

In: **Recent Advances in Canine Infectious Diseases**, L.E. Carmichael (Ed.)  
Publisher: International Veterinary Information Service ([www.ivis.org](http://www.ivis.org))

## **Canine Brucellosis Caused by *Brucella Canis*** (23 Nov 1999)

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### **Etiology and Epidemiology**

Canine brucellosis is caused by *Brucella canis* (*B. canis*), a rough, small, gram-negative, intracellular bacterium. Other *Brucella* species, i.e., *B. abortus*, *B. suis*, have occasionally caused canine infections but they are not discussed here. The canine brucella was first recognized in 1966 as a cause of abortions and reproductive failures and it has since been recognized in several countries. It is especially common in Central and South America, in the southern states of the United States, and has been diagnosed in commercial or research breeding kennels (beagle) in several other countries, including Japan and, more recently, in The People's Republic of China. The disease has been reported sporadically in Europe. Humans may be infected; however, dogs and other canine species are believed to be the only true hosts. Natural infections occur most commonly after ingestion of contaminated placental materials or aborted fetuses, vaginal discharges from infected bitches that are in heat or who abort, and during breeding. Following an abortion, organisms may be shed for several weeks or, intermittently, for months following an abortion. Males also may shed organisms in the urine, but bacterial numbers are relatively low, except when urine is contaminated with seminal or prostatic fluids. Prevalence data is meager, but seroprevalence rates appear high (20-30%) in Mexico and Central/South America. Estimates in the southern United States, Japan have been reported to be 7-8% in stray dogs. Food-producing animals are highly resistant. True prevalence rates are unknown and other epidemiological aspects of canine brucellosis are lacking.

### **Clinical Signs**

Clinical signs are associated principally with the reproductive tract. In females, the most prominent sign is abortion after 45-55 days of gestation in about 75% of the cases. Early embryonic death and resorption, or abortion 10-20 days after mating, may occur in some cases. These may go unnoticed and the female may present with the chief complaint of "failure to conceive". In males, the main sign is epididymitis of one or both testes, and infertility. Testicular atrophy and a moist scrotal dermatitis may be present. Semen from infected males usually contains large numbers of abnormal sperm and inflammatory cells, especially during the first 3 post-infection months. Chronically infected males may have no sperm, or reduced numbers of immature sperm. Autoimmune (anti-sperm) antibodies are present and probably contribute to male infertility. Nonspecific signs in both sexes include lethargy, loss of libido, premature aging and generalized lymph node enlargement. *B. canis* has been isolated from field cases of diskospondylitis, a condition that also has been reproduced experimentally in SPF dogs. Recurrent uveitis has been occasionally reported in infected dogs after several weeks of infection.

Infected males harbor organisms in the prostate gland and epididymides for several months. Bacteria are disseminated the seminal fluids and, occasionally, urine. *B. canis* is short-lived outside the dog and is readily inactivated by common disinfectants.

### **Diagnosis**

The diagnosis of canine brucellosis requires laboratory confirmation. Blood cultures are strongly recommended before declaring an animal infected. Serological tests which are presently available to most

veterinarians in the U.S. are imprecise since surface antigens of rough *Brucella*, such as *B. canis*, cross-react strongly with antibodies to several other nonpathogenic bacterial species. The most commonly used tests include:

### **Serological Tests**

Rapid Slide Agglutination Test (RSAT) - The RSAT requires brief treatment of serum with 2-mercaptoethanol (0.2M), and is available in the U.S. as an office screening test (D-Tec CB; Symbiotics Corp., Kansas City, Missouri, USA.). The antigen is rose bengal-stained *B. ovis*, which cross-reacts with *B. canis*. A negative slide test is strong evidence that the dog is not infected, but only about 40% of dogs whose sera agglutinate the slide test antigen are actually positive for canine brucellosis. Thus, dogs positive on the slide agglutination test should not be considered infected until additional serological tests are done -- blood cultures are always indicated because of the long and continual periods of bacteremia. More than 50% of infected dogs have a bacteremia lasting 1 year or longer. More specific antigens (*B. canis* M-) are now available (NYS Diagnostic Laboratory, Cornell University), but they have not been made commercially available (see (3) below).

Tube agglutination (TAT) and Agar Gel Immunodiffusion (AGIDcwa) Tests - These tests utilize cell wall antigens and are additional serologic methods available through diagnostic laboratories. However, those tests also are flawed by false-positive reactions and difficulties in interpretation, especially with 'early' sera or sera from chronically infected dogs. Results obtained by the RSAT, TAT, and AGIDcwa tests should be confirmed by more specific tests (below) and isolation attempts.

Improved Serodiagnostic Tests - Improved tests include: (1) A RSAT that employs a mutant strain (less mucoid, "M-") of *B. canis* that has high specificity (M-RSAT); (2) an agar gel immunodiffusion test (AGIDcpa) that employs cytoplasmic protein antigens extracted from the bacterial cytoplasm. The protein antigens are highly specific for the Genus *Brucella* and are useful in distinguishing between infected and noninfected dogs who possess antibodies that react in agglutination or AGID (cell wall antigens) tests, noted above (1 & 2). (3) enzyme-linked immunosorption tests (ELISA) that employ as antigen cell wall LPS extracts of *B. canis* M- or cytoplasmic proteins extracted from *B. abortus*. Published results indicate significant advantages of the improved tests noted above and the warrant serious consideration for further development for use in diagnostic laboratories or as veterinary office kits.

Indirect Fluorescent Antibody Test (IFAT) - An IFAT is used by several diagnostic laboratories in the U.S., but data on its accuracy have not been published. Results from Cornell University's Diagnostic Laboratory indicate a high rate of false positive reactions with the IFA test.

### **Blood Cultures**

Despite improvements in serologic diagnostic methods, blood cultures should always be performed when the disease is suspected. *B. canis* is readily isolated from the blood on tryptose or trypticase soy media for several months after infection. Cultures should be incubated aebically, since CO<sub>2</sub> is inhibitory. Detailed methods are described in the references (Alton et al, 1988). Many laboratories are insufficiently familiar with the interpretation of canine brucellosis diagnostic procedures, which has frequently resulted in the destruction of non-infected dogs solely on the basis of agglutination test results that, in fact, were false-positive reactions.

### **Prevention and Control**

Attempts to develop a suitable vaccine which would induce immunity, yet not provoke serological responses that interfere with the diagnosis, have not been successful. Presently, the development of a vaccine is considered undesirable since the brucella vaccines which have been studied offered only moderate protection and vaccinated dogs developed antibodies which would confuse the serodiagnosis. Prevention of infection and elimination of infected dogs should be the principal control strategy in kennels.

Prevention requires yearly testing of all breeding stock and the testing of all dogs to be introduced into a kennel. In the United States, the New York State Diagnostic Laboratory at Cornell University is recognized as the principal, and most reliable, testing lab. Only proved non-infected dogs should be bred. In the United States, females in breeding kennels are commonly tested by the RSAT before their expected estrus. At least 3 weeks should be allowed to perform further tests to ascertain whether a seropositive test indicates actual infection or a false-positive result. Two negative tests done at 4-6 week intervals should be required for all dogs to be introduced into breeding colonies. The two tests will detect dogs who may be incubating the

disease. If a bitch aborts, assume infection until it is proved otherwise. Bitches who abort should be kept isolated and the premises must be disinfected. If a male dog loses interest in mating, or develops testicular abnormalities and poor fertility, it should be examined for brucellosis.

**Treatment**

Treatment is not recommended for dogs in breeding kennels, and where dogs cannot be isolated and accurately monitored following antibiotic therapy. Treatment is expensive and cures are difficult to achieve, especially in chronically infected males. Repeated blood cultures and serologic monitoring is required for at least 3 post-treatment months before a dog can be declared negative. Recrudescence of the infection after the cessation of antibiotic treatment is common. Even if the organism can be successfully eliminated, males frequently remain sterile because of irreversible damage to the testes and epididymides. Spaying or castration is believed to reduce the risk of transmission from infected dogs; however, this hypothesis has not been tested experimentally and neutering does not eliminate organisms from the body. All neutered dogs should receive a course of antibiotics. The most successful and practical treatment results have been obtained with a combination of a tetracycline drug, e.g., tetracycline hydrochloride, doxycycline, minocycline, and streptomycin administered during the first 3 months of infection. More than 80% cure rates have been achieved in kennels, where dogs initially diagnosed as infected were euthanized and additional ('early') cases were treated. Unfortunately, dihydrostreptomycin is unavailable for treatment of dogs in the United States. A cure is more difficult to achieve in chronic infections.

If available, dihydrostreptomycin (10 mg/kg IM bid) is given for the initial 7 days of treatment together with a tetracycline antibiotic (25 mg/kg orally tid), which is continued for 4 weeks. During the last 7 days of tetracycline therapy, streptomycin is again given. In some instances where the first course failed, a second course of treatment has been successful. Since streptomycin is no longer available in the USA for treatment of dogs, gentamicin has been recommended by some clinicians as a substitute antibiotic. However, there is insufficient data to recommend gentamicin, and preliminary studies indicate that its efficacy is unsatisfactory, except in very early cases - i.e, in dogs infected for less than 1-2 months. Treatment is not recommended for breeding dogs, or when long-term (3 months) follow-up is unlikely. Treatment failures are especially common in infected males where organisms are commonly sequestered in the prostate gland and epididymides. Testing and elimination of infected dogs is the only proven method of eradication of *B. canis* from an infected kennel. An attempt should be made to identify the source of the infection - unfortunately, this has rarely been accomplished since breeders are reluctant to admit culpability.

Management of infected kennels/dogs is time consuming and expensive. Veterinarians must be prepared to address owners' concerns and render judicious advice, which may vary according to circumstances. Prevention is essential to avoid the ordeal of infection in a breeding kennel. As soon as canine brucellosis is diagnosed in a kennel, vigorous measures must be implemented until the disease has been eradicated. Infected kennels should be quarantined, even though most states/countries do not have formal regulations. Lack of such measures has led to widespread, even international, spread of *B. canis* infection.

Control Strategies	
<p><b>KENNEL DOGS</b></p> <ul style="list-style-type: none"> <li>• Positive dogs - euthanasia. Isolate dogs as much as possible.</li> <li>• Test sera from all dogs: Agglut/AGID tests (blood cultures of all suspicious animals).</li> <li>• <b>Euthanasia</b> - all infected dogs.</li> <li>• Test dogs monthly for 3 months until colony is negative on 2 successive tests.</li> </ul>	<p><b>PET DOGS - CHOICES DIFFICULT</b></p> <ul style="list-style-type: none"> <li>• Isolate dogs.</li> <li>• Spay or castrate + treatment.</li> <li>• Treatment uncertain; chances greater for success in early infections.</li> <li>• Follow-up serology for 3 months post-treatment.</li> <li>• Euthanasia should be considered: Uncertainty of treatment; cost great; disappointment common.</li> </ul>

### Public Health Significance

Humans are susceptible to *B. canis*, but infections are uncommon and they are usually mild. Approximately 40 cases of human infection have been reported in several countries, however the actual number is unknown since cases are rarely diagnosed, or reported. Symptoms are usually vague - prolonged febrile illness with lymph node enlargement. Most natural infections have been acquired through close contact with infected dogs. Laboratory infections also have been reported. Unlike the dog, infected humans usually respond rapidly to antibiotics (tetracyclines or tetracyclines + streptomycin).

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