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A Comprehensive Review of *Brucella canis*: Zoonotic Risks and Preventive Strategies

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ABSTRACT

Brucella canis, a zoonotic agent primarily infects dogs and wild Canidae. Infection is notably suspected in dogs exhibiting epididymitis, infertility or disco-spondylitis. Recent reports indicate a growing incidence of *Brucella canis* infections in dogs, particularly among those imported into the UK from Eastern Europe. In India, the first reported case of *Brucella canis* infection was documented by in 1992.Although human infections by *B. canis* are relatively uncommon, clinical manifestations are typically mild, yet severe cases can potentially lead to septicemia. The disease in humans is incurable and spreads through contact with fluids from infected animals. Various diagnostic protocols, including Real-time PCR, Rapid slide agglutination test (RSAT), and Complement fixation test (CFT), are employed for the diagnosis of canine brucellosis. *Brucella canis*-specific quantitative polymerase chain reaction (qPCR) from non-invasive samples (vaginal swab or urine sample) allows its early detection. These diagnostic tests play a crucial role in diagnosing canine brucellosis. The "gold standard" for diagnosing brucellosis involves culture of *Brucella* isolated from body fluids (such as blood, cerebrospinal fluid, and urine) or tissues. Given the potential zoonotic risks, it is imperative to consistently include *B. canis* in diagnostic algorithms for canine diseases. Veterinary professionals play a vital role in this integrated approach, contributing to the prevention and management of *Brucella canis* infections in both animals and humans.
Keywords**:** *Brucella canis*, Diagnosis, Prevention, Zoonosis.

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INTRODUCTION

Infections caused by *Brucella* spp. are widespread among various animal species including humans. Various *Brucella* species, such as *Brucella abortus* and *B. melitensis*, can infect dogs. Dogs may serve as vectors, contributing to the dissemination of these organisms among farms and posing a potential source of human contamination. *Brucella canis*

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stands as a potentially infectious agent that can have devastating effects on both animal and human populations. Canine brucellosis, attributed to *B. canis* was first identified in the United States in 1966 following episodes of abortion and reproductive failure in kennels (Batinga, 2017; Carmichael and Shin, 1996). In Europe, canine brucellosis sporadically occurs and *B. canis* was identified in the United Kingdom in a dog imported from Spain (Djokic *et al*., 2023; Dunne *et al*., 2002). In India, the disease was reported by Srinivasan *et al.* (1992), revealing a seroprevalence of 1.9% in Madras city. Bacteria belonging to the genus *Brucella* are gram-negative, non-motile, non-encapsulated, non-spore-forming, facultative intracellular coccobacilli (Carmichael *et al*., 2006; Hollett, 2006; Keid *et al*., 2017). Of the six classical *Brucella* species, four are recognized to cause diseases in dogs and humans: *Brucella canis* (with dogs as the natural reservoir), *Brucella melitensis* (sheep and goats), *Brucella suis* (pigs), and *Brucella abortus* (cattle, bison, buffalo) (Carmichael *et al*., 2006; Hollett, 2006). Serological studies in wild canids have documented positive antibody titers in foxes and coyotes (Carmichael *et al*., 2006). In contemporary times, the growing interaction between humans and pet dogs has escalated. Considering the zoonotic potential of the disease, dedicated surveys are essential to enhance our comprehension of the incidence and progression of canine brucellosis. There is limited literature available regarding the clinical progression of brucellosis in patients concurrently affected by other diseases. The primary objective of this review is to furnish comprehensive information about *B. canis* infection and its zoonotic potential. This knowledge aims to contribute to the prevention of disease transmission and the mitigation of associated complications.

ZOONOTIC POTENTIAL AND MODES OF TRANSMISSION

There are four recognized *Brucella* species with the capability to infect humans. Among these species, *B. melitensis* exhibits the highest level of pathogenicity and invasiveness in humans, followed in decreasing order of pathogenicity by *B. suis*, *B. abortus*, and *B. canis* (Carmichael *et al*., 2006; Acha *et al*., 2003). *B. canis* is considered endemic in the southern United States, Central America, and South America, with reports extending to regions in Canada, Asia, Africa, and Europe (Buhmann *et al*., 2019; Cosford, 2018; Hensel *et al*., 2018). In Australia, it is classified as exotic, while in New Zealand, it is non-existent (Rovid, 2018; Mor *et al*., 2016). In the United States, approximately 100 to 200 cases of human brucellosis, caused by various *Brucella* species, are diagnosed annually (Kazmierczak, 2012). Detecting an outbreak of brucellosis can be challenging because the initial symptoms closely resemble those of influenza (Chain, 2005; Elbehiry *et al*., 2023). The transmission of *Brucella canis* to humans primarily occurs through contact with infected dogs or their secretions, as well as direct exposure in laboratory settings (Krueger *et al.,* 2014; Carmichael and Shin, 1996). Individuals at a higher risk of contracting this zoonotic infection include those who handle breeding dogs in kennels and come into contact with reproductive tissues and fluids from infected dogs (De-Massis *et al*., 2022;Hollett, 2006). It's worth noting that the pathogenicity of *Brucella canis* is considered relatively low, which places it at a lower perceived public health risk compared to other *Brucella* species, especially *Brucella melitensis*, as well as biotypes 1 and 3 of *Brucella suis* (Kaden *et al*., 2014; Spickler, 2018).Interestingly, HIV-positive patients with appropriate CD4 counts and negative viral loads have also been diagnosed with *B. canis* infections and have received successful treatment for these infections (Lucero *et al*., 2010). Despite its lower pathogenicity in comparison to other species, *B. canis* is still capable of infecting humans and causing serious illnesses (Lucero *et al*., 2005; Marzetti *et al*., 2013). Common clinical signs associated with human brucellosis include fever (often occurring periodically and at night), fatigue, headache, weakness, malaise, chills, sweats, weight loss, enlargement of the liver (hepatomegaly), spleen (splenomegaly), and lymph nodes (lymphadenopathy) (Carmichael *et al*., 2006; Hollett, 2006; Lucero *et al*., 2010). Serious complications arising from *B. canis* infection in humans may encompass septic arthritis, vegetative endocarditis, osteomyelitis, epidural abscess, pleural effusion, oral lesions, and lower extremity aneurysms (Carmichael *et al*., 2006; Hollett, 2006; Spickler, 2018). Occasionally, additional complications may manifest, including discospondylitis, uveitis, meningitis, glomerular nephritis, and draining skin lesions (Chomel and Arzt, 2013). Deaths attributable to *B. canis* infection are infrequent, except in cases involving severe underlying infections or delayed treatment.

PATHOGENESIS

The primary modes of *Brucella* transmission occur during everyday activities in dogs, including reproductive, social, and grooming interactions, which involve contact with the genital, conjunctival, and oro-nasal mucosae (Carmichael and Greene, 2006; Makloski, 2011). The primary sources of transmission are reproductive fluids, which encompass vaginal discharges, fluids associated with the fetus and placenta, and vaginal fluids following abortion or stillbirth, as well as semen (Kaltungo *et al*., 2014; Carmichael and

Greene, 2006). *Brucella* bacteria adhere to mucous membranes, penetrate the epithelial barrier, and are subsequently engulfed by the mononuclear phagocytic system, where they get intracellularly localized. This process involves the utilization of virulence factors, potentially through the type IV secretory system, and the inhibition of the bactericidal myeloperoxidase-peroxide-halide system, accomplished by releasing 5-guanosine and adenine (Hollett, 2006; Chacon-Diaz*et al*., 2015; Davidson and Sykes, 2014). The intracellular organisms then travel through the reticulo-endothelial system to reach local lymph nodes (such as the retropharyngeal, inguinal, and superficial iliac nodes), the liver, spleen, and possibly the bone marrow. After 7-30 days of infection, bacteria enter the bloodstream, resulting in intermittent bacteremia. These organisms primarily target 'steroid-dependent' reproductive tissues, including the prostate, testicles, epididymis, gravid uterus, and placenta (Hollett, 2006). These reproductive tissues typically exhibit a mixed inflammatory response, involving lymphocytes, plasmacytes, and histiocytes (Carmichael and Greene, 2006; Hollett, 2006; Brennan *et al*., 2008). Bacteremia spreads organisms and antibody-antigen complexes to the end-arterial circulation, leading to conditions like discospondylitis in the intervertebral disk or anterior uveitis and endophthalmitis in the eye (Carmichael and Greene, 2006; Hollett, 2006). Bacteremia can persist for extended periods, as seen in experiments where infected dogs still had positive blood cultures after 5.5 years (Hollett, 2006). After 3 to 4 months, bacteremia levels decrease, but the organism remains in the blood or becomes sequestered in tissues. Typically, cell-mediated immune responses lead to self-clearance, which occurs within an average of 2 to 3 years (Carmichael and Greene, 2006).

Clinical manifestations associated with *B. canis* infection encompass reproductive failure, with abortions frequently occurring between 45 and 55 days of gestation (Carmichael, 1966). Moreover, reports indicate reproductive failure and disrupted whelping patterns in association with *B. canis* infection (Moore and Gupta, 1970). In female dogs infected through mating, early embryonic death may occur approximately 2 to 3 weeks after transmission, presenting as a failure to conceive or infertility (Carmichael and Greene, 2006). Post-abortion, vaginal discharges are prevalent, displaying variability in duration (1 to 6 weeks), amount, and exudate appearance, typically characterized as serosanguineous but occasionally viscous and grayish-green (Holst *et al*., 2012). In male dogs, Orchitis is a reported manifestation of *B. canis* infection, with testicular swelling being infrequent and often imperceptible. However, palpation may reveal pain in the testicles or epididymis, and distention of the tunica vaginalis cavity with fibrinopurulent exudate has been documented (Makloski, 2011; Moore and Kakuk, 1969). Over time, affected males may develop chronic epididymitis and, ultimately, infertility attributed to the formation of antibodies that clump sperm together and delayed-type hypersensitivity reactionsagainst spermatozoa, resulting in spermatogenic arrest (Forbes and Pantekoek, 1988).

DIAGNOSIS

Brucella pathogen often initiates a subclinical infection that can go undetected for extended periods (Lucero *et al*., 2005; Lucero *et al*., 2010). When a febrile patient presents with signs and symptoms of an unknown cause, coupled with a history of close contact with dogs, healthcare providers

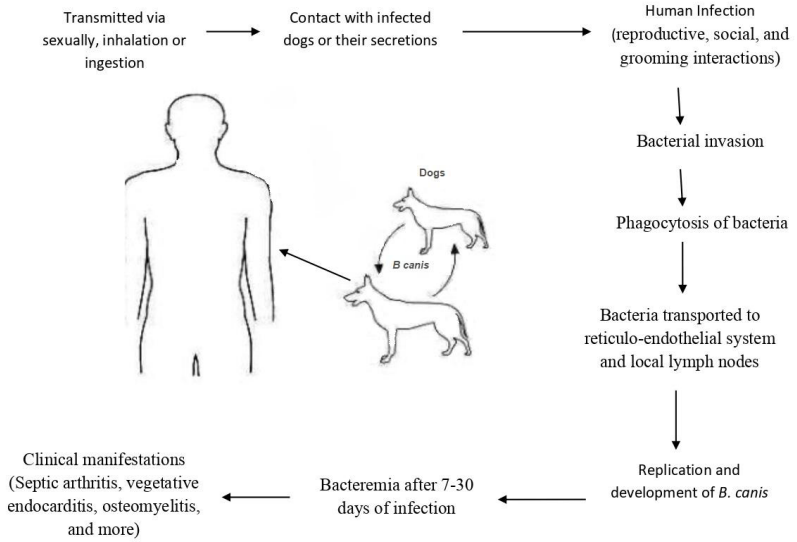

Fig. 1: Transmission and Pathogenesis of *Brucella canis* Infection

should consider the possibility of brucellosis. In such cases, it is imperative to take appropriate actions to both diagnose the condition and prevent the further spread of the infection (Nomura *et al*., 2010). Common diagnostic tests, including complete blood count (CBC), serum biochemistry profiles, and urinalysis, often produce normal results and may not be consistently effective in *B. canis* infection. On occasion, non-specific findings that suggest inflammatory disease may be observed, including leukocytosis, neutrophilia, hyperglobulinemia, and hypoalbuminemia (Lucero *et al*., 2005). The combination of patient history, clinical signs, and additional diagnostic assessments may lead to more definitive testing for *B. canis* infection. The definitive method/gold standard for diagnosing brucellosis is through direct bacteriological testing, which entails cultivating *Brucella* isolated from body fluids (e.g., blood, cerebrospinal fluid, urine) or tissues (Carmichael *et al*., 1996; Yagupsky, 1999). However, due to slow growth of *Brucella* bacteria, obtaining culture results may take several days or even weeks. Moreover, the bacteria necessitate specialized media with a carboxyphilic environment for optimal cultivation (Sabour *et al*., 2020). Commonly used serological tests include the rapid slide agglutination test (RSAT), often employed for screening, as well as the tube agglutination test (TAT). Immunofluorescent antibody tests (IFA) are also utilized to rule out infection. (Carmichael and Greene, 2006; Hollett, 2006; Keid *et al*., 2009; Lewis and Anderson, 1973). A positive screening test should be followed by a confirmatory test, such as the 2-mercaptoethanol RSAT (2ME-RSAT) or agar gel immune-diffusion assay employing an internal cytoplasmic antigen (AGIDcpa) (Carmichael and Greene, 2006; Hollett, 2006). Direct isolation and culture of *Brucella* are commonly performed. The complement fixation test (CFT), enzyme-linked immune sorbent assay (ELISA), and fluorescence polarisation assay (FPA) can be employed for detecting *B. canis*. However, many of these tests lack high sensitivity and specificity, necessitating the use of multiple techniques to enhance detection rates (Garin Bastuji *et al*., 2006). Notably, FPA has been found to be as effective as CFT and is recommended due to its accuracy, speed, and high throughput. These tests should be integral components of routine serological diagnosis for brucellosis (Skosana, 2021).

TREATMENT AND PREVENTION

The widely accepted recommendation is to discourage treatment and, instead, euthanize truly infected animals due to the risk they pose to both canine and human populations (Carmichael and Greene, 2006; Hollett, 2006; Bramlage, 2015). In cases where euthanasia is not possible due to client preferences, isolation can be considered, following thorough client education and appropriate medical record documentation. Individual with brucellosis showing no clinical signs of the disease should be isolated (Polak, 2019). Due to intracellular localization of *Brucella*, treatment is typically unsuccessful in dogs experiencing morbidity and persistent infection (Olsen and Boggiatto, 2022).Antibiotic therapy does not guarantee elimination of the organism and relapse or re-infection is believed to be common (Carmichael and Greene, 2006; Hollett, 2006; Spickler, 2018). Traditionally, a tetracycline-based antibiotic (such as tetracycline hydrochloride, doxycycline, or minocycline) is administered orally with daily or divided standard dosing for a minimum of 1 to 2 months, or a combination therapy involving doxycycline, enrofloxacin, and streptomycin, with or without rifampin may be considered (Cosford, 2018; Wanke*et al*., 2006). Monitoring using the AGIDcpa test every 2 to 6 months can potentially help in recognizing relapse and determining the duration of antibiotic therapy, with two consecutive negative results suggesting adequate therapy (Hollett, 2006).

Detailed prevention strategies for breeding facilities have been outlined by the United States Department of Agriculture (USDA) and the Georgia Department of Agriculture websites (Bramlage, 2015). The prevention strategies include the use of one-time-use protective equipment (such as gloves, goggles, masks, gowns, and boots), thorough hand washing, proper sample handling, routine disinfection (using substances like 2.5% sodium hypochlorite, quaternary ammonium compounds, or 70% ethanol with a minimum of 10 minutes contact time), drying and exposure to sunlight, staff and client education, and notifying laboratory personnel receiving specimens about the suspected diagnosis (Carmichael and Greene, 2006;Hollett, 2006; Kazmierczak, 2012; Spickler, 2018). Dogs should undergo serial screening tests performed 8 weeks apart and test negative before being admitted to a kennel or breeding program. Dogs testing positive should be isolated, and decisions should be made regarding euthanasia, treatment, and monitoring (Carmichael and Greene, 2006; Hollett, 2006).

CONCLUSIONS

Taking "One Health" approach is crucial for advancing our knowledge of canine and human seroprevalence rates, understanding pathogenesis, and developing effective management strategies. It is strongly recommended that dog breeders and charitable organizations involved in importing dogs from overseas prioritize testing for this disease. In cases where the etiology of immune system and

metabolic disorders is unclear, and there is close contact with dogs suspected of having canine brucellosis, healthcare providers should consider the possibility of human brucellosis caused by *B. canis*. Additionally, veterinarians treating imported dogs should consistently employ appropriate personal protective equipment (PPE) to minimize the risk of infection. Moreover, there is an urgent need for the standardization of infection protocols within the scientific community. This standardization is essential for deciphering and comparing the vast amount of research results published in the field of brucellosis.

CONFLICT OF INTEREST

None

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