FELINE BARTONELLOSIS AND CAT SCRATCH DISEASE

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The genus *Bartonella* is currently comprised of at least 20 species and subspecies of vector-transmitted, fastidious, gram-negative bacteria that are highly adapted to one or more mammalian reservoir hosts. On an evolutionary basis, *Bartonella henselae* and *Bartonella clarridgeae* have evolved to cause persistent intravascular infection in domestic cats and wild felid species. In contrast, *Bartonella vinsonii* subspecies *berkhoffii* has evolved to cause persistent intravascular infection in dogs and wild canines, including coyotes and foxes, but was recently isolated from a cat. Other *Bartonella* species have evolved to cause persistent blood borne infection in rodents, small mammals or ruminants, for example *Bartonella bovis*. 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. Two other *Bartonella* species, *B. bovis* and *B. quintana* 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. 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* species to cats and dogs has not been determined. As reviewed in several publications, numerous domestic and wild animals, including bovine, canine, feline, human, and rodent species can serve as chronically infected reservoir hosts for various *Bartonella* species. In addition to the large number of documented reservoir hosts, an increasing number of arthropod vectors, including biting flies, fleas, keds, lice, sandflys and ticks have been implicated in the transmission of *Bartonella* species. 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.

Once an animal is infected by a bite, a scratch or arthropod, *Bartonella* species localize to erythrocytes and endothelial cells, which provides a potentially unique strategy for bacterial persistence within the blood stream of reservoir or non-reservoir species. *In vitro* infection of human CD34+ progenitor cells with *B. henselae* suggests that these bacteria are capable of infecting bone marrow progenitor cells which may contribute to ongoing erythrocytic infection. Infection of bone marrow progenitor cells followed by non-hemolytic intracellular colonization of erythrocytes would preserve *Bartonella* organisms for efficient vector transmission, protect *Bartonella* from the host immune response, facilitate widespread vascular dispersion throughout the tissues of the body, and potentially contribute to decreased antimicrobial efficacy.

Feline Bartonellosis

The extent to which members of the genus *Bartonella* are pathogenic for cats remains to be determined. *Bartonella henselae* bacteremia can be documented in 25 to 41% of healthy cats in different regions throughout the world. Self-limiting febrile illness of 48 to 72 hours duration, mild to moderate transient anemia, and transient neurologic dysfunction was

reported in cats experimentally infected with *B. henselae* by blood transfusion. Self-limiting fever can also occur in *B. henselae* bacteremic cats following minor surgical procedures. Although unproven, it is likely that stress, such as surgery or trauma, can induce transient disease manifestations in cats, including self-limiting fever, mild anemia and neurological dysfunction. Due to the high percentage of chronically bacteremic healthy cats in the United States, establishing a cause and effect relationship between disease manifestations and bacteremia in cats has required large epidemiological studies in *Bartonella* endemic and non-edemic regions. Seroepidemiologic studies have generated contrasting results, as to whether fever, lymphadenopathy, stomatitis and gingivitis are caused by *B. henselae*. *Bartonella henselae* DNA and intrathecal antibody production has been demonstrated in cats with neurological disease. Immunosupression associated with FeLV or FIV appears to increase the pathogenicity of *B. henselae* infection in cats. In experimentally infected cats, fever, lymphadenopathy, mild neurological signs and reproductive disorders have been reported. In experimentally-infected cats, gross necropsy results are unremarkable; however, histopathological lesions can include peripheral lymph node hyperplasia, splenic follicular hyperplasia, lymphocytic cholangitis/pericholangitis, lymphocytic hepatitis, lymphoplasmacytic myocarditis, and interstitial lymphocytic nephritis. These findings would indicate that antibiotic treatment should be considered in seroreactive or bacteremic cats with these disease manifestations.

The diagnosis of *Bartonella* infection should be confirmed by culturing the organism from blood or tissues such as lymph node or heart valve (endocarditis) or by amplifying *Bartonella*-specific DNA sequences from blood or tissues using PCR. *Bartonella henselae*, *B. quintana* and *B. bacilliformis* can be visualized within erythrocytes using confocal or electron microscopy. Cell lysis, using a commercially available lysis centrifugation technique or by freezing the blood sample prior to plating, facilitates bacterial isolation of *B. henselae* from blood, but is most likely less sensitive for the isolation of other *Bartonella* spp. Although organisms within the genus *Bartonella* are fastidious and slow-growing, *B. henselae* and *B. clarridgeae* can be cultured successfully with agar plates containing 5% defibrinated rabbit or sheep blood, that are maintained at 35^{o}C in a high humidity chamber with a 5% CO_2 concentration. In our experience, bacterial colonies may not be visible until 10 to 56 days after inoculation of the agar plate. As cats maintain a higher level of bacteremia, culturing *B. henselae* or *B. clarridgeiae* from aseptically obtained blood samples is much more likely to be successful than culturing *B. henselae* from a dog or human blood sample. The recent introduction of a liquid growth medium (*Bartonella* alpha *Proteobacteria* growth medium) has facilitated the successful isolation of *B. henselae* from dog and human blood samples. Also the use of BAPGM allowed us to isolate *B. vinsonii* subsp. *berkhoffii* from the cat with osteomyelitis. The BAPGM diagnostic platform is now available from Galaxy Diagnostics (www.galaxydx.com).

Because members of the order *Rickettsiales* are cultivable only in host cells or viable tissue culture cells, cultivation of *Bartonella* species on bacteriologic media, in conjunction with DNA divergence studies, provided the justification for removal of members of the genus *Bartonella* from the order *Rickettsiales.* In tissues, *Bartonella* are small, curved, gram-negative rods that stain positively with silver stains such as the Warthin-Starry stain, although this approach is diagnostically insensitive in most instances. PCR assays targeting several *Bartonella*-specific gene sequences have been described and should be used to confirm infection with a *Bartonella* species infection in tissues or blood.

Seroconversion, detected by IFA or ELISA techniques, can be documented in people with acute disease and in cats following experimental infection. The kinetics of the serologic

response to *B. henselae* antigens in chronically-infected experimental cats is highly variable in degree and duration. A seroepidemiologic survey, incorporating 577 samples from throughout North America identified an overall prevalence of 28%, with prevalence rates ranging from a low of 4–7% in the Midwest and Great Plains region to 60% in the southeast. High seroprevalence rates correlate with warm, humid climates conducive for the environmental maintenance of cat fleas, which have been shown to be capable of transmitting *B. henselae* from cat to cat. A seroepidemiologic study of 592 cats in Baltimore, MD, identified an overall seroprevalence rate of 14.7%, with a much higher prevalence in feral cats (44.4%) as compared to pet cats donated to the City Municipal Animal Shelter (12.2%). Although providing useful epidemiologic data, serologic test results may be of limited clinical utility for several reasons. We have been unable to detect *B. henselae*-specific antibodies in some bacteremic cats, whereas in other cats we have been unable to culture *Bartonella* when antibodies are detectable. In recent prospective clinical studies, the detection of antibodies was not predictive for the detection of DNA, which is a strong indication of active infection. Negative blood cultures obtained from cats seroreactive to *B. henselae* antigens may reflect low level bacteremia or the timing of the blood culture, since experimentally-infected cats experience a relapsing pattern of bacteremia. Numerous naturally-infected cats are bacteremic for months to years, generally in conjunction with low antibody titers. In our experience, high antibody titers generally correlate with positive blood cultures or detection of *Bartonella* DNA in the blood using the BAPGM enrichment platform. The extent of serologic cross reactivity to *Bartonella* species requires additional clarification. We and others have demonstrated co-infection with *B. henselae*, *B. clarridgeiae*, *B. bovis* or *B. quintana* in cats.

Because of disparate results among studies and an overall lack of microbiologic data in clinical therapeutic trails, numerous issues related to treatment of *Bartonella* infection remain controversial. In contrast to the apparent lack of response to antimicrobial treatment in human CSD patients, bacillary angiomatosis, parenchymal bacillary peliosis, and acute *Bartonella* bacteremia appears to respond to antimicrobial treatment, even in immunocompromised (predominantly HIV-infected individuals). Doxycycline, erythromycin and rifampin are recommended antibiotics, but clinical improvement has been reported following the use of penicillin, gentamicin, ceftriaxone, ciprofloxacin, and azithromycin. Treatment for 2 weeks in immunocompetent individuals and 6 weeks in immunocompromised people is generally recommended. Relapses, associated with bacteremia, have been reported in immunocompromised people despite treatment for 6 weeks. Antimicrobial efficacy has not been established for any antibiotic for eliminating *B. henselae* bacteremia in cats. Results from our laboratory and others indicate incomplete treatment responses in cats treated for 2 or 4 weeks with doxycycline or enrofloxacin. Cats experimentally-infected with *B. henselae* do not develop protective immunity following heterologous challenge with *B. clarridgeiae* or other *B. henselae* strains. Differences in results derived from cats infected with *B. henselae* by blood transfusion from an infected cat or by flea transmission are most likely due to alterations in virulence, induced during *in vitro* culture and subsequent inoculation of cultured organisms.

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 1983, small, argyrophilic (easily impregnated with silver), gram-negative, pleomorphic bacteria were seen within blood vessel walls and macrophages in lymph nodes of patients with CSD. In 1988, a bacteria, later designated *Afipia felis*, was cultured from lymph nodes of CSD patients. In the same year, Cockerell and colleagues

proposed a possible association between epithelioid angiomatosis and CSD in a letter to *Lancet*. In 1992, Regnery and colleagues at the Centers for Disease Control, identified seroreactivity to *B. henselae* antigens in 88% of 41 patients with suspected CSD compared to 3% of controls. Similarly, a case-controlled Connecticut study of CSD patients and their cats identified a strong association with cats 12 months of age or younger, a history of a scratch or bite, contact with fleas, and seroreactivity to *B. henselae* antigen. Additional support that *B. henselae* is 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. Subsequently, we cultured *Bartonella* species from 17 of 19 cats owned by 14 patients with CSD, which indicates that bacteremia is a frequent occurrence in cats that transmit *B. henselae* to a human being. Studies to date indicate that *B. henselae* is the predominant, but not the sole cause of CSD. In 1995, Clarridge et al. isolated a novel *Bartonella* species, which was named *B. clarridgeiae,* from a cat belonging to a patient infected with HIV from whom *B. henselae* was isolated. Our studies have implicated *B. clarridgeiae* as a cause of inoculation papules, fever and regional lymphadenopathy (CSD) in 3 people, however to date, *B. clarridgeiae* DNA has not been detected in lymph nodes of CSD patients and the organism has not been isolated from blood or lymph nodes of these patients.

Historically, atypical manifestations of CSD have included tonsillitis, encephalitis, cerebral arthritis, 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 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.

Based upon recent advances in our knowledge of the zoonotic potential of members of the genus *Bartonella*, the designations cat scratch disease and cat scratch fever may be most appropriate when considering human disease manifestations from a historical perspective. 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* and potentially *B. clarridgeiae*, some patients deny the possibility of a cat scratch or bite wound, or indicate no contact with cats. Transmission from environmental sources, arthropod vectors or 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*.

Although recent research findings have substantially improved our understanding of the clinical, microbiologic and zoonotic aspects of diseases caused by *Bartonella* species, the exact mode of transmission, the relative role of various insect vectors such as fleas and

ticks, the identification of potential reservoir hosts, and the spectrum of animal and human illnesses caused by these organisms remains largely undetermined. For example, although it is well established that the human body louse transmits *B. quintana*, the reservoir and mode of transmission that results in bacillary angiomatosis in the United States has not been established. The pathogenic potential of these organisms appears to be of considerable importance in dogs, as well as immunocompromised and immunocompetent people.