Use of Brucella cytoplasmic proteins in the serodiagnosis of canine brucellosis (14-Aug-1999)

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Abstract

The most widely used serological method for canine brucellosis is the rapid slide agglutination test (RSAT) with the M-strain of *B. canis*, which mainly detects antibodies to the rough lipopolysaccharide (R-LPS) of the pathogen. This test has a false-positive rate of about 10% due to epitopes shared between *Brucella* R-LPS and the LPS from other bacteria. Our goal was to develop an alternative test based on *Brucella* cytoplasmic proteins which could improve the serology of canine brucellosis. We obtained an anti-LPS monoclonal antibody which was coupled to a gel matrix to obtain an immunosorbent. The cytoplasmic fraction of *B. abortus* was passed through the column so that the LPS was retained while the proteins eluted. The complex mixture of proteins, which we have termed CP, was used to develop an indirect ELISA that has shown good specificity both in human and animal brucellosis. By comparison with a cut-off calculated using sera from 100 normal dogs, the samples from 31 dogs with highly suspected brucellosis (i.e. compatible symptoms and a positive result in RSAT) were all positive against CP. A positive result was also obtained with 11 dogs having no symptoms of brucellosis but with a positive result by RSAT, which were detected during the serological control of kennels with recent episodes of abortion (2 with positive blood culture). During the serological follow-up of treated animals, anti-CP antibodies usually remained high while RSAT became negative. Notably, a positive culture was obtained in one of these dogs at the time of the serological discrepancy, which suggests that the ELISA has higher sensitivity than the RSAT to detect *B. canis* infection. The good results obtained with the CP antigen led us to investigate the potential diagnostic usefulness of particular cytoplasmic proteins. We obtained a monoclonal antibody (termed BI24) directed to a 18 kDa cytoplasmic protein present in all *Brucella* species. We developed an ELISA in which BI24 is adsorbed to the plate and used to capture the 18 kDa protein present in the CP antigen. This test was positive for all but 2 of the 31 sera from dogs with highly suspected brucellosis, and titers did not differ by more than one dilution from those obtained with CP. The 11 asymptomatic dogs detected by classical serology during kennel controls were also positive against the 18 kDa protein. When both ELISAs were used to perform a serological follow-up in some animals, the curves obtained were very similar. Recently we have obtained the 18 kDa protein in recombinant form in *E. coli* and have used it in an indirect ELISA which rendered essentially the same results that the capture ELISA with the native protein. To further define the specificity of the indirect assay we have performed a screening for antibodies to the recombinant 18 kDa protein and to R-LPS (by RSAT and ELISA) in 212 dogs attended in the Hospital for diverse health problems. Antibodies to R-LPS were detected by ELISA in 13 dogs (8.9%) and to the 18 kDa protein in 8 dogs (3.7%). Seven of the latter had titers equal or lower than 400. We are currently evaluating the clinical records of these dogs for relationships with current or past infections or diseases.