

This Series on
Brucellosis was
awarded the
American Kennel Club
Club Publication
Award for
Extraordinary
Achievement and
Communications
Excellence.

Brucellosis:

Transmission, Diagnosis, and Prevention—Part II

By Nancy P. Melone, Ph.D.

This article is printed in two parts. Part I, published in the June/Breeder issue, discusses the cause of brucellosis, where it is found, its clinical signs, and the risk to humans. Part II, published in the August/Specialty issue, considers how the disease is transmitted, diagnosed, and prevented.

Brucellosis effectively ends breeding careers. Both intact and castrated dogs can get it and pass it on to other dogs and their owners. There is no vaccine, no cure, and no reliable treatment. Symptoms are vague or absent. Testing is complex and some states mandate euthanasia of any affected dog. It is a growing problem.

How is *Brucellosis* Transmitted?

B. canis infects susceptible dogs by contact with the mucosal surfaces of the nose, mouth, eyes, or vagina. The typical mode of transmission is by ingestion of aborted placental tissues and/or vaginal discharges from infected bitches. Semen, urine, saliva, nasal secretions, and mammary secretions from infected dogs may also transmit the disease to others.

Dogs who do not show any clinical signs of infection can still harbor the bacteria for long periods of time. Typically, it takes about 21 days from exposure to when bacteria appear in the blood stream (i.e., bacteremia). Once bacteria appear in the blood stream, the bacteria can localize (e.g., in the

prostate) where they cause continuous and recurrent bacteremia for anywhere from a couple of months to three to four years. Both the method of transmission and the volume of bacteria present in contaminated tissues influence how readily disease is transmitted.

The *B. canis* bacteria can settle in the prostate and/or epididymis (i.e., the elongated organ on the back of the testis that stores sperm while they mature) of a male who has no symptoms of disease. If the dog is an active stud dog these infection sites act as a source for widespread infection of breeding females. According to Carmichael and Joubert (1988), "Venereal transmission appears to occur most frequently when

infected males are bred to susceptible females and somewhat less often when susceptible males are bred to infected females." A common misunderstanding among stud dog owners is that stud dogs do not require testing before each breeding. Carmichael and Joubert's research strongly suggests that before each breeding (regardless of insemination method) both the stud dog and the bitch should be tested.

The highest concentrations of *B. canis* are expelled from the body during the first six months after infection, but transmission of *B. canis* via venereal routes occurs readily even when low numbers of bacteria are shed. The bacteria can be transmitted by natural mating and also by artificial insemination (i.e., fresh, chilled or frozen semen). Furthermore, bacteria can be excreted in urine for years.

Aborted placental tissues and post-abortion vaginal fluids (which can be secreted for up to six weeks) contain high numbers of bacteria. As such, infected and aborting breeding bitches in any kennel are a significant danger to other healthy, disease-free dogs.

B. canis bacteria can survive dry environments or low temperatures, particularly temperatures below freezing. The bacteria can remain viable for several months in water, aborted fetuses, and feces if temperatures are low, humidity is high, and there is no sunlight to the area. It can survive in dust and soil and can be spread via objects such as dishes, buckets, or clothing. Disinfection is not complicated and can be accomplished with commonly available chemicals (e.g., bleach).

How is *Brucellosis* Diagnosed?

Diagnosis of *B. canis* is complicated, difficult, and expensive. It typically involves multiple tests to screen and various combinations of other tests before a definitive diagnosis can be made. Baseline laboratory tests (e.g., complete blood count) generally do not detect *brucellosis*. Dogs must not be on antibiotics when being tested.

Screening Tests for *Brucellosis*

Serologic (blood serum) testing is useful for initial screening of dogs, but generally positive results on these tests do not provide a definitive diagnosis of *brucellosis*.

***B. canis* bacteria can survive dry environments or low temperatures, particularly temperatures below freezing.**

Rapid Slide Agglutination Test (RSAT) and Tube Agglutination Test (TAT), serological tests, are typically used for initial *brucellosis* screening because they are readily available, economical, and can quickly identify dogs that are negative. Because RSAT and TAT tests are highly sensitive (i.e., produce very few false negatives), they are good initial screens. For example, on the RSAT, 95% of dogs testing negative are truly negative. If your dog's RSAT comes back negative, the tested dog is mostly likely negative (unless the dog has been exposed to the bacteria sometime during the last 8-12 weeks).

The RSAT and TAT tests offer different information to the breeder. The RSAT offers a positive or negative test result. TAT provides a titer number. A TAT titer of 1:50 indicates early infection or recovery from infection. Dogs testing between 1:50 to 1:100 are considered suspicious for infection. Titers greater than 1:200 are highly suggestive for an active *brucellosis* infection.

Unfortunately the bacterial cell wall antigen used in both the RSAT and TAT tests can cross-react with antibodies of other bacteria that are not *B. canis*. As such, the tests react to antibodies that are not *B. canis*, producing false positive results. When this happens, the tests are said to be highly sensitive, but not very specific. Only 20-50% of dogs testing positive on RSAT are truly positive. The addition of 2-mercaptoethanol to RSAT and TAT can reduce false positives somewhat, but the bottom line is that any positive test result on either the RSAT or TAT should be verified using another more specific testing method.

Stud Dog



The Enzyme-Linked Immunosorbent Assay (ELISA) test is a newer serologic test that uses a heat-soluble bacterial extract of the *B. canis* bacteria (HE antigen) as the antigen. Initial results indicate that this test is both highly specific and highly sensitive, and pending additional research it has potential for becoming a verification test. ELISA testing is usually positive 30 days after infection. Test availability may be an issue now.

Agar Gel Immunodiffusion Test (AGID) identifies antibodies to cytoplasmic antigens of *B. canis* (CPAg-AGID). They do not cross-react with antibodies to other bacteria so the test is specific and is recommended by some states as a test to verify positive RSAT or TAT tests. The AGID test becomes positive 12 weeks after infection, so breeders should be aware that dogs recently infected can have negative results during that window. On the positive side, AGID tests can remain positive for 36 months following resolution of bacteremia.

Tests for Definitive Diagnosis

A diagnosis is typically made using a process involving a combination of serologic (blood) testing, bacterial culture, and/or polymerase reaction (PCR). A definitive diagnosis is made using culture or PCR analysis.

Bacterial culture involving the isolation of *B. canis* from infected tissues is a definitive test for diagnosing *brucellosis*. The presence of bacteria in the blood stream typically occurs 2-4 weeks after infection. Semen can be used for culture during the first three months of infection when the concentration of *B. canis* is highest. Vaginal swabs, aborted fetuses, and placentas may also be cultured. Be aware that blood cultures may be negative in chronically-infected dogs because bacteremia may come and go.

Polymerase reaction analysis (PCR) can be used to detect *Brucella* in various body tissues and fluids, including semen, vaginal swabs, blood, and aborted tissues. PCR is considered the gold standard but may not be available in all areas. PCR of whole blood can be more sensitive than bacterial culture because it can detect not only living but also dead bacteria. PCR tests on semen or vaginal swabs can be positive in cases where bacteremia is no longer present and blood PCR is negative.

Continued on next page

Dogs who do not show any clinical signs of infection can still harbor the bacteria for long periods of time.

Brucellosis

Continued from page 93

Is There a Treatment for *Brucellosis*?

There is no reliable treatment for *brucellosis*. In cases in which an antibiotic cocktail has been used, bacteria have been reduced but infection

has not been fully eliminated. While castration controls transmission via genital routes, it does not prevent the infected dog from spreading the disease via other routes. Studies have shown that sexu-

ally-mature uninfected male dogs housed together with sexually-mature infected dogs contract the disease in four to six months. In general, euthanasia, repeated testing of remaining dogs, and disinfection of the contaminated areas is the only way to stop the spread of disease. Clearly, disease diagnosis can be devastating to a breeding program, but ignoring it can be worse.

How Do I Reduce Risk of Infection of My Breeding Stock

Feldman and Nelson (2004) make the following recommendations to breeders:

- New additions to a breeding program should be isolated for at least one month. These dogs should have two negative *Brucella* titers one month apart before they are admitted to the kennel.
- Dogs in active breeding programs should be tested at least once every six months. Preferably, both the dog and bitch should be tested before each breeding.
- No *Brucella*-positive dog should be bred.
- If any dogs are found to test positive, they recommend the following protocol.
- The entire kennel must be tested, and those testing positive must be isolated and retested. Dogs that test positive over several

months over several tests should be removed from the kennel.

- Some tests for *B. canis* do not become positive for weeks to months after infection. Testing must continue on a monthly basis until all animals with positive results have been eliminated. Testing may need to be done indefinitely, including on non-breeding dogs.
- Regularly disinfect kennel areas and equipment.

General Recommendations

Everyone must assess for themselves what their risk exposure is and what preventive measures they will take. The following is a summary of recommendations offered by the various veterinary medicine academic experts.

For Breeders

- Test the stud dog and bitch three weeks prior to breeding. If positive, retest.
- Test virgin dogs before breeding. Breeding is not the only way to acquire *brucellosis*.
- Consider annual testing of castrated, intact, and dogs not being bred that live/interact with breeding dogs.
- Do not place infected dogs as pets.
- Practice good kennel hygiene.

For Breeders and Pet Owners

- Do not let your dogs lick or smell the urine or vaginal discharges (e.g., at dog shows, on walks).
- Before bringing any new dog (e.g., breeding dogs, puppy, rescue/foster) into your kennel/home, test the animal for *brucellosis*.

Dogs in active breeding programs should be tested at least once every six months. Preferably, both the dog and bitch should be tested before each breeding.



For Puppy Buyers

If you are buying a puppy, ask to see the *brucellosis* test results for both the sire and dam (i.e., the test should be dated about 12 weeks before the puppy's birthday).

Resources

Carmichael L.E. and J.C. Joubert (1988). "Transmission of *Brucella canis* by contact exposure." *Cornell Vet*, 78: pp. 63-73.

Carter, T. and C. Johnson (2012, July). "Brucella Canis: a Threat to Canine and Human Health." Proceedings of the 7th International Symposium on Canine and Feline Reproduction (ISCFR, 2012). Whistler, Canada.

Feldman, E. C. and R.W. Nelson (2004). "Brucellosis and Transmissible Venereal Tumor," in *Canine and Feline Endocrinology and Reproduction* (pp. 919-924). St. Louis, MO: Saunders.

Johnston, S.D., M.V. Root-Kustritz, and P.N.S. Olson (2001). *Canine and Feline Theriogenology*. Philadelphia, PA: Saunders.

Veterinary Information Network (2014, January 10), *Brucellosis* (Zoonotic). www.vin.com.

© 2014 Nancy P. Melone

About the Author

Nancy P. Melone, Ph.D., Editor Emerita of *The Alpenhorn*, breeds under the kennel name Thorn-Creek (www.thorncreekbmds.org). She was awarded the prestigious Dog Writers Association of America's Maxwell Medallion and the Morris Animal Foundation Advances in Canine Veterinary Medicine Award. She holds a Ph.D. in Information and Decision Sciences from the University of Minnesota. Nancy lives peacefully, covered in dog hair, with her husband and six Berners on 30 acres in Butler, Pennsylvania.

Credits: Special thanks to cartoonist, Bob Seaver, for the illustrations and proof readers, Ellen Folke and Timothy McGuire, Sr., Ph.D.

Looking for the right match for your Berner?

Try *The Alpenhorn's* **b•LIST** advertising special!

THE STUD DOG DIRECTORY IS BACK

June 2015

(Breeding issue of *The Alpenhorn*)

Only \$65/ad Save 13%

Ads include four-generation pedigree and your dog's photo. Ads are 1/2 page black and white, and will be prepared by *The Alpenhorn*. They will appear in a special advertising section within the June 2015 issue of *The Alpenhorn*.

Deadline for submission is April 1, 2015.

Contact Sharon Hermann for details,

Sharon@ZeldasSong.com.