



Canine Brucellosis Guidance Document for Indiana cases

Revised July 24, 2019

Published by the Indiana State Veterinarian for the Indiana State Board of Animal Health
Discovery Hall, Suite 100; 1202 E 38th Street
Indianapolis, IN 46205
887-747-3038
www.in.gov/boah

Table of Contents

Page 2	Information on Canine Brucellosis
Page 2 – 3	Transmission of Disease
Page 3	Testing
Page 3 - 4	Treatment
Page 4	Prevention
Page 4 - 5	Quarantine and Isolation Facilities
Page 6	Cleaning and Disinfecting Facilities
Page 7 - 8	Zoonotic Potential
Page 8	Indiana Rules Regarding Moving Diseased Animals
Page 8	Re-homing Considerations for Positive Dogs
Page 9	Prevention: How to Keep Brucellosis Out of Kennels
Page 10	Responding to a Positive Diagnosis and Reestablishing a Brucellosis-Free Kennel
Appendix A	Summary Of Canine Brucellosis Tests

Information on Canine Brucellosis

Canine brucellosis is an infectious disease that mainly affects the reproductive tract of dogs. Infection is caused by a bacterium (*B. canis*) which is highly contagious and easily spreads from dog to dog. The disease is transmitted through contact with fluids from the reproductive tract, such as vaginal secretions, semen, urine or aborted tissues from infected dogs. Both males and females may transmit the bacteria during the breeding process.

Clinical signs of canine brucellosis vary between male and female dogs. The most common signs in females are failure to conceive or abortion approximately 6 weeks to 8 weeks after breeding without any signs of illness. Conception is possible for infected females, but pregnancies generally result in smaller litter sizes and/or weak, fading puppies. Males will exhibit tender, swollen or shrunken testicles, leading to infertility, poor reproductive ability and loss of libido.

The disease is zoonotic, meaning it can be transmitted to humans. Humans can acquire the infection when an infected dog's body fluids come in contact with broken skin or are accidentally ingested. Canine brucellosis in humans is characterized by flu-like symptoms, including fever, headache and lethargy.

Canine brucellosis is on the state of Indiana's reportable disease list. Laboratories, veterinarians and animal owners are required to report positive test results to the Indiana State Board of Animal Health (BOAH) within 2 business days after the diagnosis.

Transmission of disease

Transmission of canine brucellosis may occur via several routes:

- Breeding – Infected females can shed high numbers of bacteria during estrus and at the time of breeding. Infected males may shed bacteria in the semen for up to 2 years following infection. Males can possibly shed bacteria intermittently for life.
- Contact with aborted fluids, tissues or vaginal secretions—*B. canis* organisms are shed in the highest numbers in aborted material. The time following an abortion is a very infective time for kennels and aggressive cleaning and disinfection should be considered.
- Semen and urine from infected males have high concentrations of bacteria for approximately 2 months following infection. However, males may continue to shed bacteria in the urine and semen for up to 2 years or more, post-infection.

- Urine from infected females
- Exposure to other bodily fluids – This includes exposure to blood, milk, saliva, nasal discharge, feces and ocular secretions from infected dogs.
- Transmission by fomites
 - Veterinary equipment including vaginoscopy, artificial insemination (AI), medical equipment/syringes, blood transfusions
 - Husbandry equipment including kennels, crates, grooming equipment (such as clippers and brushes).
 - People may spread infection on their clothing, hands and shoes.

Testing

In general, accurate and timely testing for *B. canis* can be a challenge.

The *B. canis* organism has a rough outer coating which makes producing an effective test difficult. This rough coat can also cause cross-reactions with other common respiratory pathogens in dogs, which creates a high level of false positive results on initial screening tests. Confirmatory tests will help rule out false positives, but may take up to 7 days for results. Kennel owners should work with a veterinarian to determine which test protocol is best for the situation. While culture is considered to be a definitive test, no one test will identify every positive case.

Only veterinarians may submit samples to a laboratory for an official brucellosis test.

Refer to Appendix A (Summary Of Canine Brucellosis Tests) for interpretation and guidance about specific, available tests.

Treatment

Currently, no cure for canine brucellosis exists. This disease is considered to be a lifelong infection in affected dogs.

NO vaccine is available for the prevention of canine brucellosis.

Infected males will always have the potential to shed bacteria, even after neutering. Infected females may eventually stop shedding bacteria after being spayed; however, several months to years may pass after spaying before the female consistently tests negative for the bacteria.

Infected dogs given long-term treatment with antibiotics may test negative for infection temporarily. **It is essential to realize that antibiotic treatment does not cure brucellosis**

infection. Treatment can decrease the amount of circulating bacteria in the blood, causing the animal to test negative temporarily. However, the brucellosis bacteria remain in the animal, especially in the reproductive tract. Once antibiotic treatment is stopped, the bacteria multiply and the animal begins shedding bacteria again in large quantities. Treatment may allow brucellosis-infected dogs to continue to reproduce; however, the number of pups produced will be significantly lower than for uninfected dogs and the treated animal will continue to be a source of infection for other dogs in the kennel. Treatment can make dogs test negative for brucellosis, a method used by unethical breeders to sell positive dogs to unsuspecting buyers.

Puppies born to brucellosis-infected dams can be infected with canine brucellosis. These puppies will be infected for life, posing a health risk to people and to other dogs.

In general, any dogs that are positive for canine brucellosis should be euthanized. In rare situations involving highly trained dogs, like law enforcement or service animals, special considerations and options may be reviewed in cooperation with the local health department, to determine if the infected animal can be salvaged while still protecting human and animal health.

Prevention

The best treatment for canine brucellosis is to prevent infection in the first place.

Breeding dogs should be purchased from kennels that are actively screening and monitoring for brucellosis. All newly purchased dogs should be quarantined for 8 weeks. They should test negative for canine brucellosis prior to being moved into the quarantine area. They should be retested 8 weeks later before being moved into the breeding kennel. BOAH recommends all breeding dogs older than 6 months of age be tested annually.

Quarantine and Isolation Facilities

Proper use of a quarantine facility is an important step in keeping unwanted organisms, such as bacteria, viruses and parasites, out of a kennel. All new animals should be placed in a quarantine facility until they have met established testing requirements for entering the kennel and a knowledgeable individual has observed them for at least 60 days. Dogs showing signs of illness or testing positive for canine brucellosis should not be placed into the kennel.

Animals that become sick while in a kennel can easily spread disease or infection to other dogs. At the first sign of illness, the owner(s) should consider moving those animals to another area

of the facility, such as an isolation room or building. Dogs can be treated in an isolation area with a lower risk of spreading infection to the remainder of the kennel population.

Things to consider when setting up a quarantine facility and/or isolation facility include:

- A **quarantine facility** should be used for the temporary housing and observation of recently purchased dogs, before they are introduced to the main kennel.
- An **isolation facility** should be used for the temporary housing of dogs showing clinical signs of illness, and for the duration of their treatment.
- Isolation and quarantine spaces should be kept separate. Co-locating these functions will present a risk of exposing new or existing dogs to illness.
- The best locations of these facilities will allow animals to be easily moved into and out of the areas without exposing healthy animals.
- Access to the quarantine and isolation facilities should be limited to necessary individuals only, such as one person needed to care for the dogs. If the same person caring for dogs in the kennel is also caring for dogs in the quarantine and isolation areas, extra care should be taken for good biosecurity when moving between spaces. Care should also be taken for movements of equipment and animals.
- Consider having a sink inside each facility for hand washing, treatments and disinfection purposes.
- Air flow to these areas should be separated from other animal housing areas to avoid spreading airborne infections.
- Materials and surfaces should be easy to clean and disinfect.
- Complete separation must be maintained from all other animals' living spaces within the quarantine and isolation areas (i.e., solid walls between cages or kennels).
- Separate equipment and supplies must be used exclusively for the quarantine and isolation areas.
- Anyone entering these areas should wear personal protective equipment, such as:
 - Plastic boots, shoe covers or boots to be worn only in this area,
 - Coveralls to be worn over clothes when in this area, and
 - Gloves.
- Cleaning and caring for the animals in quarantine, then isolation, should be done only **after** tending to all of the other animals in the kennel. This reduces the risk of spreading illness or disease.
- Facilities must be cleaned and disinfected following the removal of all animals and before introduction of new animals.

Cleaning and Disinfecting Facilities

Brucellosis can be easily spread from dog to dog through birth fluids, urine and other bodily fluids. To prevent reintroduction of infection, animal owners must maintain strict cleaning and disinfecting procedures. **REMOVAL OF ALL FECAL MATERIAL, URINE AND DEBRIS PRIOR TO DISINFECTION PROCEDURES IS ABSOLUTELY ESSENTIAL.** All equipment (including waterers, food bowls, leashes and toys) and facilities on the premise should be cleaned and disinfected on a routine basis and following all infectious disease outbreaks.

Cleaning is the act of removing fecal material, urine and debris from the area using a cleaning solution or detergent such as soap.

Disinfection is the act of removing pathogens, such as bacteria, viruses and parasites, by using a disinfecting product. Most disinfecting products must be applied and left on clean surfaces for a specific amount of time to be effective in removing the pathogen. Generally, this is no less than 10 minutes of contact time. The product should then be rinsed with clean water to avoid exposure to the animals and damage to the equipment and kennels. Users must read and follow the label directions when using a disinfection product in the kennel.

The following products are considered to be effective for disinfection:

- **Household Bleach:** a 1:32 dilution is best for routine disinfection (½ cup bleach per gallon of water). A 1:10 dilution (1 ½ cups of bleach per gallon of water) may be necessary following a diagnosis of brucellosis or other disease to deep clean the kennel. This solution can have a strong odor, so users should wear a mask when using a 1:10 solution and remove all dogs from the area until the product has been rinsed.
- **Phenolic Disinfectants** – Lysol, One Stroke Environ – Follow label directions for dilution and necessary contact time.
- **Quaternary Ammonia Compounds** – Roccal D, Parvosol – Follow label directions for dilution and necessary contact time.

REMEMBER: MOST SURFACES SHOULD BE RINSED FOLLOWING PRODUCT APPLICATION BEFORE ANIMALS ARE PLACED BACK INTO THE AREA. FOLLOW THE LABEL RECOMMENDATIONS.

***Brucella canis* Zoonotic Potential**

The National Association of State Public Health Veterinarians reported in 2012 that between 100 cases and 200 cases of brucellosis are reported in people each year in the United States. These cases include six classically recognized species based on antigenic/biochemical characteristics and primary host species: *B. abortus* (cattle), *B. melitensis* (sheep and goats), *B. suis* (swine, cattle, rodents, wild ungulates), *B. ovis* (sheep), *B. canis* (dogs), and *B. neotomae* (rodents). Recently, more species have been recognized.

In the majority of canine brucellosis (*Brucella canis*) cases in humans, the infection is related to exposure to whelping females when high concentrations of bacteria occurred in birthing fluids and vaginal discharges. Humans can be exposed to the bacteria when an infected dog's body fluids come into contact with broken skin or are accidentally ingested. For this reason, people should wear protective equipment such as gloves, eyewear or face masks when performing artificial insemination, providing whelping assistance and especially when handling aborted materials and tissues. People should also be careful to wear protective equipment when cleaning and disinfecting known positive kennels as the bacteria can easily be aerosolized and inhaled during the cleaning process, especially when water and cleaners are sprayed on the area using hoses or power sprayers. Because the organism is difficult to culture, study and diagnose, the epidemiology of infections in humans is poorly understood.

In 2012, a case of canine brucellosis in humans was diagnosed in a child in New York who was infected by a puppy purchased from a pet store. The source of the puppy was a large breeding operation in Iowa that was found to have several breeding dogs positive for the organism. Transmission was documented by laboratory tests that matched the strain of brucellosis in the child and puppy with the infection found in the kennel.

The incubation period in a human can vary. Reported times vary from 2 weeks to 3 months. No published information is readily available on the communicability of the organism to and between humans. No cases of transmission from human to human have been reported.

Clinical signs in humans are non-specific and may resemble influenza. Symptoms might include fever (often periodic and nocturnal), fatigue, headaches, weakness, malaise, chills, sweats and weight loss. Symptoms might include enlargement of the liver, spleen and lymph nodes. In severe cases, more serious symptoms such as septic arthritis and heart valve abnormalities may occur.

Diagnosis in people can be challenging. Culturing the organism is considered the "gold standard" for diagnosis, but is difficult and takes a minimum of 4 weeks. Serological tests can be tried, but, again, are difficult and can produce inconclusive results.

Prevention consists of testing any symptomatic and exposed dogs, then immediately removing test-positive dogs from the site. Good hygiene when around dogs, especially during whelping and abortions, is important. Contact with birthing fluids, vaginal discharges and urine should be

minimized, especially by anyone with a compromised immune system, children, pregnant women and the elderly. Proper disposal of these bodily fluids and good cleaning and disinfection will help minimize any potential for infection.

Puppies born to infected dams are likely to be infected. Infected puppies will be infected throughout their lives, posing a health risk to people and to other dogs. Breeders should be aware of the potential for liability and damaged reputation surrounding zoonotic cases of *B. canis* entering the consumer markets through the sale of infected puppies or adult dogs.

Indiana Rules Regarding the Movement of Diseased Animals

Knowingly moving animals infected with canine brucellosis, including selling or giving away, violates Indiana law and may be subject to action by the State Board of Animal Health. Sites selling and/or receiving infected dogs may be subject to quarantine by BOAH.

Re-homing Considerations for Positive Dogs

Since canine brucellosis is not considered to be a curable disease and has zoonotic potential, the Board of Animal Health recommends humane euthanasia of these dogs. These dogs should not be re-homed. Veterinarians and rescues that attempt to re-home these animals should be aware of the potential liability associated with re-homing.

Prevention: Best Practices to Keep Brucellosis Out of Kennels

1. Only purchase dogs from known, reputable breeders who routinely test their dogs. If a purchase seems “Too good to be true”, it likely is!
2. QUARANTINE and test ALL incoming dogs prior to introduction into the kennel.
 - a. All dogs should be tested prior to arrival at the facility or within 5 days of arrival.
 - b. All dogs should be tested a second time, 8 weeks after arrival.
 - c. Only after these two negative tests have been completed should an animal be moved from quarantine into the general kennel population.
 - d. If any of the dogs in quarantine are diagnosed with brucellosis during the quarantine period, they should be removed from the premises immediately. All other dogs in quarantine should start their quarantine period over and be retested approximately 8 weeks later.
3. Breeding dogs should not leave the kennel facility other than to visit a veterinarian for treatment. Any animal that is sent out of the kennel for a purpose other than veterinary care should be quarantined and tested 60 days to 90 days after its return to the kennel. This may be challenging if the animal was bred off-site and is due to whelp while in quarantine.
4. Consider using artificial insemination (AI) to bring new genetics into the breeding program. Semen should be obtained from dogs that have proven to be negative for *B. canis* (two negative tests at least 8 weeks apart prior to the collection of the semen).
5. All visitors to the kennel should not have visited any other breeding facilities that same day. Always use good biosecurity for kennel visitors (wash hands, clean shoes, clean clothing).
6. Consider asking visitors to not touch or handle dogs or equipment while at the facility. If necessary, provide disposable gloves to the visitor to use while at the kennel.
7. All visitors should disinfect their shoes prior to entering the kennel. Owners may choose to also provide disposable shoe covers to be worn while inside.
8. Maintain proper cleaning and disinfection protocols at all times. Work with a veterinarian to determine what this protocol should be.
 - a. *B. canis* is short-lived outside of the body. It is readily inactivated by common disinfectants and sunlight.
 - b. *B. canis* is stable in the environment in the presence of organic debris (feces, dirt, bedding or debris) for up to 2 months.
 - c. *B. canis* can survive drying in the presence of organic debris, can withstand freezing and can survive in water, dust and soil; therefore, proper cleaning, prior to disinfection, is a must.
 - d. The facility should be cleaned and disinfected in the order of animal susceptibility to disease. Start first in the kennel areas housing puppies and nursing females, then clean areas containing healthy adults and lastly clean areas with unhealthy, quarantined or isolated animals.

Responding to a Positive Diagnosis and Reestablishing a Brucellosis-Free Kennel

1. Immediately move all suspect/positive animals into a quarantine or isolation area.
2. Work with a veterinarian to determine if further testing of the suspect/positive animal is necessary.
3. All dogs with a positive diagnosis of canine brucellosis should be removed from the breeding program and/or euthanized. Brucellosis is not considered to be treatable disease. All positive animals will pose a significant risk to other dogs in the kennel and should be removed as soon as possible.
4. All other dogs in the kennel should be retested using an appropriate test 4 weeks to 8 weeks following the initial diagnosis of brucellosis in the kennel. Any dogs diagnosed as suspect/positive on this test should be removed from the kennel.
5. Once brucellosis is diagnosed in a kennel, testing and removal of all positive dogs in the kennel is the only way to return to a brucellosis-free status. **All dogs in the kennel should be re-tested every 4 weeks to 8 weeks until ALL dogs test negative on two consecutive tests.**
6. Consider stopping all dog sales until a negative kennel status is achieved. If this is not possible, all dogs and puppies should test negative for *B. canis* prior to sale.
7. Clean and disinfect the kennel on a regular schedule as recommended by a veterinarian.
 - a. *B. canis* is short-lived outside of the body. It is readily inactivated by common disinfectants and sunlight.
 - b. *B. canis* is stable in the environment in the presence of organic debris (feces, dirt, bedding or debris) for up to 2 months.
 - c. *B. canis* can survive drying in the presence of organic debris, can withstand freezing and can survive in water, dust and soil; therefore, proper cleaning, prior to disinfection, is a must.
 - d. The facility should be cleaned and disinfected in the order of animal susceptibility to disease. Start first in the kennel areas housing puppies and nursing females, then clean areas containing healthy adults and lastly clean areas with unhealthy, quarantined or isolated animals.
 - e. *B. canis* can easily be aerosolized during cleaning when hoses and pressure washers are being used. Personal protective equipment should be worn when cleaning known infected kennels to avoid the transmission of infection to humans. Also be careful to avoid transmission of the infection to other dogs in the immediate area.

APPENDIX A: SUMMARY OF CANINE BRUCELLOSIS TESTS

SCREENING TESTS					
Test	Sample	Earliest Detection (Weeks PI)	Advantages	Disadvantages	Notes
Rapid Slide Agglutination (RSAT) "Slide" or "Card" Test	Serum	3 – 4 weeks	<ul style="list-style-type: none"> • Quick, high sensitivity • Rare false negatives (as long as animal is at least 1 - 2 weeks PI) • Up to 50 – 60% false positives • Good screening test 	<ul style="list-style-type: none"> • False positives possible, must confirm by other tests 	<ul style="list-style-type: none"> • If result positive, then more specific test should be ordered – dog should be isolated • If result negative – dog unlikely to have infection • Blood sample hemolysis can cause false positives
Mercaptoethanol (ME) Rapid Slide Agglutination (ME-RSAT)	Serum	3 – 4 weeks	<ul style="list-style-type: none"> • Quick, high sensitivity, few false negatives. Increased specificity over RSAT. • The ME decreases the IgM cross reaction with Bordetella and other bacteria 	<ul style="list-style-type: none"> • False positives possible, must confirm by other tests 	<ul style="list-style-type: none"> • Irish wolfhound and Old-English sheepdogs have a high percentage of false positives • 99% correlation between a negative test and a lack of infection
Tube Agglutination Test (TAT) "Tube" Test	Serum	3 – 6 weeks	<ul style="list-style-type: none"> • Result is a semi quantitative titer • Good screening test 	<ul style="list-style-type: none"> • False positives possible, must confirm by other tests • Longer testing time 	<ul style="list-style-type: none"> • Result is a titer <ul style="list-style-type: none"> ▪ < 50 – Early infection/recovering ▪ 50-100 – Suspect infection ▪ >200 – Highly presumptive of current infection
ME-TAT	Serum	5 – 8 weeks	<ul style="list-style-type: none"> • Semi quantitative titer • Increased specificity over TAT 	<ul style="list-style-type: none"> • Longer testing time (2 days to run) 	
Agar-Gel Immunodiffusion (AGID) Cell Wall (Somatic) Antigen	Serum	5 – 12 weeks	<ul style="list-style-type: none"> • Positive earlier than CPAg • Very sensitive test 	<ul style="list-style-type: none"> • Procedure and interpretation are complex, non-specific reactions • Poor availability of test (Cornell or University of Florida) 	<ul style="list-style-type: none"> • High percentage false negatives • Can still get false positives
Indirect Fluorescent Antibody	Serum	Unknown	<ul style="list-style-type: none"> • Available and convenient for diagnostic labs • Good screening test 	<ul style="list-style-type: none"> • May be less sensitive than ME-TAT as a screening test • False positives are possible 	<ul style="list-style-type: none"> • Became alternative test when RSAT and TAT tests were not available • Available at Ohio State

APPENDIX A: SUMMARY OF CANINE BRUCELLOSIS TESTS

CONFIRMATORY TESTS					
Test	Sample	Earliest Detection (Weeks PI)	Advantages	Disadvantages	Notes
Internal Cytoplasmic Protein Antigen (CPAg)-AGID	Serum	8 – 12 weeks	<ul style="list-style-type: none"> Highly specific confirmatory test utilizing highly purified cytoplasmic protein devoid of contamination with LPS 	<ul style="list-style-type: none"> Maternal antibodies prevent seroconversion in puppies – not useful in pups until 6 months post-weaning Complex Procedure 	<ul style="list-style-type: none"> Run at Cornell or Florida Lab False positive tests are not likely Most specific confirmatory test Can detect chronic cases when other tests have negative results Least-sensitive for initial screening
Indirect Fluorescent Antibody	Serum	Unknown	<ul style="list-style-type: none"> Available and convenient for diagnostic labs Good screening test 	<ul style="list-style-type: none"> May be less sensitive than ME-TAT as a screening test False positives are possible 	<ul style="list-style-type: none"> Became alternative test when RSAT and TAT tests weren't available Available at Ohio State
ELISA	Serum	30 days PI	<ul style="list-style-type: none"> Good results with Mutant <i>B. canis</i> for cell wall extracts 	<ul style="list-style-type: none"> Antigen purity and preparation are CRITICAL 	<ul style="list-style-type: none"> Became alternative test when RSAT and TAT tests weren't available.
Blood or Tissue Culture	Whole Blood or Tissue Swab	Bacteremia detectable 2 – 4 weeks PI, up to 3 years	<ul style="list-style-type: none"> Low cost Can identify the actual organism for antimicrobial sensitivity and / or DNA profiling 	<ul style="list-style-type: none"> False negative results possible Requires sterile technique of blood collection Intermittent bacteremia may require serial cultures POOR SCREENING TEST 	<ul style="list-style-type: none"> ONLY POSITIVE RESULT IS DEFINITIVE! Negative result does NOT rule out infection Contaminant overgrowth can lead to false negative Should consider a culture once every 24 hours for 3 consecutive days
PCR	Whole Blood or Tissue Swab	1.5 – 2.0 CFU / ml detected	<ul style="list-style-type: none"> 5X more sensitive than culture Rapid results 	<ul style="list-style-type: none"> False negative results are possible because the PCR depends on the presence of bacteria for positive results. The length of bacteremia is variable with this infection, so the test COULD become false negative later in the course of infection. Requires sterile technique of blood collection POOR SCREENING TEST 	<ul style="list-style-type: none"> Positive results indicate infection IF specific primers are used and contamination is excluded False-positive results occur with contamination False negative results with inadequate extraction techniques Test is run at Kansas State Lab, Dr. Bai stated that their PCR includes a culture enrichment process which makes it more sensitive than some other PCR assays. They have a detection limit of 2 CFU / ml

* Tests incorporating cell wall antigens are subject to interpretive error because Lipopolysaccharide (LPS) antigens of several bacteria can cross react with *B. canis*. Therefore, **false positives are more common than false negatives** for RSAT and TAT. The addition of Mercaptoethanol (ME) to the test will decrease this cross reaction and increase the specificity of the test (ME-RSAT and ME-TAT).

** Cross reaction can occur with the antibodies of bordetella, pseudomonas, moraxella and other gram negative bacteria.

PI = Post Infection

July 2019