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DETECTION OF *Brucella ovis* IN OVINE FROM PARAÍBA STATE, IN THE NORTHEAST REGION OF BRAZIL

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ABSTRACT

To determine the presence of *Brucella ovis* in ovine from Paraíba State, in the Northeast region of Brazil, 80 animals slaughtered in the public slaughterhouse of Patos city were used. Before slaughter, blood samples were collected by jugular venopuncture from each animal, and after slaughter, testicles, epididymus and uterus were aseptically collected. For the serological diagnosis of *B. ovis* and *B. abortus* infections, the agar gel immunodiffusion (AGID) and Rose Bengal (RBT) tests were carried out, respectively. In addition, microbiological culture and polymerase chain reaction (PCR) were performed on testicle, epididymus and uterus samples. Six animals (7.5%) tested positive for the presence of *B. ovis* antibodies and all animals tested negative for the presence of *B. abortus* antibodies. One AGID-positive animal tested positive at uterine swab culture. PCR was able to amplify DNA of *Brucella* spp. from the pool of testicle, epididymus and uterus samples from AGID-positive animals. This is the first report of isolation and detection of *B. ovis* DNA by PCR in ovine from the Northeast region of Brazil.

Key words: *Brucella ovis*, isolation, PCR, ovine, Paraíba State

Brucellosis is an important disease of animals caused by bacterial species included in the genus *Brucella*, some being zoonotic. *Brucella ovis* is a non-zoonotic, stable rough species that produces a clinical or subclinical chronic disease of sheep characterized by testicular alterations and subsequent low fertility in rams and occasional abortions in ewes (7).

In Brazil, some attention has been given to ram epididymitis caused by *B. ovis*, particularly in the Southern states where the sheep industry is more developed (12). *B. ovis* was firstly isolated in Brazil in 1972 in the State of Rio Grande do Sul (3). More recent studies were conducted and showed that the infection is spread in some states of the country, with antibody prevalences ranging from five to 35% (5,6,9,10,11,14).

In the Northeast region of Brazil, where 58% of brazilian sheep are concentrated, *B. ovis* was never isolated. The aim of this study was to report the detection of *B. ovis* in sheep in Paraíba State by microbiological culture and polymerase chain reaction (PCR).

Eighty sheep (64 males and 16 females) slaughtered in the public slaughterhouse of Patos city (7°01´S, 37°16´W) were used during September 2005 to May 2006. Before slaughter, blood samples were collected by jugular venopuncture from each animal, and after slaughter, testicles and epididymus or

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uterus were aseptically collected. Samples were stored on ice in a cooler during transport to the Federal University of Campina Grande (UFCG), Patos, Paraíba State, Brazil. Sera were separated after clotting, centrifuged, and stored in sterile cryotubes at -20º C until further analysis. For PCR analyses, testicle, epididymus and uterus samples were also stored at -20º C.

Serological diagnosis of *B. ovis* infection was carried out using the agar gel immunodiffusion test (AGID) with lipopolysaccharides and proteins antigens from *B. ovis*, strain Reo 198 (Instituto de Tecnologia do Paraná – TECPAR, Curitiba, PR, Brazil). The Rose Bengal test (RBT) using *B. abortus* 1119-3 antigen (Instituto Biológico, São Paulo, SP, Brazil) was used for the detection of serum antibodies against smooth *Brucella*. The tests were performed according to the manufacturer's instructions.

For the isolation of *B. ovis* testicle, epididymus and uterus swab samples were cultured on enriched *Brucella* agar plates with an antibiotic mixture (4) and incubated at 37 ^{o}C under aerobic conditions for seven days. *B. ovis* colonies were presumptively identified by morphological and biochemical characteristics (1).

For the PCR analysis, two samples were used (samples 1 and 2). Each sample consisted of a pool of testicle and epididymus fragments from two males, and uterus fragment from one female, all tested positive for the presence of anti-*B. ovis* antibodies. Pools were crushed in a sterile mortar and pestle, and resuspended as 10% suspension (w/v) in ultrapure water (Milli-Q, Millipore Inc.). For the DNA extraction, the proteinase K protocol was used (13).

The PCR was performed using the primers directed to the 16S-23S rRNA interspace region of *Brucella* spp. (ITS66: ACATAGATCGCAGGCCAGTCA and ITS279: AGATACCGACGCAAACGCTAC) (8). ITS66 and ITS279 primers are specific to *Brucella* spp. and the expected size of the amplification product from *Brucella* is 214 bp. The amplification reaction mixture was prepared in a volume of 50 mL containing 200 µM of each deoxynucleoside tryphosphate, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 1.5 mM $MgCl_2$, 0.5 mM of each primer, 1.5 U platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 5 mL of template DNA. The reaction was performed in a DNA thermal cycler (MJ Research PTC 200 DNA engine, Watertown, MA, USA) without mineral oil. Ultrapure water was used as negative control and *B. ovis* strain 63/290 as positive control. After an initial denaturation at 95 ^{o}C for 2 min, the PCR profile was set as follows: 30 s of template denaturation at 95 ^{o}C, 30 s of primer annealing at 62 ^{o}C and 30 s of primer extension at 72 ^{o}C, for a total of 40 cycles, with a final extension at 72 ^{o}C for 5 min. The samples were analyzed by electrophoresis in a 2% agarose gel and then stained with ethidium bromide (0.5 mg/mL). The DNA bands were visualized under UV light.

Six animals (7.5%) tested positive for the presence of anti-*B. ovis* antibodies at AGID, with four males and two females. All animals were RBT negative. One AGID- positive animal tested positive at uterine swab culture. PCR was able to amplify DNA of *Brucella* spp. from the pool of testicles, epididymus and uterus samples from AGID positive animals (Fig. 1).

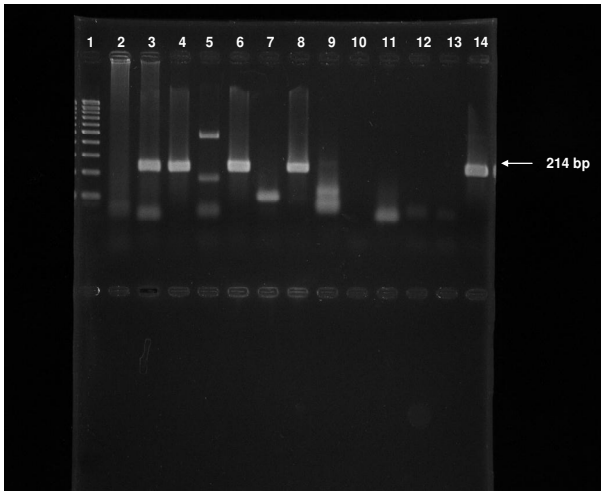

Figure 1. PCR for detection of *Brucella* spp. in pools of testicles, epididymus and uterus. Eletrophoretic separation of the amplicon into 2% agarose gel is documented across lanes 1-14. Lane 1, 100 bp DNA ladder (Invitrogen, Carlsbad, CA, USA); lane 2, pure sample 1; lanes 3-5, sample 1 diluted 1:2, 1:3 and 1:4, respectively; lane 6, sample 2 diluted 1:2; lane 7, pure sample 2; lane 8 and 9, sample 2 diluted 1:3 and 1:4, respectively; lanes 10-12, TE buffer; lane 13, ultrapure water; lane 14, *B. ovis* strain 63/290.

In the Northeast region of Brazil, serological surveys for the *B. ovis* infection were carried out in several states. In Rio Grande do Norte State, Silva *et al.* (14) used AGID test and detected 35% of seropositive rams. In Pernambuco State, Coleto *et al.* (6) found 17.5% of positive ovine at AGID test. Clementino *et al*. (5), in Sertão and Borborema mesoregions of the Paraíba State, found the prevalence of seropositive rams of 5.57% (95% CI = 3.86%-7.97%). However, *B. ovis* was never isolated from ovine in the Northeast region of Brazil. This is the first report of isolation and detection of *B. ovis* DNA by PCR in this area, and indicates that preventive measures should be taken to control and prevent the infection.

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