Canine Brucellosis: *Brucella canis*

Contagious Abortion, Undulant Fever

Last Updated: April 2012



IOWA STATE UNIVERSITY®

College of Veterinary Medicine lowa State University Ames, Iowa 50011 Phone: 515.294.7189 Fax: 515.294.8259 cfsph@iastate.edu www.cfsph.iastate.edu



INSTITUTE FOR INTERNATIONAL COOPERATION IN ANIMAL BIOLOGICS

Iowa State University College of Veterinary Medicine www.cfsph.iastate.edu/IICAB/

Importance

Canine brucellosis, caused by *Brucella canis*, is an important cause of reproductive failure, particularly in kennels. This organism causes abortions, stillbirths, epididymitis, orchitis and sperm abnormalities in dogs. Although dogs that have been spayed or neutered do not have reproductive signs, they occasionally develop other conditions such as ocular disease and discospondylitis. *B. canis* can persist in an animal even after antibiotic treatment. In kennels, infected dogs are often euthanized to prevent them from infecting other dogs or people. Canine brucellosis is sometimes difficult to diagnose with the currently available tests.

Although *B. canis* is zoonotic, its importance as a cause of human illness is still unclear. Few cases have been reported in people, and most of these infections have been mild. However, human infections with *B. canis* may be underdiagnosed. The symptoms of this disease are nonspecific, diagnostic suspicion is low among physicians, and bacterial culture is the only commonly available test for diagnosis in people.

Etiology

In dogs, brucellosis is mainly caused by *Brucella canis*, a Gram-negative coccobacillus or short rod. This organism is a facultative intracellular pathogen. Other *Brucella* species occasionally associated with disease in dogs include *Brucella abortus*, *B. melitensis* and *B. suis*. (For information on these organisms, see the factsheets titled "Bovine Brucellosis," "Ovine and Caprine Brucellosis," and "Porcine Brucellosis" respectively.)

In addition to the organisms found in dogs, humans can be infected with the less virulent M- strain of *B. canis*, which is used as an antigen for serological testing. One clinical case was reported in a person exposed to this organism in the laboratory.

Geographic Distribution

B. canis appears to be widely distributed. Locations where it has been reported include the United States (particularly the southern states), Canada, Central and South America (including Mexico), some European countries, Tunisia, Nigeria, South Africa, Madagascar, the Philippines, Malaysia, India, Korea, Japan, Taiwan and China. New Zealand and Australia appear to be free of this organism.

Transmission

B. canis occurs in the fetus, placenta, fetal fluids and vaginal discharge after an abortion or stillbirth. It can persist in vaginal discharges for several weeks after an abortion. It is also shed in normal vaginal secretions, particularly during estrus, as well as in milk. High concentrations of *B. canis* may be found in semen for weeks or months after infection, and intermittent shedding of smaller quantities can occur for years. *B. canis* is also shed in urine, and low concentrations of bacteria may be excreted in saliva, nasal and ocular secretions, and feces.

In dogs, *B. canis* primarily enters the body by ingestion and through the genital, oronasal and conjunctival mucosa, but transmission through broken skin may also be possible. Most cases are thought to be acquired by venereal transmission or by contact with the fetus and fetal membranes after abortions and stillbirths. Puppies can be infected *in utero*, and may remain persistently infected even if they appear normal. Nursing puppies can be infected from milk, but the importance of this route is controversial. Other potential sources of infection include blood transfusions and contaminated syringes.

B. canis can be spread on fomites. In conditions of high humidity, low temperatures and no sunlight, *Brucella* spp. can remain viable for several months in water, aborted fetuses, feces, equipment and clothing. *Brucella* species can withstand drying, particularly when organic material is present, and can survive in dust and soil. Survival is longer when the temperature is low, particularly when it is below freezing.

Humans usually become infected with members of the genus *Brucella* by ingesting organisms or via the contamination of mucous membranes and abraded skin. In case reports, *B. canis* infections have been described after close contact with

dogs, especially animals that recently aborted or gave birth, and after exposure to large amounts of the organism in laboratories (e.g., contact with bacterial cultures). However, the source of the organism could not be determined in some cases.

Disinfection

Brucella species are readily killed by most commonly available disinfectants including hypochlorite solutions, iodophores, 70% ethanol, isopropanol, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene; however, organic matter and low temperatures decrease the efficacy of disinfectants. Disinfectants reported to destroy Brucella on contaminated surfaces include 2.5% sodium hypochlorite, quaternary ammonium compounds, 2-3% caustic soda, 20% freshly slaked lime suspension, or 2% formaldehyde solution (all tested for one hour). Ethanol, isopropanol, iodophores, substituted phenols or diluted hypochlorite solutions can be used on contaminated skin; alkyl quaternary ammonium compounds are not recommended for this purpose. Autoclaving [moist heat of 121°C (250°F) for at least 15 minutes] can be used to destroy Brucella species on contaminated equipment. These organisms can also be inactivated by dry heat [320-338°F (160-170°C) for at least 1 hour]. Boiling for 10 minutes is usually effective for liquids. Brucella species can also be inactivated by gamma irradiation.

Infections in Humans

Incubation Period

There is little information on the incubation period for brucellosis caused by *B. canis*. Relatively few cases have been documented, and in many cases reported in the literature, exposure was ongoing or the source was unknown. The symptoms caused by other *Brucella* spp. usually appear within 2 weeks of exposure, but some cases have developed as late as 3 months.

Clinical Signs

The virulence of *B. canis* for humans has been considered to be low, as few cases have been documented and most have been mild. However, infections may be underreported; specific diagnostic tests are rarely performed, and this disease can be difficult to diagnose.

Because few descriptions of human *B* canis infections have been published, the expected clinical signs are based mainly on the syndromes caused by other species of *Brucella*. Some people infected with *Brucella* spp. remain asymptomatic. In symptomatic cases, the disease is extremely variable and the clinical signs may appear insidiously or abruptly. Typically, brucellosis begins as an acute febrile illness with nonspecific flu-like signs such as fever, chills, headache, malaise, back pain, myalgia and generalized aches. Drenching sweats can occur, particularly at night. Some patients may also have lymphadenopathy, splenomegaly or hepatomegaly. Some patients with brucellosis recover spontaneously, while others develop persistent symptoms that typically wax and wane and may include fever, weakness and other nonspecific signs. Occasionally seen complications include arthritis. spondylitis, chronic fatigue and epididymo-orchitis. Neurologic (including personality changes, signs meningitis, uveitis and optic neuritis), anemia, internal abscesses, nephritis, vasculitis, endocarditis and dermatitis have been reported with some species of Brucella. Other organs and tissues can also be affected, resulting in a wide variety of syndromes. The mortality rate for brucellosis (which includes the disease caused by highly virulent organisms such as B. melitensis) is low; in untreated persons, estimates of the case fatality rate vary from less than 2% to 5%. Deaths are usually caused by endocarditis or meningitis.

Published infections with B. canis have included a variety of clinical signs and presentations consistent with this description. They range from mild fatigue, or fatigue and intermittent fever as the only symptoms, to a febrile illness with fatigue, malaise, nausea, chills, night sweats and headache. Fever of unknown origin, sometimes prolonged, was the presenting syndrome in some individuals. Enlargement of the spleen was reported in several cases. Weight loss, enlarged lymph nodes, abdominal pain, an enlarged liver and signs of liver dysfunction have also been documented. Nausea, vomiting and diarrhea have been described, especially in children, and one individual reported persistent cough, sore throat and conjunctival burning (in addition to night sweats, headache, lethargy and myalgia). Oral lesions were found in a child concurrently infected with B. canis and cytomegalovirus, and resolved with antibiotic treatment for brucellosis. Serious syndromes including endocarditis have been reported in a few cases. B canis was associated with aortic valve vegetations and lower extremity aneurysms in one boy, and calvarial osteomyelitis, epidural abscess, pleural effusion and pulmonary nodules in another child. Some conditions seen in other Brucella infections, such as epididymo-orchitis and neurological signs, have not been reported with B. canis as of 2012. This might be due either to the few cases known, or because this species is usually less pathogenic for humans than livestock Brucella. Some illnesses caused by B. canis persisted for several months before diagnosis. A few patients also relapsed with inadequate treatment.

A laboratory worker exposed to the less virulent Mstrain of *B. canis* developed symptoms similar to those caused by wild-type strains of *Brucella*.

Two *B. canis* infections were diagnosed in people infected with HIV-1. *B. canis* was found in the blood of one patient with a febrile syndrome. In another individual, the presenting signs included high fever, malaise, arthralgia and loose stools, with a 5 month history of low-grade fever, fatigue, arthralgia, myalgia and night sweats.

Communicability

There is no information about the communicability of *B canis* in humans. Other species of *Brucella* are not transmitted between people by casual contact, and transmission by other routes is unusual, though it can occur (e.g., by blood transfusion, accidental ingestion of contaminated material, or sexual intercourse).

Diagnostic Tests

Brucellosis caused by *B. canis* can be difficult to diagnose in humans. The symptoms are nonspecific, and few diagnostic tests are available. *B. canis* can sometimes be found in blood, and some cases have been detected when this organism was unexpectedly isolated during routine blood culture. However, *B. canis* grows slowly, and it may not appear within the time that blood cultures are routinely held. Overgrowth by other organisms may also prevent its detection in samples taken from non-sterile sites. Antibiotics can inhibit the growth of *B. canis*, even when the dose or type of antibiotic is not curative in the patient.

The serological tests used routinely to diagnose human infections with B. abortus, B. suis and B. melitensis do not detect antibodies to B. canis. These tests use "smooth phase" antigens ("smooth" lipopolysaccharide in the cell wall); however, B. canis is a "rough" form of Brucella. Reagents are not produced commercially for human B. canis tests, and serological tests for this organism are not performed routinely by diagnostic laboratories. In some reports in the literature, antibodies to B. canis were detected with tests developed for this purpose or adapted from canine assays at the institution. Most of these assays were agglutination tests. They included microagglutination, tube agglutination and the rapid slide agglutination test (RSAT). One group developed an indirect enzyme-linked immunosorbent assay (ELISA), and a serological study from the 1970s used agar gel immunodiffusion (AGID) for B. canis surveillance in humans. Cross-reactions can occur between Brucella and some other microorganisms in serological tests. Nonspecific reactions can also result in false positive agglutination. Some published case reports suggest that clinical resolution is associated with declining antibody titers to B. canis.

Relying on a single type of test (i.e., either culture or serology) may miss some infections. In the literature, *B canis* was not always found in the blood of some patients identified by serology, and conversely, serology did not always detect antibodies in people who had *B. canis* in the blood.

Treatment

Brucellosis in people is usually treated with an prolonged course combining two antibiotics. Different antibiotics may be recommended, depending on the patient's age and pregnancy status. Relapses can be seen if treatment is inadequate. Most occur within four to six months after the treatment ends. There is only limited experience with the treatment of *B. canis*; however, standard antibiotic treatments for brucellosis resulted in good responses and successful resolution in published cases. Two patients who were not given brucellosis-specific treatments, but had gradually improved on other antibiotics, experienced relapses. Their illnesses resolved with standard brucellosis-specific antibiotics. Another patient experienced two relapses before an extended 4-month treatment resulted in the disappearance of antibodies to *B. canis* and no further symptoms during a two year follow-up.

Prevention

Potential hazards to people should be discussed when brucellosis is diagnosed in a dog, as antibiotics do not reliably eliminate *B. canis*, and the level of risk to human companions is currently uncertain. Euthanasia of infected animals is usually recommended in kennels, and it is also an option in pets. Some authors recommend periodic serological monitoring of treated pets, to detect rising immune responses from recrudescence. Good hygiene is also likely to decrease human exposure, especially during births and abortions, but also during contact with urine, vaginal secretions and other potential sources of *B. canis*.

Prophylactic antibiotics may be prescribed for laboratory workers in some situations.

Morbidity and Mortality

There is little information about B. canis infections in humans. Although these infections are thought to be uncommon or rare, with only 18 documented cases between 1967 and 1982, they also appear to be underdiagnosed. Case reports in the literature describe infections in immunocompetent, healthy people and in individuals infected by HIV-1. Two disease clusters were recently reported in the literature. One occurred in two pet shop workers in Japan, probably as a result of handling an aborted canine fetus without protection. No other individuals who had contact with these two patients or with six infected dogs had evidence of exposure or illness. Those examined included colleagues at the pet shop, family members, laboratory workers who handled cultures, and a veterinarian. In a cluster of cases from Buenos Aires, three young children and three adults from two households became infected after close, regular contact with a pet dog and/or her three puppies. One of the three children was ill, while two had laboratory abnormalities or enlargement of the spleen at examination. All three children were treated with antibiotics. Two adults had serological evidence of exposure, but did not require treatment, and one adult described possible symptoms but declined clinical examination. The dogs were removed from the households, and the antibody titers in the people decreased over time.

In 2011, a serological study was conducted in patients who had signs consistent with brucellosis in Turkey. Among these patients, 8.9% were seropositive for *B. canis* in a RSAT test, 3.7% were seropositive by a 2mercaptoethanol RSAT (this test detects IgG and not IgM) and 3.8% by a microagglutination test. Some of these reactions were reported to be false positives, but false negatives were also found. In another recent Turkish study, which used a slide agglutination test (SAT) confirmed by 2mercaptoethanol SAT, the seroprevalence was 1.6% among healthy blood donors.

A number of serological surveys were conducted in the 1970s and early 1980s. Most of these studies found a low rate of reactors, but a few reported higher seroprevalence rates. In a German study, less than 0.5% of serum samples from blood donors (1400 samples) had antibodies to B. canis. No samples from clinical patients (480 samples), people exposed to dogs or infectious material (35 samples), veterinarians and assistants (16 samples) or animal caretakers (19 samples) were seropositive. The samples were tested by agglutination, and positive results were confirmed by complement fixation and indirect immunofluorescence. In the U.S., the seroprevalence was reported to be 0.4% in military recruits aged 18-26 (using an experimental tube agglutination test), and 0.6% in Florida residents. In contrast, a 1975 study from the Oklahoma Health Sciences Center reported an overall seroprevalence of 68% in people "with an average exposure to dogs," 73% in veterinarians, and 57% in male blood donors, using a microtiter technique to detect B. canis antibodies. In Mexico, 13% of hospitalized patients with various illnesses had antibodies to B. canis. In Argentina, 2% of individuals were seropositive in house-to-house surveillance, using AGID with a B. ovis antigen, and 0.2% were seropositive in a survey of rural schools.

Infections in Animals

Species Affected

B. canis is only known to be important in dogs. Antibodies to this organism have been reported occasionally in wild canids including foxes and coyotes, as well as in one raccoon (*Procyon lotor*). Experimental infections have been established in chimpanzees (*Pan troglodytes*), stumptail macaques (*Macaca arctoides*), rhesus macaques (*Macaca mulatta*), mice, rabbits and guinea pigs. Sheep, swine and cattle were reported to be highly resistant to experimental infection by oral and conjunctival inoculation; however, two field infections with *B. canis* have been reported in cattle. After oral inoculation, three of 14 experimentally infected cats developed bacteremia, but agglutinating antibodies were not detected.

Incubation Period

The period between infection and reproductive signs is variable; abortions are most common at approximately 7-9 weeks of gestation. Early embryonic deaths have also been reported two to three weeks after venereal transmission.

Clinical Signs

B. canis can cause abortions and stillbirths in pregnant dogs. Most abortions occur late, particularly during the seventh to the ninth week of gestation. Abortions are usually followed by a mucoid, serosanguinous or graygreen vaginal discharge that persists for several weeks. Early embryonic deaths and resorption have been reported a few weeks after mating, and may be mistaken for failure to conceive. Some pups are born live but weak. These pups often die soon after birth. Other congenitally infected pups can be born normal and later develop brucellosis. Clinical signs occur during subsequent pregnancies in some dogs, but not in others.

The sperm may have morphological abnormalities and reduced viability in some infected males. Epididymitis and scrotal edema are common in the acute stage, and orchitis may be seen. Scrotal dermatitis can occur due to selftrauma (e.g., licking). Unilateral or bilateral testicular atrophy can be seen in chronic infections, and some males become infertile. Prostatitis may lead to pain and difficult urination and defecation.

Lymphadenitis is common in infected dogs. The retropharyngeal lymph nodes may enlarge after oral infection, and the superficial inguinal and external iliac nodes after vaginal infection. Generalized lymphadenitis is also common. Other symptoms that are occasionally reported include lethargy or fatigue, exercise intolerance, decreased appetite, weight loss and behavioral abnormalities (loss of alertness, poor performance of tasks); however, most affected dogs do not appear seriously ill. Occasionally, discospondylitis of the thoracic and/or lumbar vertebrae can cause stiffness, lameness or back pain. Chronic uveitis, endophthalmitis, polygranulomatous dermatitis, endocarditis, osteomyelitis related to hip prostheses, and meningoencephalitis/ low grade meningitis have also been reported. Fever is rare. Many infected dogs remain asymptomatic.

Dogs with brucellosis may recover spontaneously, beginning a year after infection, but recovery is more common after two to three years, and some dogs remain chronically infected for years. Deaths are rare except in the fetus or newborn.

Post Mortem Lesions

The lymph nodes are often enlarged in affected animals. The retropharyngeal and inguinal lymph nodes are often involved, but generalized lymphadenitis also occurs. The spleen is frequently enlarged, and may be firm and nodular. Hepatomegaly may also be seen. Scrotal edema, scrotal dermatitis, epididymitis, orchitis, prostatitis, testicular atrophy and fibrosis are common in males, and metritis and vaginal discharge may be seen in females. Less commonly reported lesions include discospondylitis, meningitis, focal non-suppurative encephalitis, osteomyelitis, uveitis and abscesses in various internal organs. Aborted puppies are often partially autolysed and may have evidence of generalized bacterial infection. These lesions can include subcutaneous edema, subcutaneous congestion and hemorrhages in the abdominal region, serosanguinous peritoneal fluid, and degenerative lesions in the liver, spleen, kidneys and intestines.

Communicability

B. canis is shed in semen, with the highest concentration during the first six months after infection, and it may be excreted intermittently for years. In addition to natural mating, this organism can be transmitted by artificial insemination with fresh, chilled or frozen semen. Other sources of *B. canis* include the fetus, placenta, fetal fluids and vaginal discharge after an abortion or stillbirth, as well as normal vaginal secretions, particularly during estrus. Urine and other body fluids and excretions can contain *B. canis*. Chronically infected dogs can shed this organism despite being seronegative and blood culture negative.

Uninfected dogs living with infected animals of the same sex were found to acquire the infection within 6 months.

Diagnosis

Canine brucellosis should be considered in dogs with reproductive conditions; however, some infected dogs are asymptomatic or have only nonspecific signs such as lymphadenitis. Focal signs such as discospondylitis or uveitis can also occur in spayed or neutered animals. Canine brucellosis is sometimes difficult to diagnose, and the best chance of success is if multiple techniques (e.g., culture, PCR and serology) are used in combination.

The rapid slide agglutination test (RSAT) and the tube agglutination test (TAT) are often used to detect antibodies to B. canis in dogs. The RSAT is a rapid commercial test that can be used for screening. Adding 2-mercaptoethanol (2-ME) to these assays (i.e., the 2-ME RSAT or 2-ME TAT) improves specificity by dissociating IgM, which is more likely than IgG to cross-react with other bacteria. However, this can also decrease sensitivity, especially during the early stage of the immune response when IgM predominates. Other serological tests that have been used either clinically or in research include AGID, ELISA, an indirect fluorescent antibody (IFA) test, complement fixation, a lateral flow immune-chromatographic assay (LFIA) and counter-immunoelectrophoresis. Positive reactions in some screening tests, such as RSAT, are often confirmed with a more specific assay. Titers vary between individuals and with the detection method.

A number of factors should be considered when using serological tests to diagnose *B. canis* infections, False positive reactions can be a problem, as cross-reactions can occur between this organism and other Gram-negative bacteria such as *Bordetella* and *Pseudomonas*. Nonspecific agglutination reactions can also occur. Conversely, antibodies to *B. canis* may be absent in dogs during the early stages of the infection (i.e., before the development of antibodies), as well as in chronically infected animals. Some tests, such as RSAT, have a reputation for a high incidence of false positives. However, a recent study found that some reactions which would be classified as false positives, based on culture and clinical signs alone, occur in animals that are positive by PCR.

A definitive diagnosis can be made if B. canis is cultured from an animal. Brucella spp. can be isolated on a variety of plain media, or selective media such as Farrell's medium or Thayer-Martin's modified medium. Enrichment techniques can also be used. B. canis colonies are naturally rough (R) or mucoid (M). This organism grows slowly, making detection more difficult. Bacterial overgrowth can be a problem in nonsterile samples. Blood cultures are often used to detect B. canis. Bacteremia can be intermittent, and the number of organisms in the blood may be low. Repeated culture may be necessary for isolation. Samples from the genital tract (e.g., semen, vaginal discharges, placenta) are often more useful than blood, especially in animals with reproductive signs. B. canis may also be detected in milk, urine and aborted fetuses (gastric contents, liver, spleen), as well as in tissues such as the lymph nodes, spleen, prostate, epididymis, testis, uterus, liver and bone marrow, and clinically affected vertebrae or eyes. Bacteria are not always found, especially in chronically infected dogs. Antibiotics can also interfere with culture.

B. canis colonies can be identified by phenotypic methods or genetic techniques. The 'Bruce-ladder' multiplex PCR assay, which can be used to distinguish species of *Brucella*, sometimes misidentifies *B. canis* as *B. suis*. Methods to solve this problem, including substitution of a pair of primers in the Bruce-ladder assay, and a new multiplex conventional PCR assay (Suis-ladder) have been published. Single nucleotide polymorphism (SNP) typing has also been reported to correctly identify *B. canis*.

Real-time polymerase chain reaction (PCR) assays can be used to detect *B. canis* in samples such as semen, urine, and vaginal and uterine swabs. PCR can also be attempted on whole blood.

Treatment

Some affected dogs have been treated successfully with long-term antibiotics. Treatment usually requires a combination of two different antibiotics, but enrofloxacin alone appeared to be successful in one trial. A few case reports have also documented the successful treatment of chronic or recurrent endophthalmitis caused by *B. canis*.

No treatment is certain to eliminate *B. canis*. Even when this organism seems to have disappeared, it may persist in tissues such as lymph nodes, spleen, uterus and prostate. Recrudescence is possible, especially when an animal is stressed. For this reason, euthanasia of infected animals is often recommended in kennels, and this option should also be discussed when the disease is found in a pet. Neutering can be used as an additional control measure in treated animals, if they are intact. Periodic serological monitoring may be able to detect rising antibody titers during recrudescence.

Prevention

Canine brucellosis is usually introduced into a kennel in an infected dog or semen. New animals should be isolated and tested before adding them to the general population. A second serological test, performed before release from quarantine, may detect animals that are in the early stage of the infection and seronegative on arrival. Due to the difficulties in brucellosis testing, some infected animals may not be identified. It has also been recommended that dogs be tested before allowing them to breed. Some authors recommend testing all dogs in a kennel, either annually or twice a year. This may reduce losses in the event that *B. canis* is introduced.

In infected kennels, brucellosis can be controlled by sanitation and infection control measures, together with the euthanasia, isolation or removal (e.g., to a research facility) of infected dogs. Housing in individual cages reduces the spread of the organism. Dogs from infected kennels should not be sold or used for breeding. Repeated testing and removal of infected animals, combined with quarantine and testing of newly added dogs, has been used to eradicate brucellosis from some kennels. There is no vaccine for *B. canis*.

Morbidity and Mortality

All breeds of dogs are susceptible to canine brucellosis. *B. canis* spreads rapidly in confined populations, especially during breeding or when abortions occur. Although death is rare, except in the fetus and neonate, significant reproductive losses can be seen, particularly in breeding kennels. Up to 75% fewer puppies may be weaned from affected kennels.

The prevalence of canine brucellosis is still incompletely understood. In the southern U.S., one author estimated that approximately 6% of dogs overall had antibodies to B. canis in the 1970s. Several individual surveys at that time reported that infections were more common in stray and feral dogs than in pets. In one study, 7.6% of the stray dogs and no pets on an Air Force Base in Mississippi had antibodies to B. canis. A survey from Tennessee found seropositive dogs only among free roaming animals and not those that were confined. Another survey reported that 9% of mature stray dogs but only 1% of pet dogs were seropositive. Among dogs tested in animal shelters in Illinois and Wisconsin in the 1970s, 6.7% were seropositive in a slide agglutination test, 1.5% were seropositive when confirmed by tube agglutination, and the organism was isolated from the blood of 0.2%. One recent paper mentioned unpublished work demonstrating antibodies in less than 2% of stray dogs in an animal shelter in Oklahoma, and all of these dogs were negative by culture. However, some authors report that the prevalence might be rising in some populations. In

Canine Brucellosis

Oklahoma, 2% of domesticated dogs were reported to be seropositive in 1994-1995, and 13% in 2002- 2003.

Antibodies to *B. canis* have also been found occasionally in wild canids in the U.S. In one study, 22% of the San Joaquin kit foxes (*Vulpes macrotis mutica*) tested from one region of California had antibodies to *B. canis* in 1981-82. No foxes from the same region were seropositive in 1984. Antibodies to *B. canis* have also been detected sometimes in coyotes, but several surveys in this species, including some conducted within the last two decades, did not find any seropositive animals.

In Canada, one study reported that 1.6% of dogs were seropositive by the 2-mercaptoethanol tube agglutination test in Quebec in the 1970s, and 0.6% had titers that indicated active infections. A study published in 1980 found that 5% of dogs in southwestern Ontario were seropositive by RSAT, 1.5% of these samples had suspicious titers by tube agglutination, 0.05% had a positive titer by tube agglutination, and 0.3% were positive by immunoprecipitation.

Several surveys have been published for Central and South America. One study from Mexico City reported that approximately 12% of the stray dogs tested had evidence of infection with B. canis in the 1970s. However, another survey from Mexico City found that 28% of the dogs tested were seropositive in an agglutination test, and B. canis was isolated from the blood of 1.6%. In 1980, 30.5% of stray dogs in a county of Buenos Aires were seropositive, and the organism was isolated from 6%. Additional studies of dogs in Buenos Aires were published in 2008 and 2009. One found antibodies to B. canis in 7.3% of the dogs tested in low income areas, and isolated the organism from 1.7% of these animals. The other reported that 15% of the dogs presented at a free neuter program were seropositive in the RSAT, and seropositivity was confirmed in 11% of these animals with an indirect ELISA. Almost all of the dogs at this clinic (98%) were female, and many of the seropositive animals came from areas where dogs are allowed to roam.

A few surveys have been published from countries in Asia. In 1976-1977, antibodies to *B. canis* were found in 11% of the dogs in some parts of Japan, and the organism was isolated from 37% of these seropositive animals. A 2008 study from Japan reported that 2.5% of the dogs in an animal shelter were seropositive, using a microplate agglutination test. In a Chinese study from the 1980s, the seroprevalence rates for dogs from various provinces varied from 0.5% to 43%, with higher seropositivity in older animals. In a study published in 2011 from India, the seroprevalence was 2.3% by 2-ME tube agglutination, 3% with a dot ELISA, 1.5% by AGID and 16% by indirect-ELISA.

Occasional reports of clinical cases have been published from Europe, but few surveys have been conducted. In a survey from West Germany during the 1970s, 1.8% of pet dogs had titers of 1:50, 2.9% had titers of 1:100, and 1.3% had titers of 1:200 in the tube

agglutination test. Using complement fixation, agar gelprecipitation and indirect immunofluorescence, only 0.2% of these samples were confirmed positive. In Turkey, reported seroprevalence rates in dogs were 6.3% and 6.7% in two surveys from the 1980s, and 7.7% in a survey published in 2005. Using both PCR and culture, evidence of infection was found in 8.3% of lymph node samples collected from dogs that died in a Turkish city pound in 2007-2008.

There is very little information on the seroprevalence in Africa. In a recent study from Nigeria, 0.3% of the household dogs tested had antibodies to *B. canis* by RSAT.

Internet Resources

Centers for Disease Control and Prevention (CDC). Brucellosis. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellos

is_t.htm

Food and Agriculture Organization of the United Nations. Manual for the Recognition of Exotic Diseases of Livestock, A Reference Guide for Animal Health Staff. <u>http://www.spc.int/lrd/ext/Disease_Manual_Final/index.h</u> <u>tml</u>

Public Health Agency of Canada. Material Safety Data Sheets <u>http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-</u> eng.php

The Merck Manual www.merckmanuals.com/professional/index.html

The Merck Veterinary Manual <u>http://www.merckvetmanual.com/mvm/index.jsp</u>

References

- Alton GG, Forsyth JRL. *Brucella* [online]. In Baron S, editor. Medical microbiology. 4th ed. New York: Churchill Livingstone; 1996. Available at: <u>http://www.ncbi.nlm.nih.gov/books/NBK8572/</u>. Accessed 4 Jun 2007.
- Aras Z, Uçan US. Detection of *Brucella canis* from inguinal lymph nodes of naturally infected dogs by PCR. Theriogenology. 2010;74(4):658-62.

Barkha S, Dharmendra Kumar S, Dhirendra Kumar S. Immunochemical characterization of antigens of *Brucella canis* and their use in seroprevalence study of canine brucellosis. Asian Pac J Trop Med. 2011;4(11):857-61.

- Bischof R, Rogers DG. Serologic survey of select infectious diseases in coyotes and raccoons in Nebraska. J Wildl Dis. 2005;41(4):787-91.
- Blankenship RM, Sanford JP. *Brucella canis*. A cause of undulant fever. Am J Med. 1975;59(3):424-6.

- Boebel FW, Ehrenford FA, Brown GM, Angus RD, Thoen CO. Agglutinins to *Brucella canis* in stray dogs from certain counties in Illinois and Wisconsin. J Am Vet Med Assoc. 1979;175(3):276-7.
- Boeri E, Escobar GI, Ayala SM, Sosa-Estani S, Lucero NE. Canine brucellosis in dogs in the city of Buenos Aires. Medicina (B Aires). 2008;68(4):291-7.
- Bosu WT, Prescott JF. A serological survey of dogs for *Brucella* canis in southwestern Ontario. Can Vet J. 1980;21(7):198-200.
- Brower A, Okwumabua O, Massengill C, Muenks Q, Vanderloo P, Duster M, Homb K, Kurth K. Investigation of the spread of *Brucella canis* via the U.S. interstate dog trade. Int J Infect Dis. 2007;11(5):454-8.
- Brown J, Blue JL, Wooley RE, Dreesen DW. *Brucella canis* infectivity rates in stray and pet dog populations.Am J Public Health. 1976;66(9):889-91.
- Brown J, Blue JL, Wooley RE, Dreesen DW, Carmichael LE. A serologic survey of a population of Georgia dogs for *Brucella canis* and an evaluation of the slide agglutination test. J Am Vet Med Assoc. 1976;169(11):1214-6.
- Cadmus SI, Adesokan HK, Ajala OO, Odetokun WO, Perrett LL, Stack JA. Seroprevalence of *Brucella abortus* and *B. canis* in household dogs in southwestern Nigeria: a preliminary report. J S Afr Vet Assoc. 2011;82(1):56-7.
- Carmichael LE. Canine brucellosis. Isolation, diagnosis, transmission. Proc US Livestock Sanit Ass. 1968;71:517-27.
- Carmichael LE, Shin SJ. Canine brucellosis: a diagnostician's dilemma. Semin Vet Med Surg (Small Anim). 1996;11:161-65.
- Carmichael LE, Zoha SJ, Flores-Castro R. Problems in the serodiagnosis of canine brucellosis: dog responses to cell wall and internal antigens of *Brucella canis*. Dev Biol Stand. 1984;56:371-83.
- Centers for Disease Control and Prevention [CDC]. Brucellosis (*Brucella melitensis, abortus, suis,* and *canis*). CDC; 2005 Oct. Available at:

http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis_t.ht m. Accessed 6 May 2012.

- Davis DS, Boeer WJ, Mims JP, Heck FC, Adams LG. *Brucella abortus* in coyotes. I. A serologic and bacteriologic survey in eastern Texas. J Wildl Dis. 1979;15(3):367-72.
- Diker KS, Aydfn N, Erdeger J, et al. (1987): Serologic survey of dogs for *Brucella canis* and *Brucella abortus* and evaluation of mercaptoethanol microagglutination test. Ankara Univ Vet Fak Derg. 1987;34:268–77.

Flores-Castro R, Segura R. A serological and bacteriological survey of canine brucellosis in Mexico. Cornell Vet. 1976;66(3):347-52.

Flores-Castro R, Suarez F, Ramirez-Pfeiffer C, Carmichael LE. Canine brucellosis: bacteriological and serological investigation of naturally infected dogs in Mexico City. J Clin Microbiol. 1977;6(6):591-7.

- Galphin SP Jr. A serologic survey for *Brucella canis* in dogs on a military base. J Am Vet Med Assoc. 1977;171(8):728-9.
- Gardner DE, Reichel MP. No evidence of *Brucella canis* infection in New Zealand dogs. Surveillance. 1997; 24:17-18.

Garner G, Saville P, Fediaevsky A. Manual for the recognition of exotic diseases of livestock: A reference guide for animal health staff [online]. Food and Agriculture Organization of the United Nations [FAO]; 2003. Brucellosis (canine). Available at: <u>http://www.spc.int/lrd/ext/Canine-Feline/BRUCELLOSIS%28CANINE%29E.HTM</u>. Accessed 4 Jun 2007.

- Gese EM, Schultz RD, Johnson MR, Williams ES, Crabtree RL, Ruff RL. Serological survey for diseases in free-ranging coyotes (*Canis latrans*) in Yellowstone National Park, Wyoming. J Wildl Dis. 1997 Jan;33(1):47-56.
- Gous TA, van Rensburg WJ, Gray M, Perrett LL, Brew SD, Young EJ, Whatmore AM,Gers S, Picard J. *Brucella canis* in South Africa. Vet Rec. 2005;157(21):668.
- Government of Tasmania, Department of Primary Industries and Water [DPIW]. Brucellosis in sheep [online]. DPIW; 2012 April. Available at: http://www.dpiw.tas.gov.au/inter.nsf/WebPages/CART-

6SN7UA?open. Accessed 13 Jun 2007.

- Gyuranecz M, Szeredi L, Rónai Z, Dénes B, Dencso L, Dán Á, Pálmai N, Hauser Z, Lami E, Makrai L, Erdélyi K, Jánosi S. Detection of *Brucella canis*-induced reproductive diseases in a kennel. J Vet Diagn Invest. 2011;23(1):143-7.
- Higgins R, Hoquet F, Bourque R, Gosselin Y. A serological survey for *Brucella canis* in dogs in the Province of Quebec.Can Vet J. 1979;20(11):315-7.
- Hoff GL, Bigler WJ, Trainer DO, Debbie JG, Brown GM, Winkler WG, Richards SH, Reardon M. Survey of selected carnivore and opossum serums for agglutinins to *Brucella canis*. J Am Vet Med Assoc. 1974;165(9):830-1.
- Hoff GL, Schneider NJ. Serologic survey for agglutinins to *Brucella canis* in Florida residents. Am J Trop Med Hyg. 1975;24(1):157-9.
- Hollett RB. Canine brucellosis: outbreaks and compliance. Theriogenology. 2006;66:575-87.
- Holzman S, Conroy MJ, Davidson WR. Diseases, parasites and survival of coyotes in south-central Georgia. J Wildl Dis. 1992;28(4):572-80.
- Istanbulluoglu E, Diker S. A serological analysis of *Brucella canis*. Ankara Univ Vet Fak Derg. 1983;30:14–8.
- Jiang H, Mao LL, Zhao HY, Li LY, Piao DR, Tian GZ, Di DD, Lei L, Cui BY. Reemergence and genetic comparison of *Brucella canis* in China, using a multiple-locus variablenumber tandem-repeat assay. Vet Microbiol. 2012;154(3-4):419-21.

Kahn CM, Line S, Aiello SE, editors. The Merck veterinary manual. Whitehouse Station, NJ: Merck and Co; 2011. Brucellosis in dogs. p. 1288-9.

Keid LB, Soares RM, Vasconcellos SA, Megid J, Salgado VR, Richtzenhain LJ. Comparison of agar gel immunodiffusion test, rapid slide agglutination test, microbiological culture and PCR for the diagnosis of canine brucellosis. Res Vet Sci. 2009;86(1):22-6.

Kikuchi YK, Sakuma YS, Sato T, Suzuki S, Hoshi S, Sato K, Nobunaga T, Isayama Y, Machishima Y, Ishida N. A survey of *Brucella canis* infection in dogs sheltered in Tohoku University School of Medicine. Jikken Dobutsu. 1979;28(2):279-86.

- Kimura M, Imaoka K, Suzuki M, Kamiyama T, Yamada A. Evaluation of a microplate agglutination test (MAT) for serological diagnosis of canine brucellosis. J Vet Med Sci. 2008;70(7):707-9.
- Kortepeter M, Christopher G, Cieslak T, Culpepper R, Darling R, Pavlin J, Rowe J, McKee K, Eitzen E, editors. Medical management of biological casualties handbook [online]. 4th ed. United States Department of Defense; 2001. Brucellosis. Available at: http://www.vnh.org/BIOCASU/7.html.* Accessed 16 Dec 2002.
- Koylass MS, King AC, Edwards-Smallbone J, Gopaul KK, Perrett LL, Whatmore AM. Comparative performance of SNP typing and 'Bruce-ladder' in the discrimination of *Brucella suis* and *Brucella canis*. Vet Microbiol. 2010;142(3-4):450-4.
- Lawaczeck E, Toporek J, Cwikla J, Mathison BA. *Brucella canis* in a HIV-infected patient. Zoonoses Public Health. 2011;58(2):150-2.
- Ledbetter EC, Landry MP, Stokol T, Kern TJ, Messick JB. Brucella canis endophthalmitis in 3 dogs: clinical features, diagnosis, and treatment.Vet Ophthalmol. 2009;12(3):183-91.
- Lewis GE Jr, Anderson JK. The incidence of *Brucella canis* antibodies in sera of military recruits. Am J Public Health. 1973;63(3):204-5.
- Li YK. A study on one strain of *Brucella canis* isolated from a cow at the first time. Honghua Liu Xing Bing Xue Za Zhi. 1988:9;342–4.
- López G, Ayala SM, Efron AM, Gómez CF, Lucero NE.A serological and bacteriological survey of dogs to detect *Brucella* infection in Lomas de Zamora, Buenos Aires province. Rev Argent Microbiol. 2009;41(2):97-101.
- López-Goñi I, García-Yoldi D, Marín CM, de Miguel MJ, Barquero-Calvo E, Guzmán-Verri C, Albert D, Garin-Bastuji B. New Bruce-ladder multiplex PCR assay for the biovar typing of *Brucella suis* and the discrimination of *Brucella suis* and *Brucella canis*. Vet Microbiol. 2011;154(1-2):152-5.
- Lovejoy GS, Carver HD, Moseley IK, Hicks M. Serosurvey of dogs for *Brucella canis* infection in Memphis, Tennessee.Am J Public Health. 1976;66(2):175-6.
- Lucero NE, Corazza R, Almuzara MN, Reynes E, Escobar GI, Boeri E, Ayala SM. Human *Brucella canis* outbreak linked to infection in dogs. Epidemiol Infect. 2010;138(2):280-5.
- Lucero NE, Escobar GI, Ayala SM, Jacob N. Diagnosis of human brucellosis caused by *Brucella canis*. J Med Microbiol. 2005;54:457-61.
- Lucero NE, Jacob NO, Ayala SM, Escobar GI, Tuccillo P, Jacques I. Unusual clinical presentation of brucellosis caused by *Brucella canis*. J Med Microbiol. 2005;54:505-8.
- Lucero NE, Maldonado PI, Kaufman S, Escobar GI, Boeri E, Jacob NR. *Brucella canis* causing infection in an HIV-infected patient. Vector Borne Zoonotic Dis. 2010;10(5):527-9.
- Makloski CL. Canine brucellosis management. Vet Clin North Am Small Anim Pract. 2011;41(6):1209-19.
- McCue PM, O'Farrell TP. Serological survey for selected diseases in the endangered San Joaquin kit fox (*Vulpes macrotis mutica*). J Wildl Dis. 1988;24:274-81.
- Monroe PW, Silberg SL, Morgan PM, Adess M. Seroepidemiological investigation of *Brucella canis* antibodies in different human population groups. J Clin Microbiol. 1975;2:382-386.

Moore JA, Gupta BN. Epizootology, diagnosis and control of *B. canis*. J Vet Med Ass. 1970:156: 1737-40.

Munford RS, Weaver RE, Patton C, Feeley JC, Feldman RA. Human disease caused by *Brucella canis*. A clinical and epidemiologic study of two cases. JAMA. 1975;231(12):1267-9.

Nicoletti P. Diagnosis and treatment of canine brucellosis. In Kirk RW, Bonagura JD, editors. Current veterinary therapy X. Small animal practice. Philadelphia, PA: WB Saunders; 1989. p. 1317-20.

Nomura A, Imaoka K, Imanishi H, Shimizu H, Nagura F, Maeda K, Tomino T, Fujita Y, Kimura M, Stein G. Human *Brucella canis* infections diagnosed by blood culture. Emerg Infect Dis. 2010;16(7):1183-5.

Oncel T, Akan M, Sareyyupoglu B, et al. Seroprevalence of *Brucella canis* infection of dogs in two provinces in Turkey. Turkish J Vet Anim Sci. 2005;29:779–83.

Percy DH, Egwu IN, Jonas AM. Experimental *Brucella canis* infection in the monkey (*Macaca arctoides*). Can J Comp Med. 1972;36(3):221-5.

Piampiano P, McLeary M, Young LW, Janner D. Brucellosis: unusual presentations in two adolescent boys. Pediatr Radiol. 2000;30(5):355-7.

Pickerill PA. Comment on the epizootology and diagnosis of canine brucellosis. J Am Vet Med Ass. 1970;156:1741-2.

Polt SS, Dismukes WE, Flint A, Schaefer J. Human brucellosis caused by *Brucella canis*: clinical features and immune response. Ann Intern Med. 1982;97(5):717-9.

Poulou A, Markou F, Xipolitos I, Skandalakis PN. A rare case of *Brucella melitensis* infection in an obstetrician during the delivery of a transplacentally infected infant. J Infect. 2006;53:e39-41.

Public Health Agency of Canada. Material Safety Data Sheet – *Brucella* spp. Office of Laboratory Security; 1999 Nov. Available at: <u>http://www.phac-aspc.gc.ca/lab-bio/res/psdsftss/msds23e-eng.php</u>. Accessed 4 Jun 2007.

Randhawa AS, Kelly VP, Baker EF Jr. Agglutinins to *Coxiella burnetii* and *Brucella* spp, with particular reference to *Brucella canis*, in wild animals of southern Texas. J Am Vet Med Assoc. 1977;171(9):939-42.

Rifkin GD, Supena RB, Axelson JA. Case report. *Brucella canis* bacteremia: a case with negative *B canis* agglutinins. J Med Sci. 1978;276(1):113-5.

Rousseau P. Brucella canis infection in a woman with fever of unknown origin. Postgrad Med. 1985;78(5):249, 253-4, 257.

Sauret JM, Vilissova N. Human brucellosis. J Am Board Fam Pract. 2002;15:401-6.

Sayan M, Erdenliğ S, Etiler N. Investigation of *Brucella canis* seropositivity by in-house slide agglutination test antigen in healthy blood donors. Mikrobiyol Bul. 2011;45(4):655-63.

Sayan M, Erdenlig S, Stack J, Kilic S, Guducuoglu H, Aksoy Y, Baklan A, Etiler N. A serological diagnostic survey for *Brucella canis* infection in Turkish patients with brucellosislike symptoms. Jpn J Infect Dis. 2011;64(6):516-9.

Schoenemann J, Lütticken R, Scheibner E. Brucella canis infection in man. Dtsch Med Wochenschr. 1986 3;111(1):20-2.

Shang DQ. Investigation of *B. canis* infection in China. Zhonghua Liu Xing Bing Xue Za Zhi. 1989;10(1):24-9.

Strom Holst B, Lofqvist K, Ernholm L, Eld K, Cedersmyg M, Hallgren G. The first case of *Brucella canis* in Sweden: background, case report and recommendations from a Northern European perspective. Acta Vet Scand. 2012;54(1):18.

Varela-Díaz VM, Myers DM. Occurrence of antibodies to Brucella canis in rural inhabitants of Corrientes and Neuquén Provinces, Argentina. Am J Trop Med Hyg. 1979;28(1):110-3.

Vinayak A, Greene CE, Moore PA, Powell-Johnson G. Clinical resolution of *Brucella canis*-induced ocular inflammation in a dog. J Am Vet Med Assoc. 2004;224(11):1804-7, 1788-9.

Wanke MM. Canine brucellosis. Anim Reprod Sci. 2004;82-83:195-207.

Wallach JC, Giambartolomei GH, Baldi PC, Fossati CA. Human infection with M- strain of *Brucella canis*. Emerg Infect Dis. 2004;10:146-8.

Wanke MM, Delpino MV, Baldi PC. Use of enrofloxacin in the treatment of canine brucellosis in a dog kennel (clinical trial). Theriogenology. 2006;66(6-7):1573-8.

Weber A, Brunner H. Seroepidemiological investigations on the incidence of *Brucella canis* antibodies in man. Zentralbl Bakteriol Orig A. 1977;238(2):237-43.

Weber A, Schliesser T. The occurrence of antibodies to *Brucella canis* in domestic dogs in the Federal Republic of Germany. Berl Munch Tierarztl Wochenschr. 1978;91(2):28-30.

Ying W, Nguyen MQ, Jahre JA. *Brucella canis* endocarditis: case report. Clin Infect Dis. 1999 ;29(6):1593-4.

*Link defunct as of 2007