three islands. Initially,
whole herd vaccination was conducted, including both heifers (from 4 to 12 months of age) and adults. On the following vaccination rounds in herds previously vaccinated, only heifers were vaccinated. Moreover, in Terceira and S. Miguel, in herds still infected with an intra-herd incidence of more than 10% during the previous 6 months, all adults were re-vaccinated (Fig. 2) after 6 months as were adjacent herds, (60–3000 animals revaccinated per year—data not shown).

The vaccination campaign was implemented simultaneously with routine brucellosis surveillance by the official veterinary services. Due to a lack of staff and the need to urgently vaccinate a large proportion of the population, simultaneous serological testing of all vaccinated animals was not performed in the initial stages of the programme.

2.5. Calculation of rates

2.5.1. Vaccination coverage rates (in eligible animals and herds) were calculated as follows:

- **Annual herd vaccination rate**: percentage of herds where vaccination was performed at least once during the year.
- **Annual animal vaccination rate**: number of animals present in the herd during the respective year (taking into account an average replacement rate of 20%), and vaccinated from the beginning of the programme/eligible animal population.

2.5.2. Prevalence and incidence rates (in the respective areas) were calculated as follows:

- **Annual herd incidence**: number of herds newly infected in the year/total eligible herds.
- **Annual herd prevalence**: number of infected herds during the year/total eligible herds.
- **Annual animal prevalence**: number of infected adult animals present in the herd during the year/total number of adult animals.
- **Mean within-herd prevalence**: mean number of infected adult animals per infected herd/mean number of adults per herd.

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**Table 1**

Herd and animal vaccination rates in the islands of S. Miguel, S. Jorge and Terceira during the period 2001–2007.

<table>
<thead>
<tr>
<th>Year</th>
<th>Herds</th>
<th>Vaccinated</th>
<th>Rate (%)</th>
<th>Animals</th>
<th>Vaccinated</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Under vaccination programme, N</td>
<td>N</td>
<td></td>
<td>Eligible, N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>2001*</td>
<td>6009</td>
<td>381</td>
<td>6.34</td>
<td>161,187</td>
<td>10,300</td>
<td>6.39</td>
</tr>
<tr>
<td>2002</td>
<td>6248</td>
<td>2909</td>
<td>47.93</td>
<td>155,234</td>
<td>60,267</td>
<td>38.82</td>
</tr>
<tr>
<td>2003</td>
<td>5972</td>
<td>1998</td>
<td>33.46</td>
<td>148,089</td>
<td>72,257</td>
<td>48.79</td>
</tr>
<tr>
<td>2004</td>
<td>5242</td>
<td>2806</td>
<td>53.53</td>
<td>149,371</td>
<td>112,507</td>
<td>75.32</td>
</tr>
<tr>
<td>2005</td>
<td>5492</td>
<td>3752</td>
<td>68.32</td>
<td>155,337</td>
<td>130,653</td>
<td>84.11</td>
</tr>
<tr>
<td>2006</td>
<td>5422</td>
<td>3161</td>
<td>58.30</td>
<td>156,179</td>
<td>125,760</td>
<td>80.52</td>
</tr>
<tr>
<td>2007</td>
<td>5406</td>
<td>3596</td>
<td>66.52</td>
<td>159,303</td>
<td>131,814</td>
<td>82.74</td>
</tr>
</tbody>
</table>

* August to December.

**Fig. 2.** Annual herd vaccination and revaccination rates in the islands of S. Miguel, Terceira and S. Jorge during the period 2001–2007.
3. Results

3.1. Vaccination coverage

The overall herd and animal vaccination coverage on the 2002–2007 period are given in Table 1. The herd and animal coverage rates increased rapidly, reaching a peak in 2005 and remaining around 60% and 80% respectively, until the end of 2007. The distribution of the herd coverage amongst the three islands for the same period is given in Fig. 2. In Terceira, a high rate was reached in 1 year, and rates were kept over 60% up to 2007. However, it took

![Graphs showing vaccination coverage, prevalence, and incidence.](image-url)

Fig. 3. Annual animal vaccination rate, animal prevalence, herd prevalence and incidence in the three islands (A) and in S. Miguel (B), Terceira (C) and S. Jorge (D) islands during the period 2001–2007.
longer to reach acceptable rates in the other two islands; the best rates were not attained until 2005 in S. Miguel (67%) and 2007 in S. Jorge (98%).

Coverage rates at the animal level in each island are given in Fig. 3. These rates are much higher than those observed with herds, although trends are similar (Fig. 3A). Again, Terceira discloses the highest coverage rates and is the only island where not only almost all target animals (i.e., young and adults) were vaccinated during the first year of the programme, but also where coverage rates remained above 90% up to 2007 (Fig. 3B). In S. Jorge, an 80% coverage rate could be reached only in 2006 after a slow increase in the 2002–2007 study period (Fig. 3C). This coverage rate was never reached in S. Miguel (Fig. 3D).

3.2. Herd incidence, herd prevalence and individual animal prevalence

The results obtained in terms of annual herd incidence, herd prevalence and animal prevalence of Brucella infection calculated from the serological surveillance results are shown in Fig. 3. The overall trend is a regular decrease of the three parameters from the beginning of the vaccination programme up to the end of the study period (Fig. 3A). Average herd incidence, herd prevalence and animal prevalence decreased 69.26%, 39.26% and 75.41% respectively, from 2002 to 2007 for the whole area.

Again, clear differences in the evolution of these rates can be seen among the three islands. While in S. Miguel and S. Jorge, initial herd prevalence and incidence rates were approximately 6% and 4% respectively, these rates were higher in Terceira (11% and 10% respectively). In this island, a very rapid decrease of the three parameters was observed 1 year after the beginning of the programme, with a herd prevalence close to 1% reached within 2 years. This decrease in prevalence remained regular until 2007 (zero-level reached) (Fig. 3B). In the two other islands, however, the decrease in incidence and prevalence started later, namely in 2004 for S. Jorge and 2005 for S. Miguel, and it was not as regular as in Terceira (Fig. 3C and D). The zero-level was still not reached in 2007 for any of the three parameters for these two latter islands.

The mean within-herd prevalence followed the same pattern, declining on a gradual pace until 2007, but again
with a more rapid and regular decrease for the island of Terceira (Fig. 4).

Taking into account the results of the surveillance programme on dairy herds in Terceira, obtained with the regular monthly MRT testing, a shift point can be observed on October 2002 (Fig. 5), which was 9 months after the beginning of the mass vaccination programme.

In Terceira, it should also be noted that the parcels where animals from affected herds were pastured were thoroughly spread throughout the island in September 2002 and all were free of brucellosis 5 years later (Fig. 6).

3.3. Bacteriological examination

Thus far, field strain Brucella abortus, biovar 1 has been the only Brucella strain isolated in the Azores. In only one case was RB51 isolated. This was in 2007, from a weak calf born to a vaccinated dam, which died within 48 h after birth.

During the 2001–2007 period, 298 abortions were reported and samples submitted for bacteriological investigation in Terceira and S. Miguel islands. From those, B. abortus biovar 1 was isolated 73 times, for a mean isolation rate of 24.50%. Very high isolation rates were observed at the beginning of the campaign (38 and 57% respectively in Terceira and S. Miguel in 2001). In Terceira, where the highest numbers of abortions were regularly seen, the isolation rate decreased regularly until 2007 without any change in the bacteriological procedures (data not shown).

During the study period, supramammary (N=303) and retro-pharyngeal (N=342) lymph nodes and spleen (N=298) samples from 343 sero-positive animals slaughtered due to brucellosis suspicion were submitted for culture. Brucella was isolated from 176 animals (51%). Similar isolation rates (39%) were observed for supramammary and retro-pharyngeal lymph nodes, but a lower rate was seen for spleen (7%).

3.4. Side-effects in animals and humans

During the 5-year study timeframe almost 250,000 females, of which at least 180,000 were adults, were vaccinated with the RB51 vaccine. No side-effects, such as abortion were reported (passive reporting). The only outstanding observation that could be related to vaccination was the weak newborn calf from which B. abortus RB51 was isolated (cited above).

During the 2001–2006 period, the annual number of human cases was low (≤4) and none of them was due to RB51, but to field strain B. abortus biovar 1. It was impossible to relate the disease occurrence in humans with the prevalence in animals.

4. Discussion

The aim of this report was to present the detailed results obtained in a field situation where mass RB51 vaccination was implemented in combination with a severe test-and-slaughter programme. This type of findings has not been previously reported.

Due to its socio-economic importance and to the risk factors for the spread of brucellosis in the Azores, related to (i) the favourable climate for the persistence of Brucella in the environment and (ii) with a production system based on small parcels of land with constant animal movements, vaccination was considered as the best strategy for controlling the disease on the three most important and problematic islands.

Extensive field usage of RB51 vaccine in the USA and in several countries in Central and South America since the 1980s gave apparent good results (Lord et al., 1998; Samartino, 2002; Schurig et al., 2002; Poester et al., 2006). However, as results of experimental trials are still controversial (Moriyon et al., 2004), the European Commission showed some reluctance to accept the pilot study that was proposed for the Azores.

Due to historical problems with a batch of S19 that spread infection in the Azores in 1984 (L.H. Medeiros, personal communication, 2007) this vaccine could not be used any more and the production of the 45/20 vaccine had stopped worldwide. Therefore, the only available alternative for vaccination in the Azores was to use RB51.

Despite the difficulties that exist in any field campaign performed on a large scale, namely the lack of personnel
Fig. 6. Evolution of the distribution of the parcels of land in Terceira considering the herd sanitary status for Bovine Brucellosis in September 2002 (A), June 2005 (B) and October 2007 (C).
and budget, our study shows the results of such a field campaign implemented on three different islands over a 5-year period. The general trend observed in the 3 islands is the clear decrease of the mean number of positive animals per herd, and mean disease prevalence and incidence. This is probably due to a lower rate of transmission within and between herds. However, as far as herd and animal prevalence rates are concerned, the trends were not comparable in the three islands. Terceira showed an initial within-herd prevalence rate close to 30% and the highest herd prevalence rate (close to 11%) of the archipelago. Where almost all the cattle population was covered by mass vaccination and by MRT surveillance during the duration of the study, a regular and dramatic reduction of the herd and animal prevalences were observed. Moreover, eradication was approached at the end of the 5-year period.

However, on S. Jorge, where the initial within-herd prevalence rate was high (33%) although the initial herd prevalence rate was lower than in Terceira (6%), a remarkable reduction in prevalence was observed only after 5 years. On this island, the effective rate of vaccine coverage was initially very low and remained lower than 80% until 2006 as a result of the opposition that producers showed to vaccination. In S. Miguel, where the initial within-herd prevalence rate was similar to that on Terceira (about 30%), no clear reduction in the prevalence rates were achieved during the study period. The differences in the outcome of the campaign on this island can be explained by the lack of veterinarians to perform the mass vaccination campaign.

On several occasions, the improvements achieved in Terceira were communicated to producers in S. Jorge and lead to a reinforcement of the campaign on this island. In S. Miguel, the island with the largest cattle population, the official services staff only achieved a low coverage rate; therefore contracts with private veterinarians are presently being promoted.

As the cattle population in Azores is almost exclusively dairy, the MRT has been used since 1994 for rapid detection of Brucella infection, allowing for the application of early action plans by the official services. Although the measures on the 3 islands were not modified substantially during the implementation of the programme, this could have given an advantage to Terceira versus the other islands that did not promote it with the same frequency. In the island of S. Miguel, a more frequent MRT sampling was implemented in 2007.

The results of the bacteriological testing were parallel to the evolution of the disease in the area, with high levels of isolation rates from abortion materials at the beginning of the programme followed by a regular decrease until 2007. However, considering the three islands, the number of abortions reported and samples for testing represented only 0.5–1.6% of the mean rate of abortions (1.95–6.5%) classically expected in uninfected dairy cattle (Forar et al., 1995).

As already reported by other authors, the culture of head and mammary lymph nodes sampled from seropositive animals at the abattoir was far more sensitive than the culture of the spleen (Hornitzky and Searson, 1986).

Revaccination of animals in outbreaks has been carried out in Terceira since 2002, raising the resistance of animals in higher risk areas. It can be assumed that this measure helped in reducing the prevalence of infection on that particular island. This measure started on the island of S. Miguel in 2007 and is expected to be maintained.

It may be pointed out that on S. Miguel the resistance of the producers to vaccination was linked with the belief that vaccination was causing a large number of abortions, even though this supposition was not confirmed by any epidemiological or laboratory data. In 2006, the veterinary services had to implement a massive awareness campaign to increase the vaccination coverage in the island, which subsequently improved in 2006–2007 and eventually lead to a decrease in prevalence.

It can be concluded from this study that, in conditions similar to those existing in the Azores, i.e. geographically limited territories protected from external re-introduction of infection, but in which movements and contacts between herds and animals are almost out of control, the use of massive vaccination with the RB51 vaccine in combination with a strictly applied test-and-slaughter programme, as has been the case on Terceira island, could lead to eradication in a relatively short period of time. The parallel use of monthly bulk MRT and blood-testing on this island should also be noticed as a probable factor improving the sensitivity of the surveillance programme in the rapid detection of newly infected herds.

However, the examples of S. Jorge and S. Miguel show that no definitive success could be obtained in terms of prevalence reduction when RB51 is not massively and regularly applied to almost all the cattle population, particularly in areas where contacts between herds cannot be sufficiently controlled.

It is probable that the introduction of vaccination has reinforced the commitment of breeders as well as of veterinarians to correctly applying control measures. However, it can be assumed that RB51 vaccination together with these measures played a major role. Indeed, definitive success was only obtained in Terceira, the island where the vaccine was well applied together with a test-and-slaughter programme.

Finally, it should be noted that, during the study period, there was no evidence of major side effects of the RB51, either in terms of induction of cattle abortions or in the reporting of vaccine-related human cases.

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References


