SHORT COMMUNICATION

Detection of *Brucella* spp. DNA in the semen of seronegative bulls by polymerase chain reaction

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Introduction

Cryopreservation offers the advantage of allowing for the storage of semen for long periods and facilitating its distribution, but it may also act as a vehicle of distribution of numerous pathogens (Vinodh et al., 2008). An agent that is transmissible through semen is *Brucella abortus*, which causes orchitis and may be associated with vesiculitis and epididymitis (Amin et al., 2001). However, many animals are asymptomatic carriers, which is why cattle breeders do not see brucellosis as a cause of infertility and decline in reproduction rates.

The infection of healthy cows through artificial insemination (AI) with contaminated semen may be more frequent (Langenegger, 1992; Poester, 1997) than through natural mating. This is because the semen is deposited directly in the uterus, which contains few antibodies and defence cells, and is the preferred site for bacteria, thus making AI an important transmission route and an efficient form of dissemination of the disease in cattle herds (Brasil, 2005).

In view of the above, the purpose of this work was to detect the presence of *Brucella* spp. by polymerase chain reaction. Twenty-seven samples were found to be positive, underscoring the importance of researching brucellosis in males and the need for greater care in the selection of sperm-donating bulls for semen centres.

Material and Methods

Semen samples from 88 reproductively mature bulls were used. These bulls were reproductively mature, that is, older than 18 months, and

Keywords:
brucellosis; seminal plasma; males; bovine

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Received for publication March 14, 2012
came from three cattle-breeding farms in the state of Minas Gerais, Brazil. These farms are identified here as A, B and C, while the two semen centres are identified by the letters D and E.

The semen samples were collected between January and June, 2008. At the same time, we collected blood serum samples and they were submitted to rose bengal test. The serological tests were performed in July, 2008, and all bulls were detected as negative.

Genomic DNA was extracted from the samples of seminal plasma following the method described by Regitano and Coutinho (2001). A pair of specific primers was designed for the amplification of the VirB5 gene (Hartigh et al., 2004), amplifying a sequence of 514 base pairs (bp).

The PCR assay was performed using genomic DNA from the standard S2308 strain as positive control and the samples collected for the experiment, in a total volume of 20 µl. Each reaction contained 50 ng of DNA, 0.03 mM of MgCl₂, 0.25 pmol of primer, 0.3 mM of dNTPs and 1.5 U of taq DNA polymerase. The PCR was carried out in a gradient thermal cycler (Eppendorf Mastercycler Gradient), and the temperature cycle employed consisted of 35 cycles divided into three stages: denaturation at 95°C for 60 s, annealing at 60°C for 45 seconds and extension at 72°C for 60 s. The amplified products were examined by electrophoresis at 90 V for 1 h, separation at 0.8% agarose gel, stained with Sybr Gold (Invitrogen, Carlsbad, CA, USA) and visualized with a transilluminator under ultraviolet light. The DNA extraction and PCR test were performed between September and November, 2008.

**Results and Discussion**

Twenty-seven of the bulls were positive, representing 30.68% of the 88 tested bulls. The positive bulls comprised 14 natural mating animals and 13 semen donors. Only one other article describes the use of PCR for the diagnosis of brucellosis in male bovines (Amin et al., 2001), and the authors found only seven animals out of a total of 65 positive bulls. Their results are a cause for concern on the part of animal health authorities, because all these animals tested negative in the official Brazilian assays (Brasil, 2005) that are internationally recommended (OIE Manual, 2000). This fact clearly demonstrates the need for broader studies of this disease at cattle-breeding farms, which will increase the reliability of the expanding market of Brazilian semen exports.

The main entryway for brucellosis on cattle-breeding farms is through the purchase of infected animals. However, the use of semen for AI from contaminated donors may be a hidden and little-studied risk, infecting females in their first pregnancy to genetically valuable cows. After contracting the infection, it can be detected by serological testing of the cows, which, according to recommendations, should then be destroyed (Acha and Szifres, 2003).

Considering the findings of this study, we recommend the use of PCR as a routine assay for the detection of *Brucella* spp. in bovine seminal plasma, including animals testing negative in official tests.

**Acknowledgements**

This project was financially supported by CNPq, Fapemig and Fundect, to which we are grateful. We would also like to thank the veterinary doctors who donated the samples to this project.

**References**


