Theriogenology Question of the Month

This feature is sponsored by the American College of Theriogenologists. Readers of the *JAVMA* are invited to submit contributions. Contributions should provide a learning exercise about theriogenology. A specific question should be posed for the readers. The author's answer to the question and a brief discussion should be presented. Possible topics include commonly seen problems in domestic or exotic animals. Herd problems in dairy and beef cattle, sheep, goats, horses, and exotic hoofstock, problems in kennels or catteries, or flock problems in domestic and exotic fowl also are appropriate. Please contact Dr. Craig A. Smith, Associate Editor (800/248-2862, ext 259, or FAX 847/925-1329), for further details.

History

A 4.5-year-old sexually intact female Samoyed was admitted for a prebreeding examination. The bitch was not in estrus at the time of admission. She had failed to conceive when bred 6 months previously by use of artificial insemination with fresh semen of good quality 2 days after ovulation. Day of ovulation had been determined by serial evaluation of serum progesterone concentrations.

Serologic testing for brucellosis had been performed at the time of that previous breeding. At that time, results for the **rapid slide agglutination test (RSAT)** were weakly positive without addition of **2-mercaptoethanol (2-ME)** and negative after addition of 2-ME.[a]

Physical examination did not reveal abnormalities. Serum thyroid hormone concentrations were within reference ranges.[b] Serologic testing for brucellosis was performed, using the RSAT; test results were positive, both before and after addition of 2-ME.

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Question

What additional tests should be performed to confirm your diagnosis? Please turn the page.

Answer

Agarose gel immunodiffusion (AGID) serologic testing for brucellosis

Discussion

Serologic testing is easy to do and can be quickly performed. It can be used to identify infected dogs as soon as 8 to 12 weeks after infection until 4 to 36 months after the dogs become abacteremic.[1] Two types of serologic tests, agglutination and AGID,[c] are commonly used.

In the agglutination test, Rose Bengal-stained cell-wall antigens of *Brucella canis* are mixed with a serum sample. Agglutination of the mixture is evidence that there are IgG and IgM antibodies to those cell-wall antigens in the serum sample. Determinants of cell-wall antigens are shared among *Brucella* spp and other bacteria, including mucoid *Pseudomonas aeruginosa*, mucoid *Staphylococcus* spp, and *Bordetella bronchiseptica*.[2] There can be cross-reactions when a serum sample contains antibodies to these related cell-wall antigens, yielding false-positive results.[1] False-positives results are reported for 20 to 75% of the tests conducted.[3] Specificity may be enhanced by incubating the serum with 2-ME, a compound that breaks disulfide bonds of the large IgM molecules; those larger IgM molecules cause more nonspecific agglutination within the test than do smaller IgG molecules.[1] Agglutination tests are extremely sensitive and reportedly have a predictive accuracy of > 95% in dogs that are tested > 3 weeks after infection.[3] Test results will remain positive for as long as 30 months after the dog becomes abacteremic.

Two variations of the agglutination test exist, the RSAT and the **tube agglutination test (TAT)**. Both agglutination tests are sensitive but not specific. The TAT has an advantage over the RSAT in that it is semi-quantitative; titers of ≥ 1:200 on the TAT are considered positive for infection with *B canis*.[3,4] Current recommendations are that these assays be used as screening tests; dogs that have positive results for the RSAT performed after addition of 2-ME or the TAT performed after addition of 2-ME should be reevaluated by use of an AGID test or microbial culture of a blood sample before a definitive diagnosis is made.[5]

Agarose gel immunodiffusion tests can be performed by using cell-wall antigens or cytoplasmic antigens of *B canis*. Similar to the agglutination tests, AGID tests with cell-wall antigens may yield false-positive results. Cytoplasmic antigens are shared only among *Brucella* organisms, and because infection with *Brucella* organisms other than *B canis* is rare in dogs, few cross-reactions are evident on AGID tests that use those antigens.[1,6] The AGID test that uses cytoplasmic antigens is considered the most specific serologic test currently available.[3] The test cannot detect antibodies to *B canis* until 12 weeks after infection; however, it will detect chronically infected dogs, with results remaining positive for as long as 36 months after the dog becomes abacteremic.[4] In dogs with chronic infection, *B canis* replicates within leukocytes, and antibody titers decline.[1]

Culture of *B canis* from infected tissues (eg, aborted fetuses, fetal fluids, or placental membranes; lymph nodes) or blood of dogs is a definitive diagnostic test for brucellosis and is the only method by which infected dogs can be identified during the first 8 to 12 weeks after infection.[5] However, microbial culture is time-consuming and may yield false-negative results, especially in chronically infected dogs.[1]

Because of the intracellular location of the organism, antimicrobial treatment is unrewarding.[1,7-9] None of the antibiotic regimens are 100% successful, and the disease is generally considered to be incurable. Although the seroprevalence of brucellosis in dogs in the United States is low, the economic implications for spread of this disease in dense populations of valuable dogs, such as at kennels, dog shows, and field trials, as well as the zoonotic potential of the disease, often necessitate that infected dogs be euthanatized. It is recommended that dogs in these high-risk populations be routinely screened by use of agglutination tests, and additional testing by use of AGID tests should be conducted to identify false-positive results.

Outcome and Management

The AGID test for *B canis* was performed on an aliquot of the serum used for the agglutination test described previously. The result for the AGID test was negative, and the dog was declared not infected with *B canis*.

[a]D-Tec CB, Synbiotics Corp, San Diego, Calif.
[b]Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, Mich.
[c]Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY.

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