Diagnosis of Canine Brucellosis: Comparison between Serological and Microbiological Tests and a PCR Based on Primers to 16S-23S rDNA Interspace

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ABSTRACT

A pair of primers directed to 16S-23S rDNA interspace (ITS) was designed directed to Brucella genetic sequences in order to develop a polymerase chain reaction (PCR) putatively capable of amplifying DNA from any Brucella species. Nucleic acid extracts from whole-blood from naive dogs were spiked with decreasing amounts of Brucella canis RM6/66 DNA and the resulting solutions were tested by PCR. In addition, the ability of PCR to amplify Brucella spp. genetic sequences from naturally infected dogs was evaluated using 210 whole-blood samples of dogs from 19 kennels. The whole-blood samples collected were subjected to blood culture and PCR. Serodiagnosis was performed using the rapid slide agglutination test with and without 2-mercaptoethanol. The DNA from whole blood was extracted using proteinase-K, sodium dodecyl sulphate and cetyl trimethyl ammonium bromide followed by phenol–chloroform purification. The PCR was capable of detecting as little as 3.8 fg of Brucella DNA mixed with 450 ng of host DNA. Theoretically, 3.8 fg of Brucella DNA represents the total genomic mass of fewer than two bacterial cells. The PCR diagnostic sensitivity and specificity were 100%. From the results observed in the present study, we conclude that PCR could be used as confirmatory test for diagnosis of B. canis infection.

Keywords: Brucella canis, canine brucellosis, PCR, internal transcribed spacer, blood culture, rapid slide agglutination test

Abbreviations: CTAB, cetyl trimethyl ammonium bromide; ITS, interspace; PCR, polymerase chain reaction; RBPT, Rose Bengal Plate test; R-LPS, rough lipopolysaccharide; RSAT, rapid slide agglutination test; SDS, sodium docdecyl sulphate

INTRODUCTION

Brucella canis, the aetiologcal agent of canine brucellosis, is an important cause of abortions and infertility in dogs. Canine brucellosis is currently diagnosed by serodiagnosis and the most widely used serological tests are the rapid slide agglutination test (RSAT) (George and Carmichael, 1978; Carmichael and Joubert, 1987), rapid slide agglutination test with 2-mercaptoethanol (2ME-RSAT), and agar gel immunodiffusion test (Zoha and Carmichael, 1982). The 2-mercaptoethanol is used to improve specificity of the agglutination tests as
a result of the denaturation of the less specifically reacting IgM antibodies (Carmichael, 1976; Badakhsh et al., 1982).

All these tests detect mainly antibodies directed to surface antigens of *Brucella*, of which the rough lipopolysaccharide (R-LPS) is the most important. It has been established that these tests are highly accurate in identifying non-infected animals (Carmichael, 1976; Wanke et al., 2004), but they are subject to considerable interpretative error because LPS antigens of several bacterial species cross-react with *B. canis* antigens (Carmichael et al., 1984). Therefore, false-positive reactions are very common, indicating that any positive result should be confirmed by a specific test (Carmichael, 1976; Carmichael et al., 1984).

*B. canis* infection should be confirmed using a direct method of diagnosis, such as bacterial isolation. Blood is the sample of choice for microrganism isolation because of the prolonged period of bacteraemia caused by *B. canis* infection (Carmichael and Kenney, 1970; Johnson and Walker, 1992; Carmichael and Shin, 1996).

Although bacterial isolation is considered the gold standard for *Brucella* diagnosis, the polymerase chain reaction (PCR) is a good alternative to overcome some major drawbacks of bacteriological methods (Bricker, 2002; Al Dahouk et al., 2003; Navarro et al., 2004). Bacterial isolation has the disadvantage of being time-consuming since it takes about 10 days or longer for the identification of the causative agent. In addition, microbiological methods depend on the bacterial viability and, as a consequence, may pose a threat to laboratory personnel (Wallach et al., 2004).

Detection of *Brucella* spp. DNA in whole blood by PCR has already been reported in cattle, goats, bubaline and humans (Leal-Klevezas et al., 1995, 2000; Matar et al., 1996; Queipo-Ortuño et al., 1997, 1999; Morata et al., 1998, 1999; Navarro et al., 1999, 2002; Guarino et al., 2000; Zerva et al., 2001; Al-Nakkas et al., 2002, 2005; Nimri, 2003; Efalki et al., 2005a). Nevertheless, the performance of PCR for the detection of *Brucella* spp in dogs is barely known (Baek et al., 2003).

The primer pair B4 and B5 directed to the gene encoding a 31 kDa protein of *Brucella* has been widely and successfully used for PCR detection of *Brucella* infection in blood samples (Matar et al., 1996; Queipo-Ortuño et al., 1997; Morata et al., 1998, 1999, 2002; Navarro et al., 1999, 2002; Zerva et al., 2001; Efalki et al., 2005a,b). However, primers B4 and B5 also amplify DNA of *Ochrobactrum* spp. (Velasco et al., 1998; Casanas et al., 2001), which may cause bacteraemia in humans (Gransden and Eykyn, 1992).

The rDNA array of the *Brucella* genome is present in three copies, whereas the gene coding for the 31 kDa protein, in contrast, is present only once. As reported elsewhere (Navarro et al., 2002; Al Dahouk et al., 2003), a PCR system based on multiple-copy genes may be more sensitive than other PCR systems based on amplification of single-copy genes. In fact, comparison of different primers for detection of *Brucella* spp. in human blood samples has shown that primers directed to 16S rDNA are more sensitive than primers directed to 31 kDa protein coding gene (Navarro et al., 2002).

Despite the high evolution rate of the spacer region (ITS) between 16S rDNA and 23S rDNA in closely related organisms (Hillis and Dixon, 1991), ITS sequences are essentially identical within *Brucella* genus, as one can infer by sequence analysis of ITS from *B. melitensis*, *B. suis* and *B. abortus* available in molecular databases. Therefore, the ITS region was chosen to design a primer pair potentially capable of amplifying genetic sequences from any one of the six recognized species of *Brucella* without cross-reaction with *Ochrobactrum* spp.