

CANINE AND HUMAN BARTONELLOSIS: EMERGING INFECTIOUS DISEASES

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INTRODUCTION

Bartonella species are fastidious gram-negative bacteria that are highly adapted to a mammalian reservoir host and within which the bacteria usually cause a long-lasting intraerythrocytic bacteremia.¹⁻³ These facts are of particular importance to veterinarians and physicians, as an increasing number of animal reservoir hosts have been identified for various *Bartonella* species. Among numerous other examples, *Bartonella henselae* has co-evolved with cats, *Bartonella vinsonii* subsp. *berkhoffii* has co-evolved with dogs and wild canines, and *Bartonella bovis* has co-evolved with cattle.¹⁻² Importantly, the list of reservoir-adapted *Bartonella* species, including a large number of rodent species that might serve as “pocket pets”, continues to grow exponentially, as new *Bartonella* spp. are discovered. Prior to 1990, there were only two named *Bartonella* species, whereas there are now at least 22 named and numerous unnamed or candidatus species, based upon deposited Gen Bank sequences or preliminary reports, respectively.

In the natural reservoir host, chronic bacteremia with a *Bartonella* species can frequently be detected by blood culture or PCR in outwardly healthy individuals.¹⁻³ In contrast, the diagnostic detection of a *Bartonella* spp. in a non-reservoir adapted host can be extremely difficult.⁴ Most, although not all diseases caused by *Bartonella* spp. occur in accidental hosts and these organisms are being increasingly implicated as a cause of zoonotic infections. Until recently, mechanisms that facilitate persistent *Bartonella* bacteremia in mammals were not well understood. Recent reports have identified an intra-endothelial and intra-erythrocytic localization for these bacteria, which represents a unique strategy for bacterial persistence.¹⁻⁶ Non-hemolytic intracellular colonization of erythrocytes and endothelial cells would preserve the organisms for efficient vector transmission, protect *Bartonella* from the host immune response, and potentially contribute to decreased antimicrobial efficacy.³ Other *in vitro* studies indicate that *Bartonella* spp. can infect dendritic cells, microglial cells, monocytes and CD34+ bone marrow progenitor cells.

EPIDEMIOLOGY

Bartonella vinsonii (*berkhoffii*) was isolated from a dog with endocarditis in our laboratory in 1993.^{7,8} Retrospectively, long-term administration of immunosuppressive doses of corticosteroids for a presumptive diagnosis of systemic lupus erythematosus may have facilitated the isolation of the original type strain of *B. vinsonii* (*berkhoffii*) from this dog that subsequently developed endocarditis. Due to the relatively recent recognition that dogs can be infected with *B. vinsonii* (*berkhoffii*), *B. henselae* and potentially other *Bartonella* spp., seroprevalence data is limited.⁹⁻¹² Seroprevalence was determined in 1,920 sick dogs from North Carolina or surrounding states that were evaluated at a veterinary teaching hospital.⁹ Using a reciprocal titer of >32, only 3.6% of sick dogs had antibodies to *B. vinsonii* (*berkhoffii*). Risk factors that could be associated with seroreactivity included: heavy tick exposure (Odds ratio 14.2), cattle exposure (OR 9.3), rural vs. urban environment (OR 7.1) and heavy flea exposure (OR 5.6). These data were interpreted to support the possibility that exposure to *B. vinsonii* (*berkhoffii*) was more likely in dogs in rural environments that were allowed to roam. In addition, these dogs were likely to have a history of heavy tick infestation. Using sera from dogs experimentally infected with *R. rickettsii* or *Ehrlichia canis*, cross reactivity to bartonella antigens was not detected. However, 36% of serum samples derived from dogs naturally infected with *E. canis* were reactive to *B. vinsonii* antigens. As *E. canis* is transmitted by *Rhipicephalus sanguineus*, this tick may be involved in the transmission of *B. vinsonii*. The possibility of tick transmission was further supported by two additional studies involving dogs infected with one or more *Ehrlichia* spp. from the same geographic region, in which seroreactivity to *B. vinsonii* (*berkhoffii*) antigens was 30% and 89%, respectively.^{13,14} Seroprevalence, using *B. vinsonii* (*berkhoffii*) antigens, was 10% (4/40 dogs) in dogs with suspected tick-borne illness from Israel and 36% in dogs with fever and thrombocytopenia from Thailand.^{12,15} Using an ELISA assay, 35% of 869 samples,

derived from coyotes in California, contained antibodies to *B. vinsonii* (*berkhoffii*) antigens.¹⁶ Current data indicates that exposure to *B. vinsonii* (*berkhoffii*) can be found throughout much of the United States and most tropical and subtropical regions of the world.

Based upon current evidence, *B. vinsonii* (*berkhoffii*) is considered the most frequent *Bartonella* species that causes disease in dogs. However, this conclusion may not be accurate, as sera from dogs has not been screened systematically against a large panel of *Bartonella* species antigens and minimal PCR testing has been performed. Studies from Hawaii, the United Kingdom and Japan identified *B. henselae* seroprevalences of 6.5% (2/31 dogs), 3.0% (3/100 dogs) and 7.7% (4/52)^{17,18,19} In our laboratory, *B. henselae* seroprevalence was 10% and 28% in healthy and sick dog populations, respectively. Although the pathogenicity of all *Bartonella* spp. in dogs is poorly characterized, it is becoming increasingly clear that species other than *B. vinsonii* (*berkhoffii*) can infect dogs. For example, *B. henselae* was amplified and sequenced on two independent occasions from the liver of a dog with peliosis hepatis.²⁰ This is a unique pathological lesion that is induced only by *B. henselae* infection in people.¹ Recently, *B. henselae* DNA was amplified from a dog with granulomatous hepatitis, a histopathological lesion that is reported with some frequency in children infected with *B. henselae*.²¹ Similarly, *B. clarridgeae* DNA has been amplified and sequenced from the liver of a Doberman pincher with copper storage disease and from the aortic valve of a dog with vegetative valvular endocarditis.^{21,22} *Bartonella elizabethae*, a species that infects rodents, was PCR amplified and sequenced from an EDTA blood sample obtained from a dog that had experienced chronic weight loss culminating in sudden unexplained death.²³ These observations indicate that although presumably infrequent, those *Bartonella* spp. that frequently infect cats or rodents, and are transmitted by fleas among reservoir hosts, may cause disease manifestations in dogs.

PATHOGENESIS

Although as yet unproven, *B. vinsonii* (*berkhoffii*) is presumably transmitted to dogs by the bite of an infected tick. Based upon antidotal evidence dogs may be infected with *B. henselae* by a cat bite or scratch, analogous to cat scratch disease in people. If *B. vinsonii* causes chronic intraerythrocytic and endothelial cell infections, like other *Bartonella* spp. it is presumably well tolerated by the dog for extended periods of time. Similar to other highly adapted intracellular vector-transmitted pathogens, the factors that ultimately result in these bacteria causing disease manifestations are yet to be determined. If similar to babesiosis, another intraerythrocytic pathogen, stress, hard work, parturition, or concurrent infection with other organisms may contribute to the development of pathology. Following experimental inoculation of SPF dogs with culture grown *B. vinsonii* (*berkhoffii*), there was sustained suppression of peripheral blood CD8+ lymphocytes, accompanied by an altered cell surface phenotype and an increase in CD4+ lymphocytes in the peripheral lymph nodes.²⁴ Therefore, infection with *B. vinsonii* (*berkhoffii*) might induce a degree of chronic immunosuppression that could predispose dogs to other infectious agents, resulting in a wide array of clinical manifestations in naturally-infected dogs.

The extent to which infection with bartonella influences the pathophysiology of ehrlichiosis, a disease of much longer historical venue, deserves critical reappraisal. For example, infection with *Bartonella* in dogs concurrently infected with *Ehrlichia canis* may contribute to the tendency to develop epistaxis. Of similar potential concern in both canine and human medicine is the finding of co-segregation of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* (previously *Ehrlichia equi* or human granulocytic ehrlichiosis), *Babesia microti*, and *Bartonella vinsonii* (*arupensis*) in *Ixodes scapularis* ticks in the northeastern and northcentral United States. Regional differences in tick species, accompanied by differences in the bacterial, viral and protozoan organisms that ticks transmit creates substantial challenges for the clinician in regard to diagnosis and medical management.

From an evolutionary perspective, it is obvious that vectors, vector-borne organisms, and animal and human hosts have developed a highly adapted form of interaction. In general, vectors need blood for nutrition; bacterial, rickettsial and protozoal organisms need an intracellular environment to survive, and immunologically, most hosts appear to be able to support chronic infection with many vector-borne organisms for months to years without obvious deleterious effects. These factors serve to illustrate the potential difficulty in establishing causation in dogs or people co-infected with multiple tick-transmitted pathogens.

CLINICAL FINDINGS

The spectrum of disease associated with *Bartonella* infection in dogs and most other animal species is currently unknown. Endocarditis, associated with *B. vinsonii* (*berkhoffii*) occurs in large breed dogs with a potential predisposition for aortic valve involvement.^{7,22,25} In some dogs, intermittent lameness, bone pain or fever of unknown origin can precede the diagnosis of endocarditis for several months, whereas other dogs will present with an acute history of cardiopulmonary decompensation. Cardiac arrhythmias secondary to myocarditis can be detected in dogs without echocardiographic evidence of endocarditis.

Bartonella-induced granulomatous lymphadenitis, involving the left submandibular lymph node, was diagnosed in a dog on the basis seroreactivity to *B. vinsonii* (*berkhoffii*) antigens, visualization of Warthin-Starry silver staining bacteria within the lymph node and PCR amplification followed by Southern blot hybridization.²⁶ (Figure 3) Seven days prior to enlargement of the lymph node, the owners removed an engorged tick from the left ear. This case provides the best current evidence that ticks can transmit *Bartonella* spp. to dogs and potentially to people. The granulomatous lymphadenitis in this dog would be analogous to acute bartonellosis (cat scratch disease) in people, where a scratch or bite injects the inoculum (usually *B. henselae*), rather than inoculation by the bite of a tick.¹ Based on recently obtained serologic evidence in dogs, *B. vinsonii* (*berkhoffii*) or closely related *Bartonella* species appear to contribute to the development of dermatologic lesions indicative of a cutaneous vasculitis, anterior uveitis, polyarthritis, meningoencephalitis or immune-mediated hemolytic anemia.²⁷ Additional research efforts, using carefully designed case controlled studies will be necessary to establish the frequency and extent to which *Bartonella* spp. contribute to ocular, orthopedic, neurological or hematological abnormalities in dogs.

DIAGNOSIS

Thrombocytopenia, anemia, which frequently can be immune-mediated, and neutropenia or neutrophilic leukocytosis are the most commonly detected hematological abnormalities in dogs that are seroreactive to *B. vinsonii* (*berkhoffii*) antigens. Thrombocytopenia is found in approximately half of the dogs with disease manifestations. Eosinophilia is also found in approximately one third of infected dogs. Monocytosis can also occur in *B. vinsonii*-infected dogs, particularly those with endocarditis. Hemoglobinuria, generally unaccompanied by hematuria, is a frequent finding, particularly in dogs with immune-mediated hemolytic anemia. Serum biochemical abnormalities are usually very mild or nonexistent.

As antibodies to *B. vinsonii* (*berkhoffii*) antigens is infrequently detected (<4%) in sick or healthy (<1%) dog populations in endemic regions, detection of *B. vinsonii* (*berkhoffii*) antibodies in a sick dog provides strong clinical evidence for prior exposure and potentially active infection. For this reason, treatment of seroreactive dogs or dogs from which *Bartonella* spp. DNA is detected in blood or tissue samples would be recommended. A reciprocal titer of 64 or greater is considered indicative of prior exposure to or active infection with *B. vinsonii* (*berkhoffii*) or *B. henselae* in dogs.

Attempts to isolate *B. henselae* or *B. vinsonii* from immunocompetent dogs with serologic or molecular evidence of *Bartonella* infection have not been successful in most instances. When using the currently recommended microbiologic techniques, there appears to be considerable variation in the degree of difficulty associated with the isolation of different *Bartonella* spp. from the blood of different animal species. Because conventional microbiological techniques have historically lacked sensitivity, bartonellosis was usually diagnosed by PCR amplification of organism specific DNA sequences and/or through serological testing. As described below, we have combined a culture enrichment step and a highly sensitive real time PCR assay to enhance the molecular detection or isolation of *Bartonella* species from the blood, cerebrospinal fluid, joint fluid and pleural, pericardial and abdominal effusions. We have used the BAPGM platform described below to enhance the molecular detection of *Bartonella* spp. in animal and human patient samples.

Recent serologic and molecular evidence indicates that co-infection in dogs with *Ehrlichia*, *Babesia*, *Rickettsia* and *Bartonella* spp. may be more frequent than previously realized.^{7,9,13-15,25} As certain *Borrelia*, *Ehrlichia*, *Babesia* and *Bartonella* spp. can cause chronic, insidious infection in dogs, the relative role of each organism to the pathogenesis of specific disease manifestations in a sick, naturally infected dog will remain difficult to establish in the clinical setting. When dealing with sick dogs with a history of tick exposure, clinicians should screen by serological or molecular testing modalities for a panel of tick-transmitted pathogens.

ISOLATION AND MOLECULAR DETECTION OF *BARTONELLA* SPECIES

Because conventional microbiological techniques lack sensitivity, bartonellosis is usually diagnosed by PCR amplification of organism specific DNA sequences and/or through serological testing. Recently, the development of a more sensitive isolation approach, using BAPGM (*Bartonella* alpha *Proteobacteria* growth medium) followed by real time PCR has greatly facilitated the molecular detection or isolation of *Bartonella* species from the blood of sick or healthy animals, including dogs and human beings.^{19,20} Obviously, the relative sensitivity of the diagnostic methods used to detect *Bartonella* species infection greatly influences an investigator's ability to establish disease causation or a clinician's ability to initiate appropriate treatment. The use of recently optimized microbiological techniques has facilitated the recognition of blood-borne *Bartonella* spp. infections in dogs, horses, human beings and porpoises in our laboratory.

Diagnostic testing for *Bartonella* species (serology, PCR and BAPGM Blood Culture/PCR combination testing) is available through the:

Galaxy Diagnostics, Inc.

2 Davis Drive

Research Triangle Park, NC 27709

919-354-1055

www.galaxydx.com

PATHOLOGIC FINDINGS

In dogs, pathologic findings associated with *Bartonella* spp. infection include endocarditis, myocarditis, granulomatous lymphadenitis, granulomatous hepatitis, and peliosis hepatitis. Multifocal areas of severe myocardial inflammation can be found in dogs with *B. vinsonii* (*berkhoffii*) endocarditis. Although not specific for bartonella infections, organisms can be detected in diseased tissues using silver stains, particularly in acute bartonella infections, such as acute regional lymphadenitis (cat scratch disease). During chronic infections, organisms are presumably too few in number to be detected in tissues by silver staining, unless the fulminate infection is localized to heart valves. Recently, we have documented *B. vinsonii* (*berkhoffii*) infection in a dog that developed bacillary angiomatosis following immunosuppressive drug therapy instituted by the attending veterinarian for pancytopenia. We have also isolated *B. vinsonii* (*berkhoffii*) by BAPGM blood culture from a cat with recurrent osteomyelitis.

THERAPY

To date, an optimal protocol has not been established for the treatment of bartonella infections in cats, dogs, or people.^{30,31} Regardless of the antibiotic that is used for treatment, a long duration of antibiotic administration (4-6 weeks) may be necessary to eliminate the infection. Macrolides (azithromycin) most probably represent the oral antibiotic class of choice for treating bartonella infections.^{30,31} Fluoroquinolones alone, or in combination with amoxicillin, have also elicited a positive therapeutic response in dogs, which is accompanied by a progressive decrease in *B. vinsonii* antibody titers.²⁷ Doxycycline may or may not be effective for treatment of *B. vinsonii* (*berkhoffii*), but data from cats experimentally or naturally-infected with *B. henselae* or *B. clarridgeae* indicates that a high dose of doxycycline (10 mg/kg every 12 hours) for 4-6 weeks may be necessary to eliminate bartonella infection in dogs, cats or other animal species.³² Serum antibody titers decrease rapidly (3-6 months) and are generally no longer detectable in dogs that recover following antimicrobial therapy.²⁷ Therefore, post-treatment serology may be a useful adjunct to BAPGM/PCR to determine if therapeutic elimination of bartonella infections has been achieved.

PREVENTION

Increasingly, veterinarians play an important role in advising the public as to the epidemiological and zoonotic implications associated with vector-borne pathogens. Numerous non-domestic animal species frequently serve as the primary reservoir for *Bartonella* species. For example, the coyote appears to be an important reservoir host for *B. vinsonii* (*berkhoffii*).³³ Although somewhat circumstantial, there is increasing evidence that *Bartonella* species can be transmitted by fleas and ticks to cats, dogs or human beings.^{9,25,34} Based upon scientific evidence generated during the past several decades, vector-transmitted pathogens can induce clinical manifestations ranging from acute fatal illness (i.e. Rocky Mountain spotted fever, ehrlichiosis, babesiosis and bartonellosis) to chronic debilitating disease states (ehrlichiosis, babesiosis, borreliosis, and bartonellosis). Therefore, minimizing or eliminating flea and tick exposure is perhaps of greater veterinary and

public health importance today, than during any previous time in history. When rigorous flea and tick control measures are instituted, it is highly probable that transmission of *Bartonella* species will be greatly reduced or eliminated.³⁵ Recently, we have reported a high molecular prevalence of *Bartonella* spp. infection in healthy Golden Retrievers and Golden Retrievers with lymphoma (18% of both populations were actively infected based upon PCR testing).³⁶ Interestingly, this is the second study in which routine application of acaracides decreased the risk of lymphoma in the dog study populations.

PUBLIC HEALTH CONSIDERATIONS

When a genus of bacteria is discovered or in the case of *Bartonella*, rediscovered; numerous clinical, microbiological and pathological concepts related to disease causation and microbial pathogenesis are sequentially redefined. Subsequently, the medical relevance of the genus undergoes continued maturation; as knowledge of the organism, the host immune response, diagnostic test sensitivity and specificity, treatment efficacy and epidemiology expand. Since the early 1990s, a paradigm of discovery, rediscovery and ongoing biological and medical redefinition of *Bartonella* spp. as zoonotic human pathogens has clearly applied to this genus. Due to extensive contact with a spectrum of animal species, veterinary professionals appear to have an occupational risk of infection due to frequent exposure to *Bartonella* spp., therefore these individuals should exercise increased precautions to avoid arthropod bites, arthropod feces (i.e. fleas and lice), animal bites or scratches and direct contact with bodily fluids from sick animals.^{38,40} Physicians should be educated as to the large number of *Bartonella* spp. in nature, the extensive spectrum of animal reservoir hosts, the diversity of confirmed and potential arthropod vectors, current limitations associated with diagnosis and treatment efficacy, and the ecological and evolving medical complexity of these highly evolved intravascular, endotheliotropic bacteria.

Bartonella vinsonii (berkhoffii), originally isolated from a dog, has been isolated from a human endocarditis patient and from immunocompetent veterinary professionals.^{38,40} The extent to which dogs can serve as a reservoir host for *B. vinsonii (berkhoffii)* or other *Bartonella* spp., such as *B. henselae*, *B. clarridgeae* or *B. elizabethae* is poorly characterized. Although dogs have been implicated in the direct transmission of *B. henselae* to people by a scratch or bite, this mode of transmission is as yet, poorly established. Recently, DNA from several *Bartonella* spp. was amplified and sequenced from the saliva of healthy and sick dogs.³⁹ This finding, until further clarified, suggests that veterinary professionals should limit contact with saliva from dogs or cats. Also, we have recently reported the isolation of candidatus *Bartonella melophagi*, a species adapted to sheep blood, from two woman.⁴¹

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