ORIGINAL ARTICLE

Evidence for Unapparent *Brucella canis* Infections among Adults with Occupational Exposure to Dogs

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Impacts

- Serological results suggest that zoonotic infections with *Brucella canis* are occurring more often than the occasional clinical case reports indicate.
- Medical personnel should consider *B. canis* infections in the differential diagnoses of patients with brucellosis-like symptoms, especially among persons with intense dog exposure.
- There is a critical need for serological diagnostic assays to detect human *B. canis* infections.

Keywords:
Brucellosis; zoonoses; occupational exposure; communicable diseases; emerging; seroepidemiologic studies

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Received for publication July 1, 2013

doi: 10.1111/zph.12102

Summary

Human serological assays designed to detect brucellosis will miss infections caused by *Brucella canis*, and low levels of periodic bacteremia limit diagnosis by blood culture. Recent *B. canis* outbreaks in dogs and concomitant illnesses in caretakers suggest that unapparent human infections may be occurring. With more than a quarter of a million persons in occupations involving dogs, and nearly 80 million dog owners in the United States, this pathogen is an under-recognized human health threat. To investigate occupational exposure to *B. canis*, we adapted a commercial canine serological assay and present the first controlled seroepidemiological study of human *B. canis* infections in recent years. 306 adults with occupational exposure to dogs and 101 non-matched, non-canine-exposed subjects were enrolled. Antibodies were detected using the canine D-Tec® CB rapid slide agglutination test (RSAT) kit with a secondary 2-mercaptoethanol (ME)-RSAT. Results were validated on a blinded subset of sera with an additional RSAT and indirect enzyme-linked immunoassay at the National Administration of Laboratories and Health Institutes (ANLIS) in Argentina. Seroprevalence ranged from 10.8% (RSAT) to 3.6% (ME-RSAT) among canine-exposed subjects. Kennel employees were more likely to test RSAT seropositive compared with other canine exposures (OR = 2.7; 95% CI, 1.3–5.8); however, low seroprevalence limited meaningful occupational risk factor analyses. Two seropositive participants reported experiencing symptoms consistent with brucellosis and having exposure to *B. canis*-infected dogs; however, temporality of symptom onset with reported exposure could not be determined. D-Tec® CB results had substantial agreement with ANLIS assays (Cohen's kappa = 0.60–0.68). These data add to a growing body of literature suggesting that people occupationally exposed to dogs may be at risk of unapparent *B. canis* infection. It seems prudent to consider *B. canis* as an occupational public health concern and encourage the development of serological assays to detect human *B. canis* infections.

Introduction

Brucella canis, an orally and sexually transmitted bacterium, was first recognized in dogs in 1966 and continues to plague breeding kennels today. The bacterium is readily transmitted between dogs in these environments, no canine vaccine is available, antimicrobial treatment is difficult, canine testing is typically not required by state authorities, and relapse is common (Brower et al., 2007; ISU, 2007). *B. canis* is a major source of economic loss in both large and small US dog breeding facilities. In addition to reproduction losses, outbreak control recommendations are to test and euthanize all positive animals, resulting in the culling of hundreds of dogs in large facilities (Brower et al., 2007). The true prevalence of *B. canis* infections in the United States is unclear, but studies conducted in the 1970s suggested that 6–9% of stray dogs in various states were infected (Brown et al., 1976; Lovejoy et al., 1976; Galphin, 1977; Randhawa et al., 1977; Boebel et al., 1979). However, state animal health laboratory diagnosticians in Oklahoma have reported an increasing prevalence among dogs in recent years, especially in kennel operations, with 2–3% of dogs testing positive between 1994–1999 and 14% positive by 2003 (Welsh and Dirato, 2012).

Human brucellosis is a nationally notifiable disease in the United States confirmed by culture of *Brucella* spp. or a $\geq$ 4-fold rise in *Brucella* antibody titre between paired serum specimens (CDC, 2010). Routine clinical diagnostics for human brucellosis were developed to detect antibodies against smooth *Brucella* spp. antigens (principally *B. abortus*, *B. melitensis*, and *B. suis*) and do not detect *Brucella* spp. with rough antigens (*B. canis* and *B. ovis*) (Moreno et al., 1984); therefore, clinically important human *B. canis* infections are likely to be missed and hence underreported (Polt et al., 1982). In addition to the absence of a human serologic test, lack of clinical suspicion, ill-defined clinical presentations, low levels of periodic bacteremia and the selective nature of the organism in culture further limit diagnosis in humans (Scheftel, 2007).

The 40 total reported human cases of *B. canis* infections in the English literature have chiefly resulted from contact with infected dogs or by laboratory exposures (Swenson et al., 1972; Tosi and Nelson, 1982; Young, 1983; Lum et al., 1985; Rousseau, 1985; Schoenemann et al., 1986). Human seroprevalence studies, only conducted in the 1970s (Lewis and Anderson, 1973; Hoff and Nichols, 1974; Monroe et al., 1975; Flores-Castro and Segura, 1976; Weber and Brunner, 1977; Soriano et al., 1978; Varela-Diaz and Myers, 1979), predominantly employed agglutination assays developed by Carmichael et al. (Carmichael and Kenney, 1968) or a later modification (Damp et al., 1973). Although not considered outdated, agglutination assays have since been improved and commercialized (Polt and Schaefer, 1982; Carmichael and Joubert, 1987), and new serological techniques have been developed, including an indirect enzyme-linked immunosorbent assay (IELISA) (Serikawa et al., 1989; Lucero et al., 2002, 2005). In spite of improvements in serologic testing, recent epidemiological data examining human *B. canis* antibody seroprevalence are sparse (Shin and Carmichael, 1999a; Hollett, 2006).

The dog breeding industry has grown significantly since *B. canis* was first recognized. The American Pet Products Association estimates the number of dogs owned in the United States has increased by more than 18 million in the last decade alone. There are now approximately 78.2 million dogs owned in the United States, with 40% of households owning at least one dog, 5000 US animal shelters, 2400 federally licensed dog breeders, and thousands of smaller, undocumented 'mom and pop' dog breeders. Overcrowded shelters and kennels create the perfect environment for amplified infectious disease transmission between dogs and the people who work with them.

While veterinarians are usually considered the chief at-risk occupational group for *B. canis* infection, a 1974 Florida study that included 43 veterinarians failed to bear this out, identifying no seropositivity in this group (Hoff and Nichols, 1974). Dog breeders and kennel employees are at greater potential risk of infection because they commonly come in direct contact with blood, semen and placentas of dogs, and may do so with less attention to personal protective protocols. In this cross-sectional seroepidemiological study, we sought to look for evidence of unapparent *B. canis* infections in a highly dog-exposed US adult population and to assess the usefulness of a commercially available veterinary serologic assay kit for use in humans.

Materials and Methods

Participant recruitment and enrolment

This study was approved by University of Iowa and University of Florida institutional review boards. All participants signed an informed consent form. All participants had to be at least 18 years of age and self-report no current immunocompromising conditions. Eligibility for the canine-exposed group was defined as persons exposed to multiple dogs as part of their work or hobby in the last 5 years. The target population, including breeders, kennel employees, veterinary personnel, animal shelter workers, and dog show handlers, were recruited via mailed letters, telephone calls and face-to-face encounters. Breeders, shelters and veterinary clinics were identified through Iowa and Florida state databases of licensed breeders and practicing veterinarians, as well as through internet searches. Enrolments typically occurred at the participants' home or place of employment, but also at large trade venues and dog shows.

To enrol non-canine-exposed participants, individuals affiliated with the University of Iowa and University of Florida (e.g. faculty, staff and students) were targeted via face-to-face encounters at University common areas. Non-canine exposure was defined as not having exposure to multiple dogs as part of one's work or hobby, nor pet dogs in the household, in the last 5 years. During recruitment, potential non-exposed participants were pre-screened in regard to their dog exposure. If the person met the inclusion criteria for non-canine exposure, was at least 18YO and self-reported no immunocompromising conditions, he or she was invited to enrol.

After written informed consent was obtained, participants completed a self-administered questionnaire. The enrolment questionnaire documented participants' general demographic information (gender, age), as well as the lifetime exposure to sheep and pet dogs for which total numbers of years of exposure and the average number of animals exposed to at a given time were reported. Subjects were also asked whether they had ever been exposed to a sick dog, including a dog known to be infected with *B. canis*, for which they reported the date of their most recent exposure and how many dogs were affected. Subjects were also asked whether they ever developed any illness (including symptoms consistent with brucellosis) following contact with a sick dog. Canine-exposed participants completed an additional questionnaire section to ascertain details of their occupations/hobbies that involved exposure to dogs and personal hygiene practices when working with dogs. In addition to completing the enrolment questionnaire, subjects permitted collection of a blood specimen via venipuncture at the single encounter. Whole blood specimens were transported on ice to the laboratory within a few hours of collection, where sera were separated and immediately stored at −80°C until assayed.

Serological analyses using the D-Tec® CB RSAT

To assess the seroprevalence of *B. canis* in the study population, participants' sera were tested for antibodies against rough *Brucella* spp. at the University of Florida by adapting the D-Tec® CB test kit (Synbiotics Corporation, Kansas City, MO, USA), a canine brucellosis rapid slide agglutination test (RSAT) kit used for veterinary purposes to screen canine sera for *B. canis* infections. The D-Tec® CB RSAT employs a suspension of whole *B. ovis* stained with Rose Bengal, which cross-reacts with serum IgM and IgG antibodies against *B. canis* (Escobar et al., 2010). To decrease the occurrence of false positives, the kit also includes a 2-mercaptoethanol (ME-RSAT) confirmatory procedure that blocks common non-specific agglutinins in sera, including cross-reacting IgM against other bacteria (Carmichael et al., 1984; Carmichael and Joubert, 1987). Kit instructions were followed, save for a few modifications. For the initial screen, rather than following kit instructions to use a supplied plastic dropper, a precise 60 μl each of serum and prepared antigen were pipetted and mixed with a stir stick within an oval circle as directed on the test card provided with the kit. The card was gently rocked to swirl the mixture for 2 min (kit instructed to rock for 10–15 s and lay flat for 2 min). The mixture was then observed for agglutination. If a serum sample produced a positive agglutination reaction, the ME-RSAT procedure was then completed. To accomplish this, 2ME and serum were first mixed in equal parts (60 μl) in a test tube. This mixture (sera diluted 1 : 1) was then added to the prepared antigen in equal parts (60 μl) and the subsequent RSAT steps followed. The D-Tec® CB test kit included a positive control that was employed during RSAT performance. Results were interpreted blindly by one reader as strongly positive (extensive agglutination), weakly positive (visible agglutination) and negative (absence of agglutination).

D-Tec® CB RSAT validation study

Because the D-Tec® CB RSAT had not previously been used to examine human sera, sera from 50 study participants were shared in a blinded fashion with the Brucellosis Laboratory at the National Administration of Laboratories and Health Institutes (ANLIS), Buenos Aires, Argentina. Agreement between the D-Tec® CB RSAT and ANLIS's RSAT and ELISA assays was assessed.

ANLIS RSAT

Using (M-) variant *B. canis* strain, the RSAT was performed as previously reported (Lucero et al., 2002, 2005). The results of this screening test were reported as positive, weakly positive and negative.

ANLIS IELISA

To confirm the RSAT results, an indirect enzyme-linked immunosorbent assay (IELISA) using (M-) variant *B. canis* antigen as previously reported (Lucero et al., 2002, 2005) was performed. A serum sample was classified as positive when the test's optical density (OD) was at least 27 percentage of the OD achieved with a strong control serum. Relative percentage positivity (%P) = (OD_{414} of test sample/OD_{414} of strong control serum) × 100.

ANLIS Buffered Plate Antigen Test (BPAT)

To examine whether the D-Tec® CB RSAT results were confounded by cross-reacting human antibodies against smooth *Brucella* spp. strains (e.g. *B. abortus*), the same

subset of 50 study sera were also tested by BPAT. The BPA antigen was prepared at ANLIS from a suspension of *Brucella abortus* 1119-3. The assay was run as previously reported (Lucero and Bolpe, 1998); serum (80 μl) and antigen (30 μl) were mixed on a divided glass plate and then incubated for 8 min at room temperature with intermittent mixing. Any sign of agglutination was considered positive.

Statistical methods

Assessing epidemiologic risk factors

To examine the seroprevalence of *B. canis* antibodies among the study participants, the measured outcome was serological reactivity by the D-Tec® CB RSAT kit; results for both the RSAT and ME-RSAT were considered as a dependent outcome for bivariate and multivariate modelling. Results of the qualitative RSAT were categorized as positive, weakly positive or negative and were considered in an ordinal fashion when the proportional odds assumption was met. Results were dichotomized to positive/negative when data were sparse, with weakly positive results considered as positive.

To assess independent risk factors, occupations/hobbies were subgrouped as breeder, kennel owner, kennel employee, veterinarian, veterinary staff, shelter worker, groomer, trainer, race track employee and 'other'. For each subgroup, subjects reported the number of years they had ever participated in that occupation/hobby as well as the average number of dogs they are/were exposed to in that given occupation/hobby. The primary independent variable, canine exposure, was examined in a number of ways: dichotomized, ordinal and continuous.

Questionnaire data were used to identify associations between outcome and independent risk factors using logistic regression. Odds ratio (OR) estimates and 95% confidence intervals were ascertained for simple unadjusted comparisons. We examined risk factors for bivariate associations with RSAT results using binary logistic regression and proportional odds modelling (Capuano et al., 2007). An exact method was used for sparse data, and the score test was used to evaluate the proportional odds assumption. Covariates with P values < 0.25 were considered for inclusion in multivariate models. Multivariate models were designed using manual backwards elimination. Analysis was performed using SAS v9.2 (SAS Institute, Cary, NC, USA).

Assay validation

To assess the accuracy of the D-Tec® CB kit for use with human sera, 50 study sera samples were shared with ANLIS in a random, blinded fashion to evaluate the agreement between the veterinary kit and ANLIS assays developed to test human sera for diagnostic purposes. Inter-assay agreements between the kit's results (RSAT and ME-RSAT) and ANLIS's assays (RSAT and ELISA) were calculated via Cohen's Kappa coefficient, a statistical test of inter-rater agreement for categorical outcomes. Agreement among the 2 screening tests, as well as the 2 confirmatory tests, was calculated. D-Tec® CB RSAT and ANLIS RSAT results were dichotomized to positive/negative, with weakly positive results considered as positive.

Results

Between 2007 and 2010, 306 canine-exposed adults and 101 non-canine-exposed, non-matched adults granted informed consent, completed the enrolment questionnaire and submitted a serum sample. The gender distribution was similar between exposure groups (OR = 1.0; 95% CI, 0.6–1.6), but the non-canine-exposed subjects tended to be younger than the exposed group (means of 33 YO and 43 YO, respectively) ($P < 0.001$) (Table 1). Overall, the participants were more likely to be female (68%), and 75% resided in Iowa (206) or Florida (116) where the majority of enrolments took place. Occupations involving close contact (approx. 3 ft) with dogs and their median dog-years of exposure are described in Table 2. On average, dog breeding kennels were modest in size among this study population, with breeders reporting a median of 3 breeding females in their kennels.

Seroprevalence of *B. canis* antibodies and associated risk factors using the D-Tec® CB RSAT kit

Thirty-nine subjects screened seropositive for IgM/IgG antibodies against rough *Brucella* spp. by the RSAT; 33 (10.8%) were canine-exposed participants and 6 (6.0%) were non-canine-exposed participants (OR = 1.9; 95% CI, 0.8–4.7) (Table 1). To examine specific occupational risk factors associated with seropositivity among the canine-exposed subjects, exposure data were analysed. Kennel employees were significantly more likely to test seropositive by the RSAT, compared with other occupations (OR = 2.7; 95% CI, 1.3–5.8) (Table 2). In examining other potential occupational risk factors associated with RSAT seropositivity, a multivariate risk factor model was designed (Table 3). Along with kennel employees (adjusted OR = 4.2; 95% CI, 1.7–11.2), subjects who reported exposures to breeding female dogs were more likely to test RSAT positive (adjusted OR = 2.9; 95% CI, 1.1–7.9). In addition, subjects who reported to rarely or never wash their hands after caring for a sick dog were significantly more likely to be seropositive compared with those who reported sometimes/most of the time/always washing their hands (adjusted OR = 26.5; 95% CI, 1.1–infinity).

Table 1. Comparison of demographic characteristics and *Brucella canis* serological results between canine-exposed enrollees and non-canine-exposed enrollees using Wald chi-square binary logistic regression

Covariate	*n* (*n* = 407)	Exposed (Row%) (*n* = 306)	Non-exposed (Row%) (*n* = 101)	Unadjusted OR (95% CI)[a]
Age (years)[a]				
49–78	137	121 (88.3)	16 (11.7)	**6.5 (3.5–12.1)**
30–48	138	114 (82.6)	24 (17.4)	**4.1 (2.3–7.1)**
18–29	130	70 (53.9)	60 (46.2)	Ref
Gender				
Male	129	97 (75.2)	32 (24.8)	1.0 (0.6–1.6)
Female	278	209 (75.2)	69 (24.8)	Ref
Ever in close contact with sheep[a,b]				
Yes	198	176 (88.9)	22 (11.1)	**4.8 (2.8–8.2)**
No	182	114 (62.6)	68 (37.4)	Ref
D-Tec® CB RSAT screen				
Weak/Strong positive	39	33 (84.6)	6 (15.4)	1.9 (0.8–4.7)
Negative	368	273 (74.2)	95 (25.8)	Ref
D-Tec® CB ME–RSAT confirmation				
Positive	13	11 (84.6)	2 (15.4)	1.8 (0.4–17.4)[c]
Negative	394	295 (74.9)	99 (25.1)	Ref

Significant correlations are indicated in bold ($P < 0.05$).
[a]Covariate has some missing data.
[b]Close contact defined by within 3 feet of animal includes incidental and prolonged exposures.
[c]Exact logistic regression method used.

Table 2. Subjects' reported occupations, levels of dog exposure and D-Tec® CB results comparing each occupation with the remaining canine-exposed categories aggregated together, using logistic regression

Occupation[a]	*n*	Median dog-years of exposure (IQR)[b,c]	RSAT + OR (95% CI)	ME-RSAT + OR (95% CI)
Veterinary staff	92	79 (24–200)	0.9 (0.4–1.9)[d]	0.9 (0.1–3.7)[f]
Breeder	89	60 (25–245)	1.9 (0.9–4.1)[e]	2.1 (0.5–8.5)[f]
Kennel staff	72	60 (30–300)	**2.7 (1.3–5.8)**[e]	1.9 (0.4–7.8)[f]
Veterinarian	64	140 (80–264)	0.3 (0.1–1.2)[e]	0.8 (0.1–4.2)[f]
Shelter staff	48	60 (18–155)	1.2 (0.5–3.1)[e]	0.5 (0.01–3.9)[f]
Trainer	39	50 (12–160)	1.6 (0.6–4.2)[e]	1.5 (0.2–7.9)[f]
Kennel owner	31	225 (117–520)	0.5 (0.1–2.4)[e]	0.9 (0.02–6.6)[f]
Groomer	23	50 (14–210)	0.8 (0.2–3.5)[e]	1.2 (0.03–9.5)[f]
Race track staff	16	540 (200–1560)	0.6 (0.01–4.1)[f]	g
Show handler	12	60 (26–186)	1.7 (0.2–8.5)[f]	2.6 (0.1–21.3)[f]
Owner/Hobbyist	7	50 (18–90)	g	g
Researcher	2	19 (5–32)	g	g
Pet store staff	1	180 (180–180)	g	g

Significant correlations are indicated in bold ($P < 0.05$).
[a]Subjects allowed to cite multiple occupations.
[b]Calculated as the reported number of years multiplied by the average number of dogs per day.
[c]Inter-quartile range.
[d]Proportional odds model used.
[e]Wald Chi-square test used.
[f]Exact logistic regression method used.
[g]Too few subjects to accurately calculate odds ratio.

For the confirmatory ME-RSAT, 13 (33%) of the 39 screen positives were strongly positive for IgG antibodies against rough *Brucella* spp.; 11 (3.6%) were canine-exposed participants and two (2.0%) were non-canine-exposed participants (OR = 1.8; 95% CI, 0.4–17.4) (Table 1). No exposures examined were significantly

Table 3. Occupational risk factors associated with a positive D-Tec® CB RSAT result among canine-exposed subjects, using logistic regression

Risk factor	*n* (*n* = 306)	RSAT positive (Row%)	Unadjusted OR (95% CI)	Multivariate OR (95% CI)[d]
Gender				
Male	97	13 (13.4)	1.5 (0.7–3.1)[a]	e
Female	209	20 (9.6)	Ref	e
Age (years)[b]				
49–78	121	9 (7.4)	0.5 (0.2–1.4)[c]	e
30–48	114	15 (13.2)	1.0 (0.4–2.5)[c]	e
18–29	70	9 (12.9)	Ref	e
Ever used tobacco products				
Yes	100	11 (11.0)	1.0 (0.4–2.1)[a]	e
No	187	21 (11.2)	Ref	e
Ever in close contact with sheep[b]				
Yes	176	20 (11.4)	1.2 (0.5–2.6)[a]	e
No	114	11 (9.7)	Ref	e
Kennel employee				
Yes	72	14 (19.4)	**2.7 (1.3–5.8)**[c]	**4.2 (1.7–11.2)**
No	234	19 (8.1)	Ref	Ref
Exposed to breeding female dogs				
Yes	89	14 (15.7)	1.9 (0.9–4.1)[c]	**2.9 (1.1–7.9)**
No	217	19 (8.8)	Ref	Ref
Washes hands after caring for a sick dog[b]				
Never/rarely	3	2 (66.7)	17.3 (0.9–∞)[d]	**26.5 (1.1–∞)**
Sometimes/most of the time/always	266	27 (10.2)	Ref	Ref
Experienced an illness after having close contact with a sick dog[b]				
Yes	12	3 (25.0)	2.7 (0.5–11.8)[d]	e
No	268	29 (10.8)	Ref	e
Exposed to known *B. canis*-positive dog				
Yes	23	3 (13.0)	1.3 (0.2–4.7)[d]	e
No/unknown	283	30 (10.6)	Ref	e

Significant correlations are indicated in bold ($P < 0.05$).
[a]Proportional odds model used.
[b]Covariate has some missing data.
[c]Wald Chi-square test used.
[d]Exact logistic regression method used.
[e]Variables not included in multivariate logistic regression model.

associated with the outcome when considering all 306 exposed subjects.

D-Tec® CB RSAT validation results

Comparing the D-Tec® CB kit results (RSAT and ME-RSAT) to the ANLIS assays (RSAT and ELISA), there was moderate to substantial agreement with Cohen's Kappa ranging from 0.60 to 0.68. When comparing the D-Tec® CB RSAT screen results (detecting both IgM & IgG) to the ANLISRSAT screen (IgM & IgG), the kit performed very well (Cohen's Kappa = 0.68); 8 samples were discordant. When comparing the D-Tec® CB confirmatory ME-RSAT (detecting only IgG) to the ANLIS confirmatory IELISA (IgG & IgA), the ME-RSAT performed moderately well (Cohen's Kappa = 0.60). Of the 13 ME-RSAT positive sera, all but one were identified as positive by the IELISA, while the IELISA identified eight additional sera as positive compared with the ME-RSAT. All 49 sera were negative by the BPA assay (one serum sample could not be tested due to insufficient volume).

Evidence of clinical *B. canis* infections

In examining questionnaire data, one subject reported exposure to two *B. canis*-infected dogs 6 months prior to enrolling in the study, and also reported experiencing headaches and swollen lymph nodes after coming into close contact with a sick dog. Follow-up investigations revealed the breeding kennel associated with this subject had experienced recurrent *B. canis* outbreaks. Another subject (#402) reported exposure to a *B. canis*-infected (positive) dog more than 4 years prior to testing. On the study questionnaire, this subject also reported experiencing symptoms consistent with brucellosis after being in close contact with a sick dog (fever, cough, sore throat, headaches, muscle

aches, sweats, back pain and arthritis). It is unknown why subject #402 tested seropositive by the ANLIS assays but not by the D-Tec® CB RSAT.

Discussion

Among dogs, *B. canis* is transmitted venereally. Canine infections occur most commonly after ingesting placental material, aborted foetuses or secretions from an infected female that is either in heat, aborting or breeding. Canine brucellosis typically presents as abortions in females at 40–60 days of gestation. In stud dogs, epididymitis and scrotal dermatitis are the most common clinical signs, which sometimes progress to complete scrotal necrosis (Shin and Carmichael, 1999b). Males shed bacteria in their seminal fluids and urine. The bacteria are typically cleared within a few days of treatment; however, re-infection is common and some body fluids may be infectious for weeks as prolonged bacteremia can occur. Infection is most prevalent among wild dog packs, in kennels, and in large-scale breeding facilities where often large numbers of dogs intermingle (Hollett, 2006; ISU, 2007). All breeds are susceptible to *B. canis* infections.

B. canis enters a canine host via genital, oronasal or conjunctive mucosal routes (Wanke, 2004) by penetrating the epithelial lining. Bacteria are phagocytized by macrophages and transported to lymphatic and genital organs via the circulatory system for reproduction (Carmichael and Kenney, 1970; Wanke, 2004). Its intracellular characteristic allows the bacteria to reside in the host's cells and further evade the humoral immune response. Bacteremia can be prolonged and intermittent. Because *B. canis* is transmitted through vaginal secretions, placenta material, semen, blood and urine, humans are potentially at risk of infection if they come in direct contact with infected bodily fluids and organs.

After *B. canis* was identified in dogs in 1966, several seroepidemiological studies were conducted in the 1970s to determine whether this pathogen was a zoonotic threat to humans. A 1973 study of US military recruits found a 0.4% seroprevalence of *B. canis* antibodies (Lewis and Anderson, 1973). A 1974 study examined 167 animal shelter workers and 43 veterinarians from 21 establishments in 16 counties of Florida (Hoff and Nichols, 1974; Hoff and Schneider, 1975). One of 167 shelter workers (0.59%) tested positive (agglutinin titres of $\geq$1 : 200), but no veterinarians had titres against *B. canis* antibodies. In 1975, Monroe et al. examined *B. canis* seroprevalence among a US cohort divided into 4 categories: 193 newborn infants (no exposure), 2026 hospitalized/non-hospitalized patients, hospital employees and blood donors (average dog exposure), 73 practicing veterinarians (high exposure) and 113 patients with fevers of undetermined origin (Monroe et al., 1975). Employing the modified microtitre plate agglutination technique (Damp et al., 1973), seroprevalence for the groups was 5.7%, 67.8%, 72.6% and 80.5%, respectively. With a reported 81% of febrile patients testing seropositive, results suggested that *B. canis* was eliciting clinical illness in humans. While the authors did not believe false positives were confounding their results, these findings have not been reproduced in other seroprevalence studies.

A 1976 serological study in Mexico among hospital patients found a 13.3% seroprevalence (27 of 203 patients) (Flores-Castro and Segura, 1976). Similar to the Monroe study, in 1977, Weber and Brunner examined 1915 human sera samples (1400 blood donors, 480 clinical patients and 35 persons exposed to dogs and material infected with *B. canis*) (Weber and Brunner, 1977). Three subjects were confirmed positive (0.2%), and all were healthy blood donors. Using the cross-reacting *B. ovis* antigen, a 1979 study found that 23 of 1952 (1%) of people tested in rural Argentina were positive for *B. canis* antibodies (Varela-Diaz and Myers, 1979). The most recent study, conducted in the United States in 1982, detected *B. canis* antibodies in 4 (0.3%) of 1147 human sera tested; all 4 were patients with undiagnosed febrile illness (Polt and Schaefer, 1982; Polt et al., 1982). Occasional case reports of human clinical infections with *B. canis* are presented in the literature, and most involve direct exposure to a confirmed *B. canis*-positive dog (Swenson et al., 1972; Tosi and Nelson, 1982; Young, 1983; Lum et al., 1985; Rousseau, 1985; Schoenemann et al., 1986). The first naturally acquired human infection occurred in 1970, but was the sixth reported human case (the first four were laboratory exposures and the fifth was an animal caretaker with asymptomatic serological evidence of exposure) (Swenson et al., 1972). Another report described a laboratory worker developing a clinical illness after exposure to live M-cells for *B. canis* antigen production (Wallach et al., 2004). Recently, there have been two case reports of *B. canis* causing clinical infections in HIV-positive immunocompromised patients following exposure to positive dogs (Lucero et al., 2010b; Lawaczeck et al., 2011), as well as the first documented outbreak in humans (Lucero et al., 2010a). A family of 6 (3 adults and 3 children) developed *B. canis* infections following exposure to a sick dog and her puppies. Notably, the index case was initially misdiagnosed, and it would have gone undetected if not for the subsequent positive culture. Considering all the human case reports, the most commonly reported human symptom was a recurrent fever of unknown origin.

Brucella canis infections in humans mimic brucellosis caused by other *Brucella* species and can cause both acute and chronic infections (ISU, 2007). Acute human brucellosis elicits symptoms similar to that of influenza, which include fever, sweats, headaches, back pain and physical

weakness. Brucellosis can also cause long-lasting or chronic symptoms, including recurrent fevers, joint pain and fatigue. Two of the study participants may have experienced clinical brucellosis, although the temporality of symptom onset with the reported exposure to *B. canis*-infected dogs could not be determined.

This current study expands on the *B. canis* prevalence data collected in the 1970s and suggests that humans continue to be exposed to *B. canis*, with an overall estimated seroprevalence of 3.6% (11/306) among the canine-exposed study population, based on results of the confirmatory D-Tec® CB ME-RSAT. Prior US-based seroprevalence studies among canine-exposed populations reported a much lower rate of 0.6% in Florida (Hoff and Nichols, 1974). The change may reflect independent or combined differences in the population sampled, improvements in diagnostics, or an overall increase in prevalence of human infection, but it emphasizes the importance of re-examining *B. canis* prevalence data in the United States. Interestingly, global reporting of both seroconversion- and clinical blood-culture-positive human infections with *B. canis* also appears to be on the rise (Lucero et al., 2010a, b; Nomura et al., 2010; Lawaczeck et al., 2011; Sayan et al., 2011).

Considering the specific antibodies detected with the various assays (IgM & IgG in RSAT screens, and IgG in ME-RSAT and ELISA) (Al Dahouk et al., 2003), there was a strong correlation between the D-Tec® CB kit and ANLIS assays. The likely explanation for the inter-rater discordance is the very different natures of the diagnostic tests. While a full discussion is outside of the scope of this report, the tests greatly differ with respect to purpose, detection of specific antibody types and clinical performance. Although the definitive diagnosis of brucellosis (gold standard) is the isolation of *Brucella* sp., its efficacy is low and therefore a negative blood culture cannot rule out the disease. In contrast to bacterial culture, serological tests can only indirectly prove *Brucella* infections by high or rising titres of specific antibodies. The ANLIS IELISA has shown 100% specificity and sensitivity among sera from 110 asymptomatic people with negative blood culture as well as several negative serologic assay results (including RSAT) and 17 patients with a positive *B. canis* culture or in close contact with culture-positive dogs (Lucero et al., 2005). Some discordance with the D-Tec® CB kit may be attributed to antigen preparation, because the antigen used in the ANLIS assays (M-variant *B. canis* strain) differed from that used in the D-Tec test (*B. ovis*). A further limitation of this study was having only one serum sample for each participant, as serological testing should be repeated after 1–2 weeks for suspicious cases.

While the possibility of false positives owing to cross-reacting antibodies against the *B. ovis* antigen used in D-Tec® CB test kit was considered, it is viewed as very unlikely. Infection with *B. ovis* has not been reported in man (Tsolis et al., 2009), and this forms the basis of its use in a commercially available test kit. Further, while a total of 198 subjects (49%) reported incidental or recurrent exposures to sheep, and while canine-exposed subjects were more likely to cite exposures to sheep ($P < 0.0001$), it was not statistically associated with D-Tec® CB RSAT or ME-RSAT seropositivity ($P = 0.47$ and $P = 0.66$, respectively). In addition, the BPA assay was run to determine whether smooth *Brucella* antibodies (*B. abortus*, *B. melitensis* and *B. suis*) were cross-reacting with the rough *B. ovis* antigen used in the D-Tec® CB kit; all 49 sera tested negative. Previous studies have indicated that the RSAT could be a suitable screening tool for identifying human antibodies against *B. canis* (George and Carmichael, 1974; Shin and Carmichael, 1999a; Lucero et al., 2005; ISU, 2007). The whole bacteria antigen used in the RSAT is most effective for detecting antibodies against *Brucella* in the early stages of infection (IgM antibodies). The RSAT has been reported to elicit false positives due to shared antigenic epitopes of lipopolysaccharides of other bacteria (Wanke, 2004). The addition of 2ME is intended to prevent most of this potential cross-reactivity (ISU, 2007), and more accurately measure IgG antibodies indicative of a prior infection (IgG may not be present for 3–12 weeks following infection). While this scenario is not ideal for diagnosing clinically ill dogs, the ME-RSAT provides a means to detect long-lasting IgG antibodies in humans. However, the 2ME may also block some anti-*B. canis* IgM, and even IgG, antibodies, leading to a loss of sensitivity. Diluting the serum 1 : 1 with 2ME may also reduce sensitivity. Traditionally, the RSAT is considered highly sensitive but less specific; however, one report examined the D-Tec® CB kit with canine sera and found a higher specificity (Hollett, 2006; Keid et al., 2009). The RSAT sensitivity and specificity were 70.58% and 83.34%, respectively, and the ME-RSAT sensitivity and specificity were 31.76% and 100%, respectively, when compared to a positive blood culture.

Two control subjects in this study were ME-RSAT seropositive. One subject cited 9 years of exposure to two pet dogs more than 5 years in the past. If antibodies persist longer than 5 years, this subject may have been a true positive. The second subject could not remember any personal dog exposure and is interpreted as a likely false positive, for which further confirmation by additional testing is warranted.

This study's serological results suggest that occupational infections with *B. canis* occur more often than previously thought. Hence, it seems prudent that persons occupationally exposed to breeding dogs should protect themselves with personal protective clothing, gloves and careful hand washing after exposure to canine secretions and reproductive tissues. Additionally, questionnaire data indicated that

35% of enrolled dog breeders were not performing *B. canis* testing in their kennels (including when acquiring new dogs, breeding dogs, or selling dogs). At-risk dog populations should be regularly tested for *B. canis*.

The study also demonstrated that the D-Tec® CB kit is an effective screening tool for detecting *B. canis* antibodies in human sera. While blood culture is the gold standard for *B. canis* detection in humans, sensitivity is limited due to the very low levels and periodic nature of bacteria release into the blood and the often fastidious nature of the bacteria with respect to growth in artificial media. This rapid, easy to use, commercially available kit would be valuable for resource-limited clinical diagnostic laboratories as well as for local and state health departments examining zoonotic transmission in conjunction with *B. canis* outbreak investigations in dog kennels.

Infection with *B. canis* may explain undiagnosed acute and chronic illnesses among people with brucellosis-like symptoms. Medical personnel should include *B. canis* infections in their differential diagnoses, especially among persons with intense dog exposure, and be mindful that current standard serologic testing will miss these infections. Considering the more than quarter of a million persons in the United States with occupations involving dogs, and millions of dog enthusiasts, the 3.6% seroprevalence we found suggests that unapparent *B. canis* infections are a public health concern. Efforts should be made to adapt the veterinary serological *B. canis* diagnostics for human use and to win Food and Drug Administration approval.

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