# Public Health Implications of *Brucella canis* Infections in Humans

**Summary Findings and Recommendations of the *Brucella canis* Workgroup*, March 2012**
**National Association of State Public Health Veterinarians**

## I. Introduction

Brucellosis is a zoonotic disease, resulting in 100-200 human cases reported annually in the USA. The genus *Brucella* consists of six classically recognized species (or nomen 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). More recently, other species have been recognized: *B. ceti* (cetaceans), *B. pinnipedialis* (seals), *B. microti* (voles, also isolated from soil), and *B. inopinata* (single isolate from a human).

Veterinary public health officials typically are called upon to investigate zoonotic infections with *B. abortus*, *B. melitensis*, and *B. suis.* These species are all relatively well-defined with regards to their human health implications, diagnosis, and treatment. In contrast, the public health impact of *B. canis*, also considered a zoonotic species of *Brucella*, is much less clear, as is the optimal response when human exposure occurs. The purpose of this paper is to summarize what is known about *B. canis* from the public health perspective, point out gaps in knowledge, suggest ways to address these deficiencies, and develop interim consensus recommendations for managing human exposures to this bacterium.

*Brucella canis*, first identified in 1966, is a gram-negative nonmotile aerobic intracellular coccobacillus with rough colony morphology when grown on artificial medium. Dogs and wild *Canidae* are the only animal species that serve as reservoirs of *B. canis* under natural conditions. Information on the epidemiology, clinical signs, diagnosis, treatment, and prevention of canine brucellosis is readily available.[1,2]

## II. Canine Brucellosis

Although this paper will not discuss canine brucellosis in detail, some basic information about the disease in dogs is presented here which is pertinent to the discussion of human health risks associated with this pathogen.

*Brucella canis* is transmitted among dogs by mucosal contact with infected material. Vaginal discharges, semen, and fluids and tissues associated with birth and abortion contain the highest concentrations of the bacteria, but urine, blood, milk, saliva, and feces also contain organisms.[3] Pups can be infected *in utero*, intrapartum, or during nursing. The infective dose in dogs ranges from $10^4$ for the conjunctival exposure route to $10^6$ for the oral route. Concentrations of $10^3$ to $10^6$ organisms per ml have been found in urine of infected dogs.[2] Dogs can remain bacteremic for at least five years.[4] The primary clinical manifestations in dogs consist of reproductive problems, although prostatitis, uveitis, and diskospondylitis have also been described. Optimal control measures for canine brucellosis in breeding kennel situations include the testing and removal of infected animals, breeding management changes, and environmental controls. For pets in households, control measures are not as well established, and are complicated by the

human-animal bond and the uncertainty about the actual risk that an infected dog poses to its owners.

Treatment of dogs with brucellosis can be attempted but may not always be successful. Castration or spaying of infected dogs is thought to reduce the risk of transmission, although to our knowledge, this has not been verified experimentally. After neutering, an appropriate antibiotic regimen should be given (typically doxycycline plus an aminoglycoside). Treatment failures have been reported, and are more frequent in males because of sequestration of bacteria in the prostate. Elimination of *B. canis* after neutering and treatment should not be assumed. Instead, serial monitoring of agglutination titers and blood cultures should be performed to judge treatment efficacy.[2]

In general, the United States (US) has a modest prevalence of canine brucellosis compared to Mexico and Central and South America (1-8% compared with 20-30% respectively). Within the US, southern states appear to have a higher prevalence of infection when compared with the rest of the country, and the prevalence is higher in stray dogs versus owned animals.[2]

## III. Human infection with *B. canis*

### A. *Epidemiology*

The epidemiology of *B. canis* infection in humans is poorly understood. Although human brucellosis is a nationally notifiable condition, the Centers for Disease Control and Prevention (CDC) does not receive data on the etiologic species when brucellosis cases are reported. Therefore, the proportion of reported brucellosis attributable to *B. canis* is unknown. The lack of available laboratory diagnostic tests (discussed below) poses another impediment to the understanding of the epidemiology of this agent.

The vast majority of peer-reviewed articles on human brucellosis caused by *B. canis* consist of case reports about individual cases or small family clusters. Infection has been reported in persons in close contact with infected dogs and in laboratorians working with cultured *B. canis*.[5,6] The few serosurveys in the literature are dated and contradictory. A seroprevalence study of 1,208 healthy military recruits published in 1973 identified 5 (0.4%) with serologic evidence of current or previous *B. canis* infection.[7] A second study from 1975 tested convenience samples from 513 Florida residents and found 2 (0.39%) persons seropositive for *B. canis* infection.[8] Both of these studies used the tube agglutination test employing rough lipopolysaccharide antigen. However, a 1975 report found a much higher seroprevalence using a microtiter plate agglutination technique. This study examined sera from four groups of subjects: newborns (N=193), adults drawn from the general population (N=2,026), veterinarians (N=73), and patients who had fever of unknown origin (N=113). Seroprevalence in these four groups were 5.7%, 67.8%, 72.6%, and 80.5% respectively.[9] The rates of antibody prevalence in this last survey seem unrealistic and must raise concern about the specificity of the assay used. (Leland Carmichael, Cornell University, personal communication, 2012)

Since 1973, the CDC has isolated *B. canis* from approximately 50 human specimens. A recent literature review identified 43 documented human cases in the United States and approximately 14 more internationally since 1967 (Rita Traxler, CDC, personal communication, 2012). These low numbers would indicate that human illness due to *B. canis*

is probably not a significant public health concern. However, it seems likely that *B. canis* infections in humans are significantly underdiagnosed and under-reported, primarily due to the nonspecific presentation of the disease and the lack of readily available laboratory testing.

**B. *Clinical Manifestations***

Signs and symptoms of *B. canis* infections in humans are generally similar to those of brucellosis caused by *B. abortus* and *B. melitensis*. Manifestations 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.[10,11] 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[12], aortic valve vegetations, calvarial osteomyelitis, epidural abscess, pleural effusion[10], oral lesions[13], lower extremity aneurysms,[10,14] and culture negative endocarditis.[15] 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.[16,17]

**C. *Diagnosis***

***1. Antigen detection:***

The diagnostic gold standard remains the isolation of *B. canis* from a clinical specimen. However, *Brucella* spp. are relatively fastidious and grow slowly in vitro; therefore, cultures may be prematurely discarded and considered negative due to insufficient length of incubation. If brucellosis is suspected, cultures should be maintained for at least four weeks.[18] Bacteremia is typically intermittent and of a low level which may result in negative results even in patients who have brucellosis. Empiric treatment with antibiotics will also affect the ability to culture the organism. It is important to note that *B. canis* in culture, like all *Brucellae*, poses a significant occupational risk of infection to laboratory staff. Brucellosis is among the most frequently reported laboratory-acquired infections.

Polymerase chain reaction (PCR) assays have been developed[13,19] which are able to discriminate various species of *Brucella*, including *canis* and vaccine strains. Unfortunately, PCR assays to identify *B. canis* from primary patient specimens (e.g., blood) are not readily available in the US. Instead, they are used to speciate *Brucella* already growing in culture.

***2. Serology:***

In contrast to other *Brucella* species (*B. abortus*, *B. melitensis*, *B. suis*) which grow in smooth colonies, *B. canis* naturally forms rough phase (mucoid) colonies in culture. Serologic tests that use suspensions of smooth phase *Brucellae* are useless in diagnosing *B. canis* infections.[6] To our knowledge, all commercially available human serologic assays in the United States utilize smooth phase *Brucella* species as their antigen substrates, and therefore do not detect antibodies against the rough phased *B. canis*. Unfortunately, this limitation is typically not explained by commercial laboratories to clinicians who order *Brucella* serology. The CDC does not currently perform serologic testing for *B. canis*.

As would be expected, serologic tests for veterinary use are much more readily available and utilize the rough phase *Brucella* species of *canis* or *ovis*. (*B. canis* and *B. ovis* cross-react with each other.) Most veterinary assays rely on some form of agglutination test, such as rapid

slide agglutination, tube agglutination, or microtiter agglutination. Virtually all tests using rough phase *B. canis* or *B. ovis* can have relatively high frequencies of false positive results. Indirect immunofluorescence, ELISA, and even PCR assays have also been used, but can result in false positives if rough phase *B. canis* is used as the antigen. In order to reduce nonspecific reactions on agglutination assays, sera can be treated with 2-mercaptoethanol (2-ME) to eliminate the less specific IgM antibody. A 2-ME tube test for veterinary specimens is available at the USDA National Veterinary Services Laboratories.(Steven Hennager, USDA-NVSL, personal communication, 2012) Because suspensions of wild-type *B. canis* tend to aggregate even in the absence of specific antibodies, a less mucoid mutant strain (designated M-), which does not produce autoagglutination, has been recommended for serologic diagnosis to reduce the number of false positive results.[3,6] Newer assays such as agar gel immunodiffusion and ELISA tests that use antigens extracted from cytoplasmic proteins have the potential for increased specificity, but are not widely available.[1]

The availability of veterinary diagnostic tests and the lack of available human serologic assays raise the issue of the validity of using veterinary assays on human sera for the diagnosis of *B. canis* infection. This has been done, with the presumption that the veterinary 2-ME agglutination test is valid in humans for both diagnostic purposes and for following antibody titers post-treatment.[20] (Edward Young, Baylor College of Medicine, personal communication, 2012) We found one literature reference in disagreement, which stated: "The beta-mercaptoethanol test used in veterinary laboratories for detection of bacteremic dogs that maintain high IgG titers is not satisfactory for human disease in which the important consideration is detection of an immune response independent of the presence of bacteremia."[21] The rationale behind this rather confusing statement was not provided in the paper.

To our knowledge, the only laboratory that currently accepts human serum for *B. canis* antibody testing is run by Dr. Edward Young at Baylor University in Houston, Texas. Specimens are accepted on a limited basis from persons who are clinically suspected of having brucellosis or who have sustained a probable high risk exposure. A commercial veterinary test kit is used (D-Tec CB; Symbiotics Corp., Kansas City, Missouri, USA). The Baylor laboratory stipulates that the test is experimental and not approved by CLIA, CAP or the FDA. The D-Tec test is a rapid slide agglutination test that utilizes 2-ME. One study describes the RSAT, when used to test canine serum, as having high sensitivity and low specificity: "This test is sensitive and can be performed at the early stage of infection. Since false negatives are rare, it can be used as a filter to discard negative animals and carry out more specific tests on those that are positive. There is a commercial test available (D-Tec) … that uses a suspension of *B. ovis* stained with Rose Bengal that cross reacts with *B. canis* antigen and gives a high percentage of false positives, even with the addition of 2-ME."[3]

In summary, the diagnosis of human *B. canis* infection is challenging due to the nonspecific clinical presentation, the organism's fastidiousness in culture, and the lack of available serologic assays.

**3. *Routine laboratory tests*:**
Routine laboratory tests are generally not revealing, with patient WBC counts usually being normal or low.[18,21]

## IV. Recommendations: Public Health Follow up for Human Exposure

There is little reliable information on the prevalence and severity of *B. canis* infection in humans. Because of this, the optimal public health response for a recognized human exposure to an infected dog is unknown. Nevertheless, public health staff do receive reports of infected dogs from veterinary diagnostic laboratories and should have a response plan.

### A. ***Survey of existing state practices***

In the spring of 2011, State Public Health Veterinarians were surveyed about their state policies pertaining to *B. canis* infections. Of the 31 states represented in the responses, *B. canis* infection in humans was explicitly reportable in 28, and canine brucellosis was reportable to state animal health agencies in 18. When queried about their dissemination of information to human and veterinary state health agencies, 14/28 state health departments routinely notified their animal health agency partners, and 17/18 animal health agencies routinely notified their state health department counterparts.

Only five responding states have official policies that address brucellosis in dogs and four have specific policies on *B. canis* infections in humans beyond standard communicable disease follow up. However, 23 states do have legal authority to respond to reports of canine cases. In kennel situations, authority in various states exists, including closure of the kennel or revoking its license, mandating brucellosis testing, requiring that all positive dogs be neutered or euthanized, and prohibiting the adoption/sale of non-neutered dogs from the kennel. In household situations, authority includes quarantining all dogs in the household, requiring testing of dogs, neutering any positive dogs in the household, and testing and euthanasia of positive dogs. In states where such legal authority does not exist, these above measures are typically recommended rather than mandated. The majority of responding states do provide educational materials or consultation to pet owners and kennel operators regarding the zoonotic risk associated with a positive dog.

### B. ***Response to an Exposure to B. canis***

Because of the lack of reliable data on the severity and incidence of human *B. canis* infections, any recommendations can only be based on anecdotal case reports, incomplete surveillance data, and extrapolation from human brucellosis infections caused by other species. Due to these limitations, these guidelines must be considered interim. However, there is no doubt that *B. canis* is pathogenic for humans and can, on occasion, cause significant illness. Because of this, it seems most prudent to recommend some form of public health follow up on human exposures to *B. canis* until the virulence and the epidemiology of this disease is further defined.

#### *1. Notification and Weight of Evidence Prompting a Response*

Public health action is typically triggered by a report of the diagnosis of *B. canis* in a dog. Because canine brucellosis is not reportable to veterinary health authorities in some states, it is advisable to make the condition notifiable, or for state health departments to enter into a memorandum of understanding with veterinary diagnostic laboratories to report canine brucellosis to the state health department. However public health authorities become aware of the case, the dog owner(s) and the attending veterinarian should be contacted about the zoonotic potential of *B. canis*.

One of the initial considerations pertains to how the veterinary diagnosis was made. Obviously, isolation of the organism is conclusive, and some form of public health response would be warranted.

*Risk to Laboratorians*: In the event of a report of a positive culture, the diagnostic laboratory should be contacted to ensure that all work with the isolate had been performed under the appropriate biosafety precautions. The CDC has guidelines regarding potential exposure to *Brucella* in a laboratory setting and recommendations regarding antimicrobial prophylaxis in this setting (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5702a3.htm). Currently, these recommendations are the same regardless of the species of *Brucella* isolated.

Unlike bacterial isolation, a serologic diagnosis in a dog is not necessarily definitive for the reasons outlined above, and false positive results are not uncommon. Because of this, public health practitioners need to evaluate such serologic reports on a case by case basis to decide whether follow up is warranted from the human health standpoint. Some factors to consider include the type of serologic assay used, its inherent limitations, and whether the dog has a clinically compatible illness or history suggestive of brucellosis.

***2. Nature of the Public Health Response***

Canine Brucellosis Risk Factors: In the majority of human case reports, *B. canis* infection was related to exposure to whelping females when high concentrations of the organism occur in birthing fluids and vaginal discharge. An owner or kennel owner/manager who chooses to keep an infected non-neutered dog poses some increased public health risks to household members and kennel staff, alike. Puppies or "spent breeders" from an affected kennel may be placed in households where the new owners are unaware of their new dog's brucellosis status.

To prevent human infection, when a positive dog has been identified in a home or kennel:

a) Confirm the diagnosis with the veterinarian, which may involve confirmation from the reporting commercial laboratory.

b) Supply the following risk reduction information to the veterinarian and the owner:

   i. A brief description of brucellosis in the canine, including fluids likely to contain viable microorganisms

   ii. Information on the likelihood of, and route of *B. canis* transmission

   iii. A description of the clinical signs and symptoms of *B. canis* infection in humans, and the necessity to seek medical attention, should brucellosis be suspected

   iv. The difficulty in diagnosing human brucellosis caused by *B. canis*

   v. The likelihood of more severe disease in persons who are immune compromised

   vi. Recommendations to minimize the risks of human infection. These include:

      - The option of euthanasia of infected dog(s)
      - If the animal is not to be euthanized, the three step process of neutering, antibiotic treatment, and repeat testing should be advised

- A caveat that treatment of canines is not always effective, particularly in male dogs despite castration and treatment
- Hygiene measures pertaining to contact with canine urine, feces, and reproductive fluids.

While recognizing that the legal authority to impose control measures varies among states, the following actions should be considered:

- When canine brucellosis has been identified within a breeding kennel setting:
  At a minimum, the sale or adoption of dogs that have not been tested and found negative for brucellosis should be prohibited. Consideration may be given to allow the sale/adoption of spayed female dogs that have been treated with an approved antibiotic regimen. Other options such as revocation of a kennel's license or ordering the testing of all dogs in the kennel and euthanasia of positives can be considered. Decisions about these control measures are typically made by state animal health authorities.
- When canine brucellosis is diagnosed within a private household:
  Owner education should be performed that addresses the six points listed in the above section "*Nature of the Public Health Response"*. Consideration can be given to a quarantine order restricting the positive animal(s) to the owner's premises, and/or requiring the neutering of all positive dogs. Education can be provided over the phone, by printed materials distributed through the family veterinarian, or by directing the owner to information on the internet.

## V. Recommendations for Future Action

It should be clear from the above discussion that the true public health significance of human *B. canis* infections is still unknown, and will remain so unless deliberate attempts are made to address the issue. The two primary reasons for this knowledge gap are inadequate surveillance (i.e., species is not specified in reports of brucellosis to CDC) and the lack of a validated, readily available serologic test for *B. canis*-specific antibodies. 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. Of particular interest is whether a significant proportion of "culture negative" conditions such as endocarditis, osteomyelitis, and septic arthritis are actually caused by *B. canis*. *Brucella* infection is already recognized as one of the causes of culture negative endocarditis and septic arthritis.[22,23]

Accordingly, we recommend that the National Association of State Public Health Veterinarians consider a resolution for co-sponsorship with the Council of State and Territorial Epidemiologists (CSTE) at the 2012 CSTE annual meeting which requires the reporting of the etiologic *Brucella* species to CDC whenever a case of brucellosis is reported, and for CDC to ensure that the National Electronic Telecommunications System for Surveillance has the ability to accept this new variable. Additionally, this resolution should strongly encourage CDC, the National Institutes of Health, and other potential partners to develop, or facilitate the development of, a reliable assay to detect antibodies to *B. canis* in human serum. Besides

providing a means of clinical diagnosis, such an assay would permit seroprevalence studies on subsets of the population based on occupation, animal exposure, and medical condition (*e.g.*, endocarditis, osteomyelitis). Finally, the resolution should encourage state health departments (via CSTE) and departments of agriculture (via U.S. Animal Health Association) to share information about diagnoses of *B. canis* infections in humans and animals.

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