BARTONELLOSIS: A ONE HEALTH PERSPECTIVES ON AN EMERGING ZOONOTIC INFECTIOUS DISEASE

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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 intra-erythrocytic 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. 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.[2-3] Prior to 1990, there were only two named *Bartonella* species, whereas there are now at least 36 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. In contrast, the diagnostic detection of a *Bartonella* spp. in a non-reservoir adapted host can be extremely difficult. 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.[4-8] 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.[2,3] 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. Other *in vitro* studies indicate that *Bartonella* spp. can infect dendritic cells, microglial cells, pericytes, monocytes and CD34+ bone marrow progenitor cells.

Epidemiology

Bartonella henselae was initially isolated from an HIV-infected human and subsequently from cats, dogs, horses, marine mammals and other small terrestrial mammals. *Bartonella vinsonii* subsp. *berkhoffii* was initially isolated from a dog with endocarditis in our North Carolina laboratory in 1993[9] and subsequently from cats, coyotes and human patients. Retrospectively, long-term administration of immunosuppressive doses of corticosteroids for a presumptive diagnosis of systemic lupus erythematosus with cutaneous vasculitis 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 somewhat limited.[3] Seroprevalence was determined in 1,920 sick dogs from North Carolina or surrounding states that were evaluated at a veterinary teaching hospital. 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 and flea infestations. Experimental flea transmission of *B. henselae* to dogs has now been confirmed in the laboratory (Lappin and Breitschwerdt, unpublished data). Also, cross reactivity to *Bartonella* antigens was not detected when testing sera from dogs experimentally infected with *R. rickettsii* or *Ehrlichia canis*. 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 sanguineous*, 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. 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. Using an ELISA assay, 35% of 869 samples, derived from coyotes in California, contained antibodies to *B. vinsonii (berkhoffii)* antigens. Current data indicates that canine and human exposure to *B. henselae* and *B. vinsonii (berkhoffii)* can be found throughout much of the United States and most tropical and subtropical regions of the world.

Studies from Hawaii, the United Kingdom and Japan identified *B. henselae* seroprevalences of 6.5% (2/31dogs), 3.0% (3/100 dogs) and 7.7% (4/52). In our laboratory, *B. henselae* is the most common *Bartonella* species found in sick dogs using the BAPGM enrichment blood culture platform. The pathogenicity of all *Bartonella* spp. in dogs is poorly understood; however, like humans, dogs can be infected with numerous *Bartonella* spp. *B. henselae* DNA has been amplified and sequenced from the livers of dogs with peliosis hepatis, a unique pathological lesion also reported in *B. henselae* infected people. *B. henselae* DNA was also amplified from a dog and a horse with granulomatous hepatitis, a

histopathological lesion reported with some frequency in *B. henselae*-infected children and adults. *Bartonella 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. *Bartonella elizabethae*, a species that infects rodents, was found in a dog that had experienced chronic weight loss culminating in sudden unexplained death. Based upon a large seroepidemiological, controlled study from the University of California (Davis), dogs that were seroreactive to either *B. henselae, Bartonella clarridgeae* or *B. vinsonii (berkhoffii)* were referred for evaluation of lameness, neutrophilic polyarthritis, nasal discharge, epistaxis and splenomegaly.[10]

In cats, *B. henselae* and *B. clarridgeiae* have been amplified or grown most frequently from cats and the fleas collected from cats. Flea-associated transmission has been well documented amongst cats. In flea endemic areas, *Bartonella* spp. seroprevalence rates in cats can be greater than 90% and bacteremia rates can be greater than 50%. Granulomatous myocarditis has been reported in cats, naturally or experimentally-infected with *B. henselae. B. vinsonii* (*berkhoffii)* caused recurrent osteomyelitis in a cat.[3,11]

Pathogenesis

Although as yet unproven, *B. vinsonii (berkhoffii)* may be transmitted to dogs by the bite of an infected flea or tick. Based upon antidotal evidence, dogs may also become infected with *B. henselae* by a cat bite or scratch, analogous to cat scratch disease in people. *B. vinsonii* appears to cause chronic intra-erythrocytic and endothelial cell infections dogs for extended periods of time, potentially resulting in vasoproliferative pathologies. Similar to other highly adapted intracellular vector-transmitted pathogens, the factors that ultimately result in these bacteria causing disease manifestations are likely multifactorial and as yet to be defined. If similar to babesiosis, another intra-erythrocytic pathogen, stress, hard work, parturition, concurrent infection with other organisms or therapeutic immunosuppression 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.[11] Therefore, infection with *B. vinsonii (berkhoffii)* appears to induce a degree of chronic immunosuppression that might predispose dogs to other infectious agents, resulting in a wide array of clinical manifestations in naturally-infected dogs. The pathogenesis of disease (myocarditis or endocarditis) in cats may ultimately be associated with the virulence of the specific strain. Cats infected with *Bartonella* spp. are commonly co-infected with hemoplasmas and at times, more than one *Bartonella* sp. However, whether co-infections magnify disease manifestations of either genera is unclear and in most studies co-infections did not appear to potentiate illness. 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 vector-borne organisms for months to years without obvious deleterious effects. These factors serve to illustrate the potential difficulty in establishing causation in cats, dogs or people infected with a single or co-infected with multiple tick-transmitted pathogens. Recently, we proposed an addition to Koch's postulates entitled the Postulate of Comparative Infectious Disease Causation.[11] By satisfying this postulate, *Bartonella* species appear to be able to cause, endocarditis, granulomatous inflammatory diseases, particularly involving heart, liver, lymph nodes, and spleen, persistent intravascular infections and the induction of vasoproliferative tumors in animals and human patients.[3,9,12-14]

Clinical findings

The spectrum of disease associated with *Bartonella* infection in dogs and most other animal species is currently unknown. Endocarditis, has been reported in cats, cows, dogs, humans, and wildlife infected with a spectrum of *Bartonella* spp. In some dogs, intermittent lameness, bone pain, epistasis 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.[3,9] Cardiac arrhythmias secondary to myocarditis can be detected in cats and dogs without echocardiographic evidence of endocarditis. Granulomatous lymphadenitis has been associated with *B. vinsonii (berkhoffii)* and *B. henselae* in dogs. *B. vinsonii (berkhoffii)* and other *Bartonella* species appear to contribute to the development of dermatologic lesions indicative of a cutaneous vasculitis, panniculitis, as well as anterior uveitis, polyarthritis, meningoencephalitis and immune-mediated hemolytic anemia.[3,10-15] Additional research efforts, using carefully designed case controlled studies are necessary to establish the frequency and extent to which *Bartonella* spp. contribute to dermatological, ocular, orthopedic, neurological or hematological abnormalities in dogs (and humans).

Clinically, many disease manifestations have also been attributed to *Bartonella* spp. infections in cats.[3] However, it is very difficult to prove disease associations in cats in the field because of the high prevalence rates in non-clinical carriers. In research cats that are infected by exposure to *C. felis*, fever, endocarditis, and myocarditis are the most common disease manifestations. As discussed for dogs, additional case controlled studies are needed in cats.

Diagnosis

Thrombocytopenia, anemia, which frequently can be immune-mediated, and neutropenia or neutrophilic leukocytosis are the hematological abnormalities in dogs that are seroreactive or BAPGM enrichment blood culture/PCR

positive.[12,15] Thrombocytopenia is found in approximately half, eosinophilia approximately one third of infected dogs and monocytosis frequently occurs in Bartonella endocarditis. Hematological abnormalities have been rarely reported in cats, but similar to dogs, a subset of Bartonella-infected cats are neutropenic. Serum biochemical abnormalities are usually very mild or nonexistent in both cats and dogs. In cats, *Bartonella* spp. antibodies have correlated with polyclonal hyperglobulinemia and hypoglycemia.[16]

As *B. henselae, B. koehlerae* and *B. vinsonii (berkhoffii)* antibodies are infrequently detected (<4%) in sick dogs in North America, detection of *Bartonella* spp. antibodies in a sick dog provides diagnostic support for prior exposure and potentially active infection. For this reason, treatment of seroreactive dogs or dogs from which any *Bartonella* spp. DNA is detected in blood or tissue samples would be recommended.

Isolation and Molecular Detection of *Bartonella* species

Because conventional microbiological isolation 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 enrichment culture 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, horses and human beings.[5,6,12] 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 clinicians ability to initiate appropriate treatment. Specifically, the use of this optimized microbiological approach has facilitated the recognition of blood-borne *Bartonella* spp. infections in dogs, horses, human beings and porpoises.[17] Diagnostic testing (animals and humans) for *Bartonella* species (serology, PCR and BAPGM Enrichment Blood Culture/PCR) is available through Galaxy Diagnostics, Inc. (contact@galaxydx.com). In cats, serology, PCR or culture combined with serology is recommended and can be procured at Galaxy Diagnostics Inc. and Colorado State University (www.dlab.colostate.edu)

Pathologic Findings

In dogs (and humans), pathologic findings associated with *Bartonella* spp. infection include endocarditis, myocarditis, granulomatous lymphadenitis, granulomatous hepatitis, osteomyelitis, bacillary angiomatosis and peliosis hepatitis.[3,9,12,13] 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 often too few in number to be detected in tissues by silver staining, unless a fulminate infection is localized to heart valves. The cardiac abnormalities noted in cats to date are similar to those described for dogs.[9] It seems likely that the spleen plays an important immunomodulatory role in controlling persistent *Bartonella* spp. bacteremia in animals and people.[13] The extent to which *Bartonella* spp. induce splenic pathology deserves additional research consideration.

Therapy

To date, an optimal protocol has not been established for the treatment of bartonella infections in cats, dogs, or people.[3,17] Regardless of the antibiotic(s) that is used for treatment, a long duration of antibiotic administration (at least 4-6 weeks) may be necessary to eliminate the infection. Due to the rapid development of resistance to macrolides (azithromycin), I no longer recommend these antibiotics as a sole or first-line antibiotic for treating *Bartonella* infections. Fluoroquinolones in combination with doxycycline are currently being used by the author to treat clinical cases of bartonellosis, while exploring antibiotic efficacy following natural or experimental infections. Doxycycline alone does not appear to eliminate *B. vinsonii (berkhoffii), B. henselae* or *B. clarridgeae* in cats, dogs or other animal species. Serum antibody titers often decrease rapidly (3-6 months) and are generally no longer detectable in dogs that recover following antimicrobial therapy. Therefore, post-treatment serology may be a useful adjunct to BAPGM/PCR to determine if therapeutic elimination of bartonella infections has been achieved. Whether there is clinical benefit to follow serologic or molecular assay results in cats has not been widely studied, but most treated cats do not become seronegative in the short term. However, bacteremia can resolve after treatment or resolve spontaneously in some cats, whereas other cats remain bacteremic despite four to six weeks of antibiotic (documented for several antibiotic regimens) administration, despite resolution of clinical abnormalities (such as lethargy, inappetance and fever).

Prevention

Although somewhat circumstantial, there is increasing evidence that *Bartonella* species can be transmitted by fleas and ticks to cats, dogs and human beings.[17] 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 to pets and their owners will be greatly reduced or eliminated.[18]

Public and Occupational Health Considerations

There is increasing evidence to support an important role for *Bartonella* species as a cause of a spectrum of disease manifestations in human patients.[4,17,19-25] Due to extensive contact with a variety of animal species, veterinary professionals appear to be at occupational risk for infection because of 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.[26] As *Bartonella* spp. have been isolated from cat, dog or human blood, cerebrospinal fluid, joint fluid, aqueous fluid, seroma fluid and from pleural, pericardial and abdominal effusions, a substantial number of diagnostic biological samples collected on a daily basis in veterinary practices could contain viable bacteria. The increasing number of defined *Bartonella* spp., in conjunction with the high level of bacteremia found in reservoir-adapted hosts, which represent the veterinary patient population, ensures that all veterinary professionals will experience frequent and repeated exposure to animals harboring these bacteria.[27,28] Therefore, personal protective equipment, frequent hand washing and avoiding cuts and needle sticks have become more important as our knowledge of this genus has improved and various modes of transmission have been defined. Physicians should be educated as to the large number of *Bartonella* spp. in nature, the extensive spectrum of animal reservoir hosts, and the diversity of confirmed and potential arthropod vectors, current limitations associated with diagnosis and treatment efficacy, and the ecological and the medical complexities induced by these highly evolved intravascular, endotheliotropic bacteria.

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