Canine Brucellosis Management

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KEYWORDS
• Brucellosis • *Brucella canis* • Infertility • Abortion

Infertility in dogs is a growing concern in breeding kennels. There are a number of bacteria, viruses, and husbandry practices that must be considered to determine the cause of decreased litter sizes, abortions, weak puppies, and lack of pregnancy, but brucellosis should be at the top of the differential list.

Brucella canis, the causative agent of canine brucellosis, is the leading cause of infertility in domestic canids, more specifically, breeding kennels worldwide.[1] This small, rough, gram-negative coccobacillus intracellular bacterium[2] was first isolated by Leland Carmichael in 1966.[1–3] It has had a huge impact on the canine breeding industry economically, costing some clients tens of thousands of dollars in loss of litters and breeding stock, veterinary and diagnostic costs, and reputation, in this author's experience.

B canis is an intracellular bacterium that has a predilection for steroid-producing tissues such as the testicles, epididymi, and prostate of male dogs and the uterus of female dogs. In addition to these tissues, this bacterium will also be found in the eyes, spinal column, liver, spleen, and lymph nodes on a regular basis. Due to this, canine brucellosis may be manifested as infertility as well as chronic, poorly responding uvelitis,[4,5] discospondylitis within the thoracic and lumbar vertebrae,[6–9] and meningitis.[10] These other clinical signs may be seen in spayed and neutered pets that may never present for infertility issues.

EPIDEMIOLOGY

In recent years, this disease appears to becoming more prevalent in breeding kennels across the country. Oklahoma alone has seen an increase in the domestic dog population from 2% in 1994 through 1995 to 13% in 2002 through 2003, with numbers continuing to rise today.[11] This may be due to the growing number of breeding kennels; the buying, selling, and trading of infected dogs; and the increased incidence of semen shipped around the country and world. Some reports indicate that stray and feral dogs are predominant reservoirs of the bacteria,[1,12] which may be the case in many Third World countries, but recent research from northern Oklahoma in which stray dogs from a local shelter were tested indicates that less than 2% of the stray population are serologically positive with none of the dogs having been culture positive (Makloski and colleagues, unpublished data).

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TRANSMISSION

This bacterium primarily enters the body through contact of the genital, oronasal, and conjunctival mucosa but may also enter though skin lesions. The most common mode of transmission is venereal, although dogs can become infected when they are exposed to or ingest infected fetal membranes, aborted fetuses, vulvar discharge, or urine from infected dogs.[13–15] Artificial insemination will protect male dogs from contracting the disease from infected females, but this reproductive technique will not protect the female if inseminated with infected semen. Many of the commonly used commercial semen extenders do not inhibit the growth of *B canis* even after cooling to 37°F for 5 days in some cases (Makloski and colleagues, unpublished research). Some puppies, if not infected in utero, which will most likely occur, can become infected by ingesting milk from lactating females as the somatic cell count is normally very high in canine milk[13] and this is an intracellular bacterium. Although it is rare, transmission can occur via saliva and tears.[16]

Infection can also occur via fomites such as water and food bowls, equipment, and clothing. This bacterium may survive in the environment for several months in conditions of high humidity, low temperatures, and no sunlight, especially if organic material is present. *B canis* can also withstand drying and can survive in dust and soil.[17]

CLINICAL SIGNS

The most common clinical sign associated with *B canis* is infertility. It is important to collect a thorough history from the owner and determine if there are actually fertility issues or if poor management is the culprit. In many cases, poor management may lead to a canine brucellosis outbreak in breeding kennels.

In the female, outward signs of *B canis* infection are limited. The classic symptom of canine brucellosis in the bitch is a late-term abortion (45–55 days' gestation), resulting in the birth of stillborn puppies that are often autolysed, having subcutaneous edema, congestion and hemorrhage of the subcutaneous abdominal region, serosanguinous peritoneal fluid loss with focal infiltration of lymphoid cells, and degenerative lesions in the liver, spleen, kidneys, and intestines[16] (**Fig. 1**). The bitch will continue to excrete vulvar discharge with high numbers of bacteria for several weeks after the abortion or parturition.[3] If the puppies survive, they may be weak and

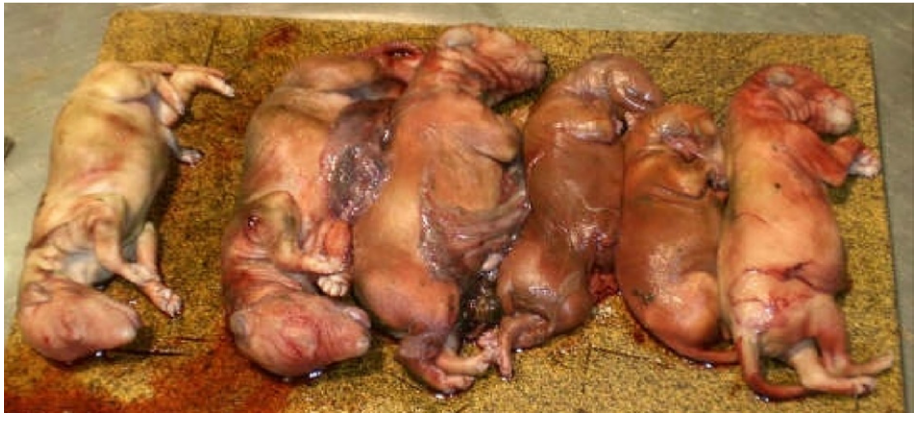

Fig. 1. Puppies from a late-term abortion (56 days' gestation).

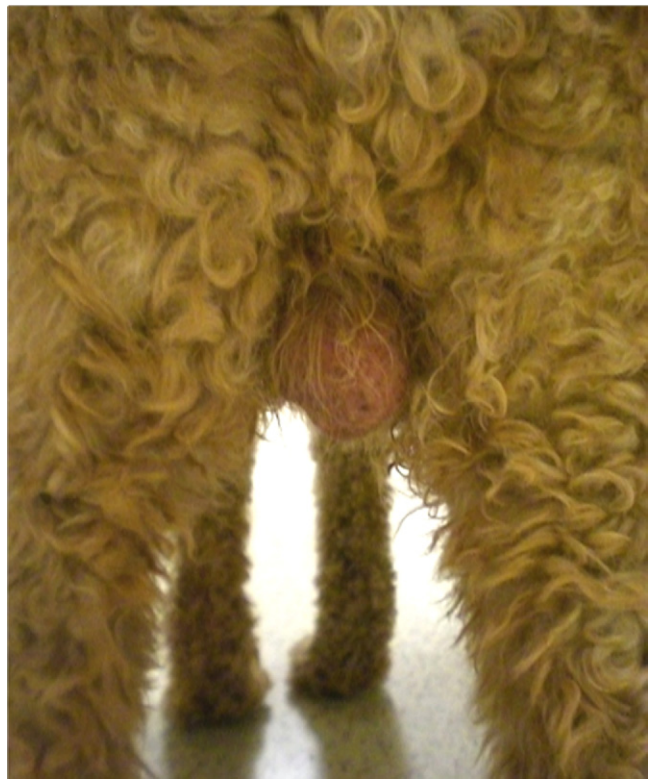

Fig. 2. Scrotal asymmetry. Note the stud dog's left testicle is smaller than the right testicle. Further diagnostics revealed a small atrophied left testicle and epididymis and the right testicle was small with an enlarged epididymis (epididymitis).

die within a few hours or weeks of birth. Some apparently normal puppies will survive but show clinical signs or test positive for the disease as they age, sometimes waiting until puberty.[18] Females may also exhibit embryo resorption or conception failure.[16]

Males may have more obvious signs of *B canis*. During the acute stages of the disease, many male dogs may have epididymitis, which results in swelling of the epididymis and leads to pain and discomfort in the scrotum. This may lead to licking of the scrotum, then scrotal edema, dermatitis, and scrotal asymmetry in unilateral cases (**Fig. 2**). Chronically, the epididymis will decrease in size, as will the testes. Orchitis is an infrequent clinical sign but will result in testicular necrosis[19] (**Figs. 3** and **4**). Testicular damage initiates the development of antisperm antibodies that may be found in the blood and prostatic fluid at about 11 to 14 weeks postinfection. Autoagglutination of the sperm can then be visualized starting at approximately 18 weeks postinfection.[20,21] *B canis* also localizes in the prostate of the male, which may lead to classic clinical signs of prostatitis (**Fig. 5**), including enlarged and painful prostate and difficulty urinating and defecating.

In addition to these clinical signs, clinicians and owners may observe chronic, unresponsive uveitis, discospondylitis, and low-grade meningitis as previously discussed.

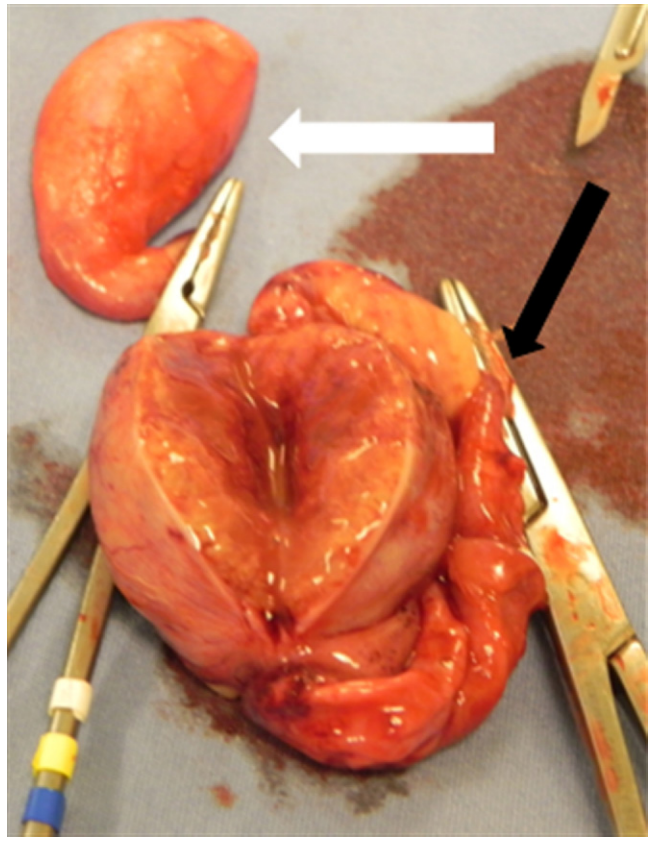

Fig. 3. Testicles that where removed due to illness, pain, and scrotal asymmetry. The patient had tested positive on RSAT prior to surgery. The confirmatory AGIDcpa was also positive and culture of the testicular tissues indicated that the testicle was infected with *B canis*. Note the normal-sized testicle indicated by the white arrow and the necrotic tissue of the affected testicle indicated by the black arrow. The spermatic cord is also engorged, as well as the epididymis.

DIAGNOSIS

Diagnosis of canine brucellosis can be difficult, and as diagnosticians, veterinarians cannot rely solely on one testing modality. The most common historical finding is infertility, but veterinarians must remember that many patients are adopted from shelters or purchased from breeding kennels as a family pet and may be spayed or neutered, so there is no known history of infertility in these cases. A thorough physical exam is necessary to gain basic information on vision, weight, locomotion, discharge, or any palpable swellings. Routine blood work and urinalysis may be collected but are often unremarkable in this disease.

Positive blood culture is a definitive diagnosis for *B canis*. Dogs are generally bacteremic starting 4 to 6 weeks after oronasal exposure and may remain bacteremic for 1 to 5 years.[22] The number of organisms in the circulating leukocytes may be low, making multiple samples necessary. Previous antibiotic therapy may make culturing difficult. Tissues from canine abortions, vaginal discharge, semen, lymph node, and bone marrow aspirates and urine are also great areas to collect culture samples. Due

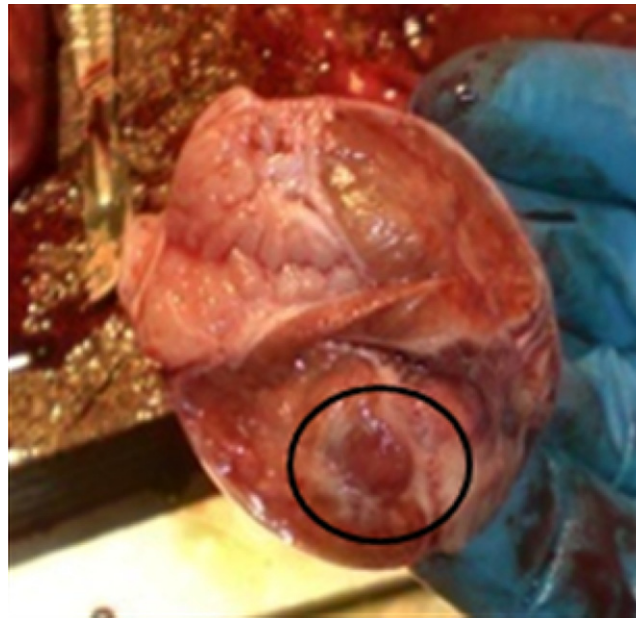

Fig. 4. Testicular abscess infected with *B canis*.

to the slow growth rate, bacterial overgrowth, and the intracellular component of this bacterium, a negative culture does not rule out the disease (**Fig. 6**).

The use of real-time polymerase chain reaction (PCR) will detect the DNA of the *B canis* organism, whether it is alive or dead.[23,24] This is an area where bacterial cultures are limited. Only live organisms may grow and replicate on culture media. If there are not enough live organisms, then the bacterial culture may be considered negative, but the patient may be harboring the organism. PCR diagnostic testing is a new tool that may be used to diagnose *B canis*. Semen, vaginal swabs, uterine swabs, and urine are appropriate samples to submit for PCR. Whole blood can also be submitted, but due to the limited time of bacteremia, this may not be an adequate sample.[25]

Serologic testing in the dog can be very challenging but can be helpful in screening for the disease. *B canis* has a rough, not smooth, plasma membrane as *B abortus, B melitensis,* and *B suis* possess.[2] The surface antigens of this bacterium make serologic tests highly sensitive, but the specificity is low, making the occurrence of false-positive results very high. Given this information, it has come as a surprise that a significant amount of false-negative results have also been encountered.[1] This may be due to the limitations of the serologic and microbiologic tests, but it may also be due to recent or chronic infection.

The serology tests include the rapid slide agglutination test (RSAT; developed in 1974), which is a rapid commercially available countertop diagnostic test that can be used in-house for a quick diagnosis or screening (**Fig. 7**). Results can be available within 2 minutes. The RSAT may cross-react with antibodies from *Bordetella, Pseudomonas, Moraxella*-type organisms, and other gram-negative bacteria. To decrease some of this cross reaction, 2-mercaptoethanol (2-ME) drops are added to increase the specificity of the RSAT; this is often referred to as

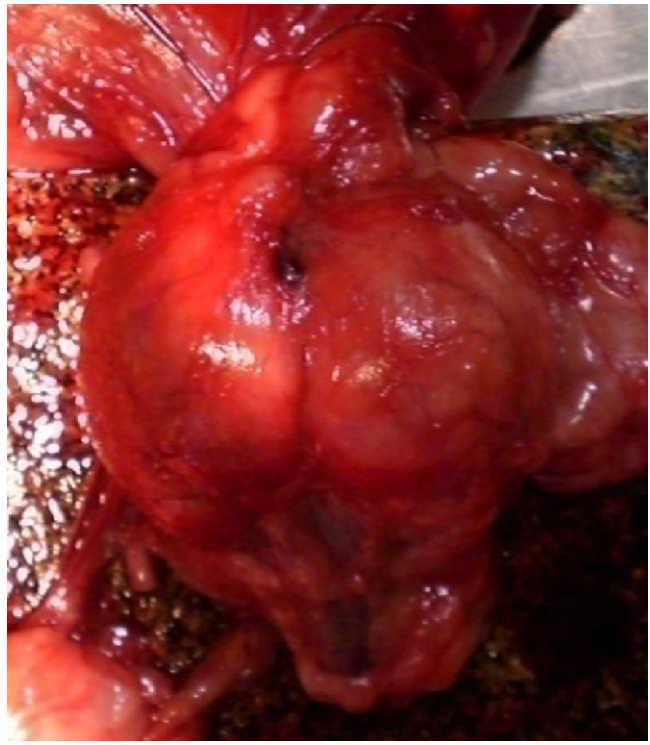

Fig. 5. Canine prostate from an infected stud dog.

the 2-ME RSAT. The tube agglutination test (TAT) detects antibodies in the serum and can be quantitative; samples with titers less than 1:200 should be retested in 2 weeks. The agar gel immunodiffusion test is used to confirm positive results from the RSAT, 2-ME RSAT, and TAT. There are 2 types of agar gel immunodiffusion tests—the first is the cell wall antigen test and the second is the more specific cytoplasmic protein antigen test. Both of these tests are more specific than the RSAT, 2-ME RSAT, and TAT and should be used to confirm any positive results before taking action.

There are 2 other types of serology tests that have been used: the indirect fluorescent antibody (IFA) test and enzyme-linked immunosorbent assay (ELISA). The IFA sensitivity is uncertain so some infected dogs may go undetected with this test. In research, the ELISA is more specific than the IFA and can detect positive dogs within 30 days of infection.[1] Unfortunately, there are no labs conducting this test commercially in the United States at this time.

B suis[26] and *B abortus* have infected dogs when the animals ingest contaminated fetal membranes or fluid or an aborted fetus.[1] These *Brucella* spp are smooth bacteria and do not cross-react with the traditional serologic tests generally used to diagnose *B canis*.

In addition to the discrepancies in the types of diagnostic tests, there is also a substantial lag time between the initial exposure and infection to seroconversion and/or a positive blood culture: 8 to 12 weeks and 4 to 6 weeks, respectively. Bacteremia may last for 1 to 5 years, while chronically infected dogs may remain

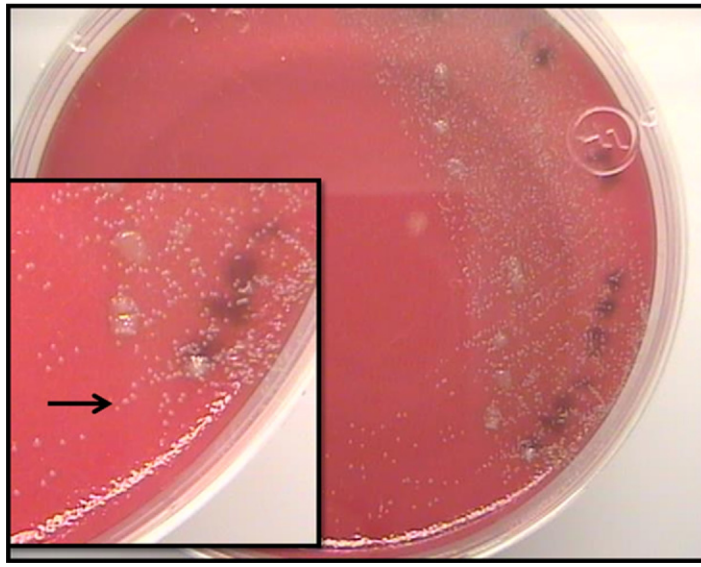

Fig. 6. Slow-growing culture and small colonies.

serologically positive for 5 years or more before dropping below detectable levels.[27] Although the chronically infected dog may be serologically negative, the organism may still be harbored within lymph nodes, liver, spleen, prostate, or other reproductive tissues and may recrudesce at anytime.

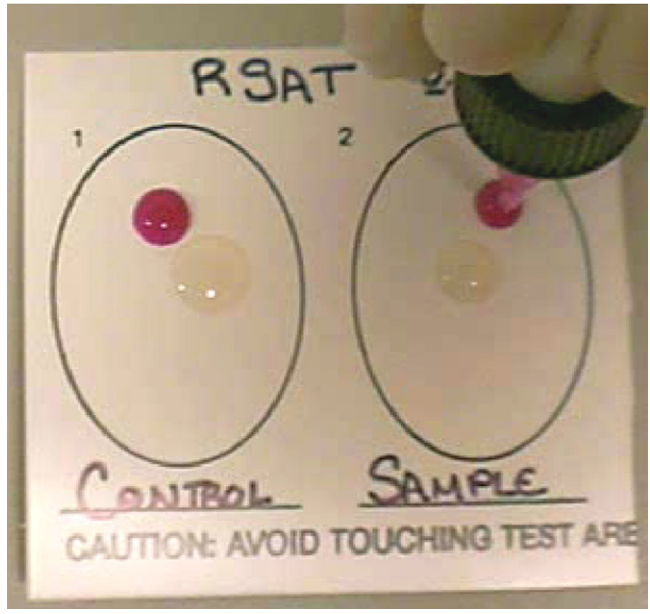

Fig. 7. RSAT (Card test).

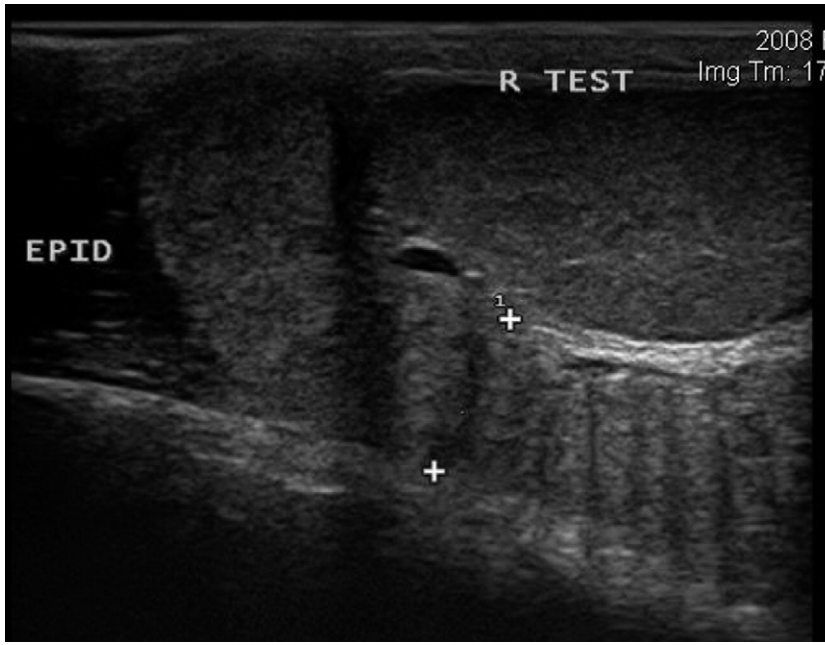

Fig. 8. Testicular ultrasound revealing epididymitis.

Diagnostic imaging such as radiography and ultrasound are modalities that can be used frequently in veterinary clinics today and may reveal lesions that may be suspicious for *B canis*. Such lesions may include unifocal or multifocal areas of inflammation of the intervertebral spaces that do not appear to affect vertebrae.[9] Occasionally, some soft tissue abnormalities in the female, such as stump pyometra,[27] may be seen on radiography or ultrasound. Real-time ultrasound of the male reproductive tract may reveal inflammation or lesions within the testicles, epididymi, or prostate (**Figs. 8** and **9**). These lesions do not provide a definitive diagnosis but should prompt the examiner to pursue further serologic or culture diagnostics.[1]

TREATMENT

Quarantine of the facility will be necessary during an outbreak and treatment. In some states, this may be state mandated, but in many, this is a voluntary quarantine. This quarantine would include that there are no new canine additions, that no dogs should be sold or relocated from the premises, and that all breeding should be suspended until the quarantine is lifted. It may be necessary to quarantine positive dogs from suspect and negative dogs on the same premises. In this event, it would be necessary to follow strict guidelines to not carry the disease from one dog to the next. This would include separate feeding and watering dishes, caring for the negative dogs first then the suspect and then the positive dogs last. It is important that the dogs do not share turnout areas as this bacteria can survive in the environment for many days and weeks.

Several antibiotic therapies have been attempted, but there are no known cures for this disease. This bacteria is sequestered inside cells and it is difficult for antibiotics to penetrate and eradicate this organism from a body. The disease may recrudesce at times of stress and the animal can be a source of infection for other dogs and

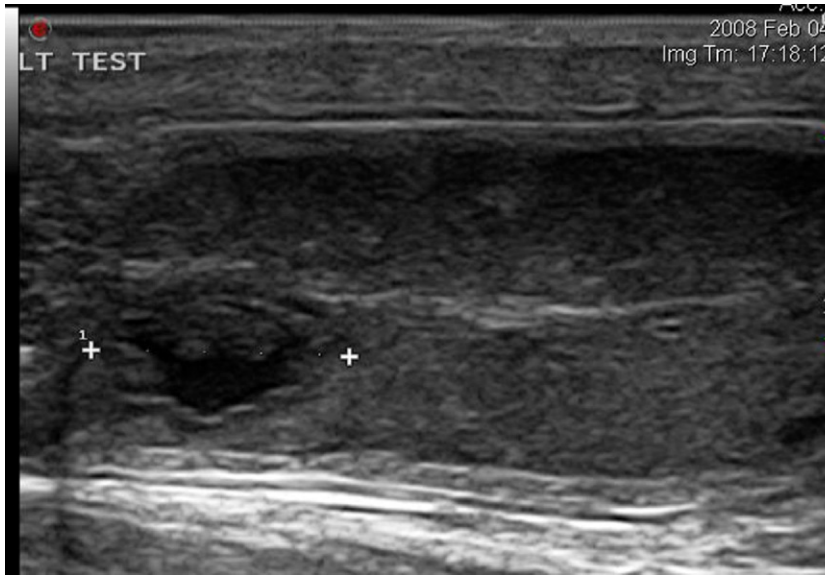

Fig. 9. Testicular abscess in a *B canis*–positive dog.

humans. This is why antibiotic therapy is not encouraged and euthanasia is the treatment of choice among veterinarians and kennel owners.

When treatment is attempted, the patients should be spayed or neutered, and studies have shown that single-antibiotic regimens are unsuccessful.[13,28,29] Combination therapy has had better results such as doxycycline (10 mg/kg po q 12 hours), gentamicin (5 mg/kg SC q 24 hours for 7 days and repeated every 3 weeks), and rifampin (5 mg/kg po q 24 hours) for 3 months.[30] Some success has been reported using enrofloxacin (5 mg/kg po q 24 hours) alone with similar efficacy to that of combination therapy.[31] After this antibiotic trial, retest and repeat until the patient has a negative test. After reaching a negative serology test, continue to test every 4 to 6 months and repeat treatment as necessary. It is also important to isolate these treated dogs from other dogs and breeding animals. The cost of antibiotic therapy and diligence of the testing protocol may deter many owners from trying to treat. It is also important to counsel owners and kennel workers that the therapy is not curative and the dog may be a risk to other dogs and humans, especially young children, older persons, and immunocompromised individuals.

PREVENTION

As with many diseases, prevention is the best treatment. It is important to quarantine and test all new additions to kennels. Due to the lag time with many of the diagnostic tests available, it is recommended that the new additions remain isolated from the general population for 8 to 12 weeks and that they be tested and found to be negative upon arrival and before coming out of quarantine.

For outside breedings, artificial insemination will decrease the male dog's exposure to the disease and should be used when possible. Artificial insemination will not protect females from the disease, so testing the male dog prior to breeding is recommended. Periodic testing of all of the dogs twice a year in kennels is recommended. This

could be during a heat cycle in the female and then on an every-6-month-period for the males. This kennel screening can help decrease exposure in the event a positive dog is introduced to the kennel and may decrease losses.

Buying dogs from and breeding to dogs in reputable kennels are encouraged, but may not decrease exposure. Routine diagnostic testing is the only way to monitor this disease in a population.

B canis is susceptible to 1% sodium hypochlorite, 70% ethanol, iodine/alcohol solutions, glutaraldehyde, and formaldehyde, and these solutions may be used to clean facilities and equipment to decrease the spread of the disease.

REPORTING

B canis is a reportable disease in many states. It is important to be aware of your state's regulations and report the disease appropriately.

ZOONOSIS

This organism is also of zoonotic concern. The symptoms manifested by this organism are not as severe as those seen with other *Brucella* organisms, such as *B abortus, B melintensis,* and *B suis*. The Centers for Disease Control and Prevention has had 30 human cases of canine brucellosis reported since this bacterium was discovered in 1966 by Carmichael.[13] The seroprevalence rates reported in humans include 13% in Mexico, 0.3% in Germany, 0.4% in US military populations, 0.6% in Florida residents, and 67.9% in Oklahoma residents according to the Center for Food Security and Public Health at Iowa State University. The high seroprevalence rate in Oklahoma was determined by testing several hospitalized and nonhospitalized individuals at the Oklahoma Health Sciences Center in the 1970s.[29] While these data may be very outdated, many believe this organism is underreported in human medicine due to the varying symptoms humans may display, ranging from flulike symptoms to endocarditis and septicemia. Unlike dogs, humans do respond well to antibiotic therapy and often clear this bacterium after long-term treatment.

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