TITLE: ISOLATION AND IDENTIFICATION OF Brucella canis IN DOGS


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ABSTRACT:

Canine brucellosis is caused by Brucella canis, which is characterized as a Gram-negative and facultative intracellular bacterium. Although the clinical signs are commonly abortion and stillbirths in female dogs, as well as epididymitis and orchitis in males, these animals could present also nonreproductive signs as lymphadenopathy, splenomegaly, diskospondylitis, osteomyelitis and uveitis, as well as asymptomatic infections. Infected dogs have intermittent bacteremia that may remain for years, acting as a continuous source of infection, including human infections, which characterized the disease as zoonosis. Affected humans have no specific symptoms. The aim of this study was to isolate and identified B. canis from blood samples in dogs with brucellosis suspicious (orchitis, diskospondylitis or osteomyelitis signals) from Porto Alegre, Rio Grande do Sul, Brazil. Fifteen blood samples from the years of 2004 to 2015, collected during bacteremia, were inoculated in Brucella agar plates and incubated at 37°C for five days in aerobic atmosphere. After incubation, Modified Ziehl-Neelsen staining was performed from the grown colonies. In addition, the DNA from the bacteria was extracted by CTAB followed phenol-chloroform method. All the samples were tested by conventional polymerase chain reaction (PCR) assay using primers targeting the S711 element of Brucella species, in order to confirm the identity of B. canis in the analyzed samples. Pure culture was obtained in all agar plates after five days of incubation, and also showed positive Ziehl-Neelsen bacilli in the staining performed. At last, all the samples (100%) were positive to the amplification of a portion of the S711 conserved element. Therefore, the fifteen dogs have confirmed infection by B. canis. As prospects, we intend to analyze the insertions and deletions in the genomes of these B. canis isolates, to perform phylogenetic analysis and temporal genome modifications analysis with all the isolates assessed. In summary, our results emphasize the occurrence and circulation of this bacterium in dogs, highlighting the importance of rapid, sensitive and specific diagnostic methods to canine brucellosis.

Keywords: molecular identification, zoonosis; brucelosis

Development Agencies: CNPq