

Diagnosis of bovine brucellosis in bulls by seroagglutination and seminal plasma agglutination tests

Diagnóstico da brucelose bovina em reprodutores através da soroaglutinação e sêmen plasma aglutinação

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Abstract

Brucellosis is a zoonotic disease with common agent is *Brucella abortus* in bovine specie. The disease in bulls is usually asymptomatic and could have absence of anti-*B. abortus* antibody in serum. It was investigated of the occurrence of bovine brucellosis in bulls, which involved testing blood serum and seminal plasma from 177 bulls used for natural mating or as semen donors. Blood was collected from all the animals by venipuncture of the jugular vein to obtain the serum samples, while seminal plasma was obtained from the animals by electroejaculation. Samples were tested in a screening test (rose bengal test). In the second stage, the samples testing positive in the RBT were subjected to the confirmatory test (2-mercaptoethanol). None of the animals tested positive when blood serum was used. However, 5.06% of the bulls were considered reagent positive when seminal plasma was tested. It also sought to determine whether there was an association between the kind of production and the destination of animals with positive results. The calculated odds ratio indicated that bulls of the kind of production dairy/both (beef and dairy) had a higher risk of brucellosis infection. It is recommended that seminal plasma agglutination and seroagglutination techniques are applied concomitantly.

Key words: Blood serum, males, natural mating, semen donor

Resumo

Brucelose é uma zoonose que nos bovinos tem como principal agente a bactéria *Brucella abortus*. A doença nos touros é geralmente assintomática e pode haver ausência de anticorpos anti- *B. abortus* no soro sanguíneo. O presente estudo teve como objetivo principal avaliar a ocorrência da brucelose bovina em reprodutores para isso foram empregados testes oficiais tendo como material o soro sanguíneo e o plasma seminal. Foram utilizados 177 machos bovinos destinados à monta natural ou doação de sêmen. O soro sanguíneo foi obtido através de venopunção da jugular e o plasma seminal através da eletroejaculação. As amostras foram submetidas ao teste de triagem (Antígeno acidificado tamponado) e na segunda etapa, apenas as amostras positivas na triagem foram submetidas ao exame confirmatório (2-mercaptoetanol). Não houveram animais positivos quando utilizado o soro sanguíneo. Para plasma seminal houve 5,06% de touros reagentes. Avaliou-se também se sistema produtivo ou a destinação

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do animal possuíam associação com resultados positivos. O cálculo de *odds ratio* demonstrou que os reprodutores bovinos do sistema produtivo misto/leite tem maior risco de infecção para brucelose. Recomenda-se que a técnica de sêmen plasma aglutinação seja feita em conjunto com a soroaglutinação.

Palavras-chave: Soro sanguíneo, machos, monta natural, doador de sêmen

Introduction

Brucellosis is a zoonotic disease whose aetiological agent, the bacterium of the genus *Brucella* is responsible for health and economic problems, particularly in the tropics and in countries where few investments are made in beef and dairy production, where its incidence is high (MATHIAS et al., 2007). The main economic losses are related decrease in production of milk and meat, sacrifice of positive animals (SELEEM et al., 2010).

In bovine species, the most common agent is *Brucella abortus* which, in bulls, is located preferentially in the testes and accessory glands. The colonisation by *Brucella* organisms in these organs were correlated to the presence of high erythritol levels (ESSENBERG et al., 2002). *Brucella* infection causes orchitis, which may be associated with vesiculitis and epididymitis (AMIN et al., 2001), although the disease is usually asymptomatic (JUNQUEIRA JUNIOR et al., 2013).

The correct identification of animals infected by *B. abortus* is one of the foundations of a bovine brucellosis control programme (BRASIL, 2006). In Brazil, the National Program for the Control and Eradication of Bovine Brucellosis (PNCEBT) recommends the rose bengal test (RBT) as a screening test and the 2-mercaptoethanol (2-ME) test as a confirmatory test, both using blood serum as the test material. This recommendation is guided by international recommendations (OMS, 1986) and it is applied in others countries with bovine brucellosis programme.

In males, the detection of antibodies in seminal plasma may be an important tool in the diagnosis of diseases that cause reproductive disorders, given the fact that some authors (VASCONCELLOS et al., 1987; JUNQUEIRA JUNIOR et al., 2009) refer

to the possibility of the absence of anti-*B. abortus* agglutinins in seroagglutination tests, although the microorganism is present in the semen.

The Seminal Plasma Agglutination (SPA) technique is based on the application of screening (RBT) and confirmatory (2-ME) tests for the detection of immunoglobulin G (Ig) and IgA in seminal plasma, which are present due to the testicular inflammatory reaction against the agent (GRASSO; CARDOSO, 1998; AGUIAR et al., 2001; JUNQUEIRA JUNIOR et al., 2009), and may serve as a complementary tool for bovine brucellosis control and eradication programmes.

The beef cattle farming, the dairy farming or the both cattle one (beef and dairy) may be presented as a risk factor to brucellosis. Dias et al. (2009), in a study developed with cows aged over 24 months in Paraná state, Brazil, found the beef cattle farming a risk factor through a univariate analysis. Negreiros et al. (2009), in a similar study in Mato Grosso state, Brazil, pointed the beef cattle farming and the both cattle one as a risk factor through a multivariate analysis. The purpose of this study was to diagnose brucellosis in bulls based on the RBT and 2-ME tests, using blood serum and seminal plasma as analysis materials and estimated the risk factors to kind of production and bull destination.

Material and Methods

The tests were performed on samples from 177 bulls of reproductive age (above 16 months of age), of various breeds, 117 from 11 farms in Brazil and 60 from semen centers. All farms and semen centers were situated in the region of Triângulo Mineiro, Minas Gerais state, Brazil. The farms were further identified according to their kind of production, i.e., Beef, Dairy or Both. The samples

were collected between June, 2006 and August, 2009. The serological tests were performed between January and July, 2010. The work was carried out in Collaborating Centre of Agricultural Protection in Central Brazil, Faculty of Veterinary Medicine of Federal University of Uberlândia.

Blood was collected from all the animals by venipuncture of the jugular vein to obtain the serum samples, while seminal plasma was obtained from the animals by electroejaculation. The first stage of the laboratory tests consisted of the screening test (RBT, rose bengal test) of the blood serum and seminal plasma. In the second stage, the samples testing positive in the RBT were subjected to the confirmatory test (2-ME, 2-mercaptoethanol). The RBT and 2-ME tests were performed according to international standards (OIE, 2012). Only reactive animal in both test it was considered positive.

The results of the frequencies found for the variable kind of production were analysed using the chi-square test as previous described (SAMPAIO, 2002), with the aid of EpiInfo version 6.04d software (DEAN, 1994). The level of significance was set at

$p < 0.05$ to reject the null hypothesis, which states that there is no association with the risk factor. The variables were compared as follows: both (dairy and beef)/dairy and beef. Dairy and both systems were grouped together due to the number of samples. For the variable of bull destination (natural mating or semen donor), the same test was applied at the same level of significance.

Results

In the blood serum RBT, only 2 of the 177 animals were found to be reactive. In the plasma seminal RBT, 19 animals were reactive, including three of semen center. No animal was positive in same time to seroagglutination and seminal plasma agglutination.

In the blood serum 2-ME test, 2 bulls showed an inconclusive reaction, both with titres of 200 in the slow tube agglutination test (SAT) and were unresponsive to 2-ME. In the 2-ME test with seminal plasma, 9 animals tested positive, including 1 of semen center. All the test results are listed in Table 1.

Table 1. Results of animals tested for brucellosis in RBT and 2-ME tests, in Uberlândia, MG, Brazil, 2010.

Farm	No. of the bull	RBT		2-ME			
		Blood serum	Seminal plasma	Blood serum		Seminal plasma	
				SAL	2-ME	SAL	2-ME
A	11	-*	+†	N‡	N	-	-
B	15	+	-	200 I§	-	N	N
B	19	-	+	N	N	-	-
B	20	-	+	N	N	200	200
B	21	-	+	N	N	200	200
B	23	-	+	N	N	-	-
C	26	-	+	N	N	50	50
C	29	-	+	N	N	-	-
F	53	+	-	200 I	-	N	N
J	106	-	+	N	N	200	200
K	108	-	+	N	N	25	25
K	109	-	+	N	N	200	100
K	110	-	+	N	N	50	25I

Continue ...

							... Continuation	
K	111	-	+	N	N	50I	50I	
K	112	-	+	N	N	25	-	
K	113	-	+	N	N	50I	-	
K	114	-	+	N	N	100	50	
K	117	-	+	N	N	200	50	
Centre A	119	-	+	N	N	50I	-	
Centre A	125	-	+	N	N	-	-	
Centre B	148	-	+	N	N	-	-	
TOTAL	-	0	19	0	0	13	10	

*Negative; †Positive; ‡Not tested; §Incomplete reaction.

No risk factor analysis was performed for the seroagglutination test results because none of the animals tested positive. For the results with seminal plasma, the odds ratio (OR) data showed no association between the bull's destination, natural mating or semen donor. The OR analysis revealed an association between the kind of production and positive reaction, with the both/dairy showing a higher risk for the occurrence of positive bulls. All the OR results are listed in Table 2.

Table 2. Results of risk factor (Odds Ratio) for bovine brucellosis in bulls, in Uberlândia, MG, Brazil, 2010.

Variable	OR	95% CI
Bull's destination	10,59	[0,60 a 185,18]
Kind of production	19,00	[2,28 a 158,21]

Discussion

According to the OIE (OIE, 2013) and recommended in PNCEBT (BRASIL, 2006), a tested animal should be considered positive in the 2-ME test when it presents a titre equal to or higher than 25 with a complete reaction. In this experiment, no bulls evaluated based on their blood serum were considered positive. However, when seminal plasma was tested, 9 bulls were detected as positives, representing 5.08% of the animals studied. These results were different those of another Brazilian study (AGUIAR et al., 2001), which found no positive animals.

In the literature, there are few data about immunoglobulin production in reproductive tract of bulls, fewer data yet about an immune response towards *Brucella* bacteria. Campero et al. (1990) performed an experimental infection with strain 19 of *Brucella abortus*, looking to verify the antibody production in serum and semen. The infection was inoculated in the seminal vesicular route or testicles. In the blood serum, the animals presented response in the first week post inoculation; however there was a fall tendency after the third week. In semen, the Ig were detected after the second week and the positive reaction to RBT remained until the end of the experiment.

Lambert et al. (1964) described a 13 months old bull with titres of 50 in blood serum and titres of 400 in seminal plasma at SAT. Yantorno et al. (1979) showed that the postvaccinal titres in bulls remained until the 7th month in serum and until the 25th month in seminal plasma. Viana et al. (1995) performed a vaccination experiment in bulls by conjunctival route and the serologic titres were transitory and equal 50 or less to SPAT (plate agglutination test). So, the higher detection of positive animals when testing seminal plasma, added to the fact that these animals did not test positive when using blood serum, confirms the statement that blood serum contains low levels of immunoglobulins, or even the absence of immunoglobulins, even if the bacterium is present in the semen (VASCONCELLOS et al., 1987; JUNQUEIRA JUNIOR et al., 2009).

Serum prevalence studies used to characterize the bovine brucellosis situation in the Brazilian States of Minas Gerais (GONÇALVES et al., 2009), Goiás (ROCHA et al., 2009); Rio de Janeiro (KLEIN-GUNNEWIEK et al., 2009) and Bahia (ALVES et al., 2009), showed, through a multivariate logistic regression, that the purchase of bulls was a risk factor to bovine brucellosis. The results of this study serve as a warning about the commerce of bulls.

International recommendations required that animals intended for breeding purposes must have a negative serological certificate for brucellosis when they are sold to another country (OIE, 2013). Therefore, the animals that tested positive in this study could be sold without any restriction and could introduce brucellosis to farms that were previously free of the disease. Even then, the producers and semen centers had been advised to slaughter the positive animals.

The OR data showed a high risk of brucellosis in both/dairy kind of production. This information is in disagreement with the literature, which argues that beef herds are at higher risk because they contain large numbers of animals and animals are replaced more frequently, and have a significant number of problems related to health control, which influence the dynamics of disease (NEGREIROS et al., 2009).

The dairy farming in Triângulo Mineiro area, place of study, has high animal density per herd (GONÇALVES et al., 2009), high number of properties with intensive and semi-intensive management systems, which shows very close contact among the animals with higher exposition to contaminated materials and abortion remains. (NICOLETTI, 1980).

Conclusions

In view of the relevant results found in this study, it was recommended that bulls should be

subjected simultaneously to seroagglutination and seminal agglutination tests. It was also suggest that veterinarians and farm owners request blood serum and seminal plasma tests for brucellosis before purchasing animals.

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