Molecular Identification of Brucella Abortus Bv5 and Strain 19 in Water Buffaloes (Bubalus Bubalis) in Northeast Argentina

Diana MARTINEZ[a*], Carolina THOMPSON[b], Ana RUSSO[c], Roberto JACOBO[a] and Susana TORIONI de ECHAIDE[b]

[a]Cátedra de Enfermedades Infecciosas, Facultad de Ciencias Veterinarias- UNNE. Sgto. Cabral 2189, 3400-Corrientes.[b]INTA, EEA, Rafaela Ruta 34 km 227, 2300-Rafaela, Santa Fe.[c] Facultad de Recursos Naturales-UNAF, CEDIVEF, Ruta Nac. 11 km 1164, 3600-Formosa.
*Correspondig author e-mail: demartinez@vet.unne.edu.ar

ABSTRACT

Buffalo (Bubalus bubalis) populations are spread across northern Argentina, and they share their habitat with bovines. Both species are susceptible to brucellosis, and they are under a National Plan of Control and Eradication. To characterize the Brucella spp. that infects buffaloes, the blood of 35 animals that tested positive to brucellosis by a complement fixation test was collected. DNA was obtained and analyzed by polymerase chain reaction using different molecular markers. The genera, species, and biovars of Brucella were established by analyzing specific regions of the genes omp31, eri, alkB, and omp2ab. Brucella spp. was identified in 15 of 35 tested buffaloes. The product of the omp31 gene identified the genera. The detection of two fragments of 297 bp and/or 1000 bp from the eri gene confirmed the presence of B. abortus S19 and wild-type B. abortus. The amplification of the alkB gene allowed the identification of B. abortus biovars characterized by fragments of 498 bp (bv1, bv2, or bv4). The simultaneous amplification of 498 bp (alkB) and 1000 bp (eri) products suggested the presence of B. abortus bv1, which is highly prevalent in the cattle of Argentina. Fragments of 827 bp and 857 bp were amplified from the omp2ab gene, and their sequences showed 100**%** identity with B. melitensis and B. abortus bv5 (GenBank). However, the 721 bp product (alkB) specific for B. melitensis could not be amplified. This is the first report indicating the presence of B. abortus bv5 in Latin America.

Keywords: brucellosis, water buffalo, molecular typification, Brucella abortus bv 5

INTRODUCTION

Buffaloes (B. bubalis) were introduced in northern Argentina in the first decade of the 20th century. The population is currently expanding, with more than 100,000 individuals that frequently share a habitat with bovines (Asociación Argentina de Criadores de Búfalos, 2006). Both species are susceptible to brucellosis, a zoonotic disease primarily caused by B. abortus and responsible for economic losses estimated at more than US$ 60,000,000 per year (García-Carrillo & Lucero, 1993). Since 2005, buffaloes have been included in the National Plan of Control and Eradication of bovine brucellosis based on the vaccination of female calves with B. abortus strain 19 (S19), serological diagnosis, and the slaughter of reactors (SENASA, 2005). The aim of this work was to identify and characterize Brucella spp. in buffaloes from NE Argentina using molecular markers.

MATERIALS AND METHODS

Buffalo samples

Thirty-five buffaloes that tested seropositive for brucellosis in a complement fixation test were selected from herds located in the provinces of Corrientes and Formosa in northeast Argentina. Blood samples were obtained from each buffalo and stored at -20°C until use. Genomic DNA (gDNA) was extracted using the standard phenol-chloroform-isoamyl alcohol method.

Polymerase chain reaction (PCR)

Different sets of primers were used to amplify Brucella gene fragments by PCR to discriminate species and biovars from blood samples (Table 1). The PCR mix included 0.2 mM dNTPs, 2.2 mM MgCl2, 1.25 U GoTaq polymerase (Promega), 0.8 mM primers, and 5 µl (0.05-0.1

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µg/µl) of DNA template. The PCR amplifications were started with a touch down at 64°C. The initial reaction was 94°C for 2 min, followed by 40 cycles at a variable hybridization temperature for 1 min, and 72°C 1 min. The final extension was performed at 72°C for 7 min. The hybridization temperature decreased by 1°C per cycle, from 64°C to 61°C during the first four cycles, and was maintained at 60°C for the remaining 35 cycles. All products were electrophoresed in 1.5% agarose gels, stained with 0.001 M ethidium bromide, and visualized by UV light. A wild-type (WT) B. abortus RN1 bv 1 and/or the vaccine strains S19 or RB51 were used as positive controls. PCR reagents without DNA were included as negative controls.

Polymorphism analyses

To assess the polymorphism among Brucella strains, PCR products were cloned into the expression vector pGEM-T easy, following the manufacturer's instructions (Promega). Sequence alignments were performed using Clustal W-BioEdit® (Hall, 1999). The generated nucleotide sequences were compared with other Brucella sequences stored in GenBank using a Blast analysis.

RESULTS

DNA from B. abortus was detected in the blood of 15 of 35 buffaloes analyzed. Fragments of 223 bp from omp31 were amplified from the blood of some buffaloes that were seropositive for brucellosis. The sequences showed 100% identity with the equivalent Brucella spp. gene stored in GenBank. An eri gene analysis identified sequences of 297 bp and 1000 bp that were specific for B. abortus S19 and WT Brucella spp., respectively. They were detected either as independent fragments or simultaneously (Fig. 1), and their sequences were confirmed in a Blast analysis.

B. abortus (bv1, bv2 or bv4) was identified by amplifying a 498 bp fragment from the alkB gene. The amplification of omp2ab using two pairs of primers showed single or double DNA fragments between 600 and 900 bp. Sequence analyses showed that the single bands of 827 bp (data not shown) and 857 bp (Fig. 2), amplified with DSF-DSR and DSF-DSR2, respectively, were 100% identical to those found in GenBank for B. melitensis and B. abortus bv5, respectively. Using the primers IS711-B. MEL(alkB), the 721 bp fragment of B. melitensis was not amplified from these buffalo samples, but a fragment of 180 bp common to B. abortus bv1, S19 and RB51 was generated instead. The omp2ab gene includes an insertion of 138 bp missing in the reference strain 2308 bv1 de B. abortus.

DISCUSSIONS

B. abortus S19 and WT strains were identified in buffaloes using different molecular markers. Detection of the vaccine strain was expected because the persistence of DNA from B. abortus S19 has been reported more than one year after the vaccination of bovines in NE Argentina, independent of the detection of antibodies (Draghi et al., 2010). Unlike B. abortus S19, which was identified by the eri gene (297 bp), identification of the WT strains required the amplification and sequencing of at least two genes to define the species and biovar. Because B. abortus S19 is also bv1, the simultaneous amplification of 498 bp (alkB) and 1000 bp (eri) fragments isolated from some buffaloes suggested the presence of the WT B. abortus bv1, which is highly prevalent in the cattle of Argentina (Lucero et al., 2008). The identity of B. abortus bv5 was established by the amplification of two fragments of 827 bp and 857 bp from the omp2ab gene (also common to B. melitensis). Notably, in these buffalo samples the expected 721 bp fragment of the alkB gene for B.melitensis was not amplified and another fragment of approximately 180 bp that was identical to those for B. abortus bv1, S19, and RB51 was obtained instead. B. abortus bv5 was often detected in buffaloes from Formosa simultaneously with S19, although only the latter was identified in Corrientes. This is the first report indicating the presence of B. abortus bv5 in Latin America. The epidemiological relevance of this strain in NE Argentina is unknown and must be investigated.

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