Update on Select Vector Borne Diseases in Dogs and Cats.

Michael R. Lappin, DVM, PhD, DACVIM
The Kenneth W. Smith Professor in Small Animal Clinical Veterinary Medicine
College of Veterinary Medicine and Biomedical Sciences
Colorado State University, Fort Collins Colorado

Abstract. In these lectures, cases will be used to show how to manage dogs and cats with flea and tick associated vector borne diseases. Anaplasmosis, babesiosis, bartonellosis, cytauxzoonosis, ehrlichiosis, hemoplasmosis and others will be discussed. Emphasis will be placed on how to recognize the agents, use of serology and PCR assays, optimal treatment, and the use of products to prevent flea and tick infestations. The objectives of the lecture are:

1. To review the major flea and tick associated infections of dogs and cats.
2. To discuss the use of serology and PCR assays in the diagnosis of vector borne agents.
3. To review the optimal treatments for vector borne agents.
4. To learn the importance of prevention vector borne infections.

Key words. Vector; tick; flea; Anaplasma; Bartonella; Ehrlichis;

There are multiple vector borne diseases in dogs; those transmitted by ticks (multiple agents), fleas (multiple agents), mosquitoes (*Dirofilaria immitis*) or sandflies (*Leishmania* spp.) are among the most common. The Companion Animal Parasite Council website is an excellent source of information (www.capcvet.org) about vector borne diseases.

The organisms of the Order Rickettsiales, in the families *Rickettsiaceae* and *Anaplasmataceae*, were reclassified in 2001 following phylogenetic analyses of the 16S rRNA and groESL gene sequences (Dumler and colleagues, 2001). Some *Ehrlichia* spp. were transferred to the *Neorickettsia* genus (including *E. risticii*) and some *Ehrlichia* spp., including *E. phagocytophila* (also called *E. equi* and human granulocytic *Ehrlichia*) and *E. platys)* were placed into the genus *Anaplasma*. The genera *Ehrlichia* and *Neorickettsia* were transferred to the family *Anaplasmataceae;* the genera of *Rickettsia* and *Orientia* remained in the *Rickettsiaceae*. The organisms in the *Ehrlichia*, *Anaplasma*, and *Neorickettsia* genera are classified genetically and by cell tropism (Monocytotropic, granulocytotropic, or thrombocytotropic).

Babesia spp., *Borrelia burgdorferi, Fransicella tularensis, Hepatozoon* spp., *Mycoplasma haemocanis*, and *Rickettsia rickettsii* are also vectored by ticks, relatively common within geographical ranges, and are associated with illness in dogs. It is also possible that some *Bartonella* spp. of dogs, which are usually flea-borne, are tick transmitted. The purpose of this proceedings is to provide attendees an update on the management of a select group of tick and flea borne disease agents that infect dogs.

CANINE GRANULOCYTOTROPIC ANAPLASMOSIS

Etiology and epidemiology. *Anaplasma phagocytophilum* (previously *E. equi*, *E. phagocytophila*, canine granulocytic *Ehrlichia*, and human granulocytic ehrlichiosis agent) is

known to infect a variety of animals, including small mammals, mountain lions, coyotes, sheep, cattle, deer, dogs, horses, and people. Small mammals and deer are natural reservoirs. The distribution of *A. phagocytophilum* is defined by the range of *Ixodes* ticks and so is most common in California, Wisconsin, Minnesota, and the northeastern states and other areas of the world with this tick genus including Europe, Asia, and Africa. Birds may play a role in spreading infected ticks and may also serve as a reservoir. *Borrelia burgdorferi* is also transmitted by *Ixodes* ticks and so co-infections can occur. The vector needs to be attached for approximately 24-48 hours in order to transmit the agent. Clinical signs usually develop approximately 1-2 weeks after infection. Neutrophils (and rarely, other leukocytes) phagocytize the organism, and once intracellular, *A. phagocytophilum* prevents phagolysosome fusion. This mechanism allows for multiplication within the phagosome, which gives the appearance of morulae in neutrophils under light microscopy. The exact pathogenesis of disease is still undetermined and it is unclear why some dogs but not others develop clinical signs of disease.

Clinical features. *Anaplasma phagocytophilum* infection appears to be primarily an acute disease in dogs. It has been associated most commonly with non-specific signs of fever, lethargy and inappetence. Stiffness and lameness consistent with musculoskeletal pain are also common and *A. phagocytophilum* has been associated with polyarthritis. Vomiting, diarrhea, difficult breathing, cough, lymphadenopathy, hepatosplenomegaly, and central nervous system signs (seizures and ataxia) have also been reported. Dogs can be chronic, subclinical carriers and so exacerbation of disease could occur in some dogs. However, chronic disease syndromes like those associated with *E. canis* infection have not been documented. In one study of valvular endocarditis, all dogs with *Bartonella* spp. associated disease were also seropositive for *A. phagocytophilum* (MacDonald and colleagues, 2004). Whether the coinfection potentiated the *Bartonella* associated disease is unknown.

Diagnosis. Morulae of *A. phagocytophilum* are commonly detected in neutrophils of most clinically affected dogs and so infection is usually confirmed during performance of a complete blood cell count. While thrombocytopenia and lymphopenia are common, neutrophil counts are usually normal. Reported biochemical panel and urinalysis abnormalities are mild and nonspecific. The morulae cannot be distinguished from those of *E. ewingii*, but the geographical range of the infections varies between the organisms and so the travel history can aid in ranking the differentials. Serologic test results (IFA and ELISA) can be used if morulae are not identified. A point of care assay that detects antibodies against *A. phagocytophilum* is available (SNAP®4Dx, IDEXX Laboratories, Portland, ME) and several commercial laboratories have antibody assays (Antech Diagnostics, Lake Success, NY). Antibody assay results can be falsely negative in acute cases and so a convalescent test 2-3 weeks later may be required to confirm exposure. As *A. phagocytophilum* infections are limited geographically, this antibody test result is not needed in the majority of the United States. Polymerase chain reaction assays performed on blood collected in EDTA can be used to confirm infection and differentiate *A. phagocytophilum* infection from other infections, but microbial DNA can also be amplified from healthy dogs. Most dogs infected by *A. phagocytophilum* have subclinical infections, most infected dogs only have an acute phase, exposure rates in endemic areas are high, and the disease syndromes associated with infection have multiple other causes. Thus, antibody test results and PCR assay results alone cannot be used to prove clinical disease associated with *A. phagocytophilum* infection.

Treatment. Several antibiotics are effective against *A. phagocytophilum* in vitro. Doxycycline administered at 5-10 mg/kg, PO, q12-24 hr for at least 10 days is recommended by most clinicians. Whether a 28 day course of doxycycline therapy as recommended for *E. canis* is needed is unknown. If tetracyclines are used, 22 mg/kg, PO, q8hr for 2-3 weeks is recommended. Chloramphenicol administered at 15-25 mg/kg, PO, q 8hr for 14-21 days may be effective in puppies and should be used to avoid dental discoloration. Most dogs respond to therapy within hours to days of initiating therapy.

Zoonotic aspects and prevention. *Anaplasma phagocytophilum* infects people as well as dogs and so the organism is zoonotic. Human infections most likely acquired by direct tick transmission, however, handling infected blood and carcasses can also lead to infection. Care should also be taken when handling ticks. There is currently no vaccine for *A. phagocytophilum* infection. Infection can be avoided by controlling ticks or prophylactic use of tetracyclines when visiting endemic areas. In one study, application of imidacloprid-permethrin prevented transmission of *A. phagocytophilum* from naturally infected *Ixodes scapularis* ticks to dogs (Blagburn and colleagues, 2004). In another study, application of permethrin 54.5% and fipronil 6.1% (Effitix; Virbac) was effective for *Ixodes ricinus* and so the product could also lessen likelihood of transmission of *A. phagocytophilum* and *Borrelia burgdorferi* (Bonneau et al, 2015). Dogs appear to be susceptible to reinfection and so tick control should be maintained at all times in endemic areas. Dogs used for blood donors that reside in endemic areas should be screened for *A. phagocytophilum* infections by serology or PCR (Wardrop et al, 2016).

CANINE MONOCYTOTROPIC EHRLICHIOSIS

Etiology and epidemiology. Organisms that are associated with monocytotropic ehrlichiosis in naturally-infected dogs include *Ehrlichia canis, E. chaffeensis,* and *Neorickettsia risticii* var *atypicalis*. An individual dog can be infected by more than one ehrlichial agent and coinfections with other tick borne pathogens are common (Kordick and colleagues, 1999).

Ehrlichia canis is the most common of these agents and causes the most severe clinical disease; it is maintained in the environment from passage from ticks to dogs. *Rhipicephalus sanguineus* and *Dermacentor variabilis* are the known vectors. The organism is not passed transovarially in the tick, so unexposed ticks must feed on a rickettsemic dog in the acute phase to become infected and perpetuate the disease. Male *R. sanguineus* can take multiple feedings and can both acquire and transmit *E. canis* in the absence of female ticks. Dogs seropositive for *E. canis* have been identified in many regions of the world and most of the United States, but the majority of cases occur in areas with high concentrations of *R. sanguineus* such as the Southwest and Gulf Coast.

Ehrlichia chaffeensis is a cause of human mononuclear ehrlichiosis. White tailed deer, voles, coyotes, and opossums are reservoirs and *Amblyomma americanum*, *D. variabilis*, and some *Ixodes* ticks are vectors. Infections by *E. chaffeensis* are detected primarily in the southeastern United States. Clinical manifestations in dogs are currently being detailed and appear to be rare.

Ehrlichia canis infection causes acute, subclinical, and chronic phases of disease. Infected

mononuclear cells marginate in small vessels or migrate into endothelial tissues, inducing vasculitis during the acute phase. The acute phase begins 1 to 3 weeks after infection, and lasts 2 to 4 weeks; most immunocompetent dogs survive. The subclinical phase lasts months to years in naturally infected dogs. Although some dogs clear the organism during the subclinical phase, the organism persists intracellularly in some, leading to the chronic phase of infection. Many of the clinical and clinicopathologic abnormalities that develop during the chronic phase are due to immune reactions against the intracellular organism. The variable duration of the subclinical phase of disease explains why *E. canis* infection does not have a distinct seasonal incidence like Rocky Mountain spotted fever (RMSF). However, acute phase disease is recognized most frequently in the spring and summer when the tick vectors are most active.

Clinical features. Clinical disease from ehrlichial infection can occur in any dog, but its severity varies depending on the organism, host factors, and presence of coinfections. Virulence is thought to vary with different field strains of *E. canis.* Dogs with depressed cell-mediated immunity develop severe disease.

Clinical findings in dogs with *E. canis* infections vary with the timing of infection. The clinical manifestations of acute phase disease are very similar to those of RMSF, owing to the development of vasculitis. Ticks are most commonly found on dogs during the acute phase of infection. Fever can occur in both clinical phases of infection but is more common in dogs with acute ehrlichiosis. Petechiae or other evidence of bleeding noted during the acute phase are generally caused by a combination of mild thrombocytopenia (consumption or immune-mediated destruction) and vasculitis; thrombocytopenia (consumption, immune-mediated destruction, sequestration, decreased production), vasculitis, and platelet function abnormalities occur in the chronic phase. The thrombocytopenia in the acute phase is generally not severe enough to result in spontaneous bleeding and so bleeding may be primarily from vasculitis and decreased platelet function.

Pale mucous membranes usually only occur in the chronic phase during the development of pancytopenia. Hepatomegaly, splenomegaly, and lymphadenopathy are from chronic immune stimulation (i.e. lymphoreticular hyperplasia) and are detected most frequently in dogs in the chronic phase. Interstitial or alveolar edema secondary to vasculitis or to inflammation, pulmonary parenchymal hemorrhage secondary to vasculitis or thrombocytopenia, or secondary infections from neutropenia are mechanisms resulting in dyspnea or cough in some dogs with ehrlichiosis. Polyuria, polydipsia, and proteinuria are reported in some dogs that develop renal insufficiency.

Stiffness, exercise intolerance, and swollen painful joints occur in some dogs with suppurative polyarthritis. Most dogs with polyarthritis from which the organism has been demonstrated have been infected with *E. ewingii* or *A. phagocytophilum.* Ophthalmic manifestations of disease are common; tortuous retinal vessels, perivascular retinal infiltrates, retinal hemorrhage, anterior uveitis, and exudative retinal detachment occur. CNS signs can include depression, pain, ataxia, paresis, nystagmus, and seizures.

Diagnosis. Neutropenia is common during acute phase vasculitis and after bone marrow suppression in the chronic phase. Chronic immune stimulation causes monocytosis and

lymphocytosis; lymphocytes often have cytoplasmic azurophilic granules (i.e., large granular lymphocytes). Regenerative anemia is from blood loss (acute and chronic phases); normocytic, normochromic nonregenerative anemia is from bone marrow suppression or anemia of chronic disease (chronic phase). Thrombocytopenia can occur with either acute or chronic ehrlichiosis, but is generally more severe with chronic phase disease. Thrombocytopathies from hyperglobulinemia potentiate bleeding in some dogs with chronic ehrlichiosis. Chronic ehrlichiosis is classically associated with pancytopenia, but any combination of neutropenia, thrombocytopenia, and anemia can occur. Changes in bone marrow cell lines associated with ehrlichiosis vary from hypercellular (acute phase) to hypocellular (chronic phase). Bone marrow plasmacytosis is common in dogs with subclinical and chronic ehrlichiosis, and the disease can be confused with multiple myeloma, particularly in those dogs with monoclonal gammopathies. Dogs with ehrlichiosis are usually not hypercalcemic and do not have lytic bone lesions.

Hypoalbuminemia in the acute phase is probably caused by third spacing of albumin in tissues because of vasculitis, whereas in chronic phase disease it is due to glomerular loss from immune complex deposition or chronic immunostimulation (i.e., monoclonal or polyclonal gammopathy). Prerenal azotemia can occur with acute or chronic disease; renal azotemia develops in some dogs with severe glomerulonephritis from chronic ehrlichiosis. The combination of hyperglobulinemia and hypoalbuminemia is consistent with subclinical or chronic ehrlichiosis. Polyclonal gammopathies are most common, but monoclonal (e.g., IgG) gammopathies can also occur.

Aspirates of enlarged lymph nodes and spleen reveal reactive lymphoreticular and plasma cell hyperplasia. Nondegenerate neutrophils are the primary cells in synovial fluid from dogs with polyarthritis caused by any *Ehrlichia* spp.; *E. ewingii* and *A. phagocytophilum* morulae can be identified in synovial neutrophils from some dogs. Bone marrow aspirates in dogs with chronic ehrlichiosis typically reveal myeloid, erythroid, and megakaryocytic hypoplasia in association with lymphoid and plasma cell hyperplasia. Morulae from *E. canis* are rarely detected in the cytoplasm of mononuclear cells. Ehrlichiosis generally causes mononuclear pleocytosis and increased protein concentrations in CSF. Antiplatelet antibodies, antinuclear antibodies (ANA), antierythrocyte antibodies (by direct Coombs' test), and rheumatoid factors are detected in some dogs with ehrlichiosis, leading to an inappropriate diagnosis of primary immune-mediated disease.

No pathognomonic radiographic signs appear in dogs with ehrlichiosis. The polyarthritis is nonerosive, and dogs with respiratory signs most commonly have increased pulmonary interstitial markings, but alveolar patterns can occur. Identification of morulae in cells documents *Ehrlichia* infection, but it is uncommon with monocytotropic strains. Examination of buffy coat smears or blood smears made from blood collected from an ear margin vessel may increase the chances of finding morulae. Some *Ehrlichia* spp. can be cultured, but the procedure is low-yield and expensive and so is not clinically useful.

Most commercial laboratories (using IFAs or ELISA) and one point-of-care diagnostic test (SNAP®4Dx, IDEXX Laboratories, Portland, ME) use reagents that detect antibodies against *E. canis* in serum. These tests are generally used as the first screening procedures in dogs suspected to have ehrlichiosis. The American College of Veterinary Internal Medicine (ACVIM) Infectious Disease Study Group suggests that *E. canis* IFA antibody titers between 1:10 and 1:80 be

rechecked in 2 to 3 weeks because of the potential for false-positive results at these titer levels.

If serum antibodies against *E. canis* are detected in a dog with clinical signs consistent with ehrlichiosis, a presumptive diagnosis of canine ehrlichiosis infection should be made and appropriate treatment begun. However, detection of antibodies alone is not diagnostic of ehrlichiosis because some dogs are subclinically infected. Additionally, negative test results do not totally exclude ehrlichiosis from the list of differential diagnoses, because clinical disease can be detected before seroconversion and not all *Ehrlichia* spp. induce antibodies that consistently detected in *E. canis* assays (Moroff et al, 2014).

PCR assays are now available commercially and can be used to detect organism-specific DNA in peripheral blood. It can be performed on joint fluid, aqueous humor, CSF, and tissues. Blood PCR results can be positive before seroconversion in some experimentally inoculated dogs, and positive results document infection, whereas positive serologic tests only document exposure (Moroff et al, 2014). However, as for serology, no standardization between laboratories currently exists, and insufficient quality control can lead to both false-positive and false-negative results. Until more information is available, the ACVIM Infectious Disease Study Group suggests using PCR with serology, not in lieu of it. Because antibiotic treatment rapidly induces negative blood PCR results, the clinician should draw the blood sample for testing and place it in an EDTA tube before treatment. In one recent study, tissues (lymph nodes, spleen, liver, bone marrow, and blood) from naturally infected dogs were assayed by PCR. Blood and lymph nodes were the most likely to be positive, but were falsely negative in approximately 30% of the samples.

Treatment. Supportive care should be provided as indicated. Several different tetracycline, doxycycline, chloramphenicol, and imidocarb diproprionate protocols have been used. The ACVIM Infectious Disease Study Group currently recommends doxycycline (5 mg/kg, PO, q12hr or 10 mg/kg PO q24h for at least 28 days). In one study of experimentally infected dogs, ticks still could acquire *E. canis* from feeding on dogs previously treated with doxycycline for 14 days (Schaefer and colleagues, 2007). Clinical signs and thrombocytopenia should rapidly resolve. If clinical abnormalities are not resolving within 7 days, other differential diagnoses should be considered. Results of studies using imidocarb diproprionate (5 to 7 mg/kg IM or SQ repeated in 14 days) to treat canine ehrlichiosis have been variable. In one study, thrombocytopenia persisted and infection was not cleared in experimentally inoculated dogs (Eddlestone and colleagues, 2006). Some patients develop pain at the injection site, salivation, oculonasal discharge, diarrhea, tremors, and dyspnea after administration of this drug. Quinolones are not effective for the treatment of *E. canis* infections in dogs.

Positive antibody titers have been detected for up to 31 months after therapy in some naturally infected dogs. Dogs with low (< 1:1024) antibody titers generally revert to negative within 1 year after therapy. Dogs with antibody titers greater than 1:1024 often maintain positive antibody titers after therapy. It is undetermined whether these dogs are persistent carriers of the organism. Based on these findings, antibody titers are considered to be ineffective for monitoring response to therapy. The ACVIM Infectious Disease Study Group recommends monitoring resolution of thrombocytopenia and of hyperglobulinemia as markers of therapeutic elimination of the organism.

It is currently unknown whether ehrlichial infections are cleared by treatment. If PCR is to be used to monitor treatment, the ACVIM Infectious Disease Study Group recommends the following steps be taken: The PCR test should be repeated 2 weeks after stopping treatment. If still positive, treatment should be reinstituted for 4 weeks and retesting performed. If PCR results are still positive after 2 treatment cycles, an alternate anti-*Ehrlichia* drug should be used. If PCR results are negative, the test should be repeated in 8 weeks, and if still negative it can be assumed therapeutic elimination is likely. In one study, PCR assay performed on splenic aspirates was superior to blood PCR to document elimination of infection (Harrus and colleagues, 2004).

Whether to treat seropositive, healthy dogs is controversial. Arguments for and against testing or treating healthy dogs were reviewed by the ACVIM Infectious Disease Study Group. The primary reason to treat a seropositive, healthy dog is to try to eliminate infection before development of chronic phase disease. However, treatment of healthy dogs is controversial for at least six reasons: (1) it is unknown whether treatment halts progression to the chronic phase; (2) not all seropositive dogs are infected; (3) not all seropositive dogs progress to the chronic phase; (4) it is unknown whether treatment eliminates infection; (5) even if infection is eliminated, reinfection can occur; and (6) treatment of healthy carriers may result in antimicrobial resistance. Because further data are needed to make definitive recommendations, owners should be given the pros and cons and asked to make treatment decisions.

The prognosis is good for dogs with acute ehrlichiosis, and it is variable to guarded for those with chronic ehrlichiosis. Fever, petechiation, vomiting, diarrhea, epistaxis, and thrombocytopenia often resolve within days after initiation of therapy in acute cases. Bone marrow suppression from chronic phase ehrlichiosis may not respond for weeks to months, if at all. Anabolic steroids and other bone marrow stimulants can be administered but are unlikely to be effective because precursor cells are often lacking. Immune-mediated events resulting in the destruction of red blood cells or platelets are likely to occur with ehrlichiosis, leading to the recommendation to administer anti-inflammatory or immunosuppressive doses of glucocorticoids to acutely affected animals. Prednisone (2.2 mg/kg PO divided q12h during the first 3 to 4 days after diagnosis) may be beneficial in some cases.

Zoonotic aspects and prevention. Dogs and people are both infected by *Ehrlichia canis*, *E. ewingii*, and *E. chaffeensis*. Although people cannot acquire ehrlichiosis from handling an infected dog, dogs may be reservoirs for these agents and may play a role in the human disease by bringing vectors into the human environment. Ticks should be removed and handled with care.

Tick control should be maintained at all times as reinfection can occur. Products that repel ticks are likely to be the best products for prevention of *E. canis* infection as the transmission times after tick attachment may be as short as 3 hours. In one study comparing topically applied permethrin/imidacloprid to 2 orally administered acaracides, the topical product was superior for blocking *E. canis* transmission (Jongejan et al, 2016). Use of collars (examples Seresto; Bayer Animal Health; Preventic; Virbac) that also repel ticks can be beneficial for blocking transmission of vector borne agents with short transmission times and can increase compliance.

Because *Ehrlichia canis* is not passed transovarially in the tick, it can be eliminated in the environment by tick control or by treating all dogs through a generation of ticks. *Rhipicephalus* can only transmit *E. canis* for approximately 155 days; if tick control is not feasible, tetracycline can be administered (6.6 mg/kg PO daily for 200 days). During this time, infected dogs will not infect new ticks and previously infected ticks will lose the ability to transmit the organism. Doxycycline given at 100 mg/dog per day was also used successfully as a chemopreventative (Davoust and colleagues, 2005). Dogs used as blood donors should be screened serologically yearly and seropositive dogs should not be used.

CANINE GRANULOCYTOTROPIC EHRLICHIOSIS

Etiology and epidemiology. *Ehrlichia ewingii* forms morulae in neutrophils and eosinophils and has been detected in dogs and people that reside in the southern and southeastern United States. *Ehrlichia ewingii* has been detected in a number of ticks, but *Amblyomma americanum* is the only proven vector to date. Deer are infected and serve as a reservoir. The incubation period after tick exposure is approximately 13 days. Pathogenesis of disease is unknown, but is likely similar to other *Ehrlichia* spp. In general, clinical signs of *E. ewingii* infection are less severe thnt those of *E. canis*. Concurrent disease or infections may play a significant role in the pathogenesis of *E. ewingii* infection.

Clinical Features. Non-specific signs of *E. ewingii* infection include fever, lethargy, anorexia, depression, and signs consistent with polyarthritis, such as stiffness. Other clinical signs include vomiting, diarrhea, peripheral edema and neurological signs like ataxia, paresis, and vestibular disease. Clinical signs can be mild, self-limited, or inapparent. Similar to *R. rickettsii*, acute disease seems to be most common and so *E. ewingii* infection should be highest on the list of differential diagnoses from the spring through autumn when *A. americanum* is most active.

Diagnosis. Suppurative polyarthritis is most common. Other clinicopathologic findings typically associated with acute *E. canis* infection, such as mild to moderate thrombocytopenia and anemia, also occur. Morulae can be detected in neutrophils and eosinophils in peripheral blood and in neutrophils from synovial fluid. However, presence of morulae is transient and so easily missed cytologically. The organism has not been cultured to date and so a specific serological test is not available. However, because the organism is closely related to *E. canis*, antibodies against *E. ewingii* can often be detected in some *E. canis* assays. However, *E. ewingii* antibodies to not bind to the *E. canis* peptide used in one commercial assay (Antech Diagnostics) and so this assay cannot be used to screen dogs for *E. canis* infection (Moroff et al, 2014). PCR assays are now used to differentiate between members of the *Ehrlichia*, *Anaplasma*, and *Neorickettsia* genera and should be performed on blood collected in EDTA before administration of antibiotics.

Treatment. Supportive care should be provided as indicated. The tetracycline, doxycycline, and chloramphenicol protocols recommended for *E. canis* infections are generally effective. The

ACVIM Infectious Disease Study Group currently recommends doxycycline (5 mg/kg, PO, q12h or 10 mg/kg PO q24h for at least 28 days) for *Ehrlichia* spp, infections of dogs.

Zoonotic aspects and Prevention. Dogs and people are both infected by *Ehrlichia canis*, *E. ewingii*, and *E. chaffeensis*. Although people cannot acquire ehrlichiosis from handling an infected dog, dogs may be reservoirs for these agents and may play a role in the human disease by bringing vectors into the human environment. Ticks should be removed and handled with care. Dogs used as blood donors should be screened serologically with *E. canis* IFA tests yearly and seropositive dogs should not be used.

ROCKY MOUNTAIN SPOTTED FEVER

Etiology and epidemiology. Rocky Mountain spotted fever (RMSF) is caused by *Rickettsia rickettsii.* Other members of the genus also infect dogs in the United States; however, they are not associated with clinical disease but can induce antibodies that cross-react with *R. rickettsii* (see Diagnosis). In another study of dogs coinfected with several tick-borne pathogens, infection with an uncharacterized rickettsial agent commonly induced cross-reacting antibodies to *R. rickettsii* (Kordick and colleagues, 1999). Canine RMSF is recognized predominantly in the southeastern states from April through September when the tick vectors are most active. *Dermacentor andersoni* (i.e., American wood tick), *Dermacentor variabilis* (i.e., American dog tick), and *Amblyomma americanum* (i.e., Lone Star tick) are the principal vectors, host, and reservoir of *R. rickettsii.* Recently, there has been a reemergence of RMSF in the southwestern states and *R. sanguineous* ticks are the vector (Demma and colleagues, 2005).

The organism is maintained in nature in a cycle between ticks and small mammals like voles, ground squirrels, and chipmunks, and it is transmitted transovarially in ticks, so nymphs and larvae can be infected without feeding. *R. rickettsii* replicates in endothelial tissues (causing vasculitis) and so can lead to diverse and sometimes severe clinical manifestations of disease as soon as 2 to 3 days after exposure. Antiplatelet antibodies can be detected in many infected dogs, suggesting an immune-mediated component to the thrombocytopenia that is frequently present.

Clinical features. Any dog not previously exposed to *R. rickettsii* can develop RMSF. Frequently, the tick has fed and left the dog before the development of clinical signs. In one study, only 5 of 30 owners knew their dogs had been infested by ticks (Gasser and colleagues, 2001). After infection, the majority of dogs are subclinical; some develop acute disease with a clinical course of approximately 14 days. No age or sex predilection exists.

Fever and depression are the most common clinical signs. Interstitial pulmonary disease, dyspnea, and cough occur in some dogs and gastrointestinal signs occur in some acutely infected dogs. Because the disease is generally acute, lymphadenopathy and splenomegaly are not as common as in dogs with ehrlichiosis. Petechiae, epistaxis, subconjunctival hemorrhage, hyphema, anterior uveitis, iris hemorrhage, retinal petechiae, and retinal edema occur frequently. Cutaneous manifestations can include hyperemia, petechiae, edema, and dermal necrosis. Hemorrhage results from vasculitis, thrombocytopenia from consumption of platelets at sites of vasculitis, thrombocytopenia from immune destruction, and in some dogs, disseminated intravascular coagulation. Central nervous system (CNS) signs include vestibular lesions

(nystagmus, ataxia, head tilt), seizures, paresis, tremors, changes in mentation, and hyperesthesia. Fatal RMSF is generally secondary to cardiac arrhythmias and shock, pulmonary disease, acute renal failure, or severe CNS disease.

Diagnosis. Clinicopathologic and radiographic abnormalities are common but do not definitively document RMSF. Neutrophilic leukocytosis, with or without a left shift and toxic cells, is found in most clinically affected dogs. Platelet counts are variable, but in one study, 14 of 30 dogs had less than 75,000 platelets/µl without evidence of disseminated intravascular coagulation (Gasser, 2001). In other dogs, hemostatic abnormalities consistent with disseminated intravascular coagulation occur. Anemia occurs in some dogs, primarily from blood loss. Increased activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, as well as hypoalbuminemia from blood loss or third spacing of albumin in tissues secondary to vasculitis occur frequently. Because *R. rickettsii* does not result in chronic intracellular infection like ehrlichiosis, hyperglobulinemia is rare. Renal insufficiency in some dogs causes azotemia and metabolic acidosis. Serum sodium, chloride, and potassium concentrations decrease in many dogs with gastrointestinal tract signs or renal insufficiency. In contrast to dogs with chronic ehrlichiosis, chronic proteinuria from glomerulonephritis is rare. Positive direct Coombs' test results occur in some dogs.

Nonseptic, suppurative polyarthritis occurs in some dogs. CNS inflammation usually causes increased protein concentrations and neutrophilic pleocytosis in CSF; some dogs may have mononuclear cell pleocytosis or mixed inflammation. No pathognomonic radiographic abnormalities are associated with RMSF, but both experimentally- and naturally- infected dogs commonly develop unstructured pulmonary interstitial patterns.

A presumptive diagnosis of canine RMSF can be based on the combination of appropriate clinical, historical, and clinicopathologic evidence of disease, serologic test results, exclusion of other causes of the clinical abnormalities, and response to anti-rickettsial drugs. Documentation of seroconversion or an increasing titer 2 to 3 weeks after initial serologic testing suggests recent infection. Diagnostic criteria used in one recent study included a fourfold rise in antibody titer or a single titer of greater than 1:1024 if the initial titer was submitted 1 week or more after initial onset of clinical abnormalities (Gasser and colleagues, 2001). Positive serum antibody test results alone do not prove RMSF because subclinical infection is common. In addition, positive serum antibody tests do not document infection by *R. rickettsii* because infection with nonpathogenic spotted fever group agents can induce cross-reacting antibodies. Demonstration of *R. rickettsii* by inoculating affected tissues or blood into susceptible laboratory animals or by documenting the organism in endothelial cells using direct fluorescent antibody staining leads to a definitive diagnosis of RMSF but are not clinically practical. Polymerase chain reaction (PCR) can be used to document the presence of rickettsial agents in blood, other fluids, or tissues and can be used to document infection. However, some apparently healthy dogs have had *Rickettsia* spp. DNA amplified from blood and so positive PCR assay results may not always correlate to RMSF (Kordick and colleagues, 1999).

Treatment. Supportive care for gastrointestinal tract fluid and electrolyte losses, renal disease, disseminated intravascular coagulation, and anemia should be provided as indicated. Overzealous fluid therapy may worsen respiratory or CNS manifestations of disease if vasculitis

is severe.

Tetracycline derivatives, chloramphenicol, and enrofloxacin are the antirickettsial drugs used most frequently. Tetracycline (22 mg/kg PO q8h for 14 to 21 days) was commonly used historically. Doxycycline (5 to 10 mg/kg PO q12h for 14 to 21 days) is an alternative to tetracyclines; GI absorption and CNS penetration are superior to tetracycline, owing to increased lipid solubility. Chloramphenicol (22 to 25 mg/kg PO q8h for 14 days) can be used in puppies less than 5 months of age to avoid dental staining associated with tetracyclines. Enrofloxacin (3 mg/kg PO q12h for 7 days) is as effective as tetracycline or chloramphenicol. In one study of 30 dogs with RMSF, all dogs survived and there were no apparent differences in response rate between tetracycline, doxycycline, chloramphenicol, or enrofloxacin (Gasser and colleagues, 2001). Fever, depression, and thrombocytopenia often begin to resolve within 24 to 48 hours after starting therapy.

Administration of prednisolone at antiinflammatory or immunosuppressive doses in combination with doxycycline did not potentiate RMSF in experimentally infected dogs. The prognosis for canine RMSF is fair; death occurs in less than 5% of affected dogs.

Zoonotic aspects and prevention. Because RMSF has not been reported twice in the same dog, permanent immunity is likely. Infection can be prevented by providing strict tick control. In a recently completed study, dogs in Oklahoma that were negative for select vector borne agents on Day 0 were randomized to be administered a topical product with repellent activity (permethrin 44.88% and fipronil 6.01%; Effitix; Virbac) or an orally administered product for 4 months while rechecked monthly for evidence of exposure to an *Ehrlichia* spp. or a *Rickettsia* spp. In that pilot study, 2 of the 21 dogs (9.5%) of the dogs administered the oral product develop antibodies against a *Rickettsia* spp. in contrast to 0 of 14 dogs administered the topical product (Lappin MR. Unpublished data, 2017).

It is unlikely that people acquire *R. rickettsii* from contact with dogs, but dogs may increase human exposure to RMSF by bringing ticks into the human environment. Two dogs and the owner all died of RMSF in one study (Elchos and Goddard, 2003). As in dogs, RMSF in people is most commonly recognized from April to September when the tick vectors are most active. Untreated RMSF is fatal in approximately 20% of infected people.

OTHER RICKETTSIAL INFECTIONS

Rickettsia felis was originally detected in a commercial cat flea (*Ctenocephalides felis*) colony and was has been shown to belong in the spotted fever group. Fever, headache, myalgia, and macular rash in humans have been attributed to *R. felis* infection in several countries around the world. The organism has been detected in *C. felis*, *C. canis,* and *Pulex irritans;* these fleas have a worldwide distribution. *Ctenocephalides felis* is a biological vector for *R. felis*; the organism can be transmitted transovarially and transtadially within the flea. *Rickettsia felis* DNA has been amplified from *C. felis* collected from cats in the United Kingdom, France, Israel, New Zealand, Australia, Thailand, and the United States but not in the blood of cats. Recently it was discovered that dogs are the likely reservoir but clinical illness has not been documented to date (Hii et al, 2011). However, it is possible that antibodies against *R. felis* cross react with those

against *R. rickettsii*, confusing the diagnosis of RMSF. Use of flea control product would theoretically lessen risk of transmission to people.

Neorickettsia helminthoeca (i.e., salmon poisoning) causes enteric signs of disease in dogs from the Pacific Northwest. *Coxiella burnetii* infection is associated with parturient or aborting cats and is primarily a zoonotic disease. *Haemobartonella canis* has been reclassified as a *Mycoplasma* and has 2 species; *Mycoplasma haemocanis* and '*Candidatus* M. haematoparvum'.

CANINE BARTONELLOSIS

Etiology and epidemiology. *Bartonella vinsonii* subsp. *berkhoffii* was initially isolated from a dog with endocarditis in North Carolina (Breitschwerdt and colleagues, 1995). Since that time, dogs in multiple areas of the world have been shown to seroreact with *B. vinsonii* (*berkhoffii*) antigens. *Bartonella vinsonii* (*berkhoffii*) is thought to be tick-borne. Serum of some infected dogs also seroreacts with *B. henselae* and *B. clarridgeiae* antigens; these *Bartonella* species are transmitted by fleas. *Bartonella* species that have been isolated from dogs or from which DNA has been amplified from blood or tissues include *B. vinsonii* (*berkhoffii*), *B. henselae*, *B. clarridgeiae*, *B. washoensis*, *B. quintana*, and *B. elizabethae*. Each of these organisms potentially can induce illness in dogs. Dogs infected with a *Bartonella* species are commonly coinfected with other agents like *Anaplasma* spp. or *Ehrlichia* spp. which may play a role in the pathogenesis of disease.

Clinical features. Clinical findings or syndromes most frequently attributed to *Bartonella* spp. infections of dogs include endocarditis, fever, arrhythmias, hepatitis, granulomatous lymphadenitis, cutaneous vasculitis, rhinitis, polyarthritis, meningoencephalitis, thrombocytopenia, eosinophilia, monocytosis, immune-mediated hemolytic anemia, epistaxis, and uveitis. *Bartonella vinsonii* (*berkhoffii*) and *B. henselae* seem to be the most likely species to be associated with clinical disease. In one study of valvular endocarditis, all dogs with *Bartonella* spp. associated disease were also seropositive for *A. phagocytophilum* (MacDonald and colleagues, 2004). Whether the coinfection potentiated the *Bartonella* associated disease is unknown. Both *B. vinsonii* and *B. henselae* have been associated with endocarditis in dogs in Colorado and Wyoming (Fenimore et al, 2011) suggesting transmission from contact with fleas infesting coyotes and possibly fox.

Diagnosis. Serum antibodies can be detected in both healthy and clinically ill dogs, and so the presence of antibodies does not always correlate to illness. Some *Bartonella* species, in particular *Bartonella vinsonii* (*berkhoffii*), can be difficult to culture and so amplification of DNA by PCR assay with or without culture is often needed to confirm infection (Duncan et al, 2007). If positive test results are detected in a clinically ill dog and there is no other obvious explanation for the illness, treatment is indicated.

Treatment. As many cases of bartonellosis in dogs have been apparently resistant to administration of doxycycline, some clinicians believe that azithromycin is the treatment of choice. Fluoroquinolones, alone or in combination with amoxicillin, were apparently effective for the treatment of some dogs with suspected clinical bartonellosis. Rifampin may be required

for resistant cases. No matter which drug is used, a minimum of 4-6 weeks of treatment is usually needed.

Zoonotic aspects and prevention. *Bartonella vinsonii* (*berkhoffii*) and *B. henselae* have been detected in both dogs and humans and cat scratch disease has been documented in a humans after exposure to dogs and by blood contaminated needles. Care should be taken to avoid bites or scratches while handling or treating infected dogs. Flea control is known lessen transmission of *B. henselae* amongst cats (Bradbury and Lappin, 2010). Flea and tick control is likely to lessen transmission of *Bartonella* species between dogs and perhaps from dogs to people.

Update on Feline Vector Borne Diseases

Michael R. Lappin, DVM, PhD, DACVIM
The Kenneth W. Smith Professor in Small Animal Clinical Veterinary Medicine
College of Veterinary Medicine and Biomedical Sciences
Colorado State University, Fort Collins Colorado, 80523

There are multiple vector borne diseases in cats; those transmitted by ticks (multiple agents), fleas (multiple agents), mosquitoes (*Dirofilaria immitis*) or sandflies (*Leishmania* spp.) are among the most common. The Companion Animal Parasite Council (www.capcvet.org), European Scientific Counsel Companion Animal Parasites (www.esccap.org/guidelinies/)m and the Companion Vector Borne Diseases (www.cvbd.com) are excellent sources of information about vector borne diseases.

There are multiple tick-borne agents that have been grown or amplified from blood or have induced serum antibodies in the serum of normal cats or those with clinical signs like fever. Most of the tick-borne diseases diagnosed in dogs have now been found in cats. In some countries, thorough evaluation of cats for tick-borne disease agents has not been completed. In those situations, dog results can be used as evidence for the presence of individual agents in the region that could potentially infect cats. Results of studies from regional ticks can also be used as evidence for risk in cats (Skarphédinsson et al, 2007; Smith and Wall, 2013; Pennisi et al, 2015). The purpose of this review is provide an update on the diagnosis and management of feline flea and tick borne diseases of significance. *Anaplasma phagocytophilum*, *Bartonella* spp., *Borrelia* spp., *Cytauxzoon* spp., *Ehrlichia* spp., hemoplasmas, and *Rickettsia* spp. will be discussed. It is less clear how important *Hepatozoon* spp. infections are in cats (Díaz-Regañón et al, 2017) and how often *Francisiella tularensis* infections are transmitted to cats by ticks and so they are not presented in depth.

Feline granulocytotropic anaplasmosis. Canine anaplasmosis has been recognized for many years. Cats have shown to be susceptible to *A. phagocytophilum* infection after experimental inoculation (Lappin et al, 2015). DNA of *A. phagocytophilum* DNA has been amplified from blood of naturally exposed cats in multiple countries (Björsdorff et al, 1999; Lappin et al, 2004; Adaszek et al, 2013; Bergmann et al, 2016; Lee et al, 2016; Savidge et al, 2016). The easiest way to remember the distribution of *A. phagocytophilum* infections in cats is to remember the range of *Ixodes* spp. or Lyme disease in people or dogs. In the United States, *Ixodes scapularis* transmits both *A. phagocytophilum* and *B. burgdorferi* but some of the current evidence suggests that *A. phagocytophilum* is the more likely cause of the clinical and laboratory abnormities.

While the pathogenesis of disease associated with *A. phagocytophilum* in cats is unknown, some cats experimentally inoculated with *A. phagocytophilum* developed anti-nuclear antibodies and increased IFN-gamma mRNA suggesting that an immune pathogenesis of disease may contribute to the clinical findings (Foley et al, 2003). Fever, anorexia, and lethargy are the most common clinical abnormalities in naturally infected cats (Savidge et al, 2016). Whether or not this agent is associated with chronic recurrent fever in cats is unknown. In a recent experimental study, cats infected with *A. phagocytophilum* by exposure to wild caught adult *Ixodes scapularis* from Rhode Island remained clinically normal over the 70 day study period in spite of being PCR positive for *A. phagocytophilum* DNA in blood for several weeks (Lappin et al, 2015). In a larger unpublished study, we infested 10 cats with *I. scapularis* twice and induced *A. phagocytophilum* or *Borrelia burgdorferi* infection in all 10 cats (Lappin et al, 2017). While repeated or new infections with both organisms occurred, all cats remained clinically normal. Since both studies were performed using ticks from the same region, it is possible a less pathogenic strain of the organism was present (Rejmanek D et al, 2013).

Cats with fever in endemic areas can have blood smears examined cytologically but morulae are not always detected in cats with clinical signs of anaplasmosis. Some commercial laboratories offer serologic testing or PCR assays to amplify *A. phagocytophilum* DNA from blood. In experimental infections, DNA is amplified from blood prior to seroconversion for most cats (Lappin et al, 2015). Approximately 30% of cats with proven clinical infections induced by *A. phagocytophilum* are seronegative when first assessed serologically, but most of the proven cases evaluated to date have ultimately seroconverted. Therefore, cats with suspected anaplasmosis may need convalescent serum samples to prove infection. Alternately, antibody testing could be combined with PCR testing of whole blood in acute cases. The SNAP4DX Plus (IDEXX Laboratories) has been shown to be accurate for the detection of *A. phagocytophilum* antibodies in cats but is not labeled for this purpose (Lappin et al, 2015). In addition, another peptide (P44-4) than the one used on the commercial assays detected antibodies even sooner.

Several antibiotics have been administered to naturally infected cats, but most cats treated in the field become clinically normal within 24 to 48 hours after initiation of tetracycline or doxycycline administration and recurrence has not reported in any cat to my knowledge (Lappin et al, 2014; Savidge et al, 2016). While clinically normal, the organism DNA can still be amplified from the blood of some cats which suggests that treatment with tetracyclines for 21 to 30 days may be inadequate for eliminating the organism from the body. In one of our recent studies, the fact that an owner paid for a tick control product was not associated with decreased risk of having *A. phagocytophilum* antibodies in serum (Hoyt et al, 2017). These results suggest lack of compliance or lack of efficacy. As repeat new infections can occur, it is imperative to maintain tick control at all times, even in cats that have been previously infected (Lappin et al, 2017).

DNA homologous with *A. platys* has been amplified from the blood of cats in some countries with *Rhipicephalus sanguineus* (Lima et al, 2010; Qurollo et al, 2014). Further studies will be required to determine if disease associations exist with this agent in cats.

Feline bartonellosis. A number of *Bartonella* spp. including *B. henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. quintana* and *B. bovis* have been cultured or amplified from client-owned cats with

fever. Fever following experimental inoculation with *B. henselae* has been documented in a number of studies including a recent study in our laboratory where the CSU-1 strain of *B. henselae* induced significant fever in three of six cats after exposure to infected *C. felis*. None of the six cats administered imidacloprid-moxidectin in that study became infected or febrile. However, not all strains or *Bartonella* spp. induce fever in all cats; for example in the imidacloprid-moxidectin study, cats inoculated with the same strain intravenously failed to develop fever. Whether fever will occur during *Bartonella* spp. infection is likely a complex interaction that is influenced by both host and organism factors.

As *B. henselae*, *B. clarridgeiae*, *B. koehlerae* are transmitted by fleas, bacteremia and antibody positive rates can be very high. For example, serum antibodies were detected in 93% of cats housed in a North Carolina shelter and *Bartonella* spp. DNA was amplified from the blood of > 50% of cats housed in Alabama or Florida shelters. The majority of these cats were thought to be normal which emphasizes that fever from bartonellosis cannot be documented by test results alone. In one study of pair matched cats with or without fever, serum *Bartonella* antibodies detected by ELISA or Western blot immunoassay were not correlated to the presence of fever. In addition, serum antibody test results are negative in between 3 and 15% of bacteremic cats. Thus, if a cat with fever is to be evaluated for *Bartonella* spp. infection the combination of blood culture or PCR assay on blood, and serologic testing will detect the greatest number of cats that are currently or previously infected. Febrile cats that are seronegative and negative for *Bartonella* spp. in blood by culture or *Bartonella* spp. DNA in blood are unlikely to have the organism as the cause of fever. However, addition of blood culture using BAPGM media is more sensitive that routine culture (www.galaxydx.com).

Fever, lymphadenopathy, uveitis, endocarditis, myocarditis, osteomyelitis, and hyperglobulinemia appear to be the most common manifestations of bartonellosis in cats. Osteomyelitis has recently been documented in infected cats. Upper respiratory tract disease, stomatitis, conjunctivitis, and pancreatitis do not seem to be associated with feline bartonellosis. If fever or other acute signs from bartonellosis is suspected in a cat, administration of doxycycline is usually effective but does not eliminate infection. The AAFP Panel Report on Bartonellosis (www.catvets.com) recommended doxycycline at 10 mg/kg, PO, daily for 7 days as the initial therapeutic trial. If gastric irritation is occurring, dividing the drug into 2 doses per day is acceptable. If a positive response is achieved, continue treatment for 2 weeks past clinical resolution of disease or for a minimum of 28 days. If a poor response is achieved by day 7 or doxycycline is not tolerated and bartonellosis is still considered a valid differential diagnosis, fluoroquinolones are appropriate second choices. In experimental or field studies, administration of enrofloxacin or orbifloxacin have led to rapid resolution of fever in cats with presumed bartonellosis. Azithromycin is now considered contraindicated because of rapid induction of resistance. Pradofloxacin (Veraflox; Bayer Animal Health) at 7.5 mg/kg, PO, once daily is considered by many to be the optimal drug for treatment of clinical bartonellosis as it is the least likely to induce resistant strains. Use of imidacloprid containing products (Advantage Multi [Advocate] or Seresto Collars; Bayer Animal Health) have been shown to block transmission of *B. henselae* amongst research cats.

Frequent contact with animals infested with *C. felis* is likely a common way to people to acquire bartonellosis and veterinarians have increased risk. Cat scratch disease has been the greatest

concern over the years but is actually not the most important manifestation in veterinarians. It is now recognized that *Bartonella* spp. infections of people is associated with endocarditis and many chronic inflammatory disease syndromes that can be confused with other infection or immune mediated diseases like polyarthritis. Neurobartonellosis with headaches and blurred vision is common. If an animal care provider has an undiagnosed chronic inflammatory disease, they should be tested for bartonellosis. The most sensitive techniques include culture and PCR (www.galaxydx.com).

Feline borreliosis. *Borrelia burgdorferi* is the cause of Lyme disease and is transmitted by *Ixodes* spp. Clinical illness in dogs and people is most common in the United States. While *B. burgdorferi* antibodies have been detected in the serum of cats for years, whether the agent induces illness in cats is still controversial (Burgess EC, et al 1992; Levy et al, 2003; Magnarelli et al, 2005; Krupka and Straubiner, 2010).

Recently, 2 manuscripts have attempted to ascribe clinical illness to *B. burgdorferi* infection in cats (Pantchev et al, 2016; Hoyt et al, 2018). The cats that were positive for *B. burgdorferi* antibodies in Belgium, Sweden and Germany had weakness, ataxia and lameness as the most common clinical signs and doxycycline was apparently effective for treatment (Pantchev et al, 2016). The biggest limitation in that study was the failure to report results of assays for other feline disease agents that may be responsive to doxycycline, in particular *A. phagocytophilum*. The cats in Maine with suspected borreliosis were seropositive to *B. burgdorferi* C6 peptide but negative for *A. phagocytophilum* antibodies, had fever, weakness, lameness, lethargy and inappetance as clinical signs, and had apparent responses to doxycycline (Hoyt et al, 2017). The biggest limitations in that study was the failure to perform *A. phagocytophilum* PCR or other diagnostic assays to evaluate for other feline disease agents that may be responsive to doxycycline. Recently, use of cefovecin was shown to be effective for the treatment of borreliosis in dogs (Wagner et al, 2015). Whether this will prove to be true for cats needs to be determined.

Borrelia garinii and *afzelii* have been amplified from ticks collected from cats in the United Kingdom (Unpublished data, Richard Walls, ISFM Congress 2017). Whether these agents are associated with clinical disease in cats is unknown.

There are currently no feline *B. burgdorferi* vaccines. In dogs, use of acaracides can block transmission of the agent and repeat infections can occur in cats (Honsberger et al, 2016; Lappin et al, 2017). Thus, use of acaracides is imperative for the control of this agent.

Feline cytauxzoonosis. Cats in the United States and Europe are infected by *Cytauxzoon* spp. (Carli et al, 2012; Díaz-Regañón et al, 2017). Excellent review articles from European authors (Lloret Aet al, 2015) and American authors (Sherrill and Cohn, 2015) are recently available.

It is apparent that *Cytauxzoon felis* infections in the United States (transmitted by *Amblyomma americanum*) can be very pathogenic when compared to the *Cytauxzoon* spp. infections occurring in cats in other countries. This may represent different species in different countries (Gallusová M et al, 2016). However, *C. felis* strain variations also play a role in whether clinical disease occurs within countries as well. For example, while fatal *C. felis* infections are common

in some regions in the United States, cats that survive or have subclinical infections are also common (Meinkoth et al, 2000; Rizzi et al, 2015). A recent study showed the *C. felis* could be transmitted between 36 and 48 hours of tick attachment and ingestion of *A. americanum* did not induce infections (Thomas et al, 2017).

In the United States, clinical infections are recognized most commonly in the spring, summer and fall. Non-specific complaints of lethargy and anorexia are reported frequently by owners. The infected cats have fever or hypothermia if presented in the final shock phase. Common physical examination findings that might lead to consideration of this agent as a differential diagnosis include pale mucous membranes, icterus, splenomegaly, and hepatomegaly. Discomfort, clinical evidence of central nervous system disease including seizures, tachypnea with or without respiratory distress, and sudden death on manipulation all occur in some cats.

Piroplasmas can be seen on the erythrocytes frequently, but can be falsely negative in the acute stages of illness. The serious clinical signs of disease relate to the development of the shizonts in tissues. The syndrome can be diagnosed by cytological demonstration of the piroplasmas on erythrocytes, cytological demonstration of shizonts in spleen, liver, or bone marrow samples, or by PCR of *Cytauxzoon* spp. DNA in blood or tissue aspirates (Sherrill and Cohn, 2015).

To date, clinically affected cats have the best response to the combination of azithromycin at 10 mg/kg, PO, q24 hours and atovaquone at 15 mg/kg, PO, q8 hours (Cohn et al, 2011; Schreeg et al, 2015) with approximately 60% of treated cats responding. This combination is superior to diminezene or imidocarb protocols (Cohn et al, 2011; Lewis et al, 2014). Minimal restraint techniques should be used during administration of supportive care to lessen the likelihood of sudden death.

The poor overall treatment responses in clinical cytauxzoonosis cases is a perfect example of why tick control can be so important. It is always better to prevent a vector borne disease rather that attempt to treat it after illness has begun. Use of acaracides appropriately should lessen the risk of transmission of this agent (Reichard et al, 2013).

Feline monocytotropic ehrlichiosis. While canine ehrlichiosis is well characterized, less is known about the agents associated with disease in cats. It is likely that any country that has *E. canis* infections in dogs, has *E canis* infections in cats. Naturally exposed cats have been shown to have *Ehrlichia*-like bodies or morulae in peripheral lymphocytes or monocytes, have had DNA consistent with *E. canis* amplified from the blood or tissues, and have had antibodies that react to *E. canis* morulae or peptides in many countries (see select reference list). However, in 2 separate experimental studies, we have failed to amplify monocytotropic *Ehrlichia* spp. from blood or detect seroconversion in cats inoculated SQ with different strains of cultured *E. canis* (Lappin and Breitschwerdt, unpublished observations, 2007; Lappin and Little, unpublished observations, 2010). These results indicate the *E. canis*-like DNA amplified from naturally-infected cats may be from a different *Ehrlichia* spp. more infective to cats, not all *E. canis* stains will infect cats, not all cats are susceptible to infection by *E. canis*, or SQ inoculation is not an effective method for infecting cats with *E. canis*. In addition, we have had field cases that have been positive for DNA identical to *E. canis* at 2 genes that never seroconverted (Breitschwert et al, 2002). It is likely that cats at greater risk for *Rhipicephalus sanguineous* infestation are more

likely to have higher prevalence rates for *E. canis* in cats like in Brazil where 9.4% of cats were PCR positive in 1 study (Braga et al, 2014). In Sicily, *E. canis* DNA was amplified from ticks collected from some cats (Pennisi et al, 2015).

Fever, lethargy, and inappetance are commonly reported clinical abnormalities detected in cats with suspected ehrlichiosis and so testing may be indicated in these cats. Thrombocytopenia, anemia, and monocytosis appear to be the most common clinical laboratory findings in naturally infected cats (Bouloy et al, 1994; Peavy et al, 1997; Beaufils et al, 1999; Braga et al, 2013). Almost every abnormality noted in dogs with clinical ehrlichiosis has been detected in cats, including monoclonal gammapathy (Neer et al, 2002).

A validated serological assay is not currently available and some cats with *E. canis*-like DNA in blood were seronegative (Breitschwert et al, 2002). Positive serologic test results occur in both healthy and clinically ill cats, and so a diagnosis of clinical ehrlichiosis should not be based on serologic test results alone. *Ehrlichia* spp. PCR and gene sequencing can be used to confirm infection and should be considered the tests of choice at this time.

Clinical improvement after therapy with tetracycline, doxycycline, or imidocarb dipropionate was reported for most cats with suspected mononcytopic ehrlichiosis. However, for some cats a positive response to therapy was a criterion for the diagnosis of ehrlichiosis. The current recommendation of the ACVIM Infectious Disease Study Group (www.acvim.org) is to administer doxycycline (10 mg/kg PO q24h or 5 mg/kg PO q12h for 28 days). For cats with treatment failure or those intolerant of doxycycline, imidocarb diproprionate can be administered (5 mg/kg IM or SQ twice, 14 days apart). Salivation and pain at the injection site are the common adverse effects and imidocarb efficacy is in question for the treatment of canine monocytotropic ehrlichiosis.

Pancytopenia occurs in cats with ehrlichiosis and when occurs in dogs, may not respond to treatment (Breitscherdt et al, 2002). This is another example of why acaracides should be used to attempt to avoid infection with vector borne disease agents.

Feline hemoplasmosis. Fever or hemolytic anemia are the most common manifestations of *Mycoplasma haemofelis*, *'Candidatus* Mycoplasma haemominutum', or '*Candidatus* M. turicensis'. In multiple studies of experimentally infected cats, *M. haemofelis* is apparently the most pathogenic species. Dual infection with hemoplasmas may potentiate pathogenesis of disease. In one study, cats with chronic *'Candidatus* Mycoplasma haemominutum' infection had more severe anemia and longer duration of anemia when experimentally infected with *M. haemofelis* when compared to cats infected with *M. haemofelis* alone. In one abstract, we reported an association between *M. haemofelis* and fever in cats without anemia. Clinical signs of disease depend on the degree of anemia, the stage of infection, and the immune status of infected cats. Direct transmission may occur with the hemoplasmas and so the agents should be on the differential list for cats with a history of fighting.

Diagnosis of hemoplasmosis is based on demonstration of the organism on the surface of erythrocytes on examination of a thin blood film or by PCR assay results. Organism numbers fluctuate and so blood film examination can be falsely negative up to 50% of the time. The

organism may be difficult to find cytologically, particularly in the chronic phase. Thus, PCR assays are the tests of choice due to sensitivity.

Doxycycline is often administered as a flavored suspension (to avoid esophageal strictures) at 10 mg/kg, PO, every 24 hours for a minimum of 7 - 10 days. In cats intolerant of doxycycline, enrofloxacin given at 5 mg/kg, PO, every 24 hours for 14 days was tolerated by cats and is equally effective or more effective than doxycycline. Administration of marbofloxacin or orbifloxacin gives similar results. Pradofloxacin (Veraflox; Bayer Animal Health) at 7.5 mg/kg, PO, once daily is considered the optimal drug for treatment of clinical hemoplasmosis as it is the only antibiotic shown to clear *M. hemofelis* bacteremia (Dowers et al, 2009). However, negative PCR assay results were not achieved in all cats. Doxycycline followed by marbofloxacin was also effective in one study (Novacco et al, 2018). Azithromycin was not effective for the treatment of hemoplasmosis in one study. Most drug protocols have failed to eliminate infection and so at this time there is no clinical utility to repeat PCR testing. The owners should be warned that recurrences may occur but are unusual.

Feline rickettsiosis. *Rickettsia spp.* are obligate intracellular gram negative bacteria that are divided into the spotted fever group and the typhus group. Cats can be infected by *Rickettsia felis*, the primary flea associated *Rickettsia* spp., and have been shown to have antibodies against *R. rickettsii*, which is tick borne. *Rickettsia felis* DNA has been amplified from *C. felis*, *C. canis,* and *Pulex irritans;* these fleas have a worldwide distribution. *Ctenocephalides felis* is a biological vector for *R. felis*; the organism can be transmitted transovarially and transtadially within the flea. Rickettsial infection is suspected to a cause of fever in cats but this has not been well documented. While we have commonly amplified *R. felis* from *C. felis* (67.4% of flea extracts in one study), we have not amplified the organism from the blood of healthy cats or cats with fever. It is now known that dogs are a more important reservoir for this agent.

In one study of cats with fever we showed *R. felis* and *R. rickettsii* antibody prevalence rates in cats in the USA to be 5.6% and 6.6%, respectively but neither organism was amplified from blood. In Spain, *R. conorii* and *R. massiliae* antibodies were found in cat serum and DNA amplified from cat blood, suggesting cats could play a role in the life cycles of these agents, or be clinically affected (Segura et al, 2014). These results prove that cats are sometimes exposed to spotted fever group organisms but further data are needed to determine significance of diseases associations. Because clinical illness in cats has not been documented, optimal treatment is unknown. However, based on results in dogs with *R. rickettsia* infection, doxycycline or a fluoroquinolone would be logical choices.

Summary. Tick control is warranted for cats as well as dogs. Products with efficacy against fleas should also be used as fleas can be vectors for several *Bartonella* spp., potentially the hemoplasmas, potentially *Coxiella burnetii*, (Cypress), *R. felis* and *Yersinia pestis*.

References available on request to mlappin@colostate.edu