Best Practices for *Brucella canis* Prevention and Control in Dog Breeding Facilities

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Disclaimer: This document presents a compilation of knowledge garnered from currently available science, technology, population medicine, and biosecurity strategies surrounding the management of *Brucella canis* infections. It should be considered a work in progress, because the understanding of this important disease continues to evolve through advances in scientific knowledge. The information contained herein is not meant to supersede any local, state, tribal, or federal laws, regulations or policies concerning brucellosis in dogs. The recommendations are intended to be considered best practices, are not meant to be exclusive management approaches, and may require adaptation to meet the specific needs of individual animals and particular circumstances.

Cover photo by Don Bramlage.

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Canine Brucellosis

Canine Brucellosis, caused by *Brucella canis*, is a significant reproductive disease of dogs. It is caused by an intracellular bacterium and often found in breeding kennels throughout the United States. *B. canis* is a zoonotic organism that can infect humans and is currently considered to be underreported in human medicine.[1]

The true prevalence of the disease in dogs is not well understood. The prevalence rates mentioned in currently available review articles and information sheets reference results obtained in the 1970's and 1980's with reported rates of infection of1 – 8%. Within the past few years it has become apparent that the incidence of *B. canis* infection within the dog breeding industry has been on the rise.[2,3.4] The disease may have also become more recognizable due to increased awareness coupled with improved diagnostic testing capabilities.

There is much confusion about the disease among kennel operators and veterinarians alike. Brucellosis, while historically thought of as a disease that causes abortions, has many clinical signs that are often misinterpreted. These include but are not limited to early abortions, testicular swelling, uveitis and spinal arthritis. Misunderstanding about the tests available for Brucellosis, often leads to the disease in kennels being misdiagnosed or under diagnosed. Routine blood work and urinalysis are often normal.

It is possible for infected females to raise infected puppies that can enter consumer markets. A 2011 survey of State Public Health Veterinarians reported that *B. canis* infection is a reportable disease in at least 28 states.[1] Because the disease is reportable in many states there is a small but important "underground" that tries to avert the reporting and thus serves as a continuum for the disease.

Additionally, it is important to emphasize that in dogs it is not a curable disease, which means that carrier animals MUST be removed from the breeding population in a kennel situation. Attempts at treatment have been very disappointing with relapses commonly occurring. Attempted treatment can mask diagnostic testing and has been shown to be yet another important contributing factor in the spread of the disease.

Pathogenesis and Clinical Signs

Natural transmission of canine brucellosis can occur by several routes. *B. canis* organisms are shed in the highest numbers in aborted material and vaginal discharge. Infected females transmit canine brucellosis during estrus, at breeding, or after abortion through oronasal contact of vaginal discharges and aborted materials. Shedding of *B. canis* may occur for up to six weeks after an abortion. Semen, seminal fluid and urine from infected males have also been documented as sources of infection. The rate of isolation from the semen of *B. canis* is usually high for the first six to eight weeks after infection but males can have intermittent shedding of the organism in low numbers for up to two years. Both males and females may shed the organism in the urine for at least three months after becoming infected. The organism can also be present in blood, milk, saliva, nasal and ocular secretions, and in the feces. Brucellosis has also been documented to be spread via fomites during vaginoscopy, artificial

insemination, blood transfusion, or by the use of contaminated syringes or other medical and husbandry equipment (do not share kennels, crates or other equipment). Hands and clothing can also act as fomites and increase the spread of the organism within a kennel (do not visit other kennels without washing clothes, disinfecting shoes and washing your hands). The use of high pressure spraying and cleaning equipment and improper wash-down techniques within kennels have also been shown to facilitate the spread of the disease. Puppies can become infected *in utero*, during birth, while nursing, by contact with contaminated surfaces, or by fomite spread.

Canine brucellosis is considered a lifelong infection in dogs. Clinical signs of canine brucellosis are extensive and variable and depend on the sex and age of the dog affected. The disease often exhibits no clinical signs that are obvious to the owner or veterinarian, or it can present as an abortion storm affecting many dogs. *B. canis* is a major cause of infertility and abortion in breeding dogs and therefore should be one of the first diseases to be ruled out when investigating reproductive complaints.

Several months after infection, there will be evidence of conception failures and infertility, such as late-term abortion with prolonged vaginal discharge, stillbirths, decreased litter size, and decreased puppy survivability. As the disease progresses adult dogs may develop epididymitis, orchitis, testicular swelling or atrophy, sperm abnormalities, uveitis, meningioencephalitis, spinal arthritis, weight loss, poor hair coat, listlessness, swollen lymph nodes, and behavioral changes. Some chronically infected dogs may be totally asymptomatic and still be a source of infection for other animals and humans. The clinical signs of infection can mimic many other diseases, and for that reason, Canine Brucellosis has been described as "The Great Imposter".

In the female the disease is usually thought of as a cause of abortions in affected breeding dogs. Most abortions occur between 45 and 55 days of gestation, and the aborted fetuses appear autolyzed. Early embryonic death may lead to resorption and result in perceived conception failure. Endometritis is usually seen in cases of uterine infections. It is also common to observe a prolonged vaginal discharge either following an abortion or during proestrus. The female may also have puppies that are born dead, born alive and soon die, or in some cases puppies that live to become adults.

In the male *B. canis* usually infects the prostate, testicles and epididymis and clinical signs are associated with these areas. Enlargement of one or both of the testicles may be seen. In long standing cases atrophy of the testicles may occur. Enlargement and inflammation of the epididymis (epididymitis) is a commonly observed clinical sign. Scrotal dermatitis and swelling is usually a source of intense licking by the affected male. Infertility may be seen, but these infected males may also breed successfully. The organism's effect on spermatozoa is well described by Greene and Carmichael.[6] Sperm abnormalities can be identified starting five weeks post infection (PI) and become pronounced by eight weeks. These early abnormalities include immature sperm, deformed acrosomes, swollen midpieces, and retained protoplasmic droplets. By 15 weeks PI bent tails, detached heads, and head-to-head agglutinations are seen. By 20 weeks PI 90% of sperm are abnormal. Aspermia without inflammatory cells may develop and is associated with testicular atrophy.

Generalized signs may be seen in dogs of any sex or age and include lethargy, lymphadenitis, ocular disease and vertebral pain. Loss of libido and unwillingness to breed are common signs seen in breeding stock. *B. canis* should be considered whenever reproductive problems occur, whether acute or chronic.

Zoonotic Information concerning *Brucella canis*

To decrease the possibility of human exposure to *B. canis* veterinarians should encourage their dog breeding clients to wear single-use protective examination gloves during assistance with whelping including the handling of newborn puppies, placentas, fetal membranes, or exposure to urine or vaginal secretions. Extreme caution should be taken when handling any aborted materials including dead or partially developed puppies, their fetal membranes and placentas. Protective gloves should also be used during assistance with any natural or artificial breeding. Appropriate use of personal protective equipment beyond just wearing gloves (respiratory and ocular protection) may be required to prevent human infection during cleaning and disinfection and animal handling in brucellosis positive kennels during quarantine and isolation situations. Caution should be taken when collecting and handling blood, serum, fluids or tissues for laboratory analysis by the veterinarian, paraprofessional staff, the client, and laboratory personnel if brucellosis is suspected.

Veterinarians should discuss with their dog breeding clients the potential for liability and damaged reputation surrounding zoonotic cases of *B. canis* entering consumer markets through the sale of infected puppies or adult dogs. These puppies or adult dogs commonly come into contact with children, older adults and other immunocompromised individuals. A recent example is a 2012 New York City case involving a 3 year old girl which is the first documented case of transmission of *B. canis* from a puppy to a child in the United States. This human case of *B. canis* infection resulted in a multistate, multi-agency investigation to determine the source of the child's infection, origin of the puppy, location of the puppy's littermate in another state (PA), additional human exposures, and an investigation of the Midwest breeding facility (personal communication with the CDC and the New York City Department of Health and Mental Hygiene).

The symptoms of *B. canis* infections in humans are generally similar to those of brucellosis caused by the other *Brucella* species (e.g., *B. abortus* and *B. melitensis)*. They are frequently non-specific, and may include one or more of the following: fever (often periodic and nocturnal), fatigue, headache, weakness, malaise, chills, sweats, weight loss, hepatomegaly, splenomegaly, and lymphadenopathy.

A thorough review of the current understanding of *B. canis* infection and the diagnostic challenges in humans can be found in the 2012 National Association of State Public Health Veterinarians (NASPHV) document "Public Health Implications of *B. canis* Infections in Humans" [1] from which the following two quotes have been extracted:

"Although there are multiple statements in the literature that *B. canis* infections tend to cause a milder illness compared to other *Brucella* spp**.**, serious manifestations have been described. These include septic arthritis, aortic valve vegetations, calvarial osteomyelitis, epidural abscess, pleural effusion, oral lesions, lower extremity aneurysms, and culture negative endocarditis. There are at least two reports describing *B. canis* infection in HIV-infected patients. The disease in both patients was well-controlled with regard to viral load and CD4 counts, and each had typical clinical presentations of brucellosis with good responses to treatment. "

"Although the low numbers of known human cases imply that the impact of *B. canis* on human health is minimal, it seems likely that a lack of clinical suspicion of the infection, its nonspecific clinical presentation, the non-availability of approved serologic tests, and the organism's fastidiousness in culture all result in the underdiagnosis, and subsequently the underreporting, of this infection".

Population Medicine and Biosecurity Considerations

Ideal control measures for canine brucellosis in breeding kennels centers around sound population medicine and biosecurity practices. Gaining negative Brucellosis status involves testing and removal of infected animals, sourcing new additions to the breeding population from proven negative kennels, and isolation and repeat testing of new additions to prove them brucellosis negative. Practicing strict biosecurity, continual disease surveillance with proper breeding management and maintaining strict environmental controls (cleaning, disinfection, proper temperature and humidity) are required to maintain Brucellosis free status. Particular care should be taken to properly clean and disinfect whelping sites and nurseries on a daily basis**.**

Once a breeding dog population is proven to be free of brucellosis the best way to keep brucellosis out of the kennel is to **isolate** and **test all** incoming dogs, proving them negative prior to placing them into the general kennel population. This is best accomplished by isolating newly purchased dogs in a separate building or facility, away from the general population for a minimum of eight weeks. All incoming dogs should be tested for *B*. *canis* on arrival and again at eight weeks. Only after these two negative screening tests have been done on **all dogs** in the isolation facility can they be safely moved into the established kennel. If during this eight week isolation period **any** of the dogs in isolation are diagnosed with brucellosis they should be eliminated from the premises immediately. The eight week isolation period is then restarted for the remaining dogs in isolation. This isolation and testing approach has proven to be the safest way to introduce new dogs into an established breeding population without the fear of introducing brucellosis or other infectious organisms.

Ideally, breeding dogs should never leave the breeding kennel facility other than to visit a veterinarian for necessary treatment (e.g., C-section, serious injury or illness). It is best to maintain a closed population of breeding dogs in the kennel and not send females out to be bred. A female sent out for breeding purposes is a risk and any dog leaving the breeding kennel facility for any reason other than a C-Section should be tested 90 days after its return to confirm its negative status. It is best to isolate that dog from the rest of the kennel population upon its return, but doing so may not be practical because it

would require isolation during gestation and whelping, which could be problematic in some kennel situations.

An alternative approach for bringing in new genetics would be to use artificial insemination (AI) for breeding females, with semen obtained from outside stud dogs that have been proven to be negative for *B*. *canis* (two negative tests at least eight weeks apart prior to collection of the semen). If male dogs are to be used for outside breeding, the safest approach would be to only offer this service through the use of AI, with the semen collected on the premises and then shipped to the outside kennel without the female ever coming to the facility.

Visitors (including the breeding client themselves) should not have visited any other breeding facilities that day, should be wearing clean clothing, should disinfect their shoes, should wear disposable protective shoe covers, and should adequately wash their hands. Ideally visitors should not touch or handle any dogs or equipment. Some accomplish access without direct contact using video viewing of dam and sire as well as video of the litter through the nursing period. This eliminates most direct disease transfer risk until the puppy is moved to the new home.

Cleaning and Disinfection

B. canis is relatively short-lived outside the body and is readily inactivated by common disinfectants as well as by sunlight. *B. canis* is stable in the environment in the presence of organic debris for up to two months (proper cleaning and disinfecting is therefore a must). *B. canis* can withstand drying in the presence of organic debris, can withstand freezing, and can survive in water, dust and soil. The combination of organic debris, high humidity, low temperatures and little or no sunlight favors survival of the organism (winter conditions in most dog kennels).

An important and often overlooked part of kennel management is the proper cleaning and disinfection of a kennel. When dealing with Brucellosis or other diseases cleaning and disinfection serves to limit the spread of the disease and is a critical component in disease prevention. Proper cleaning and disinfection takes time and must be done correctly for a kennel to be considered truly disinfected. It is important to remember that a clean kennel is not always a disinfected kennel. The consulting veterinarian must be certain that their clients thoroughly understand the entire cleaning and disinfection process including proper dilution and storage of the cleaners and disinfectants, and the fact that required contact times and appropriate rinsing are absolutely necessary. The required frequency (daily vs. weekly) for cleaning and disinfection should be thoroughly discussed with the client. The facility should always be cleaned and disinfected in order of animal susceptibility to disease starting first with the kennel areas housing the most susceptible animals (puppies and nursing bitches), followed by healthy adults and lastly areas housing any unhealthy or isolated animals. Clients should be educated on the specific biosecurity procedures that would be required during isolation or quarantine situations.

If there is a “good” aspect of *B. canis,* it is that the organism is very sensitive to most disinfectants when they are used properly. The use of a biodegradable enzyme based kennel degreaser is a must before disinfection. A properly used degreaser will effectively remove the biofilm and other organic debris (e.g., residues from feces, urine, vaginal or preputial discharges, oil from the skin and haircoat, other body

fluids) that build up in all kennels and can "hide" the offending pathogen(s). The degreaser can be applied directly with a spray bottle or conveniently by the use of a hose end foamer. The foamer allows for ease of application and increases the surface area covered. It can also help to ensure that all surfaces are covered. The use of degreasers that leave a soapy residual film that can contribute to the biofilm is discouraged. It is important to rinse all surfaces well after using any degreaser. Once a kennel has undergone degreasing the final step in the cleaning and disinfection process requires the proper application of an appropriate disinfectant to kill the offending pathogens. A properly functioning hose end foamer supplying the correct concentration of disinfectant is an excellent way to apply the disinfectant to all surfaces that the dogs will come in contact with. It is important to remember that all disinfectants have a minimal ten minute contact time with the surfaces to be effective (follow product labels and instructions supplied with disinfectant). Most disinfectants require adequate rinsing with clean water after the appropriate contact time has occurred. After following these procedures we can be assured that we have reduced the biofilm and disinfected the kennel.

Diagnosis of Brucella canis

B. canis monitoring may be achieved with a variety of tests which have been well described by Hollett [5] and by Greene and Carmichael. [6] See the test comparison chart below for specific information on available tests. The chart was compiled from various sources including peer reviewed publications [5,6], available product inserts [7], and information obtained from reputable veterinary diagnostic laboratories. Because of the zoonotic potential of Brucella, caution should be exercised when collecting and handling blood, serum, fluid or tissues.

Comparison of Diagnostic Procedures for *B. canis*				
Antibody Detection Methods				
Test	**Sample**	**Earliest Detection (weeks PI)**	**Advantages**	**Disadvantages**
Rapid Slide Agglutination Test (RSAT)	Serum	1- 4 weeks* 3-4 weeks**	Quick, high sensitivity, few false negatives. Good Screening Test.	False positives possible, must confirm by other tests.
Mercaptoethanol (ME) rapid slide agglutination test (ME-RSAT)	Serum	3 – 4 weeks***	Quick, high sensitivity, few false negatives. Increased specificity over RSAT.	False positives possible, must confirm by other tests.
Tube agglutination test (TAT)	Serum	2-4 weeks** 3-6 weeks***	Semiquantitative titer. Good Screening Test.	False positives possible, must confirm by other tests. .
ME-TAT	Serum	2-4 weeks** 5-8 weeks***	Semiquantitative titer. Increased specificity over TAT	Longer testing time (2 day test).
Agar-gel immunodiffusion (AGID) cell wall (somatic) antigen	Serum	8-12 weeks** 5 – 10 weeks***	Positive earlier than CPAg Very sensitive test.	Procedure and interpretation complex, nonspecific reactions, poor availability.
Internal cytoplasmic protein antigen (CPAg)-AGID	Serum	8 – 12 weeks***	Highly specific confirmatory test utilizing highly purified cytoplasmic protein devoid of contamination with LPS.	Maternal antibodies prevent seroconversion in puppies; so not useful until 6 months post weaning. Complex procedure.
Indirect Fluorescent antibody	Serum	Unknown	Available and convenient for diagnostic labs *** Good Screening Test.	May be less sensitive than ME-TAT as screening test *** False Positives Possible.
ELISA	Serum	30 days***	Good results with mutant (M-) B canis for cell wall extracts, or B abortus for CPAg ***	Antigen purity and preparation critical***

* D-Tec® CB Canine Brucellosis Antibody Test Kit directions insert; Zoetis Animal Health, Florham Park NJ, USA

** Hollett, R.B., 2006; Canine Brucellosis: Outbreaks and Compliance; *Theriogenology* 66: 575-587

*** Greene, Craig E. and Leland E. Carmichael, 2012: Chapter 38: Canine Brucellosis; in *Infectious Diseases of the Dog and Cat*, Fourth Edition, Craig E. Greene (editor).

Comparison of Diagnostic Procedures for *B. canis*				
Organism/Antigen Detection Methods				
Test	**Sample**	**Earliest Detection (weeks PI)**	**Advantages**	**Disadvantages**
Blood or tissue culture	Whole Blood/ FULL Blue Top Tube, or vaginal swab	Bacteremia detectable, 2-4 weeks PI.**	Low cost. Can identify actual organism for antimicrobial sensitivity testing and/or DNA profiling	Fastidious organism. False negative results possible. Requires sterile technique of blood collection. Contaminant overgrowth can lead to false negative results. Intermittent bacteremia may require serial blood cultures. POOR SCREENING TEST.
PCR	Whole Blood/ FULL Blue Top Tube, or vaginal swab	1.5 CFU/ml detected.****	5x more sensitive than culture	False negative results possible. Requires sterile technique of blood collection. POOR SCREENING TEST.

* D-Tec® CB Canine Brucellosis Antibody Test Kit directions insert; Zoetis Animal Health, Florham Park NJ, USA

**Hollett, R.B., 2006; Canine Brucellosis: Outbreaks and Compliance; *Theriogenology* 66: 575-587

***Greene, Craig E. and Leland E. Carmichael, 2012: Chapter 38: Canine Brucellosis; in *Infectious Diseases of the Dog and Cat*, Fourth Edition, Craig E. Greene (editor).

****Kansas State University Veterinary Diagnostic Laboratory

Screening a Kennel for Brucellosis

A whole kennel screening test consists of testing all canines over six months of age at least once every 12 months. If there are any reproductive concerns within the breeding population, *B. canis* screening should be done.

If a positive result is obtained on any test, the dog should be isolated and classified as a *Brucella* suspect dog. Because of the possibility of false positives on some tests, select a different diagnostic test to validate the initial results. If the second test does not agree with the initial test, a third diagnostic test should be performed eight weeks after the second test to rule in or rule out Brucellosis. Because of variability in state regulations, veterinarians should check with their state veterinarian or appropriate public health official on how to handle Brucellosis positive test results.

If a female or male dog has left the kennel for any reason other than a C-Section or veterinary procedure, the dog should be tested for *B*. *canis* eight weeks after returning to the kennel.

Testing Considerations for Brucellosis Positive Kennels

Once Brucellosis is diagnosed (two positive tests), testing and removal of all positive dogs in the kennel is the only way to gain negative status. It is highly recommended that all dog sales be stopped until negative status is achieved. Veterinarians should check with their state veterinarians or public health officials to verify if a suspect or positive kennel is placed under a strict no-sale quarantine or only a partial quarantine, in which only pups from Brucellosis positive females are prohibited from being sold, traded, or bartered. Even If a state does not have regulations concerning quarantine procedures for Brucellosis positive dogs, it is still recommended that the sale, trade, or bartering of Brucella positive dog or puppies from Brucellosis positive dams be discontinued.

Test all remaining dogs and repeat every four weeks until there are two consecutive negative whole kennel tests. Two negative tests should eliminate the organism from the kennel population. Testing should be done with the knowledge of the state veterinarian and with a test recognized by the state the kennel is physically located in.

Re-homing Considerations for Dogs Positive for *Brucella* canis

It is important to emphasize that brucellosis is currently **not** considered to be a curable disease in dogs. Attempts at treatment have been very disappointing, with relapses commonly occurring. Attempted treatment can mask diagnostic testing, and it has been shown to be an important contributing factor in the spread of the disease. What this fact currently means for kennel owners is that animals infected with any of the ***Brucella*** species MUST be removed from the breeding population.

Because of the zoonotic potential of *Brucella,* confirmed Brucellosis-positive dogs should NOT be rehomed.

Decisions concerning the possible re-homing of Brucellosis positive dogs should only be made with the approval and knowledge of the appropriate public health official(s) within the state of origin as well as within the state of destination if being transferred across state lines. If the decision is made to re-home

Brucellosis positive dogs, they should undergo an ovario-hysterectomy or castration and be placed on appropriate long-term antibiotics, with proper supervision by a licensed veterinarian that should include lifetime periodic laboratory testing for *B. canis*. Brucellosis is considered a lifelong infection in dogs, and even after undergoing surgical sterilization and long term antibiotic use, both male and female dogs may continue to intermittently shed the organism. New owners should be made aware of the ongoing potential risk that these dogs carry for infecting humans, other dogs and other susceptible animal species that they come in contact with (see zoonotic section above).

Re-homing Considerations for Retired Dogs from Kennels with an Unknown *Brucella canis* Status

Any dogs that are re-homed or transferred to any individual, entity or organization should have a negative diagnostic test for *B*. *canis* prior to change of ownership and tested again 8 weeks later. The new owner or organization should be educated in the possible zoonotic risks associated with the disease.

Summary

This document contains a compilation of knowledge garnered from currently available science, technology, population medicine, and biosecurity strategies surrounding the management of *B. canis* in dog breeding kennels. It should be considered a work in progress, because the understanding of this important disease continues to evolve through advances in scientific knowledge. It is the hope of the authors that this information will serve as a guide for veterinarians and their clients with the goal of bringing consistency and clarity to the diagnosis, prevention and management of *B. canis* infection in dog breeding kennels. Only through continued advancement of science and the dissemination of knowledge can we hope to control this devastating disease.

Recommendations

There is an ongoing need for continued basic science and clinical research surrounding prevention, diagnosis, and the identification of effective treatment options aimed at the control of this important disease. Further study will be required to clarify the epizootiology of *B*. *canis* with respect to disease pathogenesis, transmission and true prevalence rates in both dogs and in humans. Immunologic research directed towards the development of an effective *B. canis* bacterin to be used in the management and control of this disease in dogs would be welcomed. The authors recommend that laboratory standards be developed for all currently utilized and future diagnostic tests used to identify *B*. *canis* infection in dogs. We recommend the implementation of quality control proficiency testing of the currently available tests to validate accuracy, consistency, sensitivity and specificity.

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Cornell Animal Health Diagnostic Laboratory

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National Veterinary Services Laboratory

Internet Resources

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State Public Health Veterinarians 2015:

http://www.nasphv.org/Documents/StatePublicHealthVeterinariansByState.pdf

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(6) *Infectious diseases of the dog and cat*, Fourth Edition 2012 / [Edited by] Craig E. Greene ; Chapter 38 Canine Brucellosis, Craig E. Greene and Leland E. Carmichael

(7) D-Tec® CB Canine Brucellosis Antibody Test Kit directions insert; Zoetis Animal Health Florham Park NJ, USA; https://www.zoetisus.com/contact/pages/product_information/msds_pi/pi/Canine_Brucellosis_Antibody_Test_Kit_D-TEC_CB.pdf (accessed 6/26/2015).