Preventive Veterinary Medicine 126 (2016) 105–110

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Efficacy of antibiotic treatment and test-based culling strategies for eradicating brucellosis in commercial swine herds

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 25 January 2016
Accepted 31 January 2016

Keywords:
Brucella suis
Eradication
Reproduction ratio
Pigs
Epidemiology
Antibiotherapy

ABSTRACT

Swine brucellosis caused by *Brucella suis* biovar 2 is an emerging disease in continental Europe. Without effective vaccines being available, the European Food Safety Authority (EFSA) recommends the full depopulation of infected herds as the only strategy to eradicate *B. suis* outbreaks. Using data collected from 8 herds suffering natural swine brucellosis outbreaks, we assessed the efficacy of four control strategies: (*i*) oxytetracycline treatment only, as a default scenario, (*ii*) oxytetracycline treatment combined with skin testing and removal of positive animals, (*iii*) oxytetracycline treatment combined with serological testing (Rose Bengal test—RBT—and indirect ELISA -iELISA-) and removal of seropositive animals and (*iv*) oxytetracycline treatment combined with both serological (RBT/iELISA) and skin testing and removal of positive animals. A Susceptible-Infectious-Removal model was used to estimate the reproduction ratio (R) for each strategy. According to this model, the oxytetracycline treatment alone was not effective enough to eradicate the infection. However, this antibiotic treatment combined with diagnostic testing at 4-monthly intervals plus immediate removal of positive animals showed to be effective to eradicate brucellosis independent of the diagnostic test strategy used in an acceptable time interval (1–2 years), depending on the initial number of infected animals.

1. Introduction

Swine brucellosis due to *Brucella suis* biovar 2 is an infectious disease causing long term reproductive failure in pigs and important economic losses as well as trade restrictions. *B. suis* biovar 2 is restricted to Western and Central Europe and it is considered to have a low pathogenicity for human beings but to be the major cause of swine brucellosis (EFSA, 2009; Olsen et al., 2012), especially in pigs reared in outdoor breeding systems (Garin-Bastuji et al., 2000; Cvetnic et al., 2009; Muñoz et al., 2010). Although sporadic outbreaks in domestic pigs occur likely as spill-over from wild boar—which is the main natural reservoir (Godfroid and Käsbohrer, 2002; Godfroid et al., 2005; Cvetnic et al., 2009; EFSA, 2009)—the EU countries are considered officially free from swine brucellosis. In consequence, official surveillance in the EU is only performed for trade and semen production purposes.

As no suitable vaccines are available, the full depopulation of infected holdings is the only strategy recommended to eradicate swine brucellosis outbreaks in the EU (EFSA, 2009). However, such a strategy is both economically devastating and socially undesirable if outbreaks occur in large intensive holdings or in outdoor breeding systems based on rare endangered pig breeds. In these cases, the use of alternative control strategies based on effective antibiotic treatments combined with partial culling could be a more cost-effective and socially acceptable option (Olsen et al., 2012). Oxytetracycline administered continuously in feed minimizes the clinical impact and spread of brucellosis in infected holdings, but does not eliminate the infection (Olsen et al., 2012; Dieste-Pérez et al., 2015a). However, this antibiotic treatment, combined with the removal of infected pigs might result in an effective eradication of the disease from infected herds. Infected animals can be identified using *Brucella* O-polysaccharide (O/PS)-based serological tests such as the Rose Bengal test (RBT) and the indirect enzyme-linked Immunosorbent assays (iELISA) (EFSA, 2009; OIE, 2012; Muñoz et al., 2012). However, an important proportion of pigs in brucellosis-free holdings may show false positive results in these tests (Jungersen et al., 2006; McGiven et al., 2012; Dieste-Pérez et al., 2014, 2015b) due to infections caused by other gram-negative bacteria (*Yersinia ente-*

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http://dx.doi.org/10.1016/j.prevetmed.2016.01.033
0167-5877/

rocolitica O:9 being the most frequent) sharing common epitopes with *Brucella* O/PS (Thibodeau et al., 2001; EFSA, 2009). As an alternative, a skin test based on O/PS free *Brucella* cytosolic proteins can be used for diagnosing *Brucella* infection and differentiating these false positive serological reactions (Dieste-Pérez et al., 2014, 2015c). Comparison of such alternative control strategies in a quantitative way is of interest for decision-making regarding control of swine brucellosis outbreaks.

The reproduction ratio (R) is a suitable parameter to determine whether or not an infection will go extinct (De Jong and Kimman, 1994). If $R > 1$, the infection spreads resulting in either major or minor outbreaks; in contrast, if $R < 1$ the infection will fade (Diekmann et al., 1990; Velthuis et al., 2007). Thus R can be used as a measure to compare the efficacy of the various control measures implemented. Any effective strategy should result in R values below 1 (Velthuis et al., 2007). The aim of this study was to assess the efficacy, using estimates of R, of an antibiotic treatment given alone and in combination with several test-based culling strategies to eradicate *B. suis* biovar 2 outbreaks in swine herds.

2. Materials and methods

2.1. Background data for the estimation of model parameters

Data were available from 8 commercial multiplier Landrace × Large White herds suffering natural outbreaks of *B. suis* biovar 2 between 2008 and 2013. *B. suis* biovar 2 infection was confirmed in all herds by bacterial isolation from swabs of aborted material. Briefly, swabs were cultured on Farrell's and CITAís selective media under the incubating conditions described by De Miguel et al. (2011), and suspected colonies identified by standard (Alton et al., 1988) microbiological methods (oxidase, urease and agglutination with monospecific anti-A and anti-M sera). Bacterial DNA was extracted using QIAamp DNA minikit (QIAGEN, Hamburg, Germany) and Bruce-ladder multiplex PCR (García-Yoldi et al., 2006) was performed to identify *B. suis*. A multiplex PCR designed to differentiate between the five *B. suis* biovars (López-Goñi et al., 2011) was also used.

To estimate transmission parameters, information about herd structure, swine brucellosis apparent prevalence and diagnostic tests results were needed. Data on herd structure, i.e. average herd size, age distribution, annual replacement rate and farrowing interval, were obtained from the farmer's breeding records. Data on apparent within-herd prevalence and diagnostic tests results were obtained from veterinarianís surveillance records; surveillance was performed at different time intervals (i.e., some herds were sampled every month, others less frequently). The specific time intervals applied at each farm were taken into account in the data analysis. The main characteristics of the herds, the outbreaks as well as the control measures applied are shown in Table 1.

Briefly, after confirmation of infection an antibiotic treatment based on oxytetracycline administration to all animals as pelleted feed (2000 ppm), at approximately 20 mg/kg body weight daily (Dieste-Pérez et al., 2015a), was applied until eradication was achieved. This treatment was combined with various diagnostic tests and removal measures. In some herds (herds 1–4), a skin test with O/PS free *Brucella* cytosolic extracts (Dieste-Pérez et al., 2014, 2015c) was used to identify infected animals. In herd 8, only serological tests (RBT and iELISA, Ingezim Brucella Porcine. INGENASA S.L, Madrid, Spain) were used as described previously (Muñoz et al., 2012). In the remaining herds (herds 5–7), both serological and skin tests were used to identify infected animals. According to previous studies in similarly infected herds, diagnostic sensitivity (Se) of these tests were estimated as 100% (95% confidence interval (CI) 92.8–100) for skin test, 93.2% (95% CI 88.2–96.6) for RBT and 95.1% (95% CI 90.5–97.8) for the iELISA, while the specificities (Sp.) were 100% (95% CI 98.5–100), 98.5% (95% CI 96.8–99.5) and 99.8% (95% CI 98.6–100), respectively (Dieste-Pérez et al., 2014, 2015b). If initial prevalence within a herd was higher than 10%, only the aborting, infertile and old (after 4–5th farrowing cycle, depending on the farm) positive sows were removed at weaning. The other animals were kept and removed gradually when meeting the previous requirements. Once within-herd prevalence reached 10% or less, all positive animals in any test were removed. In herds 6 and 7 all positive animals were removed irrespective of prevalence, since within-herd prevalence in these herds was close to 10% at the beginning of the outbreak.

2.2. Modelling

2.2.1. Control strategies modelled

Four control strategies were evaluated:

(*i*) *Oxytetracycline treatment only*. This strategy did not include any further testing and it was assumed that infected sows were removed exclusively on the basis of the normal average annual replacement rates (ARR) as estimated over the 8 farms included in the study (48.7%, Table 1). This intervention was considered as a default scenario for comparison, but note that this strategy was not applied in any of the study herds.

(*ii*) *Oxytetracycline treatment combined with the removal of skin test positive animals*. This strategy was modelled with an increasing testing interval from 1 to 25 months. It was assumed that skin test positive animals were removed immediately after testing.

(*iii*) *Oxytetracycline treatment combined with the removal of RBT and/or iELISA (RBT/iELISA) positive animals.* In this strategy, both serological tests were applied in parallel and positive animals to either RBT or iELISA were removed immediately after testing. Removal rate was modelled as described in *ii.*

(*iv*) *Oxytetracycline treatment combined with the removal of RBT and/or iELISA and/or skin test positive animals (RBT/iELISA/skin test).* In this strategy, the three diagnostic tests were applied in parallel and positive animals to at least one of the tests were removed immediately after testing. Removal rate was modelled as described in *ii.*

2.2.2. Data analysis

A deterministic susceptible-infected-removed (SIR) model (Velthuis et al., 2007) was used to estimate the transmission rate of *B. suis* biovar 2 infection within the herds. In this model, susceptible sows become infected with a rate of $\beta \times S \times I/N$, where β is the transmission rate parameter, S and I are the number of susceptible and infectious sows respectively, N the herd size and thus I/N is the prevalence within a herd. Animals were classified as I when reacting positive to at least one of the tests applied. Sows were considered S if the test(s) applied showed negative results. According to previous studies and experience (Olsen et al., 2012), infected sows in intensive multiplication farms were deemed to be infectious until removal or death.

The expected number of new cases ($E(C)$) per time interval (Δt) depends directly on β, S and I/N as follows (Velthuis et al., 2003):

$$E(C) = S \times \left(1 - e^{-\beta \times \frac{I}{N} \times \Delta t}\right)$$

Based on the replacement rate in each cycle, new cases (C) were estimated as the difference between the number of infected sows observed at the end and the start of each time interval. If this difference was negative, C was set to zero. The number of infected sows that caused these new infections was estimated on the basis of within-herd prevalence at the previous time interval. Underlying assumption was that there is no age-dependent susceptibility.

Table 1
Background data from 8 farrowing herds infected naturally by *B. suis* biovar 2: herd's structure and control measures implemented.

Herds	Herd sizes	Annual replacement rates (%)	Farrowing interval (days)	Date of outbreak confirmation	Initial prevalence (%)	Date of outbreak eradication	Measures[a]
1	2350	49	152	02/2010	35	12/2013	• Oxytetracycline given continuously in feed (20 mg/kg BW/daily) • Skin test as diagnostic strategy • When prevalence was higher than 10%, aborted, infertile and old skin test positive animals were removed at weaning. When prevalence was lower than 10%, all positive animals were removed at weaning
2	2350	49	152	03/2010	42	12/2013	
3	500	47.5	156	07/2011	18	10/2013	
4	350	47.5	155	11/2008	45	06/2011	
5	1800	46	159	03/2012	35	09/2013	• Oxytetracycline given continuously in feed (20 mg/kg BW/daily) • RBT and iELISA sometimes combined with skin test as diagnostic strategy • When prevalence was higher than 10%, aborted, infertile and old positive animals to at least one test were removed at weaning. When prevalence was lower than 10%, all positive animals to at least one test were removed at weaning. In herds 6 and 7, all positive animals to at least one test were removed independently of the prevalence
6	240	54.9	150	09/2011	12	01/2013	
7	420	50	146	08/2011	8	01/2013	
8	1700	47.5	152	08/2009	>10	12/2013	• Oxytetracycline given continuously in feed (20 mg/kg BW/daily) • Only serology (RBT and iELISA) as diagnostic strategy • When prevalence was higher than 10%, aborted, infertile and old positive animals to at least one test were removed at weaning. When prevalence was lower than 10%, all positive animals to at least one test were removed at weaning

[a] RBT, Rose Bengal Test; iELISA, indirect enzyme-linked Immunosorbent assay (I.B Porcine, Ingenasa S.A, Madrid, Spain).

Data from the 8 herds were statistically analyzed with Stata (version 13.0) using a Generalized Linear Model (GLM) in order to estimate the transmission rate parameter (β) representative of all herds included in the study. The GLM was performed as a binomial process with C as the number of successes (number of new cases) and S as the number of trials (number of susceptibles), a complementary log–log link function and log ($I/N \times \Delta t$) as offset variable. The relationship between the expected value of the number of new cases and the independent variable(s) is presented in the following basic statistical model (Velthuis et al., 2003).

$$c\text{loglog}(E\left(\frac{C}{S}\right) = \log\left(\beta\right) + \log\left(\frac{I}{N} \times \Delta t\right)$$

Exponentiation of log (β) gives the transmission rate parameter β. The reproduction ratio (R) is the average number of secondary infections arising from one infected individual during its infectious period in a susceptible population (Diekmann et al., 1990). R can then be calculated by multiplying β with the average length of the infectious period T.

$$R = \beta \times T$$

R was calculated for each strategy evaluated. The infectious period (T) was estimated from the average of the length of the period that an infectious sow remained in a herd. In the default strategy, T was calculated from the survival rate based on the average of the annual replacement rate in the studied farms (being $1/0.487 \times 365 = 749$ days). For strategies *ii*, *iii* and *iv*, T was estimated from the survival rate as calculated from the testing intervals (ranging from 1 to 25 months) and of the Se of the diagnostic test(s), as follows:

$$T = \frac{1}{\text{testSe}} \times \text{testing interval(days)}$$

In order to be prudent, the lowest Se values of the serological tests were selected from previous studies (Dieste-Pérez et al., 2015b), being 93.2% and 95.1% for RBT and iELISA, respectively. According to previously published Se values (Dieste-Pérez et al., 2014) and to prevent overestimation, the midpoint of the 95% CI (96.4%) for the skin test was used in the model. For the sensitivity estimation of tests combinations, conditional dependence between tests was considered according to Gardner et al. (2000). The conditional covariance between RBT and iELISA test sensitivity was estimated as 0.009 based on Dieste-Pérez et al. (2015b) and thus sensitivity for this combination was estimated as 98.8%. Sensitivity of the RBT/iELISA/skin test combination was estimated as 100% assuming independence of skin test and serological tests.

Then, the efficacy of the control strategy was defined as the number of samplings (test rounds, n) required having less than one new infected animal in the herd's subsequent testing interval. It was calculated as:

$$I_0 \times R^n < 1 ==> n > -\frac{\ln(I_0)}{\ln(R)} \quad (5)$$

where I_0 is the number of infected animals in the herd at start of the eradication program.

3. Results

The estimated transmission rate parameter (β) of swine brucellosis under oxytetracycline treatment including

Table 2
Reproduction ratios (R) and their 95% confidence intervals (CI) for *B. suis* biovar 2 eradication strategies based on oxytetracycline treatment combined with either skin test (strategy *ii*), RBT/iELISA (strategy *iii*) or RBT/iELISA/skin test (strategy *iv*) followed by removal of positive animals, at varying testing interval (months).

Testing interval (months)	Skin test (*ii*)			RBT/iELISA (*iii*)[a]			RBT/iELISA/Skin test (*iv*)		
	R[b]	95%CI		R	95%CI		R	95%CI	
1	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0.05	0.06
2	0.12	0.11	0.12	0.11	0.11	0.12	0.11	0.11	0.12
3	0.18	0.17	0.18	0.17	0.16	0.18	0.17	0.16	0.18
4	0.24	0.22	0.25	0.23	0.22	0.24	0.23	0.22	0.24
5	0.29	0.28	0.31	0.29	0.27	0.30	0.28	0.27	0.30
6	0.35	0.34	0.37	0.35	0.33	0.36	0.34	0.33	0.36
7	0.41	0.39	0.43	0.40	0.38	0.42	0.40	0.38	0.42
8	0.47	0.45	0.49	0.46	0.44	0.48	0.45	0.43	0.48
9	0.53	0.51	0.56	0.52	0.49	0.54	0.51	0.49	0.54
10	0.59	0.56	0.62	0.57	0.55	0.60	0.57	0.54	0.60
11	0.65	0.62	0.68	0.63	0.60	0.66	0.63	0.60	0.66
12	0.71	0.67	0.74	0.69	0.66	0.72	0.68	0.65	0.72
13	0.77	0.73	0.80	0.75	0.71	0.78	0.74	0.70	0.78
14	0.82	0.79	0.87	0.81	0.77	0.85	0.80	0.76	0.84
15	0.88	0.84	0.93	0.86	0.82	0.90	0.85	0.81	0.90
16	0.94	0.90	0.99	0.92	0.88	0.96	0.91	0.87	0.95
17	1.00	0.96	1.05	0.98	0.93	1.03	0.97	0.92	1.01
18	1.06	1.01	1.11	1.04	0.99	1.09	1.02	0.98	1.07
19	1.12	1.07	1.18	1.09	1.04	1.15	1.08	1.03	1.13
20	1.18	1.12	1.24	1.15	1.10	1.21	1.14	1.08	1.19
21	1.24	1.18	1.30	1.21	1.15	1.27	1.19	1.14	1.25
22	1.30	1.24	1.36	1.27	1.21	1.33	1.25	1.19	1.31
23	1.36	1.29	1.42	1.32	1.26	1.39	1.31	1.25	1.37
24	1.41	1.35	1.48	1.38	1.32	1.45	1.36	1.30	1.43
25	1.42	1.35	1.49	1.42	1.35	1.49	1.42	1.35	1.49

[a] RBT, Rose Bengal Test; iELISA, indirect enzyme-linked Immunosorbent assay (I.B Porcine, Ingenasa S.A, Madrid, Spain).

[b] R was calculated as $R = \beta \times T$ where $T = \left(1/\text{testSe}\right) \times \text{testinginterval(days)}$. Sensitivity (Se) values used in this formula were 96.4% for skin test, 98.8% for RBT and iELISA combined in parallel and 100% for RBT, iELISA and skin test combined in parallel.

data from all 8 herds was 1.896×10^{-3} per day (95% CI $1.807 \times 10^{-3} - 1.989 \times 10^{-3}$). The reproduction ratio resulting from multiplying β by the infectious period (749 days) was 1.42 (95% CI 1.35–1.49). Reproduction ratios for strategies *ii*, *iii* and *iv* are shown in Table 2. Although no significant differences between the diagnostic strategies were present, R values slightly differ between strategies, being those of strategy *ii* greater than those of the other two strategies (*iii* and *iv*). In all cases, R increased with increasing testing interval until a maximum of 1.42, corresponding with the R for the default strategy. A testing interval below 17 months resulted in R smaller than 1 no matter the diagnostic strategy used, and as a consequence, the infection tended to fade.

The effect of the testing interval on the efficacy of culling strategies based on skin test (strategy *ii*), serological tests (strategy *iii*) or a combination of the three tests (strategies *iv*) is shown in Fig. 1 for three situations: 70, 200 and 800 infected animals at the beginning of the eradication effort.

Overall, the number of test rounds required to eradicate the infection increased with increasing testing interval and initial number of infected animals. If the testing interval was shorter than 8 months, the number of test rounds required to eradicate the infection remained under 10, no matter the initial number of infected animals (Fig. 1), and all-diagnostics-based strategies were similarly effective. If the frequency decreased, from once per 8 months to once per 17 months, the number of test rounds increased above 300 samplings (i.e. requiring more than 400 years) (results not shown). In addition, if increasing the testing interval, differences between strategies became greater; the three tests combination based strategy (*iv*) being most effective followed by the serology based strategy (*iii*) and finally the skin test based strategy (*ii*).

For a practical interpretation, the number of test rounds required to eradicate the infection in the three hypothetical situations (70, 200 and 800 infected animals at the beginning of eradication effort) is presented in Table 3. All strategies were similarly effective and only in some cases the strategy based on the removal of skin test positive animals required one more round than the other two (strategies *iii* and *iv*). Treating infect holdings with oxytetracycline and using a reasonable testing interval (i.e. 4 months), eradication could be achieved in 3–5 rounds depending on the initial number of infected sows, and no matter the diagnostic test strategy used.

4. Discussion

To the best of our knowledge, this is the first approach to estimate the efficacy of several strategies for eradicating brucellosis from swine herds using R. Since *B. suis* infection is usually lifelong (Olsen et al., 2012) and the life expectancy of sows can be calculated on the basis of annual replacement rates, the efficacy of a default strategy (oxytetracycline treatment and removal of old, infertile and aborted sows) could be determined. The reproduction ratio estimated for this default strategy was 1.42, proving that the antibiotic treatment alone is not effective enough to fully eradicate the infection. This is in close agreement with the inability of this treatment to cure the infection (Dieste-Pérez et al., 2015a) although the clinical impact of brucellosis in oxytetracycline treated holdings is reduced (Olsen et al., 2012). Thus, oxytetracycline-based treatment could be used to minimize the main clinical impact of the disease, but it should be combined with a test and removal strategy if eradication is the goal. We have demonstrated previously that the RBT, iELISA and skin tests are suitable methods for diagnosing *B. suis* biovar 2 infection (Dieste-Pérez et al., 2014, 2015b) and that combining these tests in parallel improved slightly the overall diagnostic sensitivity. This is in close agreement with the results in this paper (Table 2) showing that any of the diagnostic strategies can be effective to achieve eradication. However, if the initial within-herd prevalence is low or the infection is close to eradication after implementation of control measures, culling based on skin testing is advised as the best diagnostic strategy because its higher specificity compared to both serological tests, especially in presence of

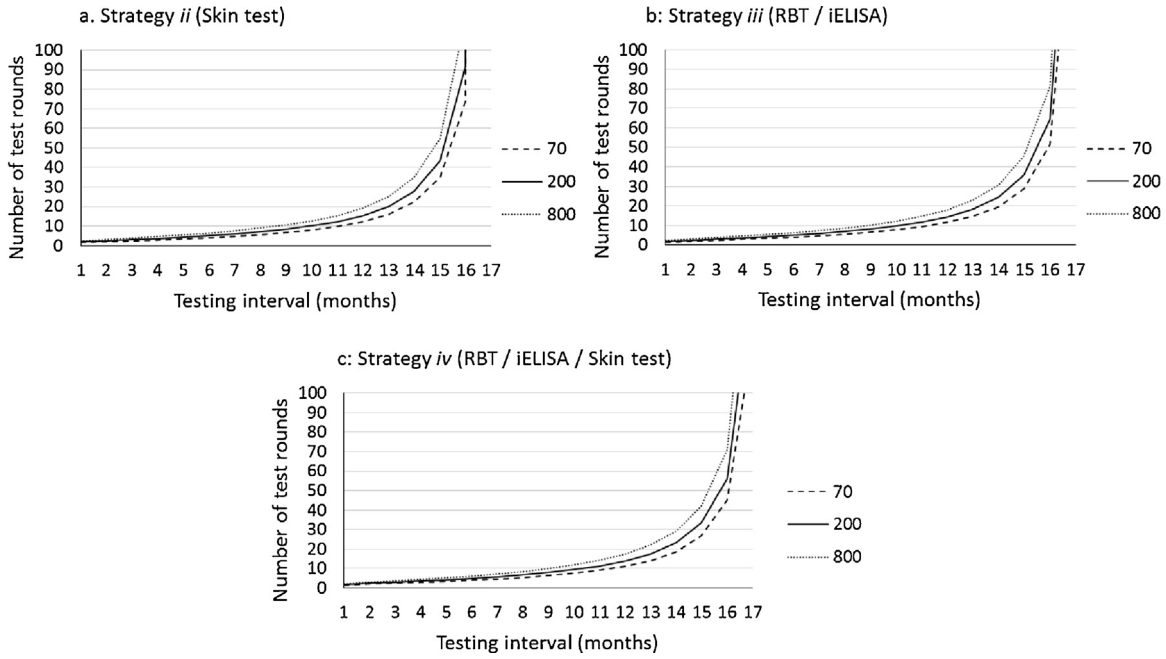

Fig. 1. Number of test rounds needed to eradicate brucellosis from a swine herd when applying oxytetracycline treatment combined with skin test (a), RBT/iELISA (b) or RBT/iELISA/Skin test (c) and removal at testing intervals varying from 1 to 17 months, at 70, 200 and 800 initial number of infected sows [1].
[1] RBT, Rose Bengal Test; iELISA, indirect enzyme-linked Immunosorbent assay (I.B Porcine, Ingenasa S.A, Madrid, Spain). The number of test rounds (n) needed to have less than one new infected animal in the herd's subsequent testing interval was calculated as $I_0 \times R^n < 1 ==> n > -\left(\ln(I_0)/\ln(R)\right)$ where I_0 is the number of infected animals in the herd at start of the eradication program and R the reproduction ratio.

false positive serological reactions (Jungersen et al., 2006; EFSA, 2009; OIE, 2012; Olsen et al., 2012; Dieste-Pérez et al., 2014).

Our results suggest that the number of test rounds required to eradicate the infection decreases when decreasing the testing interval and the initial number of infected animals. Test and removal with a 4 monthly sampling interval allows to achieve eradication in 3 rounds (equivalent to 1 year) in oxytetracycline treated herds with few number of infected animals (i.e. 70), no matter the diagnostic test used (Table 3). Moreover, when enduring situations with high number of infected animals (i.e. 800), the same testing interval still requires a relatively short period of time (less than 2 years) to achieve the eradication (5 rounds × 4 months). Due to the null (or very low) zoonotic potential of *B. suis* biovar 2 and the important reduction of abortions in infected sows treated with antibiotics, both the sanitary and economic consequences of some delay (1–2 years) in recovering the brucellosis free status are acceptable. Taking into account also the relatively high costs of each sampling round, we consider 4 months a reasonable sampling interval for implementing a suitable eradication program. However, whenever possible, shorter testing intervals can be recommended to return to free status as soon as possible.

The impact of the testing and removal measures also depends on the initial within-herd prevalence of the infection. When testing each 4 months, an outbreak with 200 initial infected animals would be eradicated in 4 tests rounds irrespective of the farm size; however, the impact of the removal of all infected animals is greater in smaller farms (i.e. 300 sows) than in big farms (i.e. 1800 sows). If farms encounter a high initial prevalence (as in the first situation), a progressive removal of aborting and old sows is advisable instead of culling all positive animals.

The environmental persistence of *Brucella* spp. is thought to be of little epidemiological significance since transmission of the infection generally requires direct or close contact with infected animals (EFSA, 2009; Olsen et al., 2012). However, the survival time of *Brucella* in soil, water and vaginal discharges or offal may increase at cold temperatures and moisture conditions up to several months

Table 3
Number of test rounds needed to eradicate brucellosis from a swine herd when applying oxytetracycline treatment combined with skin test (strategy *ii*), RBT/iELISA (strategy *iii*) or RBT/iELISA/Skin test (strategy *iv*) and removal at testing intervals varying from 2 to 8 months, at 70, 200 and 800 initial number of infected sows. Test rounds are rounded up.

Initial number infected	Testing interval (months)	Number of testing rounds per strategy[a]		
		ii	*iii*	*iv*
70	2	2	2	2
	4	3	3	3
	6	5	4	4
	8	6	6	6
200	2	3	3	3
	4	4	4	4
	6	6	5	5
	8	8	7	7
800	2	4	4	4
	4	5	5	5
	6	7	7	7
	8	9	9	9

[a] The number of testing rounds (n) needed to have less than one new infected animal in the herd's subsequent testing interval was calculated using the formula: $I_0 \times R^n < 1 ==> n > -\left(\ln(I_0)/\ln(R)\right)$ where I_0 is the number of infected animals in the herd at the beginning of the eradication program and R the reproduction ratio.

(Crawford et al., 1990; Bercovich, 1998; Castro et al., 2005). Therefore, it is advisable to perform one or two additional test rounds to detect potential re-infections from environmental origin after the first test round showing negative results. In addition to hygienic measures, selecting replacement animals from officially certified brucellosis free holdings and testing the breeding boars used for insemination are critical to avoid the introduction of infection in brucellosis free herds (Olsen et al., 2012). For these purposes, and after a previous screening with appropriate serological tests (RBT and/or well validated iELISAs), the skin test with O/PS free *Bru-*

cella cytosolic protein extracts are specific enough for an adequate diagnosis (Dieste-Pérez et al., 2015b).

In conclusion, once the impact of the disease has been minimized by antibiotic treatment, the time required to eradicate swine brucellosis will depend mainly on the testing interval and the number of infected animals at the beginning of the outbreak rather than on the diagnostic test used. The combination of antibiotic treatment and removal of test positive animals every 4 months could be a practical strategy to eradicate swine brucellosis in less than 2 years no matter the initial number of infected animals and the diagnostic test used.

Conflicts of interest

None

Acknowledgements

This research was funded by projects INIA-RTA2011-00103-00-00, consolidated group from Aragon Government A14 and supported by a Ph.D. grant (L. Dieste-Pérez) from the Aragón Government (2011–2015).

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