Evaluation of clinical usefulness of tests for canine brucellosis by calculation of predictive value

Margaret V. Root Kustritz
University of Minnesota College of Veterinary Medicine, St. Paul, MN, USA

Abstract
Canine brucellosis is an uncommon disease in many regions of the United States. Prevalence is increasing in the southeastern states and increased movement of dogs between states may increase risk to dogs elsewhere in the country. Because canine brucellosis is not curable with antibiotic therapy, euthanasia often is recommended after a positive diagnosis; making accuracy of diagnostic testing paramount. The commonly used RSAT is a good screening test for canine brucellosis. Dogs suspected to be infected with canine brucellosis, including those testing positive with the RSAT, should be tested using the AGID test. When PCR testing becomes commercially available, it will be the test of choice both for screening and differential diagnosis.

Keywords: Brucellosis, canine, AGID, PCR, RSAT

Introduction
As veterinarians, we are trained to use diagnostic tests to rule-out differentials for disease in individuals, and to screen for disease in populations. Tests differ in their sensitivity and specificity. Sensitivity is the ability of a test to accurately identify all infected animals. Specificity is the ability of a test to accurately identify all uninfected animals (Figure 1). Sensitive tests are best for screening tests because they are associated with a low false negative rate such that veterinarians will miss diagnosis and treatment of very few infected animals. However if a test is not specific, it may yield false positives; all positive results must be rechecked to ensure uninfected animals are not treated unnecessarily.

If the incidence of a given disorder in the population is known, one can determine how likely a given test is to be able to accurately identify infected individuals (positive predictive value). This is of great value in permitting differentiation between differential diagnoses. Conversely, one may wish to determine how likely a given test is to be able to accurately identify uninfected individuals (negative predictive value). A high negative predictive value for a test is valuable if it is to be used a screening test.

Canine brucellosis, caused by the bacterium Brucella canis, is a contagious, zoonotic disease that causes infertility in male and female dogs. Excellent reviews of the transmission, pathogenesis of disease, diagnosis, and control of brucellosis are available. Canine brucellosis is considered an emerging disease in some parts of the country, with increased interstate movement of dogs potentially contributing to that increase.

Dogs housed in kennels that test positive for brucellosis often are euthanized. Antibiotic therapy is described but no good long-term studies have demonstrated complete remission with antibiotic therapy. Consequences to individual dogs and to kennels are large if brucellosis is diagnosed; accuracy of diagnosis is vital.

Culture rarely is performed. Although it is definitive and may identify dogs that are negative on serologic tests, it may be difficult to identify good samples for culture. The organism is difficult to grow and there are biohazard concerns for the laboratory personnel. Serologic testing is most common, with polymerase chain reaction (PCR) tests, agarose gel immunodiffusion (AGID) testing, agglutination tests, and enzyme linked immunosorbent assay (ELISA) reported in the literature. A review of reported prevalence data and reported sensitivity and specificity data may permit better definition of which tests to use when screening populations of dogs for brucellosis.
Materials and methods
A comprehensive literature search was performed and all studies reporting prevalence data for canine brucellosis in the United States or sensitivity and specificity data for various tests for canine brucellosis were reviewed. Prevalence data by test type were used, with reported sensitivity and specificity for that test, to determine positive and negative predictive values for PCR testing of serum, semen or vaginal swabs, AGID, rapid slide agglutination test (RSAT), and tube agglutination test (TAT) with a titer of 1:200 or greater considered indicative of infection. Positive and negative predictive values were calculated as follows. Incidence was multiplied by the population size (chosen as 200) to calculate A (number of diseased animals testing positive; see Figure 1). The equation for sensitivity was used to calculate C (the number of diseased animals that tested negative). The total number of non-diseased animals was than calculated from the equation for incidence, and that total number broken down into the components of B (non-diseased animals testing positive) and D (non-diseased animals testing negative) using the equation for specificity. Positive and negative predictive values were calculated directly from the values for A, B, C, and D.

Figure 1: Calculations for sensitivity, specificity, and positive and negative predictive values

<table>
<thead>
<tr>
<th></th>
<th>DISEASED ANIMALS</th>
<th>NON-DISEASED ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE TEST RESULT</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>NEGATIVE TEST RESULT</td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

Sensitivity = A/(A+C) = true positive test results  
Specificity = D/(B+D) = true negative test results  
Positive predictive value = A/(A+B)  
Negative predictive value = D/(C+D)

Results
Reported prevalence varied by study and by diagnostic testing method used, varying from 0.2% by blood culture to 26.0% by PCR testing of serum (Table 1). Calculations for positive and negative predictive values were completed for PCR, AGID, RSAT, and TAT testing (Table 2). There were no reports of prevalence of disease in any population as measured by ELISA, nor were there any published values for sensitivity and specificity of blood culture.

Table 1: Reported prevalence of canine brucellosis using various diagnostic testing methods

<table>
<thead>
<tr>
<th>TEST TYPE</th>
<th>REPORTED PREVALENCE (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>26.0% (196)</td>
</tr>
<tr>
<td>AGID</td>
<td>3.0% (2535)</td>
</tr>
<tr>
<td>RSAT</td>
<td>6.7% (2572)</td>
</tr>
<tr>
<td>TAT with titer of 1:200 or greater</td>
<td>4.7% (3460)</td>
</tr>
<tr>
<td>Blood culture</td>
<td>0.2% (2572)</td>
</tr>
</tbody>
</table>

Discussion
Because many dogs infected with brucellosis are asymptomatic, screening is common.8,19,20 A good screening test is one with a high negative predictive value. This review suggests that most of the tests commonly used, including the RSAT and TAT (with titer of 1:200 or greater considered indicative of infection) are good screening tests. The AGID test also has a high negative predictive value. This test is available at Cornell, Florida, and Georgia only, and has a longer turn-around time than the RSAT. PCR testing has a high predictive value and will be a great boon should it become routinely available; again, turn-around time will be longer than for the RSAT.
Dogs symptomatic for brucellosis should be evaluated using a test with a high positive predictive value, to ensure brucellosis is being adequately differentiated from other diseases with a common clinical presentation. This analysis suggests that the best tests to use for diagnosis of symptomatic animals are AGID and PCR tests. PCR testing of reproductive tissues (semen or vaginal swabs) is superior to testing of serum. Again, AGID is commercially available at Cornell, Florida, and Georgia. PCR tests are not yet routinely available.

Until such time as PCR testing becomes widely commercially available, RSAT and TAT testing are good for screening populations of dogs at risk for canine brucellosis. AGID testing is the preferred confirmatory test for those testing positive at this time.

Table 2: Positive and negative predictive values of various diagnostic tests for canine brucellosis

<table>
<thead>
<tr>
<th>TEST TYPE</th>
<th>POSITIVE PREDICTIVE VALUE (%)</th>
<th>NEGATIVE PREDICTIVE VALUE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR – SERUM</td>
<td>88.1</td>
<td>100.0</td>
</tr>
<tr>
<td>PCR – SEMEN</td>
<td>100.0</td>
<td>94.5</td>
</tr>
<tr>
<td>PCR – VAGINAL SWAB</td>
<td>100.0</td>
<td>83.1</td>
</tr>
<tr>
<td>AGID</td>
<td>54.5</td>
<td>100.0</td>
</tr>
<tr>
<td>RSAT</td>
<td>20.6</td>
<td>97.8</td>
</tr>
<tr>
<td>TAT</td>
<td>15.0</td>
<td>97.9</td>
</tr>
</tbody>
</table>

References