

HUMAN BARTONELLOSIS: PERSPECTIVES OF A VETERINARY INTERNIST

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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 24 named and numerous unnamed or candidatus species, based upon deposited Gen Bank sequences or preliminary reports, respectively, seventeen *Bartonella* spp. including *B. alsatica*, *B. bacilliformis*, *B. clarridgeiae*, *B. doshiae*, *B. elizabethae*, *B. grahamii*, *B. henselae* (Houston 1 and San Antonio 2 strains), *B. koehlerae*, *B. melophagi*, *B. quintana*, *B. rochalimaea*, *B. tamiae*, *B. vinsonii* subsp. *berkhoffii* (Genotypes I, II and III), and *B. washoensis* have been associated with an expanding spectrum of human diseases.

Epidemiological evidence and experimental flea transmission studies support an important role for fleas in the transmission of *B. henselae*, *B. clarridgeae* and most likely *B. koehlerae* among cats.¹ Three other *Bartonella* species, *B. bovis*, *B. quintana* and *B. vinsonii* subsp. *berkhoffii* have been isolated from cat blood, but the modes of transmission and the reservoir potential of these species in felids has not been definitively established. Recently, we isolated *Bartonella vinsonii* subsp. *berkhoffii* from a cat with recurrent osteomyelitis spanning an eighteen month time period.² Thus, cats can maintain a chronic bacteremia with at least six *Bartonella* spp., of which five are known zoonotic pathogens.¹⁻³ In addition to fleas, an increasing number of arthropod vectors, including biting flies, keds, lice, sandflies and ticks have been implicated in the transmission of *Bartonella* species. Although there is clinical and epidemiological evidence to support tick transmission of *B. vinsonii* subspecies *berkhoffii* to dogs and coyotes, the mode of transmission of this *Bartonella* subsp. to cats and dogs has not been determined. Recent evidence supports tick transmission of *B. henselae* by *Ixodes scapularis* and *Ixodes ricinus*. Considering the diversity of *Bartonella* species and subspecies, the large number of reservoir hosts and the spectrum of arthropod vectors, the clinical and diagnostic challenges posed by *Bartonella* transmission in nature may be much more complex than is currently appreciated in human and veterinary medicine.

In the natural reservoir host, such as a cat or rodent, 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, such as a dog, horse or human patient, 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 It is important to recognize that strains of a *Bartonella* sp. vary in their virulence. Therefore, highly pathogenic strains of *B. henselae*, for which the cat is the primary reservoir, can induce granulomatous myocarditis in cats, presumably following flea transmission. Until recently, mechanisms that facilitate persistent *Bartonella* bacteremia in mammals were not well understood. Recent reports have identified an intra-endothelial, as well as intra-erythrocytic localization for these bacteria, which represents a unique strategy for bacterial persistence. Non-hemolytic intracellular colonization of erythrocytes in conjunction with the ability to invade and

replicate within endothelial cells would preserve the organisms for efficient vector transmission, protect *Bartonella* from the host immune response, and potentially contribute to decreased antimicrobial efficacy. Although the clinical implications are not understood, other *in vitro* studies indicate that *Bartonella* spp. can infect dendritic cells, microglial cells, monocytes and CD34+ bone marrow progenitor cells.

CAT SCRATCH DISEASE

For over a century regional lymphadenopathy has been associated with animal contact, particularly cat scratches. Over the years, numerous microorganisms were implicated as the cause of CSD. In 1992, Regnery and colleagues at the Centers for Disease Control, identified seroreactivity to *B. henselae* antigens in 88% of 41 human patients with suspected CSD compared to 3% of controls.² Subsequently, additional support for *B. henselae* as the predominant cause of CSD was provided when *Bartonella* DNA was amplified from lymph node samples of 21 of 25 (84%) patients with suspected CSD, using a polymerase chain reaction assay. A similar study from Sweden identified *B. henselae* DNA, but failed to identify *A. felis* DNA, in a large number of patients with suspected CSD. Prior to the recognition of *B. henselae* as the cause of CSD, *Afipia felis*, named for the Armed Forces Institute of Pathology, was considered the sole cause of CSD. Subsequently, we blood cultured *B. henselae* or *B. clarridgeae* from 17 of 19 cats owned by 14 patients with CSD, which indicated that bacteremia is a frequent occurrence in cats that transmit *B. henselae* to a human being.¹⁻²

Historically, atypical manifestations of CSD have included tonsillitis, encephalitis, cerebral arteritis, transverse myelitis, granulomatous hepatitis and/or splenitis, osteolysis, pneumonia, pleural effusion, and thrombocytopenic purpura. With the advent of specific diagnostic techniques, (culture, serology, and PCR), there has been a dramatic increase in reports describing human patients with “atypical” manifestations of CSD. Osteomyelitis, granulomatous hepatitis and granulomatous splenitis have been increasingly recognized in children infected with *B. henselae*, who frequently lack the classical lymphadenopathy of CSD. Previously, *Bartonella* infection would not have been considered a likely differential diagnosis by the physician in patients lacking a history of lymphadenopathy or animal contact. As evidenced by reports in the past four years, the spectrum of human disease associated with the genus *Bartonella* continues to expand, requiring periodic reassessment as new information becomes available. On a comparative medical (“One Health”) basis, our research group has documented many of the same CSD atypical manifestations in cats or dogs, including encephalitis, transverse myelitis, granulomatous hepatitis, osteolysis, pleural effusion, and thrombocytopenic purpura. In this context, a highly prevalent, naturally-occurring human disease (CSD) can be used as a “model” to determine the potential behavior of these bacteria in companion animal patients.

Because cat scratch disease generally denotes a self-limiting illness characterized by fever and lymphadenopathy and because the recognized spectrum of human disease manifestations associated with *Bartonella* infections (which may not include fever or lymphadenopathy) has expanded considerably in recent years, it is becoming obvious that the designation CSD lacks clinical, microbiologic and zoonotic utility. Although cats are a major reservoir for *B. henselae*, *B. clarridgeiae*, and *B. koehlerae*, some patients deny the possibility of a cat scratch or bite wound, or indicate no contact with cats. Transmission from environmental sources, various arthropod vectors, perinatally or by other animal hosts is probable and the more inclusive term bartonellosis may facilitate enhanced future understanding of diseases caused by members of the genus *Bartonella*. As physicians have been taught that CSD is self-limiting, there is an ongoing lack of appreciation that *B. henselae* can cause chronic, asymptomatic or intermittently symptomatic illness, accompanied by persistent bacteremia in people. In this context, the documentation of chronic, relapsing bacteremia in cats, dogs and other animal species provides a “model” for better understanding human bartonellosis.

BARTONELLA ENDOCARDITIS

Endocarditis can be induced by a spectrum of *Bartonella* species in dogs and human patients and is the best example of documented disease causation for this genus. Historically, *Bartonella*

species have been a cause of culture-negative endocarditis in people and dogs because the diagnostic methods used by microbiology laboratories were not adequate to isolate these bacteria. Now, by using specialized techniques, a spectrum of *Bartonella* species have been identified in research and diagnostic laboratories in different parts of the world—in heart valves or in blood cultures from dogs and people with endocarditis.³ It is important for physicians and veterinarians to recognize that some of these *Bartonella* species are found in the blood of cats, dogs, rats, ground squirrels, and rabbits.

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 PCR has greatly facilitated the molecular detection or isolation of *Bartonella* species from the blood of sick or healthy animals, including cats, dogs, horses and human beings. Most importantly, the use of this enrichment growth medium prior to PCR testing has allowed our research group to confirm that immunocompetent human patients, in particular veterinarians and veterinary technicians, can have chronic intravascular infections with *Bartonella* spp.^{4,5} Information relative to this EnrichmentPCR™ testing platform for animal and human patients is available at www.galaxydx.com.

It is increasingly clear that no single diagnostic strategy will confirm infection with a *Bartonella* sp. in the immunocompetent patient population. As described in studies from our NCSU laboratory, *B. henselae*, *B. koehlerae* and *B. vinsonii* subsp *berkhoffii* seroreactivity was found in only 58.6% of the patients in which *Bartonella* spp. infection was confirmed by EnrichmentPCR™ and sequencing. Therefore, Bartonella serology lacks sensitivity and can only be used to implicate prior exposure to a *Bartonella* sp. Even when serum from cat scratch disease patients, which is caused by *B. henselae*, is used in various diagnostic laboratories for IFA testing, test sensitivities have ranged from 14 to 100%.

EVOLVING IMPLICATIONS OF CHRONIC *BARTONELLA* SPP. BACTEREMIA IN IMMUNOCOMPETENT PEOPLE

Previously, we described *B. quintana* bacteremia in a woman who was tested following the development of an infected cat bite lesion involving the hand.⁶ Two months later, the feral cat that had induced the bite wound was captured and was also shown to be *B. quintana* bacteremic. In a cumulative study involving 392 patients with occupational animal contact or extensive arthropod exposure 31.9% were bacteremic with one or more *Bartonella* spp., when blood, serum and BAPGM enrichment culture PCR results were combined. Although this high prevalence of bacteremia is biased by testing at risk, sick individuals, it clearly demonstrates that intravascular infection with *Bartonella* sp. is much more common in immunocompetent patients, than was previously suspected. By IFA testing, only 75 out of 128 (58.6%) PCR positive patients were seroreactive to a panel consisting of five *Bartonella* sp. test antigens.

In a recent study, *Bartonella vinsonii* subsp. *berkhoffii*, *Bartonella henselae* or DNA of both organisms were amplified and sequenced from blood, BAPGM enrichment blood cultures or autopsy tissues from four family members.⁷ Historical and microbiological results derived from this family support human perinatal transmission of *Bartonella* species. To date, there have been a limited number of studies that address the potential impact of intravascular infection with a *Bartonella* sp. on reproductive performance, however, studies involving experimentally-infected cats, rodents and naturally-infected cows with various *Bartonella* sp. have identified decreased reproductive performance involving both males and females. The parents of these children had attempted to conceive children for several years prior to resorting to in vitro fertilization.

We have also described a veterinarian, who experienced a needle stick while obtaining a fine needle aspiration sample from a cutaneous histiocytic neoplasm.⁸ Subsequently symptoms, including

headaches, fatigue and intermittent paresthesias developed. This patient seroconverted to *B. vinsonii* subsp. *berkhoffii* genotypes I and III and *B. vinsonii* subsp. *berkhoffii* genotype I DNA was amplified and sequenced from sequentially obtained blood samples, whereas genotype III DNA was amplified from the cytological specimen. All symptoms resolved following antibiotic treatment.

It is increasingly evident that dogs can serve as a source for human infection with *B. vinsonii* subsp. *berkhoffii*. *Bartonella vinsonii* subsp. *berkhoffii* genotype II was amplified and sequenced from a liver biopsy from a patient with epithelioid hemangioendothelioma, after which the organism was isolated by BAPGM blood culture.⁹ The unique capability of *Bartonella* to invade and induce long lasting intraerythrocytic and intraendothelial infections, in conjunction with the ability of at least three *Bartonella* spp. (*Bh*, *Bq*, and *B. bacilliformis*) to induce VEGF-mediated vasoproliferative disease in immunocompromised or immunocompetent individuals suggests that these novel emerging bacterial pathogens might contribute to the development of vascular tumors.

Bartonella koehlerae bacteremia was documented in eight immunocompetent patients by PCR amplification and DNA sequencing, either prior to or after BAPGM enrichment blood culture.¹⁰ Presenting symptoms most often included fatigue, insomnia, joint pain, headache, memory loss, and muscle pain. Four patients were also infected with *Bartonella vinsonii* subsp. *berkhoffii* genotype II. *Bartonella koehlerae* antibodies were not detected (titers < 1:16) in 30 healthy human control sera, whereas five of eight patient samples had *B. koehlerae* antibody titers of 1:64 or greater. Studies are needed to determine if *B. koehlerae* is a cause or cofactor in the development of arthritis, peripheral neuropathies or tachyarrhythmias in human patients. Co-infection with *B. henselae* and two hemotropic *Mycoplasma* variants resembling *Mycoplasma ovis* were also found in the blood of a veterinarian with a historical diagnosis of multiple sclerosis.¹¹

PUBLIC AND OCCUPATIONAL HEALTH CONSIDERATIONS

Due to extensive contact with a spectrum of animal species, veterinary professionals appear to have an occupational risk of 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. 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. 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, 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.

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