VECTOR BORNE INFECTIOUS DISEASES IN NORTH AMERICA: CLINICAL AND ZOONOTIC IMPLICATIONS

Edward B. Breitschwerdt, DVM, Diplomate, ACVIM
Comparative Medicine Institute
Department of Clinical Sciences, College of Veterinary Medicine
Comparative Medicine Institute

College of Veterinary Medicine, North Carolina State University, Raleigh, NC

In North America, fleas, mosquitoes and ticks are considered the most important vectors for a spectrum of infectious agents that can induce disease in dogs. In regard to canine vector-borne diseases, the vector competence of fleas for the transmission of several potential vector-borne pathogens has not been extensively studied. Fleas are known to carry and potentially transmit *Bartonella henselae, Bartonella clarridgeae, Bartonella quintana, Mycoplasma hemofelis, Rickettsia felis, Wolbachia* species and *Dipylidium caninum*.[1] Recently, ticks have been shown to be vector competent for transmission of a *Bartonella* species in the laboratory and *B. henselae* DNA has been amplified and sequence from *Ixodes* sp. ticks from around the world. Fleas act as an intermediate host for the tapeworm *D. caninum,* and can be a source of infection for cats, dogs and human beings, following ingestion of the flea. *Bartonella henselae* is also a zoonotic organism that can be transmitted to cats (and perhaps to dogs) by ingestion of flea feces containing viable *B. henselae* organisms. Surprisingly, *B. henselae* can remain viable in flea feces for periods up to at least 15 days.[2] *Bartonella henselae* causes cat scratch disease, endocarditis, regional or generalized granulomatous lymphadenitis and numerous other disease manifestations in people.[3,4] Although cat bite or scratch transmission of *B. henselae* to dogs has not been proven, we have isolated the organism from young dogs with polyarthritis and a history of cat scratches. If true, this would suggest that flea infested cats are a risk factor for transmission of this organism to dogs, as well as humans. In dogs, both *B. henselae* and *B. clarridgeae* have been shown to cause endocarditis; therefore, both of these flea-transmitted organisms can be pathogenic in dogs.[3] Recently, our research group has documented chronic intravascular infection with *Bartonella* spp. in people (veterinarians, veterinary technicians and wild life biologists) with extensive arthropod exposure and animal contact.[5] Ultimately, it may be proven that flea infestations in dogs, as has been shown for cats, leads to a persistent relapsing *B. henselae* bacteremia, with transmission to people via bite (salivary inoculums) or scratch (flea feces inoculums).[6]

In recent years, there has been a rapid expansion of clinically relevant information relative to canine, feline and human hemoplasma infections. Hemotropic mycoplasmas (previously known as *Haemobartonella* or *Eperythorozoon* species) can cause a hemolytic anemia in cats, dogs and other animal species.[7] As *Mycoplasma* species are cell-wall deficient bacteria, these organisms are difficult or impossible to culture from patient samples. With the advent of PCR assays that allow for the diagnostic targeting of organism-specific gene sequences in patient samples, infection with hemotropic *Mycoplasma* species is now known to be a prevalent finding in healthy cats and dogs, as well as those with anemia. Similar to *B. henselae*, these bacteria can most likely be transmitted by fleas, induce chronic intravascular infections and potentially participate as the primary or cofactor in disease expression (including anemia). Unique *Mycoplasma* spp., including *M. hemocanis* and 'Candidatus *Mycoplasma hemoatoparvum*' infect dogs, and occasionally a feline-adapted *Mycoplasma* species can be found in the blood of a dog.[8] Human infections with *Mycoplasma hemofelis,* Candidatus *Mycoplasma hemoatoparvum*' and *Mycoplasma ovis* have been reported in human patients in North or South America. As with other vector-borne organisms, hemotropic *Mycoplasma* species have evolved to induce persistent intravascular infection, without inducing disease. Therefore, co-infection, sequential infection or concurrent development of a non-infectious disease is necessary for a given *Mycoplasma* species to induce a hemolytic crisis. Also similar to *B. henselae,* there is increasing experimental evidence to support direct salivary transmission of *Mycoplasma* species among cats. Similar to FeLV, aggressive interactions among male cats may be an important means of transmission. Transmission via saliva or vectors is poorly characterized in dogs. As has long been established mosquitoes transmit *Dirofilaria immitus*, the

cause of heartworm disease, to dogs. Recently, a *Wolbachia* endosymbiont has been identified within both adult and microfilaria of *D. immitus*.[9] Evolving evidence suggests that these organisms may play a role in the pathogenesis of the vascular injury, which has been historically attributed solely to the adult worms or microfilariae. Recent studies have shown that both dirofilarial and *Wolbachia* antigens, induce interactions within the host that result in the development of the pathology and in the regulation of the host's immune response during canine, feline and human heartworm infections. The above observations and others support comprehensive evaluation of the type, frequency and extent of vector exposure. Obtaining a comprehensive vector exposure history is an important component of the annual examination process for healthy or sick dogs.

The expanding number of known tick-borne organisms, the broad geographic distribution of many tick species, the ability of tick-borne organisms to induce chronic intravascular infections, and the highly pathogenic potential of some tick-borne organisms makes tick-borne infections the most important subset of canine vector-borne infectious diseases in North America. The remainder of this lecture will highlight new information related to various tick-borne pathogens. During the past decade, tick-transmitted infectious diseases have become increasingly important in the United States and throughout much of the world. Several factors, including the ongoing Lyme Disease epidemic, suburbanization of tick habitat, the rapid increase in deer numbers as well as other wildlife populations that reside within the peri-domestic environment, the recognition that same species and strains of tick-borne pathogens can induce disease in pets and their owners, and the widespread availability of safe and effective acaracides have all contributed to enhance the awareness of tick-borne infections among both professionals and non-professionals. In conjunction with the above factors, there has also been a concurrent discovery of new tick-borne organisms, for which clinical, epidemiological and pathological data is minimal or lacking, particularly in regard to disease causation in animals. Examples include *Borrelia lonestari, Borrelia turicatae, Rickettsia felis, Rickettsia amblyommi.*

For nearly two decades, our research group has contributed to the development of diagnostic, therapeutic and preventive strategies for the management of infections caused by tick-transmitted intracellular organisms. As a result of these research efforts, and those of many other investigators throughout the world, we continue to gain an increasingly unique perspective on the clinical and immunopathological consequences of tick-borne infectious diseases. It has been stated that: “Ticks are only interested in nutrition (a blood meal) and sex (i.e. perpetuation of the species).” Although the tick might object to this simplistic view of its complex lifestyle, bacteria, protozoa and viruses have used the predictable behavior of all tick species to facilitate their transmission and therefore the perpetuation of their species. Transmission of a tick-borne organism is most frequently accomplished when the tick obtains a blood meal; however, transmission can occur when the tick is inadvertently ingested by a dog (*Hepatozoon canis* or *Hepatozoon americanum*).[10] In those instances, when tick-transmission is the sole means by which an organism such as *Ehrlichia canis* is transmitted from one infected dog to a previously non-infected dog, and when the dog is the only known reservoir host for *E.canis*, it becomes obvious that *E. canis* would evolve to be efficiently transmitted by a tick (*R. sanguineus*) for which all three tick life cycle stages (larvae, nymph and adult) preferentially involve feeding on dogs.[11] It is equally obvious that *E.canis* would seek to induce long-lasting infection, accompanied by minimal pathogenicity to the dog (do not destroy the home you live in) and the organism would infect a cell (the monocyte) that would facilitate transfer of *E. canis* to additional blood seeking ticks. This evolutionary arrangement benefits *E. canis*, but does not appear to benefit the dog, which can develop disease manifestations ranging from epistaxis to pancytopenia. Although it is difficult to determine the factor(s) that induce disease causation when a dog is infected with a highly adapted vector-borne organism such as *E. canis*, it is certain that sequential or simultaneous infection with another vector-borne organism can contribute to more severe hematological or immunological aberrations and a more severe course of illness. Unpublished data from our laboratory indicates that many dogs appear to immunologically eliminate *E. canis* following tick-transmission, as opposed to developing chronic infection.[12] Tick-borne organisms such as *Anaplasma phagocytophilum* and *R. rickettsii* typically induce acute, severe illness, whereas other organisms such as *Babesia canis, Babesia gibsoni Bartonella vinsonii* subsp. *berkhoffii* and *E. canis* can induce chronic, insidious illness. As described briefly above, specific tick species preferentially transmit different pathogenic organisms, dogs can be sequentially or simultaneously infested with more than one tick species, and a single tick can transmit more than one organism leading to co-infection. Both the tick species and the organisms that they transmit can vary substantially within and between various geographic regions. For example, infection with *R. rickettsii*, transmitted by *Dermacentor variablis* in the state of North Carolina, occurs

much more frequently in the piedmont region (central part of the state) as compared to the eastern costal plain or the western Appalachian mountain range. All of the above factors make the diagnosis and medical management of tick-borne infectious diseases a complex and challenging task for the practicing veterinarian. Without question, the old adage "An ounce of prevention is worth a pound of cure" is applicable to any discussion of tick-borne infectious diseases. The advent of new, safe and long-lasting acaracides that can repel and kill ticks makes the prevention of tick-borne diseases an important priority for veterinarians and pet owners throughout the world. Based upon experimental infection studies, using tick attachment models, application of acaracide products can decrease the risk of transmission of *Borrelia burgdorferi*, the cause of canine Lyme borreliosis.[13,14] There is the need for additional studies to define the extent to which commercially available acaracides can prevent infection with various tick-borne organisms in North America.

SPOTTED FEVER GROUP RICKETTSIAE AND ROCKY MOUNTAIN SPOTTED FEVER

Spotted fever group (SFG) rickettsiae have been described from all continents.[14] In North America, *Rickettsia rickettsii* is the most important SFG rickettsiae because this tick-transmitted organism can cause serious or fatal illness in dogs and people.[15] The SFG group includes numerous closely related species including *R. rickettsii* (the type species), *R. africae*, *R. akari, R. australis, R. conorii, R. felis, R. montana, R. parkeri, R. rhipicephali* and *R. sibirica*, although many other SFG rickettsiae have been described. The typhus group rickettsiae, which includes *Rickettsia typhi* and *Rickettsia prowazekii,* have not been implicated as a cause of illness in dogs and experimental infection of dogs with typhus group rickettsiae in our laboratory did not result in disease. Throughout various regions of the world, spotted fever group rickettsiae are transmitted by *Amblyomma, Dermacentor*, *Haemaphysalis*, *Ixodes* and *Rhipicephalus* tick species. Regardless of the strain or species of SFG rickettsiae, these organisms generally induce an acute febrile illness secondary to endothelial cell damage, which results in vasculitis, altered vascular permeability, edema and necrosis.[16] To date, chronic infection with a SFG rickettsiae has not been confirmed in dogs or human beings. Although it seems likely that other SFG rickettsiae could induce disease in dogs, only *R. rickettsii* in North America and *R. conorii* in southern Europe have been documented as canine pathogens.[16,17] Historically in North America, only *Dermacentor variabilis* (south and eastern United States), and *Dermacentor andersonii* (north western US and Canada) were known to transmit *R. rickettsii* to dogs or human beings. There is now strong epidemiological evidence that a recent outbreak of RMSF in a nonendemic area of Arizona was caused by *Rhiphicephalus sanguineus* (The Brown Dog Tick).[18] This tick species prefers to spend all three life cycle stages (larvae, nymph and adult) on a dog. When the environment is infested with large numbers of brown dog ticks or if dogs are removed from tick infested house, human blood becomes an acceptable, if not an attractive alternative. In this setting, both *R. rickettsii* and *Ehrlichia canis* can be transmitted to human beings. In the context of morbidity, mortality and severity of disease, RMSF is the most important tick-borne infection of dogs in the United States. Due to variation in the severity and location of vascular lesions among different patients, veterinarians should anticipate a spectrum of disease manifestations following naturally-occurring infection with *R. rickettsii.* Much of the United States is considered endemic for the ticks (*Dermacentor variabilis*, *Dermacentor andersoni, Rhipicephalus sanguineus*) that transmit *R. rickettsii. Rickettsia rickettsii* infection ("Rocky Mountain spotted fever") also occurs in areas of Central and South America, where several outbreaks of fatal human illness has been reported. Clinical abnormalities associated with RMSF include fever, anorexia, depression, mucopurulent ocular discharge, scleral injection, tachypnea, coughing, vomiting, diarrhea, muscle pain, neutrophilic polyarthritis, and a diverse group of neurologic signs including hyperesthesia, ataxia, vestibular signs, stupor, seizures, and coma. In some dogs weight loss is very severe, considering the short duration of illness. Poorly localizing joint, muscle and/or neurologic pain suggestive of polyarthritis, polymyositis, or meningitis may represent the only or most prominent clinical finding. Retinal hemorrhages are a consistent finding, but may be absent early in the course of the disease. Epistaxis, melena, hematuria, and petechial to ecchymotic hemorrhages occur in some dogs, but may not develop unless diagnosis and treatment are delayed for five or more days after the onset of clinical signs. Scrotal edema, hyperemia, hemorrhage, and epididymal pain are frequently observed in male dogs. Signs associated with cardiovascular collapse, oliguric renal failure or brain death can develop in the terminal stages of the disease. Gangrene affecting the distal extremities, scrotum, mammary glands, nose or lips is associated with severe vascular obstruction and can induce substantial tissue loss, necessitating reconstructive surgery.[6] Clinical manifestations in dogs are identical in most instances to manifestations reported in human patients.

From a public health perspective, the dog is an environmental sentinel for RMSF and therefore it is important that veterinarians recognize and accurately diagnose RMSF. Diagnostic confirmation of RMSF in a dog allows the veterinarian to discuss the risk of *R. rickettsii* transmission in the peri-domestic surroundings.

CANINE AND HUMAN EHRLICHIOSIS

At least three *Ehrlichia* spp.; *E. canis, E. chaffeensis* and *E. ewingii*, are capable of infecting dogs and people in North America.[19] For various reasons dogs play a seemingly minor role in the acquisition of human infections, perhaps with the exception of human *E. canis* infections in South America (and other regions of the world in which large peri-domestic *Rhipicephalus sanguineous* populations are found).[20] Studies performed decades ago indicated that nonhuman primates could be infected with *E. canis* and more recently, an organism genetically related (or identical) to *E. canis* was isolated from a veterinarian and subsequently detected by PCR testing in other people from Venezuela.[20,21] In the United States, both *E. chaffeensis* and *E. ewingii* can cause serious disease manifestations in people, including meningoencephalitis, acute renal failure and acute respiratory failure. In addition to *E. canis,* both *E. chaffeensis* and *E. ewingii* can induce ehrlichiosis in dogs.

Investigators from South Africa have obtained molecular evidence (16S rDNA sequencing) that supports infection of dogs and people with an organism that is identical or closely related to *E. ruminantium* (previously *Cowdria ruminantium*).[22] The implications of this recent finding could prove to be of great importance, if *E. ruminantium*, the organism that causes Heartwater in cattle in Africa, were introduced into the United States by way of dog transport. Recently, a genetically similar organism (i.e. an *E. ruminatium* strain) has been found in deer from the southeastern US, and in a human from Georgia, diagnosed with ehrlichiosis.[23] *Ambylomma americanum* ticks from a park in the metropolitan area of Atlanta, GA, were used to experimentally infect a goat, which developed an acute febrile illness and remained infected (and competent to infected naïve ticks) for at least 5 months. Although not yet confirmed, infection of goats, deer and a human being suggests that this organism is most likely pathogenic for dogs.

In recent years, there have been considerable advances in defining the efficacy of various antibiotics for treatment of canine ehrlichiosis. It is now clear that 2 weeks of doxycycline is not an effective treatment for *E. canis* infection, whereas 4 weeks of therapy (doxycycline 5mg/kg every 12 hours) eliminates *E. canis* in both naturally and experimentally infected dogs.[12] The short term prognosis following treatment for canine ehrlichiosis is generally very good. Dramatic clinical improvement usually occurs within 24 to 48 hours after initiation of doxycycline or tetracycline in dogs with acute phase or mild chronic-phase disease. Rapid clinical improvement is frequently noted in chronically infected dogs; however, periods up to a year may be necessary for complete hematological recovery. The long term prognosis following treatment is much more variable, potentially related to failure to diagnose concurrent infections. Undiagnosed infection with a *Babesia* or *Bartonella* spp. can be misinterpreted as an ineffective therapeutic response when treating ehrlichiosis, as doxycycline is generally an ineffective treatment for babesiosis and bartonellosis.[24] Experimentally, enrofloxacin will suppress the clinical manifestations of *E. canis* infection and may result in hematological improvement, but does not eliminate the infection. Although imidocarb dipropionate has gained clinical acceptance in some endemic regions for treating severe, chronic, or presumed refractory cases of ehrlichiosis, lack of efficacy has been demonstrated in treating both naturally and experimentally infected dogs.

MOLECULAR DIAGNOSTIC TESTING AND VECTOR-BORNE DISEASES

Molecular diagnostic approaches have begun to facilitate a “modern day” revolution in our understanding of the interactions of single or multiple infectious agents in our patients. In addition, PCR has enhanced the detection of polymicrobial infections which emphasizes the complexity of disease expression induced by acute or chronic infection, and has resulted in the redefinition of “previously understood” vector-borne diseases such as babesiosis and leishmaniasis. PCR amplification of organism-specific DNA sequences can be accomplished in a matter of hours and in most instances the detection of a PCR amplicon confirms active infection with a specific organism. This approach has distinct advantages over culture, which can require incubation times ranging from days, to weeks, to months depending on the organism. In most instances, approaches that provide rapid culture results select for only a few more easily grown organisms. Although serology or examination of the cell mediated immune response will remain an important component of infectious disease diagnosis, these tests identify evidence of immune recognition

of a pathogen, but do not confirm active infection. Cross reactivity among various infectious agents, failure to confirm active infection, and for an increasing number of infections (babesiosis, bartonellosis, leishmaniasis and others) the serological response to the organism may be minimal or non-existent, despite documentation of chronic, active infection by PCR are limitations to the interpretation of serological test results in a given patient.[5]

Because most vector-borne pathogens are difficult, if not impossible to culture from patient samples and because many animals achieve immunological clearance of the organism following transmission of the organism, the use of PCR to document active infection prior to or at the time of initiation of therapy or as an aid to document therapeutic elimination of the infection is gaining acceptance among veterinary clinicians. PCR testing for *Anaplasma, Babesia, Cytauxzoon* (cats) *Ehrlichia, Leishmania* and *Rickettsia* species is available through the:

Vector-borne Diseases Diagnostic Laboratory
NCSU-CVM Rm 462A
4700 Hillsborough Street
Raleigh NC 27606
Phone: 919-513-8279
www.cvm.ncsu.edu/docs/ticklab.html

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 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. Information relative to this testing platform for animal and human patients is available from:

Galaxy Diagnostics Inc.
7020 Kit Creek Road Suite 130
Durham, NC 27709
www.galaxydx.com
919-313-9672

SIMULTANEOUS INFECTION WITH MULTIPLE VECTOR-TRANSMITTED PATHOGENS

Recently, simultaneous infection with more than one tick-borne pathogen has been recognized with increasing frequency in human and canine patients.[26,27] Obviously, simultaneous infection with more than one tick-transmitted pathogen has important diagnostic, therapeutic and prognostic implications for the individual patient. The pathophysiologic consequences of co-infection in dogs with various combinations of bacteria, rickettsia and protozoa have not been characterized clinically or experimentally. Although retrospective seroepidemiologic studies suggest that dogs may experience simultaneous infection with multiple tick-borne pathogens, microbiologic (culture) or molecular (PCR) evidence of simultaneous infection in dogs is currently limited. In nature, the risk of exposure to ticks, fleas, mosquitoes and biting flies is far greater for dogs than for human beings. In addition, dogs can be infested with hundreds of ticks, and at times infestation may involve different tick species. Therefore, the unknown influences of concurrent infection with multiple tick-borne pathogens, including *Anaplasma, Ehrlichia, Rickettsia, Babesia* and *Bartonella* species, on factors such as pathophysiology, diagnosis, prognosis or therapeutic outcome could be more readily characterized in dogs. Of 27 dogs that were investigated in a kennel due to increased mortality, 25 were seroreactive to an *Ehrlichia* sp., 20 to a *Bartonella* sp., 17 to a *Babesia* sp. and 22 seroconverted to *R. rickettsii* antigen.[27] Based upon PCR analysis, several dogs were co-infected with multiple *Ehrlichia* species, as well as a *Bartonella, Babesia* or *Rickettsia* species. Prospective evaluation of sick dogs, managed in our teaching hospital, has yielded molecular evidence of co-infection with multiple tick-transmitted pathogens.[26] Our recent experience indicates that dogs with heavy tick exposure can be infected at a high rate with multiple, potentially zoonotic, tick-borne pathogens.

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,* hemotropic *Mycoplasma* spp. and potentially other tick borne pathogens, such as *Anaplasma platys*, 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.[28,29] For example, 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 flea and tick borne pathogens, 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 flea and tick borne pathogens 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 and endotheliotropic bacteria.

References:

1. Rolain, JM, Franc M, Davoust B, Raoult D, Molecular detection of *Bartonella quintana, B. koehlerae, B. henselae, B. clarridgeiae, Rickettsia felis, and Wolbachia pipientis* in cat fleas, France. Emerg Infect Dis 2003;9:338-342.
2. Finkelstein JL, Brown TP, O'Reilly KL, Wedincamp J Jr, Foil LD, Studies on the growth of *Bartonella henselae* in the cat flea (*Siphonaptera: Pulicidae*). J Med Entomol 2002;39:915-919.
3. Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB, Factors associated with the rapid emergence of zoonotic Bartonella infections. Vet Res 2005;36:383-410.
4. Chomel BB, Boulouis HJ, Maruymama S, Breitschwerdt EB, *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis 2006;12:389-394.
5. Breitschwerdt EB, Maggi RG, Duncan AW, Nicholson WL, Hegarty BC, Woods CW, *Bartonella* species in blood of immunocompetent persons with animal and arthropod contact. Emerg Infect Dis 2007;13:938-941.
6. Kordick DL, Breitschwerdt EB, Relapsing bacteremia after blood transmission of Bartonella henselae to cats. Am J Vet Res 1997;58:492-497.
7. Willi B, Boretti FS, Meli ML, et al., Real-time PCR investigation of potential vectors, reservoirs, and shedding patterns of feline hemotropic mycoplasmas. Appl Environ Microbiol 2007;3:3798-3802.
8. Sykes JE, Ball LM, Bailiff NL, Fry MM, 'Candidatus Mycoplasma haematoparvum', a novel small haemotropic mycoplasma from a dog. Int J Syst Evol Microbiol 2005;55:27-30.
9. Kozek WJ, What is new in the *Wolbachia/Dirofilaria* interaction? Vet Parasitol 2005;133:127-132.
10. Baneth G, Samish M, Shkap V, Life cycle of *Hepatozoon canis* (Apicomplexa: Adeleorina: Hepatozoidae) in the tick *Rhipicephalus sanguineus* and domestic dog (*Canis familiaris*). J Parasitol 2007;93:283-299.
11. Neer TM, Breitschwerdt EB, Greene RT, Lappin MR, Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. J Vet Intern Med 2002;16:309-315.
12. Breitschwerdt EB, Hegarty BC, Hancock SI. Sequential evaluation of dogs naturally infected with *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia equi*, *Ehrlichia ewingii*, or *Bartonella vinsonii*. J Clin Microbiol 1998;36**:**2645-2651.
13. Littman MP, Goldstein RE, Labato MA, Lappin MR, Moore GE, ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. J Vet Intern Med 2006;20:422-434.
14. Parola, P, Paddock CD, Raoult D, Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 2005;18:719-756.

15. Kidd L, Hegarty BC, Sexton DJ, Breitschwerdt EB, Molecular characterization of *Rickettsia rickettsii* infecting dogs and people in North Carolina. Ann N Y Acad Sci 2006;1078:400-409.
16. Gasser AM, Birkenheuer AJ, Breitschwerdt EB, Canine Rocky Mountain spotted fever: a retrospective study of 30 cases. J Am Anim Hosp Assoc 2001;37:41-47.
17. Solano-Gallego L, Kidd L, Trotta M, et al., Febrile illness associated with *Rickettsia conorii* infection in dogs from Sicily. Emerg Infect Dis 2006;12:1985-1988.
18. Demma LJ, Traeger MS, Nicholson WL, et al., Rocky Mountain spotted fever from an unexpected tick vector in Arizona. N Engl J Med 2005;353:587-594.
19. Chapman AS, Bakken JS, Folk SM, et al., Diagnosis and management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever, Ehrlichioses, and Anaplasmosis--United States: a practical guide for physicians and other health-care and public health professionals. Morbidity and Mortality Weekly Report. March 31, 2006:55(RR04);1-27.
20. Perez M, Rikihisa Y, Wen B, *Ehrlichia canis*-like agent isolated from a man in Venezuela: antigenic and genetic characterization. J Clin Microbiol 1996;34:2133-2139.
21. Perez M, Bodor M, Zhang C, Xiong Q, Rikihisa Y, Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. Ann N Y Acad Sci 2006;1078:110-117.
22. Allsopp MT, Allsopp BA, Novel *Ehrlichia* genotype detected in dogs in South Africa. J Clin Microbiol 2001;39:4204-4207.
23. Loftis AD, Reeves WK, Spurlock JP, et al., Infection of a goat with a tick-transmitted Ehrlichia from Georgia, U.S.A., that is closely related to *Ehrlichia ruminantium*. J Vector Ecol 2006;31:213-223.
24. Breitschwerdt EB, Hegarty BC, Maggi RG, Hawkins E, Dyer P, *Bartonella* species as a potential cause of epistaxis in dogs. J Clin Microbiol 2005;43:2529-2533.
25. Neer TM, Eddlestone SM, Gaunt SD, et al., Efficacy of enrofloxacin for the treatment of experimentally induced *Ehrlichia canis* infection. J Vet Intern Med 1999;42:362-368.
26. Diniz PPVP, Maggi RG, Schwartz DS, et al., Canine bartonellosis: Serological and molecular prevalence in Brazil and evidence of co-infection with *Bartonella henselae* and *Bartonella vinsonii* subsp. *berkhoffii*. Vet Res 2007;38:697-710.
27. Kordick SK, Breitschwerdt EB, Hegarty BC, et al., Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. J Clin Microbiol 1999;37:2631-2638.
28. Maggi RG, Mascarelli PE, Havenga LN, NAidoo V, Breitschwerdt EB. Co-infection with *Anaplasma platys, Bartonella henselae* and *Candidatus* Mycoplasma hematoparvum in a veterinarian. *Parasites and Vectors* 6:106, 2013.
29. Maggi RG, Compton SM, Trull CL, Mascarelli PE, Mozayeni BR, Breitschwerdt EB. Infection with hemotropic *Mycoplasma* sp. in people with and without extensive arthropod and animal contact. J Clin Microbiol. In press.