Update on canine brucellosis*

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Abstract
Brucellosis continues to be ‘a diagnostician’s dilemma’ and a leading cause of canine infertility. Infections from *Brucella canis* are reported worldwide. Whether it be the classic case of an asymptomatic, healthy female who suddenly aborts her litter or the young field trial champion who develops testicular atrophy, this intracellular bacteria quietly disrupts both the pedigreed and the stray dog populations through its wide range of clinical manifestations. Diagnosis begins with a serologic screening test and continues with confirmation of the organism by blood culture or a laboratory assay with greater specificity. False negatives and positives are common with some tests. Annual serological examination and removal of infected animals remain priorities for controlling this health hazard to both dogs and humans, especially in more endemic areas of the world. Education and compliance should take place before rather than after an outbreak.

Keywords: Brucellosis, *Brucella canis*, dog, infertility

Introduction
Since the first reported case of *Brucella suis* in 1931 to a more recent case in 1986, *Brucella spp* have diminished canine reproductive potential in many countries. In 1952, *B. abortus* was isolated from an intact farm dog that was housed in a barn with cows known to have had *Brucella* abortions. In 1966, *Brucella canis* was identified as the Gram negative bacteria linked to canine abortions.5-8

Intact male and female dogs serve as reservoir hosts for this transmissible disease. Spread occurs through natural and artificial breeding, by oronasal contact, ingestion of infected discharge, and through contaminated urine and milk. With a variety or absence of prominent clinical signs, infected dogs go unnoticed until an open display of pain, an unexpected abortion or poor performance during a show is observed. An infected canine can fail to conceive following insemination with a proven sire, have palpable lymphadenopathy, develop a swollen scrotum from bacterial epididymitis or orchitis, experience noticeable testicular atrophy, or be totally asymptomatic. The advent of interstate and international movement of dogs for purchase, training and breeding has magnified the disease’s potential for transmission.9 A single pet, show dog or field trial contestant are each susceptible to this diagnostically elusive organism. Treatments with one or a combination of antibiotics can lower bacterial titers and minimize pathological damage to the dog. Neutering decreases severity by eliminating the major target organs, however, the likelihood for bacteremic relapse after surgery still puts children, pregnant women and immunosuppressed individuals at risk. The treatment of choice is removal of the dog from its facility followed by euthanasia. The kennel or premises should be disinfected and remain under quarantine until a monthly series of negative results has been completed. Prevention is accomplished through the screening of new arrivals to a kennel and negative serological tests prior to breeding or shipment. Some state regulatory agencies list canine brucellosis as a reportable disease. Importation of infected breeding stock and the inability to totally manage stray or feral dogs foster zoonosis.10

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Epidemiology

The popularity of the beagle5,11-14 as a research animal and in hunting or field trials prompted the first reports on brucellosis. Today, no breed is immune from exposure or infection.10 Any sexually mature, reproductively active dog is susceptible15,16 especially since stray and feral dogs remain mobile reservoirs for this disease.17,21

The list of regions and countries harboring *B. canis* is growing. Epidemiological studies and outbreaks in the dog and human population have affected Asia,13,21-23 Europe,24,25 Canada,26,27 Latin18,19,28 and South America.29,30 The United States has long been a recognized location for positive cases31-43 and importation of a dog from the southeastern USA led to a Canadian outbreak.44 Canine brucellosis has been found in Saskatchewan,45 Argentina,29,46 and South Africa.47 A few publications describe illness among the human population that has had close contact with family pets20,29 or infected breeding stock. Because the symptoms seen in people mimic other treatable diseases and canine brucellosis is not high on the differential problem list, reported cases in the literature are scant.

Millions of organisms are shed in a dog’s reproductive secretions via post abortion lochia, prostatic fluid, and during the estrous cycle. A common route of exposure from the infected dog to a naïve animal is through venereal transmission. Males housed for an extended period of time with a shedding kennelmate either become positive48,49 or are not infected.50 Urine is a less important route of transmission.23,48,51

Pathophysiology

*Brucella canis* organisms attach to appropriate receptors on macrophages that enter the lymphatic drainage after penetration of mucous membranes in the nose, mouth, conjunctiva and genitalia. The infection gains severity as more bacteria reach the membranes and are either engulfed or ingested. Phagocytosis persists. The newly embedded organisms move to regional lymph nodes during the next two to four weeks. Lymphadenitis of the retropharyngeal and inguinal nodes was described in one review.42 A delay in clinical signs occurs after the initial absorption of organisms until signs appear as a result of the dog’s cellular recognition of *B. canis*. The spleen undergoes enlargement by diffuse lymphoid and reticuloendothelial cell hyperplasia that produces the typical granulomatous reaction to *Brucella* organisms. The bacteria replicate within these tissues, and bacteremia develops within seven to 30 days. A persistent increase in circulating bacteria without an observed fever or clinical signs is typical for this disease. Dogs can harbor the infection for years and continue to be asymptomatic.

Steroid dependent tissues such as the reproductive organs are then targeted by this intracellular organism which results in unpredictable episodes of infertility lasting from months to several years. The disease spreads to the prostate, testicle, and epididymides in the stud dog and the fetus, gravid uterus, and placenta in the bitch. In the male, the cellular edema and lymphocytic infiltration from inflamed epididymides or testes induce a sperm granuloma from the leakage through surrounding tunics of the formerly immune privileged blood-testis barrier. Sperm agglutination and increased numbers of abnormal sperm or absence of any sperm are diagnosed clinically as oligospermia or azoospermia, respectively.52,53 Inflammation of the dog’s scrotal skin causes granulomatous, nodular dermatitis. In the female, pregnancy loss as early as 20 days suggests early embryonic death. Spontaneous abortion in a healthy pregnant bitch occurs between 45 (mid) to 59 days (late) of gestation. Unborn pups swallow the amniotic fluid and bacteria in utero41 and the female may deliver partially autolyzed, stillborn or normal pups that die within hours. Any surviving pup is bacteremic for several months. The aborted placenta shows focal coagulative necrosis of the chorionic villi, necrotizing arteritis, and numerous bacteria in the trophoblastic epithelial cells. The aborting bitch has a characteristic, persistent vaginal discharge for weeks with millions of organisms being shed per milliliter of fluid.51 *Brucella canis* has been cultured from the milk of infected lactating bitches7,54 and spread by blood transfusions, vaginoscopy, AI, or contaminated syringes.7,55 One author states that environmental conditions
of low temperature, absence of sunlight and high humidity allow extended viability of the bacteria in water, aborted fetuses, feces, equipment and clothing. Blood cultures from dogs can remain positive for five and one-half years. Once phagocytosis starts, bacteremia may be evident within two to three weeks but declines after three to four months. Some serologic responses may reflect a persistent increase of bacteria in organs and gonads, but the dog later becomes abacteremic with low agglutination titers of 1:25 or 1:50. A negative blood culture correlates with a decreased serum agglutination titer even when intracellular B. canis reservoirs remain in body tissues. Spontaneous recovery was reported in one dog within five years after the initial onset.

Clinical signs

Nonspecific signs for this disease include lethargy, fatigue or exercise intolerance during a field trial, weight loss, poor hair coat, hindlimb lameness, back pain, lymphadenopathy, and behavioral changes (i.e., not alert, poor performance of trained tasks). Signs associated with reproductive loss in females are infertility, apparent failure to conceive even with proper breeding management, early embryonic death or fetal resorption, failure to whelp, and primarily late term abortion. The bitch can abort two to three litters in succession, continue to be bred and whelp a normal litter from her next pregnancy. Canine brucellosis does not alter the exhibition of estrus.

The male has scrotal enlargement and associated pain from acute inflammation and a purulent discharge from the preputial orifice. Constant licking of the scrotum causes local moist dermatitis. A breeding soundness evaluation reveals decreased libido with reluctance to breed or failure to achieve intromission, unwillingness to allow manual collection of semen, decreased volume of ejaculate, and poor semen quality or successful ties without a pregnancy. After three months, Brucella canis can be isolated from the semen of infected dogs. After four months, head-to-head sperm agglutination appears on stained morphology slides. Chronic infection leads to uni- or bilateral testicular atrophy. No clinical sign(s) is/are pathognomonic for canine brucellosis, but the disease should always be a primary consideration in any dog examined for reproductive failure or infertility. Fever is not common because bacterial endotoxins from B. canis are absent.

Discospondylitis of thoracic and/or lumbar vertebrae may be seen on radiographs taken after owner complaints of the dog’s stiffness, visible hind limb lameness or paraspinal pain with paresis or paralysis. Infections with B. canis have been linked directly to previously treated orchitis, a case of osteomyelitis after total hip replacement, dogs with endophthalmitis and recurrent uveitis and even diagnosed in a spayed female. Canine brucellosis also produced low-grade, nonsuppurative meningitis and an abscessed uterine stump three years after a hysterectomy.

The highest risk for dogs and humans is the prolonged exposure to a viscous, serosanquinous vaginal discharge that may last from one to six weeks after an abortion. The extremely high bacterial content found in this lochia should prompt precautions against the ingestion, inhalation and direct contact by people caring for affected animals.

Diagnosis

Histories from confirmed cases include infertility, abortion, enlarged lymph nodes(s), swollen scrotum or tail of the epididymis, abnormal sperm, testicular atrophy or no apparent clinical signs. A differential diagnoses for infectious infertility includes canine herpes virus, Toxoplasma gondii, many miscellaneous microorganisms (e.g., Neospora caninum, Mycoplasma, Ureaplasma, E.coli, Streptococcus, Salmonella, Campylobacter) and, of course, Brucella canis. A definitive diagnosis requires identification of the specific bacterium from fetal tissue, a vaginal swab or discharge, blood, semen, infected vertebra, or eye lesion. A negative blood culture does not rule out canine brucellosis as the cause.
After a physical examination, samples for hematology, serum chemistry and urinalysis are submitted. As part of this disease’s diagnostic dilemma, results from these tests may be within the laboratory’s normal reference ranges. More evidence of infection is sought from a vaginal culture and cytology, vaginoscopy and transabdominal ultrasound which may also be normal. Examination of the male may reveal scrotal dermatitis, epididymitis, or irregular testicular shape and consistency caused by orchitis. If pain is elicited during palpation, further evaluation would include digital rectal examination, and testicular and prostatic ultrasonography. Attempts to collect semen may reveal diminished libido or pain. If semen collection is successful, however, the sample may have reduced sperm motility (or asthenozoospermia) with proximal and distal cytoplasmic droplets and acrosome deformities within the first two months after infection. Damage to the epithelial cells of the testis, epididymis and prostate and subsequent leakage of sperm leads to production of autoantibodies to sperm. Head-to-head agglutination of normal sperm and white blood cells are observed in the ejaculate after four months. Necrotizing vasculitis affects the testicular parenchyma. With the loss of seminiferous tubules, the anatomically defining septae shrink and produce testicular atrophy and azoospermia. Measurement of alkaline phosphatase may be beneficial to determine if ejaculation was complete and cystocentesis could rule out retrograde ejaculation. Fine-needle aspiration or testicular biopsy should be delayed until results of serological tests and culture are known. If laboratory tests identify an infected dog, further medical or surgical treatment is not recommended.

Radiographs will reveal unifocal or multifocal inflammation of intervertebral disks in the case of discospondylitis. Skeletal limb abnormalities are imaged for detection of osteomyelitis. Ultrasonography can be used to detect stump pyometra and ophthalmologic examination would reveal uveitis.

**Serology**

*Brucella abortus, suis, and mellitensis* react with smooth surface antigens. *B. canis* and *ovis* agglutinate with rough surface antigens. The availability, sensitivity and specificity of serological tests varies. False negatives occur, and false positives happen in chronic cases, therefore, additional testing is recommended. Direct techniques for the diagnosis of *B. canis* are blood culture, blood polymerase chain reaction (PCR), or PCR of semen or vaginal swabs. Testing is more accurate near or during estrus since the bacteremia is elevated under hormonal influence.

A commercially available rapid slide agglutination test (RSAT) or card test (D-Tec® CB, Synbiotics Corp., San Diego, CA, USA) is used as a screening test and provides results in minutes. The RSAT is considered highly sensitive (i.e., detects truly infected) but not specific (i.e., difference between true positive and true negative). False negatives are rare but false positives do occur owing to the cross reaction with surface antigens of *Bordetella, Pseudomonas, Moraxella*-type organisms and other gram-negative bacteria. If the screening test result is negative, the dog is unlikely to have brucellosis. A modified screening test adds 2-mercaptoethanol (2-ME) to inactivate IgM and thereby increase the specificity of the test. A dog can remain bacteremic for years and maintain a positive agglutinin titer. An elevated titer identified by a tube agglutination test (TAT) is positive two to four weeks following exposure. A titer of 1:200 or greater is semiquantitative evidence of an active infection and a good correlation has been seen with the recovery of the organism from a blood culture obtained at the same time. Animals with titers less than 1:200 should be retested in two weeks.

The agar gel immunodiffusion test (AGIDcwa) confirms suspected cases using a cell wall or a cytoplasmic protein antigen from *B. canis* or *abortus*. The AGIDcwa is a highly sensitive test. Reaction is positive at eight to 12 weeks after infection and negative results are seen either early or from three to four years after infection. The sensitivity of the indirect fluorescent antibody (IFA) test is sometimes uncertain and some infected dogs have gone
undetected. The ELISA test uses a common cytoplasmic antigen for *Brucella* spp. but no commercial tests are marketed.

Because *B. canis* evokes little humoral antibody response, some serological tests lack sensitivity. Advances in molecular biology and DNA enzyme replication have improved detection of *B. melitensis* in bovine and *B. ovis* in ovine semen. Primers for the PCR allow a rapid, sensitive and specific method for testing prior to natural mating, artificial insemination or travel. Positive results were reported in whole semen collected from stud dogs that were negative when screened by serological, microbiological and blood PCR tests. Seropositive dogs that were negative by blood culture may be in a different phase of bacteremia or in a later stage of infection when antibodies are still present but bacteria are absent or reduced in number. The PCR has been utilized for detection of the organism in blood, semen and vaginal swabs. Another molecular technique of cellular fatty acid profiling (CFAP) has been examined as a possible tool to monitor outbreaks related to the interstate dog trade. Chronically infected dogs can be seronegative or abacteremic. False results from infected dogs will mislead owners and, therefore, these dogs can be potential shedders in breeding kennels.

**Bacterial culture**

A positive identification of the causative organism from blood culture is the definitive proof for brucellosis. Blood cultures may yield results as early as two to four weeks post infection with dogs remaining bacteremic for years. The number of organisms circulating in the leukocyte portion of the blood is often small, therefore, multiple samples of whole blood may be required. A delay in serum titer reaction is possible for eight to 12 weeks after exposure, and the titer can fluctuate even with persistent bacteremia. This fastidious organism may not be detected in a blood sample if the animal has received previous antibiotic therapy. The stage of disease is not reflected in the magnitude of the titer. Since bacteremia occurs during the first two weeks of infection, the sensitivity of tests may decrease during the early weeks as antibody production changes from IgM to IgG. Organisms can be isolated from milk, vaginal fluids after abortion, placental and fetal tissue, semen, lymph nodes, bone marrow, urine, eye lesions or a uterine stump.

**Treatment**

*Brucella canis* is sensitive to a select group of antibiotics because of its intracellular location and its residence in multiple sites and tissues. Some drug choices result in medical failures and allow relapses. When the bacteria are phagocytized and held within cells, single antibiotic regimes are usually not curative. One author did report continued fertility in dogs following the oral use of enrofloxacin for a month. Antimicrobials do not eradicate the organism nor are they completely efficacious, but improvement or cessation of clinical signs can occur. The combination of tetracyclines (i.e., tetracycline HCl, chlorotetracycline, doxycycline, minocycline) and dihydrostreptomycin was the classic choice. Due to the unavailability of dihydrostreptomycin and the need for repetitive intramuscular administration, gentamicin has been substituted if renal function is evaluated beforehand. Rifampin has been suggested for its intracellular activity, but side effects are possible. Any combination of drugs should warrant a review of interactions and safety especially if the dog is receiving other medications, special diets, or supplements. The requirement for extended treatment increases the owner’s expense and leads to declining compliance and inconsistent results. Two or three courses of antibiotic therapy followed by serological testing are necessary, and serological rechecks should continue indefinitely. Antibiotics will reduce the number of bacteria, but negative serologic test results may lead to a false assumption that the disease has been cured. Once antibiotic treatment is stopped, a relapse is possible and the antibody titer rises again as bacteria harbored in lymph nodes and spleen replicate and are released.
**Prevention**

Any dog that tests positive must not be bred. The dog should be isolated and removed from the kennel by euthanasia. Monthly tests are performed on all remaining dogs until every positive dog has been removed. Any new dog should be isolated from the resident population for one month and have two negative tests before being placed with other breeding stock. It is safe to repopulate a kennel after all test results are negative. If a positive animal is retained, it should be spayed or neutered and given long-term antibiotic treatment. Screening tests and intermittent physical examinations could detect bacteremia, but dogs can have a positive titer for several years.

Separation in kennel housing by partial walls or wire fences that allow contact is inadequate. Stray and feral dogs can infect unsupervised dogs through incidental mating or exposure and, therefore, contact must be controlled. Females that abort should be isolated immediately and kept away from other dogs for weeks or until signs of vaginal discharge have cleared. Her whelping area should be disinfected with quaternary ammonium, 1% sodium hypochlorite or bleach, or an iodophor solution. *B. canis* does not survive in sunlight or at high temperatures. Gloves and protective clothing are worn during examinations and while working in the bitch’s quarters. Females that have aborted a litter may subsequently whelp a normal litter but can transmit the bacteria to their offspring. Older pups from potentially infected litters should be tested.

The loss from an outbreak includes the animals that are euthanized, the sizeable expense of diagnostic tests, and the inability to breed, show or move any animal during a three month quarantine. Dogs intended for breeding should be tested annually, even if artificial insemination is used. The same diagnostic laboratory should be used for sample submission to maintain consistency and dialogue.

**Public health**

Transmission of *Brucella canis* to humans is underdiagnosed worldwide. Because of its infrequent occurrence and protean manifestations, the medical profession may not consider the disease during the initial examination of a patient. If the disease is not detected initially, a positive response does occur following treatment with most antibiotics. *Brucella* causes undulant fever in humans or non-specific signs of recurrent fever, headache and weakness, which may mimic many other human diseases. Laboratory workers have tested positive after being exposed to a less virulent M-strain that is used as an antigen for serological tests. One pet owner became infected through close contact with the family dog. Another case involved a 15-year-old boy who was admitted to a hospital for a viral infection after developing persistent fever, weakness, adenopathy and enlarged spleen. One month later, *B. canis* was isolated from a blood culture after it was learned that the patient had been in close contact with three dogs, one being a stray.

Canine brucellosis has greater impact on people who are immunosuppressed (e.g., cancer patients, people infected with HIV or transplant recipients), children and pregnant women. Complications from the disease include ocular lesions, endocarditis, and fevers of unknown origin. A heightened awareness of *B. canis* infections among the human population could prevent contact and potential transmission as more cases are being described in the literature and by physicians. The appropriate serological tests should be included in the evaluation of humans exhibiting symptoms consistent with canine brucellosis and a history of contact with intact dogs.

**References**

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