Review Article Compte rendu

Brucella canis: An update on research and clinical management

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Abstract — In Canada, *Brucella canis* remains a potentially devastating infectious agent that is still considered uncommon, despite the increasing international movement of dogs. There may be a growing risk to the Canadian canine population due to a reliance on outdated seroprevalence studies and the lack of federal regulation. With the complex diagnostic and management challenges associated with *Brucella canis,* a One Health approach is necessary to address the need for ongoing research, including updating canine and human seroprevalence rates in Canada, elucidating the pathogenesis, and determining the most appropriate treatment and prevention strategies. Clinical management decisions are often complicated by currently available treatment protocols, and health risks to both canine and human populations. This article integrates recent research focusing on the pathogenesis, diagnosis, and treatment of *Brucella canis,* and outlines current clinical management approaches.

Résumé — *Brucella canis :* mise à jour sur la recherche et la gestion clinique. La brucellose canine, causée par un agent infectieux important et potentiellement dévastateur, est toujours considérée rare au Canada malgré l'arrivée croissante de chiens provenant de régions ayant une prévalence supérieure de l'infection par *Brucella canis.* Il y a un risque grandissant pour la population canine canadienne parce que l'on se fie à des études de séroprévalence désuètes et qu'il existe une absence de règlements fédéraux. En raison des défis complexes liés au diagnostic et à la gestion de *Brucella canis,* l'approche Une seule santé est nécessaire afin d'aborder le besoin de poursuivre la recherche, y compris la mise à jour des taux de séroprévalence canine et humaine au Canada, la clarification de la pathogénèse, la définition de l'éventail potentiel de manifestations cliniques et la détermination du traitement et des stratégies de prévention les plus appropriés. Les décisions de gestion clinique sont souvent compliquées par les protocoles de traitement actuellement disponibles et les risques pour la santé des populations canine et humaine. Cet article intègre de la recherche récente portant sur la pathogénèse, le diagnostic et le traitement de *Brucella canis* et présente les approches de gestion clinique actuelles.

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Introduction

Although endemic to Canada, *Brucella canis* is an elusive infectious agent of unknown significance to most practitioners. Clinical disease attributed to *Brucella canis* infection occurs sporadically, reinforcing the perception that the disease is uncommon in Canada compared with other regions of North America such as Mexico and the southeastern USA. Seroprevalence rates in the southeastern USA are estimated to be 7% to 8% (1). Earlier reports from Quebec (1970's) and southwestern Ontario (1980) still serve as the main Canadian seroprevalence data with rates of 1.6% and 0.3%, respectively (2,3). There is currently a paucity of seroprevalence studies in western Canada, but outbreaks have been observed in a Saskatchewan kennel and the Calgary, Alberta area (4,5).

With the unprecedented rates of animals moving across international borders and the lack of federal regulation, canine brucellosis may be changing its geographical distribution. In 1988, a *Canadian Veterinary Journal* article documented the identification of 2 strains, an American type strain RM66 and a Mexican strain Mex 51, in 11 *Brucella canis* isolates from Canadian dogs (6). Characterization of the current circulating strains is warranted. Until this information is available, Canadian veterinarians should be aware of the agent and consider it as a reasonable differential diagnosis in appropriate cases, regardless of historical information, or neuter status.

Establishing a diagnosis can be challenging due to the wide spectrum of clinical manifestations reported and the limitations of available diagnostic tests. The intent of this article is

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to familiarize clinical, public health, and research veterinarians with the etiology, transmission, pathogenesis, course of infection, clinical manifestations, diagnosis, treatment, prevention, and public health aspects of the disease.

Etiology

Bacteria in the genus *Brucella* are nonmotile, nonencapsulated, non-spore-forming, facultatively intracellular Gram-negative coccobacilli or short rods (7,8). Four of the six classical *Brucella* species are known to cause disease in dogs and humans: *Brucella canis* (natural reservoir animal is the dog), *Brucella melitensis* (sheep, goats), *Brucella suis* (pigs), and *Brucella abortus* (cattle, bison, buffalo) (7,8). The remaining 2 of the 6 classical *Brucella* species [*Brucella neotomae* (rodents, desert rats) and *Brucella ovis* (sheep)] are not associated with disease in dogs. Additional *Brucella* species including both terrestrial forms *(B. microti, B. inopinata)* and marine forms *(B. maris, B. pinnipediae, B. ceti)* are of uncertain pathogenicity to dogs.

Brucella canis was discovered in 1966–1967 during an investigation of abortion in beagles, in which the organism was isolated from aborted tissues and vaginal discharge (9–12). *Brucella canis* was initially thought to be a biotype of *Brucella suis* based on genotypic and phenotypic similarities (13). The significance of this distinction is paramount to the Canadian swine industry and the Canadian Food Inspection Agency (CFIA) as Canada is considered free of *Brucella suis* biovar 3 (4). Differentiation between *Brucella canis* and *Brucella suis* biovar 3 can be challenging (4). A multiplex conventional polymerase chain reaction (PCR) has been optimized to differentiate between these *Brucella* species (14).

The host range for *Brucella canis* is predominantly domestic dogs, but other species have been investigated. Serologic studies of wild canids have documented positive antibody titers in foxes and coyotes (7). Experimental studies involving conjunctival and oral inoculation of cattle, swine, and sheep with *B. canis* showed that these host species were highly resistant to *B. canis* infection, despite 2 field reports of *B. canis* in cattle (7). Similarly, oral experimental infection of cats documented transient bacteremia in 3/14 but none developed agglutinating antibody titers (7).

Transmission

Major routes of transmission for this venereally transmitted agent are genital, conjunctival, and oronasal mucosae, as occurs during normal reproductive, social, and grooming activities in dogs (7,8,15,16). The primary sources of transmission are reproductive fluids: vaginal discharges and semen. Tissues and fluids associated with the fetus, the placenta, and the vagina after abortion or stillbirth have approximately 10^6 organisms/mL (7). Shedding of the organism occurs in vulvar secretions for up to 6 wk after abortion and during estrus (7,8). In males, semen has high concentrations of the bacterium for 6 to 8 wk after infection (7). The agent is then shed intermittently for up to 2 y at lower concentrations in semen which remains an important source of infection for other dogs (7). The minimum infectious dose is approximately 10^6 organisms/mL *via* the oral route and 10^4 to 10^5 organisms/mL by the conjunctival route (7). Minor routes of transmission include *in utero,* broken skin, blood transfusions, feces, milk, and fomites such as contaminated syringes, vaginoscopes, and artificial insemination equipment (7,8,15,16).

Pathogenesis

Current paradigm

Brucella bacteria attach to mucous membranes, penetrate the epithelial barrier, and are taken up by the mononuclear phagocytic system, where they reside intracellularly. This is accomplished by utilizing virulence factors presumably *via* the type IV secretory system, and inhibiting the bactericidal myeloperoxidase-peroxide-halide system through the release of 5-guanosine and adenine (8,17,18). The intracellular organisms then travel through the reticuloendothelial system to local lymph nodes (retropharyngeal, inguinal, superficial iliac), liver, spleen, and possibly bone marrow. After 7 to 30 d, the bacteria move into the blood stream to cause intermittent bacteremia. The organism targets "steroid-dependent" reproductive tissues, including the prostate, testicles, epididymides, gravid uterus, and placenta (8). Evaluation of a Saskatchewan kennel outbreak of brucellosis found that the progestational, non-gravid uterus was also a reservoir for the bacterium (4). A mixed inflammatory response consisting of lymphocytes, plasmacytes, and histiocytes has been observed in these reproductive tissues (4,7,8). Focal coagulative necrosis of the chorionic villi, necrotizing arteritis, and numerous bacteria in trophoblastic epithelial cells can be found in the aborted placenta (4,8).

Non-reproductive body systems become affected as the bacteremia spreads organisms and antibody-antigen complexes to the end-arterial circulation of the intervertebral disk (discospondylitis), or the eye (anterior uveitis or endophthalmitis) (7,8). Interestingly, in experimentally infected dogs, immunosuppression with glucocorticoids or anti-lymphocyte serum may increase susceptibility to initial infection, but does not appear to alter the severity of disease or the course of infection (7). Elucidation of this organism's role in idiopathic inflammatory conditions such as meningoencephalitis, panniculitis, lymphadenitis, hepatitis, and splenitis should be given due consideration by future research initiatives.

Recent research

A murine study has confirmed the pathogenic strategy of *B. canis* as an intracellular bacterium, with an intracellular trafficking route indistinguishable from that of *B. abortus* (17). The study documented a less robust response in mice infected with *B. canis* compared with *B. abortus* in terms of proinflammatory cytokines (TNF-alpha, IL-6, IL-12), IFN-gamma levels, splenic inflammation, and hepatic granulomas (17). It appears that *B. canis* may be less pathogenic than other *Brucella* species in this murine model, which supports clinical observations.

Another study in mice and dogs support a Th1 immune response as essential for protection from *B. abortus* infection (19). VirB proteins are virulence factors that are part of the type IV secretory system. VirB proteins are presumably on the outer surface of the *Brucella* bacterium and are believed to promote intracellular survival. Anti-VirB antibodies promote

complement-dependent bacteriolysis (19). Immunization of mice with VirB proteins resulted in increased IFN-gamma and undetectable IL-4 in VirB-vaccinated individuals compared to the placebo, which is a pattern consistent with a Th-1 response (19). In addition, VirB-vaccinated mice challenged intraperitoneally with live *B. abortus* had a splenic bacterial load of 1 log lower than the placebo (19). Similarly, peripheral blood mononuclear cells of VirB-vaccinated dogs produced significantly higher levels of IFN-gamma than in the placebo; and *in vitro* complement-dependent bacteriolysis was significant in VirB-vaccinated dogs *versus* the placebo (19). Further studies evaluating vaccination against the virulence factor, VirB, are warranted.

A new perspective on rough and smooth colony morphologies has recently been proposed. Colony morphologies have been classified into smooth and rough forms, based on the respective presence or absence of the most external antigen, O-polysaccharide, within the lipopolysaccharide (LPS) of the cell wall. Traditionally, smooth *(B. melitensis, B. abortus,* and *B. suis)* and rough *(B. canis,* and *B. ovis)* forms were believed to represent laboratory artifacts that occur with Gram-negative bacterial colonies in culture (7,20). Recent research suggests that the loss of O-polysaccharide results from the spontaneous excision of the *wbkA* glycosyltransferase gene (21). This phenomenon is referred to as smooth to rough dissociation (20,21). The significance of colony morphology and *Brucella* LPS genetics remains controversial, but a potential link to virulence might exist (20,21).

Different strains or isolates of *B. canis* have also been reported. A less mucoid (M-) laboratory strain is maintained in the laboratory as the antigen source for serology assays (22). Interestingly, this M-strain is believed to be avirulent in dogs, but has been reported to infect a laboratory worker in a similar fashion to wild-type *Brucella canis* (22). A Swedish outbreak investigation documented differences within prophage gene content of American, African, and European isolates compared to Asian strains (23). The significance of these strains in terms of their relative pathogenicity remains unclear.

Course of infection

Bacteremic episodes can last for years as experimentally infected dogs can have positive blood cultures for 5.5 y (8). The animal seroconverts as early as 2 to 4 wk but this can be as long as 8 to 12 wk after infection (1,7,8). After 3 to 4 mo the degree of bacteremia declines, but the organism remains persistently in the blood or sequestered in tissues. The current paradigm with respect to the outcome of *B. canis* infection is that cell-mediated immune responses typically result in self-elimination within 2 to 3 y on average (7). Alternatively, humoral immune responses do not eliminate the organism resulting in persistently infected dogs (7).

Experimentally infected dogs allowed to recover naturally were immune to subsequent oral or intravenous rechallenge for up to 4 y (7). In contrast, infected dogs that did not self-eliminate the organism were susceptible to oronasal challenge 12 wk after completion of antimicrobial therapy (7). Antibiotic therapy is widely believed to be unsuccessful at eliminating persistent infection in dogs.

Clinical manifestations

Brucella canis is typically associated with reproductive abnormalities but a wide range of non-reproductive signs can occur (1). The organism has been given the nickname "the Great Imposter" to illustrate this point (1). It is important to remember that most infected dogs do not appear seriously ill. Deaths are rare except *in utero,* in newborns, and in animals with severe illness (7).

Female dogs infected venereally experience early embryonic death 2 to 3 wk after transmission, which looks like failure to conceive or infertility (7). If the pregnancy progresses spontaneous abortion occurs most commonly between 7 and 9 wk of gestation (45 to 55 d), which is referred to as a late stage spontaneous abortion (7). Normal canine gestation is 57 to 72 d (24). Mucoid, serosanguinous, or gray-green vaginal discharge persists for 1 to 6 wk after abortion (7). Endometritis has also been observed (7). Some *B. canis* infected bitches can give birth to litters that appear clinically normal. These puppies are born infected and can manifest disease later in life (7).

During the acute stage, venereally infected male dogs may initially experience epididymitis and scrotal edema, while orchitis occurs less frequently. Scrotal dermatitis also occurs due to self-induced irritation from licking. The disease can then progress to a chronic stage characterized by testicular atrophy (unilateral or bilateral) and infertility. Affected males develop chronic epididymitis and, ultimately, infertility due to anti-sperm agglutinating antibodies and delayed-type hypersensitivity reactions against the spermatozoa, leading to spermatogenic arrest (6). In male dogs that develop chronic epididymitis, 90% of sperm are abnormal at 20 wk after infection (1). Some male dogs do not develop spermatic abnormalities and infertility, but still spread the organism most likely through prostatic fluid. Prostatic disease manifestations, such as prostatitis, have also been observed (7).

Non-reproductive manifestations of *B. canis* infection most commonly include chronic uveitis, endophthalmitis, and discospondylitis. Infected dogs with ocular involvement can present with blepharospasm, aqueous flare, constricted pupils, synechiae, hypopyon, and hyphema. Dogs with discospondylitis can present with stiffness, back pain, lameness, exercise intolerance, paresis, and possibly paralysis due to spinal compression. Other manifestations of *B. canis* infection include lymphadenitis (common), pyogranulomatous dermatitis (rare), endocarditis (rare), appendicular osteomyelitis (rare), and meningoencephalitis (unknown frequency) (7). Various nonspecific signs have been associated with *B. canis* infection, including fever (rare), lethargy/fatigue, exercise intolerance, decreased appetite, weight loss, and behavioral anomalies such as loss of alertness and poor performance of tasks (7).

Diagnostics

Routine diagnostics such as complete blood (cell) count (CBC), serum biochemistry profile, and urinalysis are often normal. Occasionally, nonspecific findings supportive of inflammatory disease are identified, such as leukocytosis, neutrophilia, hyperglobulinemia, and hypoalbuminemia (18). In cases with suspected discospondylitis, imaging with plain radiography or computed tomography is indicated to identify end vertebral

Table 1. Comparison of traditional serologic assays for the diagnosis of brucellosis in dogs.

Test	Antigen	Sensitivity	Specificity	How to use test
Rapid Slide Agglutination Test (RSAT)	*B. ovis* (27,28) (M-) strain *B. canis* (29)[a]	Moderate to high sensitivity — older studies suggest high (30,31) — newer study suggests 70.58% (32)	Low to moderate specificity — older studies suggest 40%–50% (27,30) — newer studies suggest 83.34% (32)	Screening test (1,7,8)
2-MercaptoEthanol Rapid Slide Agglutination Test (2ME-RSAT)	(M-) strain *B. canis* (8)	Lower sensitivity than RSAT 31.76% *versus* 70.58% (32)	Higher specificity than RSAT 100% *versus* 83.34% (32)	Confirmatory test (1,7,8)
Tube Agglutination Test (TAT)	*B. canis* (8)	High sensitivity (1,8)	Low specificity (1,8)	Screening test[b] (1,7,8)
Indirect Fluorescent Assay (IFA)	Anti-canine immunoglobulin (Ig)G directed against antibodies to *B. canis*	Unknown sensitivity (7)	Unknown specificity	Screening test (1,8)
Agar Gel Immunodiffusion Assay using Cell Wall Antigen ($AGID_{cwa}$)	Lipopolysaccharide antigen from the cell wall of *B. canis* (8,36)	High sensitivity (1,7,8)	Lower specificity than $AGID_{cpa}$ (37)[c]	Screening test (1,8)
Agar Gel Immunodiffusion Assay using CytoPlasmic Antigen ($AGID_{cpa}$)	LPS-free, soluble, internal cytoplasmic proteins extracted from *B. canis* or *B. abortus* (8,36)	Low sensitivity — 52.94% sensitive (32) — 47.06% false negatives (32)	High specificity 100% (32,37)[d]	Confirmatory test (1,8)

[a] False positives — 10% using *B. canis* antigen *versus* 50% using *B. ovis* antigen.
[b] Results are semiquantitative (8,30,33–35) with a titer of : > 1:200 — has a good correlation with the organism being recovered from blood culture; 1:200 — presumptive of active infection; 1:25, 1:50 — recovery or chronic infection.
[c] False positives occur due to nonspecific cross reactions with cell wall antigenic complexes.
[d] Reacts with antibodies against *Brucella* spp. *(B. canis, B. abortus, B. suis);* therefore, specific to the *Brucella* genus but not individual species.

body osteomyelitis. Similarly, magnetic resonance imaging (MRI) along with cerebrospinal fluid (CSF) analysis and bacterial culture are performed in cases of suspected meningoencephalitis. Uveitis or panophthalmitis may warrant taking aqueous or vitreous humor aspirates for cytology and culture, under an ophthalmologist's care.

History, clinical signs, and ancillary diagnostics may prompt more definitive testing for *B. canis.* A positive culture can be definitive but low sensitivity leads practitioners and researchers to serology and PCR. Definitive testing for *B. canis* has been plagued by many pitfalls including sensitivity, specificity, quality control, and availability.

Blood culture

The traditional gold standard diagnostic test for *B. canis* has been culture of blood, urine, vaginal discharge, semen, or aborted fluids/tissues (1,7,8,25,26). Samples should be collected sterilely in a standard aerobic culture vial or a green top (heparinized) tube, stored on ice (not frozen) and shipped within 24 h to the laboratory, where Farrell's medium or Thayer-Martin's modified medium can be used for culture (7,25,26). Unfortunately, our ability to detect this organism is limited due to low levels of bacteria; intermittent shedding; poor sample choice for submission; inappropriate handling of sample; slow growing, fastidious forms; and incorrect culture media (7). A negative culture should not rule out infection, as the low sensitivity corresponds to an unacceptable number of false negatives. Although culture is an inappropriate screening test, it is the ideal confirmatory test.

Traditional serologic assays

Traditional serologic assays for *B. canis* are summarized in Table 1. Rapid slide agglutination tests (RSAT), tube agglutination tests (TAT) and immunofluorescent antibody tests (IFA) are typically used as initial screening tools to rule out infection (7,8,32). False negatives can occur as a result of testing prior to seroconversion, and low circulating antibody titers in some chronically infected dogs (7). False positives are the predominant concern with these serology assays due to both nonspecific and specific cross reactions with shared surface antigens on *Pseudomonas aeruginosa, Bordetella bronchiseptica, Actinobacillus equuli, Streptococcus, Staphylococcus, Moraxella*-type organisms and Gram-negative bacteria (7,8,25,38). A screening test must be followed with a confirmatory test such as 2-mercaptoethanol RSAT (2ME-RSAT) or agar gel immunodiffusion assay using an internal cytoplasmic antigen ($AGID_{cpa}$) (7,8,32). The more specific confirmatory test addresses the high rate of false positives associated with the screening tests.

ELISAs and PCR

Research into new diagnostics for *B. canis* is focused on enzyme-linked immunosorbent assays (ELISAs) and PCR, which are summarized for researchers in Tables 2 and 3, respectively. Quality control and assurance are paramount with these assays, especially PCR, to ensure accuracy of the test result, given the potential impact of a positive or negative result on an individual dog, the canine population, an individual client or a kennel operator. Assuming accurate test results, the benefits of PCR are species and sometimes biovar identification; improvements

Table 2. ELISA assays for the diagnosis of brucellosis in dogs.

Antigen	Sensitivity	Specificity
Lipopolysaccharide-free cytoplasmic proteins of *B. abortus* (39)	92%	96.7%
Hot-saline extract of *B. canis* containing outer membrane antigens (39)	92%	94.3%
Luminaze synthase of *Brucella* sp. (39)	81%	96.7%
18 kDa cytoplasmic protein of *B. canis* (40)	87%[a]	98%[b]
Bacterial whole cell extract from wild isolate of *B. canis* used as solid phase antigen (41)	95%	91%
Heat soluble bacterial extract from wild isolate *B. canis* (42)[c]	91.18%	100%
M-strain *B. canis* antigen (43)	100%	98.8%
B. ovis strain #11 antigen (43)	100%	98.8%
B. abortus RB51 strain antigen (43)	100%	98.8%

[a] Sensitivity not reported as percentage, which was calculated from the data set as 26/30 known cases tested positive with this ELISA.
[b] Specificity not reported as percentage, which was calculated from the data set as 2/103 animals tested falsely positive with this ELISA in the healthy population.
[c] Heat soluble extracts were more useful than ultrasonic homogenates of bacterial isolates to generate candidate capture antigens, as sonicated antigens were associated with more cross reactivity and, therefore, false positives in both ELISA and Western blot.

in sensitivity and specificity; minimal biological containment requirements; relatively short turnover time for results; and genetic fingerprinting to facilitate epidemiological studies and disease control (38).

Most PCR assays reported in the literature for detection of *B. canis* are genus-based not species-specific. In the past, multiplex PCRs have been used to differentiate between some *Brucella* species (14). In more recent years, *Brucella canis* — specific PCRs have been developed (23,50,51). These assays have yet to undergo extensive evaluation in canine populations to evaluate sensitivity and specificity. Until then, PCR should be used in conjunction with clinical information and serology.

Treatment

The generally accepted recommendation is that treatment should be discouraged, and truly infected animals should be euthanized due to the risk to canine and human populations (1,7,8,25,26). Disease due to *B. canis* is not currently reportable in Canada, which leaves the decision-making process to the client and the veterinarian. Euthanasia serves as a strict approach, but if this is not possible due to client opinion, then isolation can be considered after appropriate client education and medical record documentation. Patients with no clinical manifestations of the disease should be isolated and allowed to self-eliminate the organism if possible. If an adequate Th1 response occurs, the patient might spontaneously recover in 2 to 3 y on average (7,8).

Although significant illness is rare, those patients experiencing clinical signs that warrant intervention will have to be either euthanized or treated. Treatment is notoriously unsuccessful as dogs experiencing morbidity have had a Th2 response leading to persistent infection. It is important for clinicians to remember that it is not only the antimicrobial therapy, but also the individual's immune response that works in concert to determine the outcome of infection. Antibiotic therapy does not guarantee elimination of the organism, with relapse or re-infection believed to be common (1,7,8,25,26).

Original studies have demonstrated the superiority of combination antibiotic therapy over a single agent protocol. Traditionally, a tetracycline-based antibiotic (tetracycline hydrochloride, doxycycline, minocycline) is administered orally with daily or divided standard dosing for a minimum of 1 to 2 mo. The second antibiotic is an aminoglycoside (dihydrostreptomycin, streptomycin, or gentamicin) administered parenterally with daily standard dosing for either the initial 7 to 14 d of treatment, or a 7-day period every 3 to 4 wk (7,8,18,25,30,52). Aminoglycosides have significant limitations: nephrotoxicity monitoring and possible hospitalization with intravenous fluids; parenteral administration; streptomycin availability; and inadequate ocular and central nervous system penetration (18).

A recent report documents the successful treatment of 3 dogs with chronic or recurrent uveitis using combination antimicrobial therapy (doxycycline, enrofloxacin, and streptomycin, with or without rifampin) (54). All 3 dogs in this report responded in terms of clinical factors like resolution of ocular inflammation and conversion to seronegativity. Negative serology was attained after a median of 96 wk (range: 36 to 112 wk) of therapy (54).

Another article documents the response of 12 dogs in a breeding facility that was experiencing infertility and spontaneous abortions. A novel single agent enrofloxacin treatment protocol consisted of 5 mg/kg body weight (BW) orally every 12 h for 30 d with additional courses administered to females during all subsequent estrual and luteal cycles (range: 0 to 2 cycles) (55). Fourteen months later, the dogs in this study did not have any further abortions, transmission to offspring was not observed, vaginal secretions were culture negative after subsequent births, fertility was maintained, and titers declined (55). Veterinarians will appreciate the significant antimicrobial resistance concerns that might arise with respect to long-term, intermittent fluoroquinolone use.

In these studies, clinical improvement and declining antibody titers were observed with antibiotic therapy, but definitive elimination of the organism was not demonstrated (53,54). The intermittent nature of clinical disease manifestations, particularly those involving reproductive performance abnormalities, makes definitive comments about treatment efficacy impossible without an untreated group. Ethical concerns make a negative control group in clinical patients unlikely in future research endeavors.

Unfortunately, the treatment and monitoring protocols are often lengthy and time consuming, leading to escalating expense and declining client compliance (8). To the author's knowledge, there is no universally accepted treatment protocol especially in terms of treatment duration, which has involved 1 to 2 mo of therapy, 90-day treatment cycles separated by 1 to 2 mo, or indefinite antimicrobial use (8,18). Monitoring the $AGID_{cpa}$ every 2 to 6 mo can potentially help guide both the recognition of relapse, and the duration of antibiotic therapy with 2 consecutive negative results suggesting adequate therapy (8). Monitoring is indefinite and relapse necessitating retreatment is considered likely (8).

Table 3. PCR assays for the detection of *Brucella* antigen in dogs.

Primers directed to	Detection: Genus or Species	Sample	Sensitivity	Specificity
16S-23S rDNA Interspace region (44)	*Brucella* genus	Whole blood	100%	100%
16S-23S rDNA Interspace region (45)[a]	*Brucella* genus	Vaginal swabs	Not available	Not available
16S-23S rDNA Interspace region (46)[a]	*Brucella* genus	Semen	Not available	Not available
16S rRNA sequence (47)	*Brucella* genus	Whole blood	100%	100%
16S-23S rRNA Interspace region (48)	*Brucella* genus	Inguinal lymph node	100%	100%
16S-23S rRNA Interspace region (49)	*Brucella* genus	Whole blood Serum	Whole blood: 97.14% Serum: 25.71%	Whole blood: 100% Serum: 100%
Intergenic spacer IS711 fragment (23)[b]	*Brucella* genus	Males: preputial swab, semen, or urine Female: vaginal swab	Not available	Not available
Gene fragment on chromosome 1 (23)[b]	*Brucella canis* species-specific	As immediately above	Not available	Not available
B. canis outer membrane protein 25 DNA quantitative PCR (50)	*Brucella canis* species-specific	Vaginal swabs Whole blood	Vaginal swabs: 92.31% Whole blood: 16.67%	Vaginal swabs: 51.92% Whole blood: 100%
BCAN_B0548-0549 region in chromosome 2 of *Brucella canis* (51)[c]	*Brucella canis* species-specific	Whole blood Buffy coats	Not available	Not available

[a] PCR on vaginal swabs and semen in these studies correlated with blood PCR, and blood culture as assessed by a Kappa co-efficient and the McNemar test.
[b] Both *Brucella* genus-based and *Brucella canis* specific PCRs used in Swedish outbreak investigation.
[c] *Brucella canis* inoculated samples; PCR on buffy coats separated from whole blood was approximately 100 times more sensitive than from whole blood.

Prevention

Although not universally standardized, detailed prevention strategies in breeding facilities have been proposed by the USDA and the Georgia Department of Agriculture websites (1,26). Dogs should test negative on serial screening tests performed 8 wk apart prior to admission to a kennel or a breeding program. Dogs testing positive should be isolated and decisions made about euthanasia, or treatment and monitoring. An important preventative measure will involve sterilization.

Prevention also involves rigid attention to biosecurity. Principles of infection control will include: one-time-use protective equipment (gloves, goggles, masks, gowns, boots); thorough hand washing; appropriate sample handling; routine disinfection (i.e., 2.5% sodium hypochlorite, quaternary ammonium compounds or 70% ethanol with a minimum of 10 min contact time); biofilm prevention (minimize organic material); drying and exposure to sunlight; education of staff and clients; and notification of laboratory personnel receiving specimens as to the suspected diagnosis (1,7,8,25,26,55).

Public health

Approximately 100 to 200 cases of human brucellosis (all *Brucella* species) are diagnosed annually in the USA (55). One case report that has received a lot of attention is that of a 3-year-old female toddler who is believed to have acquired *B. canis* infection from a Yorkshire terrier puppy in New York City (56). In addition, HIV-positive patients with appropriate CD4 counts and negative viral loads have also been diagnosed and successfully treated for *B. canis* infections (55,57).

The pathogenicity of *Brucella canis* is considered relatively low, making it less of a perceived public health concern than other *Brucella* species, in particular *Brucella melitensis,* and biotypes 1 and 3 of *Brucella suis* (23,25,55). It is important to remember that *B. canis* is not reportable in Canadian provinces or territories, and *Brucella* is not routinely tracked beyond the genus level at the Center for Disease Control (CDC). The absence of a structured regulatory program for *B. canis* means that we do not know if human infection is underdiagnosed, especially when considering the nonspecific clinical signs, and the variable incubation period of 2 wk to 3 mo (25,55). Diagnostic limitations are also a complicating factor, necessitating research into ELISA and PCR technologies.

Currently reported clinical signs associated with human brucellosis include fever (often periodic and nocturnal), fatigue, headache, weakness, malaise, chills, sweats, weight loss, hepatomegaly, splenomegaly, and lymphadenopathy (7,8,25,55). Serious complications from *B. canis* infection in humans include septic arthritis, aortic valve vegetations, calvarial osteomyelitis, epidural abscess, pleural effusion, oral lesions, lower extremity aneurysms, and culture negative endocarditis (7,8,25,55). Deaths are rare except with serious underlying sites of infection or delayed treatment. Unlike in dogs, treatment of human *B. canis* infections has been associated with elimination of the organism (7,8,25,55)

In conclusion, *Brucella canis* should be considered as a potential differential diagnosis for small animal and public health practitioners in Canada. A One Health approach is essential to update our understanding of canine and human seroprevalence rates, pathogenesis, and management options. CVJ

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Answers to Quiz Corner
Les réponses du test éclair

1. **C)** Cattle have a limited bone marrow reserve and have a limited degree of neutrophilia in response to inflammation.

C) Les bovins possèdent une réserve de moelle osseuse limitée et présentent une faible quantité de neutrophiles en réponse à l'inflammation.

2. **D)** *Candida albicans* is a yeast which infects intact mucous membranes, most commonly the tongue and the esophagus. The infection is most common in young ungulates, and is usually associated with an underlying primary debilitating condition (i.e., thrush is a secondary disease). *Streptococcus pyogenes* and *Corynebacterium pyogenes* are bacteria which trigger a suppurative inflammatory response (i.e., pus formation or abscess formation); these agents most commonly affect the upper respiratory tract. *Actinobacillus lignieresii* is the cause of "wooden tongue," a granulomatous condition resulting from opportunistic invasion of damaged lingual tissue by the causative bacterium. *Histophilus somni* is associated with respiratory, neurologic, and reproductive infections in the cattle.

D) *Candida albicans* est une levure qui infecte les muqueuses intactes, le plus communément la langue et l'œsophage. L'infection est plus commune chez les jeunes ongulés et est habituellement associée à une affection débilitante primitive sous-jacente (le muguet est une atteinte secondaire). *Streptococcus pyogenes* et *Corynebacterium pyogenes* sont des bactéries qui déclenchent une réponse inflammatoire suppurée (p. ex., formation de pus ou d'abcès); ces agents affectent le plus communément les voies respiratoires supérieures. *Actinobacillus lignieresii* est la cause de la «langue de bois», une affection granulomateuse résultant d'une invasion opportuniste des tissus linguaux endommagés par la bactérie causale. *Histophilus somni* est associée aux infections des systèmes respiratoire, neurologique et reproducteur chez les bovins.

3. **A)** Serum IgM titer is the best test for diagnosis of an active infection.

A) Le titre d'IgM sérique est le meilleur test pour le diagnostic d'une infection active.

4. **E.)** Valvular endocardiosis is an age-related, degenerative change in which there is accumulation of a myxomatous connective tissue matrix within the valve leaflets, causing nodular thickening. The suffix "-osis" implies a degenerative condition; bacterial infection of the heart valves would lead to valvular endocarditis, or inflammation of the valves. Since endocardiosis most commonly affects the atrioventricular valves (and the mitral valve more commonly than the tricuspid), the condition may be associated with a systolic heart murmur.

E) L'endocardiose valvulaire est un changement dégénératif associé à l'âge dans lequel il y a une accumulation d'une matrice de tissu conjonctif myxomateux dans les festons des valves, causant un épaississement nodulaire. Le suffixe «ose» implique une condition dégénérative; l'infection bactérienne des valves cardiaques conduira à de l'endocardite valvulaire, soit l'inflammation des valves. Puisque l'endocardiose affecte le plus communément les valves atrioventriculaires (la valve mitrale plus souvent que la valve tricuspide), l'affection peut être associée à un souffle cardiaque systolique.

5. **D)** The flehmen response occurs in stallions following exposure to mares in estrus. The stallion will curl its upper lip and drop its penis.

D) Le flehmen est un comportement observé chez les étalons en présence de juments en chaleur. L'étalon retroussera la lèvre supérieure et rabattra le pénis.