Comparação de quatro testes sorológicos para o diagnóstico de brucelose suína

Comparison of four serological tests for the diagnosis of swine brucellosis

Comparación de cuatro pruebas serológicas para el diagnóstico de brucelosis porcina

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Resumo

O objetivo do presente artigo é apresentar resultados de uma pesquisa na qual se fez a comparação de resultados de quarto testes sorologicos para o diagnóstico de brucelose suína em porcos de rebanhos brasileiros nos quais ocorreu o surto em relação a outros livres de brucelose. Rosa Bengala (TRB), Teste Fixação de Complemento (TFC), Teste de Aglutinação com 2-mercaptoetanol (TA+2-ME), e Teste de Polarização Fluorescente (TPF) foram utilizados para testar 333 soros (271 porcas e 62 suínos em terminação) de um rebanho com suínos infectados com *Brucella suis* e 1.100 soros de suínos livres de brucelose colhidos em um abatedouro. Considerando infectadas todas as 271 porcas do surto e interpretando os resultados do TFP de acordo com as instruções do fabricante, as sensibilidades observadas foram de 95,94% para o TFP, 94,83% para TRB, 93,73% para TFC e 92,25% para TA + 2-ME. As especificidades dos testes foram TFC e TA + 2-ME, 100%; TFP, 99,55%; e TRB 99,27%. Os resultados indicaram um bom desempenho de todos os testes e a concordância entre eles foi quase perfeita.

Palavras-chave: Polarização fluorescente; Fixação complemento; Rosa bengala; Tubo de aglutinação; *Brucella suis*.

Abstract

The purpose of this article is to present results of a research in which the results of four serological tests were compared for the diagnosis of swine brucellosis in pigs from Brazilian herds in which the outbreak occurred in relation to other brucellosis-free. Rose Bengal (RBT), complement fixation (CFT), agglutination plus 2-mercaptoethanol test (TAT+ME), and commercial fluorescence polarization assay (FPA) were used to test 333 sera (271 sows and 62 finishing pigs) from a *Brucella suis* infected swine herd and 1,100 swine sera from

brucellosis free pigs taken at a slaughterhouse. Considering infected all the 271 sows from the outbreak, and interpreting results of the FPA according to the manufacturer directions, sensitivities observed were 95.94% for FPA, 94.83% for RBT, 93.73% for CFT, and 92.25% for TAT+ME. Considering infected all the pigs from the infected herd with a positive result in at least one test, sensitivities observed were 98.92% for RBT, 97.13% for CFT, 96.06% for FPA, and 94.98% for TAT+ME. Specificities of the tests were CFT and TAT+ME, 100%; FPA, 99.55%; and RBT 99.27%. Results indicated a good performance of all the tests, and the agreement among them was almost perfect.

Keywords: Fluorescence polarization; Complement fixation; Rose bengal; Tube agglutination; *Brucella suis.*

Resumen

El objetivo de este artículo es presentar los resultados de una investigación en la que se compararon los resultados de cuatro pruebas serológicas para el diagnóstico de brucelosis porcina en cerdos de rebaños brasileños en los que se produjo el brote en relación con otros sin brucelosis. Rosa Bengala (TRB), fijación de complemento (TFC), prueba de aglutinación con 2-mercaptoetanol (TA + 2-ME) y prueba de polarización fluorescente (TPF) se usaron para analizar 333 sueros (271 cerdas y 62 cerdos de finalización) de un rebaño con cerdos infectados con Brucella suis y 1.100 sueros de cerdos sin brucelosis recolectados en un matadero. Considerando todas las 271 cerdas del brote infectadas e interpretando los resultados de la TFP de acuerdo con las instrucciones del fabricante, las sensibilidades observadas fueron 95.94% para la TFP, 94.83% para TRB, 93.73% para TFC y 92 , 25% para TA + 2-ME. Los detalles de las pruebas fueron TFC y TA + 2-ME, 100%; TFP, 99,55%; y TRB 99.27%. Los resultados indicaron un buen desempeño de todas las pruebas y el acuerdo entre ellas fue casi perfecto.

Palabras clave: Polarización fluorescente; Fijación del complement; Rosa de bengala; Tubo de aglutinación; *Brucella suis*.

1. Introduction

Brucellosis is one of the most important infectious diseases of swine due to economic losses, restriction to international trade and risk of human infection. Changes in swine management practices and sanitary programs contributed to the reduction of swine brucellosis prevalence rates, but occasional outbreaks still occur in Brazil (Meirelles-Bartoli et al., 2012).

Programs to control or eradicate swine brucellosis depend on the diagnosis of the infection, which is usually performed using serological techniques. However, serological tests usually fail in detecting all infected animals, and the bacterial culture is the most sensitive diagnostic method. Beside this, false positive results may occur mainly associated with cross-reactions induced by microorganisms that share common epitopes with smooth *Brucella* antigens (Macmillan, 1999; World Organization for Animal Health [OIE], 2009).

Several serological tests have been evaluated for the diagnosis of swine brucellosis, most of them initially developed for the diagnosis of bovine brucellosis. In Brazil, the Rose Bengal test (RBT), the complement fixation test (CFT), the standard tube agglutination test plus the 2-mercaptoethanol test (TAT+ME) and fluorescence polarization assay (FPA) are used for the diagnosis of brucellosis in cattle, and are also used for the diagnosis of swine brucellosis (Brazil, 2017).

The RBT is used as a screening test, but it lacks specificity for discriminating reaction caused by smooth *Brucella* from those caused by cross-reacting bacteria (OIE, 2009). The CFT is used in Brazil as a confirmatory test, but it has a reduced sensitivity for diagnosing *B. suis* infection and is not capable of eliminating all reactions caused by cross-reacting bacteria. OIE (2009) suggests that the CFT may be used as a complementary test for porcine brucellosis diagnosis.

The fluorescence polarization assay (FPA) was initially developed for bovine brucellosis diagnosis (Nielsen et al., 1996) and after that evaluated for the diagnosis of brucellosis in several other animal species. In swine some investigations revealed a very good performance of the test, combining high sensitivity and high specificity (Nielsen et al., 1999; Silva Paulo et al., 2000; Di Febo et al., 2012). However, investigations in chronically infected animals indicated a low sensitivity of the FPA, as well as other serological tests (Stoffregen et al., 2007; Musser et al., 2013). The FPA has also been evaluated using a Bayesian approach to estimate the sensitivity and the specificity (Praud et al., 2012).

The purpose of this article is to present results of a research in which the results of four serological tests were compared for the diagnosis of swine brucellosis in pigs from Brazilian herds in which the outbreak occurred in relation to other brucellosis-free.

2. Methodology

Research is carried out with the purpose of bringing new knowledge to society as stated by Pereira et al. (2018). In the present study of a quantitative nature, serorological research was carried out.

Serum samples

Serum samples of 333 animals (271 sows and 62 finishing pigs) came from a swine herd where *Brucella suis* biovar 1 was cultivated (Meirelles-Bartoli et al., 2012). Other 1,100 swine serum samples were taken from brucellosis free swine herds at a slaughterhouse in the state of São Paulo, Brazil. The number of samples from brucellosis free herds was determined according to OIE recommendation (Jacobson, 1998).

Serological techniques

All the serum samples were analyzed by Rose Bengal test (RBT), tube agglutination test plus 2-mercaptoethanol test (TAT+ME), complement fixation test (CFT), and fluorescence polarization assay (FPA).

RBT, TAT, ME, and CFT were carried out according to standard procedures (Alton et al., 1988). For the interpretation of SAT+ME, sera that reacted at the dilution 1:25 or above in the ME and in the TAT were considered positive; sera that not reacted in the ME but reacted at the dilution 1:50 or above in the TAT were considered inconclusive. For the CFT, sera were diluted in double dilutions from 1:2 to 1:128, and sera that reacted at the dilution 1:4 or above were considered positive.

The FPA were performed with the "*Brucella abortus* Antibody Test Kit" (Diachemix, USA), using polarimeter Sentry 100 (Diachemix, USA). Swine sera were tested at a 1:25 dilution (Silva Paulo et al., 2000). The interpretation of the results followed the manufacturer directions: samples reading up to 10 mP (millipolarization units) above the negative control were considered negative; samples reading between 10 and 20 mP above the negative control, suspect; and samples reading more than 20 mP above the negative control were considered positive. The FPA was also interpreted with a single cut off, choosing the value in mP that provided the best combination of sensitivity and specificity, obtained by the maximum sum of both values (Dohoo et al., 2009).

Data analysis

The agreement between tests was obtained through the statistic Kappa, interpreted as proposed by Landis and Kock (1977). MacNemar χ^2 was used to compare the results of tests two by two. Both analysis were performed using software R (R Core Team, 2013).

The sensitivity of the tests was calculated based on the proportion of positive results observed in animals of the infected herd, and the specificity was calculated by the proportion of negative results in animals from brucellosis free herds. Confidence intervals for sensitivity and specificity were calculated as recommended by Thrusfield (2005).

3. Results

When all the 271 sows of the infected herd from which *Brucella suis* was cultivated were considered infected, the highest sensitivity were showed by the fluorescence polarization assay, 95.94% (95% CI: 92.88% - 97.72%).

The sensitivities of the other tests were 94.83% (95% CI: 92.2% - 97.47%) for the RBT, 93.73% (95% CI: 90.48% - 96.61%) for the CFT, and 92.25% (95% CI: 89.07% - 95.43%) for the TAT+ME. Considering infected all the pigs from the infected herd with a positive result in at least one of the tests, RBT, TAT+ME or CFT, the sensitivities observed were 98.92% (95% CI: 96.89% - 99.63%) for the RBT, 97.13% (95% CI: 94.44% - 98.54%) for the CFT, 96.06% (95% CI: 93.08% - 97.78%) for the FPA, and 94.98% (95% CI: 92.42% - 97.54%) for the TAT+ME (Table 1).

Table 1 - Values of sensitivity and specificity, and confidence interval (CI), of fluorescence polarization assay (FPA), Rose Bengal test (RBT), tube agglutination plus 2-mercaptoethanol test (TAT+ME), and complement fixation test (CFT) for the serological diagnosis of swine brucellosis.

Test	Sensitivity			Specificity		
	Value (%)	95% CI	n	Value (%)	95% CI	n
RBT	94.83	92.2 – 97.47	271[1]	99.27	98.57 – 99.63	1,100[3]
TAT+ME	92.25	89.07 – 95.43	271[1]	100.0	99.65 – 100.0	1,100[3]
CFT	93.73	90.48 – 96.61	271[1]	100.0	99.65 – 100.0	1,100[3]
FPA	95.94	92.88 – 97.72	271[1]	99.55	98.94 – 99.81	1,100[3]
RBT	98.92	96.89 – 99.63	279[2]	--------------	--------------------	---------
TAT+ME	94.98	92.42 – 97.54	279[2]	--------------	--------------------	---------
CFT	97.13	94.44 – 98.54	279[2]	--------------	--------------------	---------
FPA	96.06	93.08 – 97.78	279[2]	99.54	98.92 – 99.80	1,083[3,5]
FPA[4,5]	98.84	96.64 – 99.60	258	99.38	98.72 – 99.70	1,127
FPA[4,6]	98.85	96.68 – 99.61	261	97.82	96.80 – 98.52	1,145
FPA[4,7]	97.70	95.59 – 99.18	261	99.39	98.74 – 99.70	1,145
FPA[4,8]	98.85	96.68 – 99.61	261	98.06	97.74 – 99.14	1,145

n = number of samples
[1] Considering infected all the sows of the infected herd.
[2] Considering infected all the pigs of the infected herd with a positive result in at least one of the tests RBT, TAL+ME or CFT.
[3] Considering not infected all the pigs from the slaughterhouse.
[4] True condition determined by the combination of the tests RBT, TAL+ME and CFT
[5] Discarding the suspect samples.
[6] Considering positive all the suspect samples.
[7] Considering negative all the suspect samples.
[8] Single cut off = 85.9 mP (millipolarization units).
Source: Own Research.

Considering uninfected all the pigs from the slaughterhouse, the specificities of the tests were CFT and TAT+ME, 100% (95% CI: 99.65% - 100%), FPA, 99.55% (95% CI: 98.94% - 99.81%), and RBT 99.27% (95% CI: 98.57% - 99.63%).

When evaluating the performance of the FPA interpreted according to the manufacturer directions, against the combination of the results of RBT, TAT+ME, and CFT, the sensitivity and the specificity depended on how to deal with the samples suspected in the FPA. However, the results were not very different; sensitivity varied from 97.7% to 98.85%, and specificity varied from 97.32% to 99.39% (Table 1).

The results of the FPA compared with the true condition determined by the results of RBT, TAT+ME, and CFT showed that the best cut off was 85.9 mP. In that situation, the sensitivity of the FPA was 98.85% (95% CI: 96.68% - 99.61%), and the specificity was 98.06% (95%CI: 97.74 - 99.14%), as can be seen in Table 1.

The comparisons among the tests are displayed in Table 2.

Table 2 - Comparison of the results of fluorescence polarization assay (FPA), Rose Bengal test (RBT), tube agglutination plus 2-mercaptoethanol test (TAT+ME), and complement fixation test (CFT) for the serological diagnosis of swine brucellosis.

Tests	Nº of samples	Proportion of concordance	Kappa	95% confidence interval	P (MacNemar)
RBT x TAT+ME	1,426[1]	98.95%	0.9659	0.9140 – 1.0	0.0019
RBT x CFT	1,433	98.67%	0.9599	0.9085 – 1.0	0.0059
CFT x TAT+ME	1,426[1]	99.44%	0.9815	0.9296 – 1.0	0.7237
RBT x FPA	1,410[2]	98.23%	0.9439	0.8917 – 0.9961	0.4237
TAT+ME x FPA	1,404[1,2]	98.93%	0.9653	0.9130 – 1.0	0.0388
CFT x FPA	1,410[2]	98.79%	0.9612	0.9090 – 1.0	0.1456
RBT x FPA	1,433[3]	96.93%	0.9051	0.8534 – 0.9569	0.05002
CFT x FPA	1,433[3]	97.42%	0.9189	0.8672 – 0.9706	0.000019
RBT x FPA	1,433[4]	97.98%	0.9356	0.8838 – 0.9873	0.1374
CFT x FPA	1,433[4]	98.60%	0.9547	0.9030 – 1.0	0.5023

[1] Discarding the samples inconclusive in TAT+ME.
[2] Discarding the samples suspect in the FPA.
[3] Considering positive the results suspect in the FPA.
[4] Considering negative the results suspect in the FPA.
Source: Own Research

All the comparisons revealed almost perfect agreement, with kappa above 0.90. The highest kappa, 0.9815 (95% CI: 0.9296 - 1.0) was observed in the comparison between CTF and TAT+ME discarding the inconclusive results in TAT+ME. The best agreement for the FPA, discarding suspect results, was observed with the CFT, Kappa 0.9612 (95% CI: 0.909 - 1.0). In spite of the good agreement, MacNemar test revealed significant difference between RBT and TAT+ME, discarding the inconclusive results in the TAT+ME (P = 0.0019), RBT and CFT (P = 0.0059). TAT+ME and FPA discarding the inconclusive results in the TAT+ME and the suspect in the FPA (P = 0.0388), and between CFT and FPA considering positive the results suspected in the FPA (P = 0.000019).

4. Discussion

The routine diagnosis of swine brucellosis is usually carried out using serological techniques that although are adequate as herd tests, they are not so reliable for the identification of infected individual pigs.

In spite of this consideration, all the tests evaluated in this study had shown good performance, and the agreement among than was almost perfect. Different results were obtained by Stoffregen et al. (2007) who observed lower sensitivities by using card test, which is similar to Rose Bengal test, standard tube test and fluorescence polarization assay, to diagnosis brucellosis in feral swine, enzootically infected and captured in the United States of America. According to them, the lack of sensitivity may be associated with the chronicity of infection in the animals in that study, different from the situation of the animals in the present study obtained from an acute outbreak of swine brucellosis. Musser et al. (2013) also observed feral pig in the USA that was culture positive, but serologically negative in the card test and in the FPA.

Several serological tests were evaluated for serological diagnosis of porcine brucellosis, among which, there were the tests analyzed in the present study. All tests analyzed in this investigation are used in the Brazilian sanitary program for swine and cattle brucellosis.

The FPA was originally evaluated for porcine brucellosis diagnosis by Nielsen et al. (1999) who observed a sensitivity of 93.52% and a specificity of 97.24%. For the CFT, they detected a sensitivity of 93.27% and a specificity of 95.48%. Their results are not very different from the results observed in the present investigation. In another research carried out in Argentina, a relative sensitivity of 93.8% and a specificity of 98.3% were observed. These results are not different from the performance observed in this investigation, but in sera from culture positive pigs they observed a sensitivity of 80.0% for the FPA (Silva Paulo et al., 2000). This difference may be related to the fact that in our work, we tested sera from an acute outbreak of swine brucellosis. A good performance for the FPA was also observed by Di Febo et al. (2012), who observed 100% sensitivity and specificity, and a cut off of 99.5 mP. On the contrary, Stoffregen et al. (2007) observed that the FPA detected only 26 (42.6%) from 61 culture positive feral pigs.

Praud et al. (2012) used a Bayesian approach to evaluate five serological tests for the diagnosis of swine brucellosis and observed a sensitivity of 87.6% and a specificity of 95.1% for the RBT, and a sensitivity of 93.7% and a specificity of 93.0% for the FPA, values that are

below the results observed in our investigation. However, the difference in methodology and the assumptions assumed in the model adopted by those researchers must be taken into consideration.

The complement fixation is considered a low sensitive test for diagnosing *Brucella suis* infection (OIE, 2009), but we observed a sensitivity of 93.73% considering infected all the sows of the infected herd, and a sensitivity of 97.13% considering infected the pigs of the outbreak with a positive result in at least one of the serological tests. This result is similar to that observed by Nielsen et al. (1999) who obtained a sensitivity of up to 93.27%.

The results of the present study showed values of sensitivity and specificity for the RBT, CFT and FPA that are coherent with the results observed in other investigations. Although sensitivity and specificity of a particular diagnostic test are attributes inherent to it, the values observed in the investigations may vary since studies are carried out in different conditions and examined in different animal populations. As emphasized by Stoffregen et al. (2007), a very important detail of the study is if the animals are chronically or acutely infected.

The agreement observed among the tests in our investigation was almost perfect, and the tests well performed in the condition were carried out. However, as observed by other researchers (Stoffregen et al., 2007; Praud et al., 2012), none of the evaluated serological techniques are sensitive or specific enough to be used as a single test for the individual diagnosis of swine brucellosis. This may be related to the difficulty in detecting animals in all the infection phases and to the occurrence of false positive results due to cross-reactions.

5. Conclusion

The comparison of the serological tests for the diagnosis of swine brucellosis evaluated in this investigation revealed almost perfect agreement among them, with high kappa values.

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