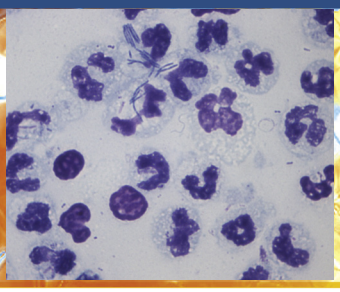

SECTION 2

Bacterial Diseases

CHAPTER 28

Ehrlichiosis

Jane E. Sykes

Overview of Ehrlichiosis

First Described: *Ehrlichia canis* was first described in 1935 (Algeria).[1] *Ehrlichia ewingii* was described in 1992 (United States).[2] *Ehrlichia chaffeensis* was described in 1991 (United States).[3]

Cause: *E. canis* (canine monocytic ehrlichiosis), *E. ewingii* (canine granulocytic ehrlichiosis), and *E. chaffeensis* (human monocytic ehrlichiosis); an *E. muris*-like organism also may infect dogs in the United States.[4]

Affected Hosts: *E. canis* causes disease in dogs. *E. canis* or a closely related organism may cause disease in cats and humans. *E. ewingii* causes disease in dogs, humans, and goats. *E. chaffeensis* causes disease in humans, possibly dogs and goats. The *E. muris*-like agent causes disease in humans and possibly dogs.

Geographic Distribution: *E. canis* is present worldwide, but especially in tropical and subtropical regions. *E. ewingii* is primarily found in the south-central and southeastern United States. Most reports of *E. chaffeensis* infection are from the southern and south-central United States. The *E. muris*-like agent has been found in the upper Midwest.

Mode of Transmission: *Rhipicephalus sanguineus* ticks (*E. canis*), *Amblyomma americanum* ticks (*E. ewingii* and *E. chaffeensis*).

Major Clinical Signs: The major clinical signs of *E. canis* infection are fever, lethargy, inappetence, weight loss, mucosal hemorrhages, uveitis, pallor, edema, and sometimes neurologic signs. *E. ewingii* primarily causes fever, lethargy, inappetence, and signs of polyarthritis.

Differential Diagnoses: Other tick-borne diseases (such as granulocytic anaplasmosis, Lyme borreliosis and babesiosis), bartonellosis, leptospirosis, lymphoma, multiple myeloma, systemic primary immune-mediated disease

Human Health Significance: All four ehrlichial species can infect and cause disease in humans. The most important human pathogen is *E. chaffeensis*, but the *E. muris*-like agent may also be important.

The ehrlichioses are a group of tick-transmitted diseases caused by intracellular, gram-negative bacteria that include *Ehrlichia canis*, *Ehrlichia ewingii*, and *Ehrlichia chaffeensis*. An organism related to *Ehrlichia ruminantium*, the cause of heartwater disease in cattle, has also been detected in ill dogs from South Africa,[5] and an organism that resembles *E. muris* has been detected in an ill dog and humans from the upper Midwest of the United States.[4] These organisms form morulae (Latin for "mulberry"), a cluster of bacteria, within phagosomes of circulating leukocytes. *Ehrlichia canis* infects monocytes and causes canine monocytic ehrlichiosis (CME), one of the most important infectious diseases of domestic dogs that are exposed to ticks worldwide. *Ehrlichia ewingii* is an unculturable bacterium that infects granulocytes and causes canine granulocytic ehrlichiosis. *Ehrlichia chaffeensis* causes human monocytic ehrlichiosis; dogs are a proposed reservoir for this organism. The geographic distribution of each pathogen is generally restricted to that of their vectors and mammalian reservoir hosts.

Organisms from the genus *Ehrlichia* are grouped within the family Anaplasmataceae. Also within this family are the bacteria *Anaplasma platys* and *Anaplasma phagocytophilum*, which cause canine thrombocytic and granulocytic anaplasmosis, respectively (see Chapter 29); and organisms belonging to the genera *Neorickettsia* (see Chapter 31). *Rickettsia rickettsii*, the cause of Rocky Mountain spotted fever (RMSF), and other spotted fever group rickettsiae belong to a separate family, the Rickettsiaceae (Chapter 30). The families Rickettsiaceae and Anaplasmataceae are phylogenetically related through the order Rickettsiales (Table 28-1). The recent availability of complete genome sequences for these organisms has helped

TABLE 28-1

Members of the Order Rickettsiales of Clinical Importance in Dogs and Cats

Family		Anaplasmataceae		Rickettsiaceae
Genus	*Ehrlichia*	*Anaplasma*	*Neorickettsia*	*Rickettsia*
Species	*E. canis* *E. chaffeensis* *E. ewingii*	*A. phagocytophilum* *A. platys*	*N. helminthoeca* *N. risticii*	*R. rickettsii*

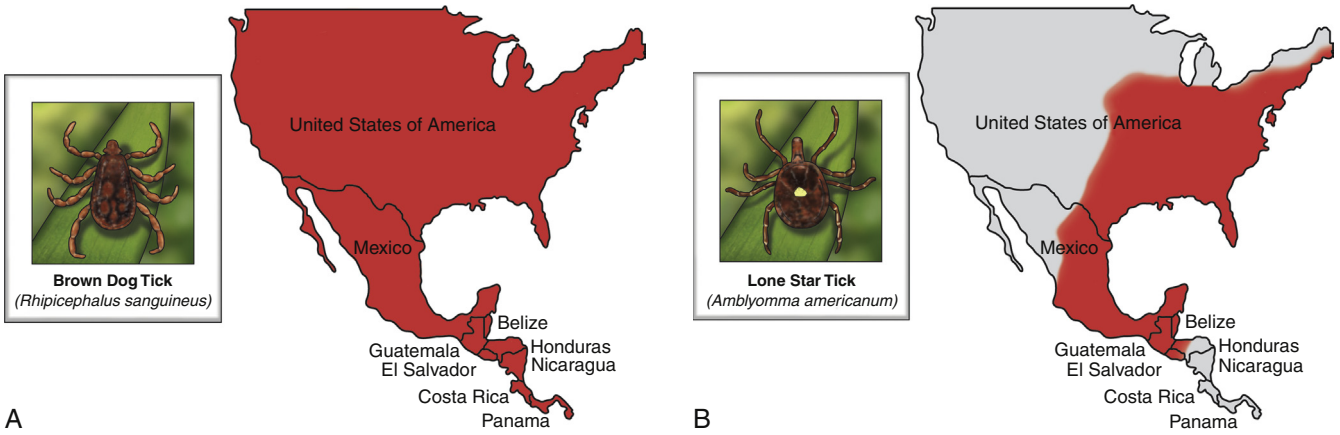

FIGURE 28-1 **A,** Distribution of *Rhipicephalus sanguineus*, which transmits *Ehrlichia canis,* in the United States, Mexico and Central America. **B,** Distribution of *Amblyomma americanum*, which transmits *Ehrlichia ewingii* and *Ehrlichia chaffeensis*. The distribution of *E. ewingii* infections in dogs more closely matches that of *A. americanum* than the distribution of *E. canis* matches that of *R. sanguineus* (highest prevalence in the southern states). This is because of chronic *E. canis* infections that occur in dogs with travel histories to southern states, where the climate is warmer and *R. sanguineus* is more prevalent.

to elucidate mechanisms of pathogenesis and host-pathogen interactions.[5-7]

The severity of clinical signs in animals with ehrlichial infections depends on factors such as the size of the inoculum, host immunity, and organism species and strain. Because of shared arthropod vectors and/or concurrent exposure to multiple vector ticks, co-infections with more than one rickettsial pathogen, as well as other arthropod-borne pathogens such as *Babesia* spp., and *Bartonella* spp., occur commonly in dogs and may complicate the clinical picture.

Ehrlichia canis Infection

Etiology and Epidemiology

E. canis is transmitted primarily by the brown dog tick *(Rhipicephalus sanguineus),* one of the most widely distributed ticks worldwide. Infection has been reported in dogs from Asia, Africa, Europe, and the Americas. Australia appears to be free of *E. canis* infection although occasionally seroreactivity to *E. canis* has been identified in dogs. The DNA of *E. canis* has been detected in other tick species, which include other *Rhipicephalus* species,[8,9] *Ixodes ricinus*,[10] *Haemaphysalis* spp. ticks,[11] and *Dermacentor* spp. ticks[11]; experimental transmission has been accomplished with *Dermacentor variabilis* ticks.[12] Different strains of *E. canis* exist that may vary in virulence. Although *R. sanguineus* is found throughout the United States, it prefers warm climates, and so disease is diagnosed most frequently in dogs living in the southeastern and southwestern states (Figure 28-1). In Europe, *R. sanguineus* is primarily found in Mediterranean regions, but its

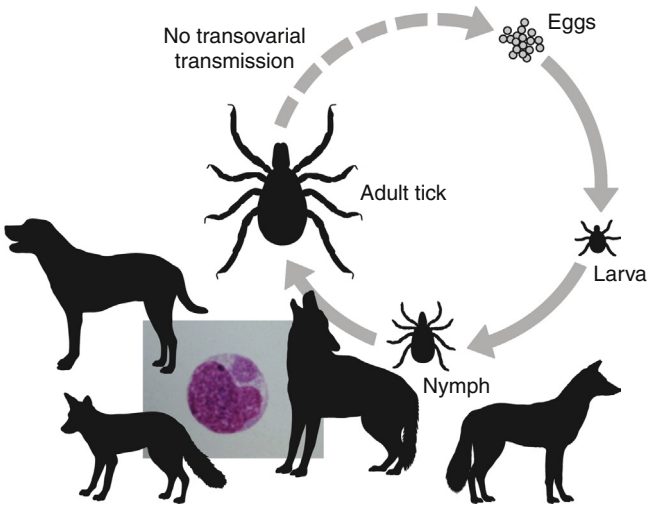

FIGURE 28-2 Life cycle of *Ehrlichia canis*. The organism is transmitted only transstadially (from larva to nymph to adult) within the tick. Jackals, foxes, and possibly coyotes also act as reservoir hosts. A morula is shown within the cytoplasm of a monocyte as seen on a blood smear.

distribution appears to be moving northward, and CME has been reported in dogs that lack travel history as far north as the Netherlands.[13] *E. canis* is the most common pathogen detected in ticks in Israel.[8] Because of chronic, subclinical infection, dogs can be transported to non-endemic regions and subsequently develop disease years later. Tick larvae or nymphs acquire infection when they feed on infected dogs. Jackals, foxes, and possibly coyotes may also act as reservoir hosts. *E. canis* is transmitted transstadially

(i.e., from larva to nymph to adult) within the tick (Figure 28-2).[14] No clear age or sex predilection for CME exists, but German shepherds are reportedly more susceptible, and prognosis may be poorer in this breed. Cross-bred dogs may be less likely to develop disease.[15] Although natural infection of cats with *E. canis* (or one or more closely related organisms) has been described in North and South America,[16-18] clinical ehrlichiosis is rarely reported.

Clinical Features

Signs and Their Pathogenesis

The course of CME has been divided into acute, subclinical, and chronic phases, although in naturally infected dogs, these phases may not be readily distinguishable. Clinical signs of acute disease occur 8 to 20 days after infection. The organism multiplies by binary fission within vacuoles of mononuclear phagocytes; rupture of infected host cells leads to infection of new cells. Immune-mediated mechanisms are important in the pathogenesis of disease, and the presence of the spleen appears to contribute to disease severity.[19] The clinical manifestations vary considerably among dogs, which may reflect factors such as *E. canis* strain variation, host immune response, stage of disease, and concurrent infections. Lethargy, inappetence, fever, and weight loss are most common. Replication of the organism in reticuloendothelial tissues is associated with generalized lymphadenopathy and splenomegaly. Ocular and nasal discharges, peripheral edema, and, less commonly, mucosal and cutaneous petechial and ecchymotic hemorrhages can also occur. Bleeding tendencies result from thrombocytopenia and platelet dysfunction,[20] which may reflect immune-mediated platelet damage.[21] Neurologic signs may result from meningeal inflammation or hemorrhage. Dogs can recover spontaneously from the acute phase within 2 to 4 weeks, after which time they may eliminate the infection or remain subclinically infected. Sequestration of organisms within the spleen may occur, and the organisms may evade the host immune system through antigenic variation.[5] This subclinical phase may persist for months to years.

Chronic CME develops in only some infected dogs. Factors that influence the development of chronic disease are unclear, but genetics may play a role. The presence of pancytopenia typifies the severe chronic form of ehrlichiosis, and results from hypoplasia of all bone marrow cells.[22] Clinical signs range in severity and include lethargy, inappetence, bleeding tendencies, mucosal pallor, fever, weight loss, lymphadenopathy, splenomegaly, dyspnea, anterior uveitis, retinal hemorrhage and detachment, polyuria/polydipsia, and edema.[15,22-24] Polymyositis occurs in some dogs, which can be manifested by diffuse muscle wasting and tetraparesis.[25] Secondary opportunistic infections such as viral papillomatosis, protozoal infections, and bacterial urinary tract infections can also develop, although the precise underlying mechanism of immunosuppression, and how it relates to successful persistence of *E. canis*, has not yet been elucidated (see Figure 26-5).[26,27] Marked granular lymphocytosis and bone marrow plasmacytosis may occur, sometimes accompanied by a monoclonal gammopathy, which may lead to misdiagnosis of lymphocytic leukemia or multiple myeloma, respectively. This has led to the recommendation that all dogs with well-differentiated lymphocytosis or otherwise unexplained monoclonal gammopathy be tested for *E. canis* infection.[28] Protein-losing nephropathy may develop as a result of immune-complex glomerulonephritis.

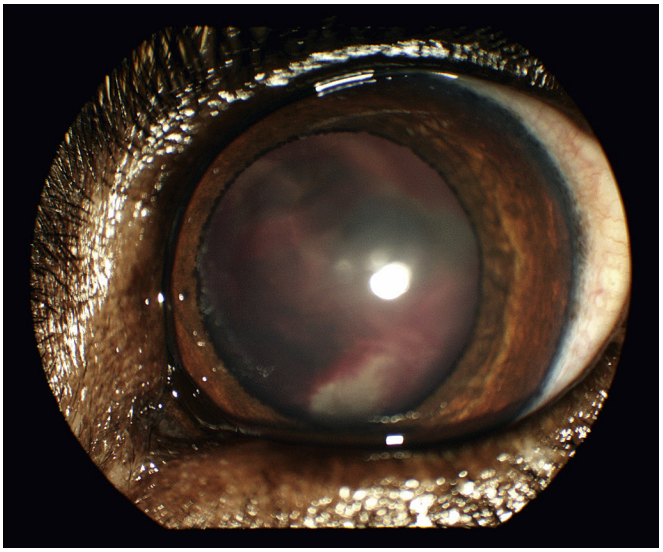

FIGURE 28-3 Retinal hemorrhage and detachment in a 4-year old female spayed Rhodesian ridgeback mix with canine monocytic ehrlichiosis. The dog also had moderate to severe, poorly regenerative, macrocytic hypochromic anemia (16.4% with a reticulocyte count of 49,000), lymphopenia (94 cells/μL), thrombocytopenia (26,000 platelets/μL), and large numbers of circulating nucleated red blood cells (75/100 WBC). Systolic blood pressure was within normal limits. (Courtesy of University of California, Davis Veterinary Ophthalmology Service.)

In reports of feline ehrlichiosis, clinical and laboratory findings have generally been similar to those in dogs; one cat had polyarthritis.[16]

Physical Examination Findings

Common physical examination findings in dogs with CME are lethargy, fever, peripheral lymphadenopathy, and splenomegaly. Ocular and nasal discharge, mucosal petechial hemorrhages, epistaxis, peripheral edema, and/or neurologic signs may be evident. Ocular abnormalities include anterior uveitis, hyphema, retinal hemorrhage, retinal detachment, and optic neuritis, with anterior uveitis being most common (Figure 28-3).[24,29] Neurologic signs include twitching, ataxia, seizures, vestibular signs, hyperesthesia, and cranial nerve defects. Dogs with chronic ehrlichiosis may have thin body condition or diffuse muscle atrophy and mucosal pallor. Findings in cats with ehrlichiosis have been similar to those in dogs, and include lethargy, splenomegaly, lymphadenopathy, petechial hemorrhages, and retinal detachment.[16,17]

Diagnosis

Laboratory Abnormalities

Complete Blood Count

Thrombocytopenia and occasionally mild leukopenia and a nonregenerative anemia occur 1 to 4 weeks after infection with *E. canis*. Mild thrombocytopenia, with increased mean platelet volume, may persist during the subclinical phase. Classically, dogs with chronic CME are pancytopenic, but more commonly, nonregenerative anemia and thrombocytopenia are noted. In some dogs, regenerative anemia or leukocytosis due to a neutrophilia and band neutrophils are present. Lymphopenia occurs in most affected dogs, but in some dogs, moderate to marked granular lymphocytosis (up to 17,000/μL) can occur. Normoblastosis, that can exceed 50 nucleated RBC per 100 WBC, may be present.

In some dogs, morulae are visualized within circulating monocytes (Figure 28-2). The finding of morulae within monocytes using cytologic evaluation of blood smears is insensitive, especially in dogs with chronic infection, and does not distinguish between *E. canis* and *E. chaffeensis* infection. Use of buffy coat smears, thin smears of blood collected from the margin of the pinna, or splenic aspirates increases the sensitivity for detection of morulae. In one study, after careful searching, morulae were found in only 2 of 19 dogs with CME.[22]

Serum Biochemical Tests

Serum chemistry abnormalities in chronic ehrlichiosis include variable hypoalbuminemia, hyperglobulinemia, and elevated ALT and ALP activities. Most often the hyperglobulinemia is due to a polyclonal gammopathy.[15] Monoclonal gammopathy can also develop. Less commonly, increases in serum urea nitrogen and creatinine concentrations are present.[22]

Urinalysis

Transient proteinuria, with urine protein:creatinine ratios that exceed 20 (reference range, <1) have been reported in dogs with acute CME. This can resolve by 6 weeks after infection.[30,31] Dogs with chronic CME may also have evidence of proteinuria. Pyuria, hematuria, and cylindruria may also be present.

Coagulation Profile

In addition to thrombocytopenia, coagulation abnormalities in dogs with CME include prolongation of the buccal mucosal bleeding time (BMBT), decreased platelet aggregation, and prolongation of the APTT.[32,33]

Cerebrospinal Fluid Analysis

Dogs with central nervous system (CNS) involvement may have increased CSF protein concentrations and lymphocytic pleocytosis.[24] Although rarely found, morulae may be detected in cells within the CSF.[34]

Bone Marrow Analysis

In dogs with chronic CME, bone marrow findings include hypoplasia or aplasia of all bone marrow elements, decreased iron stores and marrow plasmacytosis (Figure 28-4). Bone marrow mastocytosis has also been described.[35] Myelofibrosis does not typically develop in chronic CME.[36] Some dogs have normal or hypercellular marrows.

Diagnostic Imaging

Plain Radiography

Thoracic radiographs in dogs with CME often show no significant abnormalities, but sometimes bronchointerstitial infiltrates are present. This may reflect an underlying interstitial pneumonia.

Sonographic Findings

Findings on abdominal ultrasonography are nonspecific and include splenomegaly, alterations in splenic echotexture, enlargement and hypoechogenicity of the abdominal lymph nodes, and scant peritoneal effusion. Increased renal echogenicity and decreased corticomedullary definition may occur in dogs with glomerulonephritis.

Microbiologic Tests

Available diagnostic assays for ehrlichiosis in dogs and cats are listed in Table 28-2.

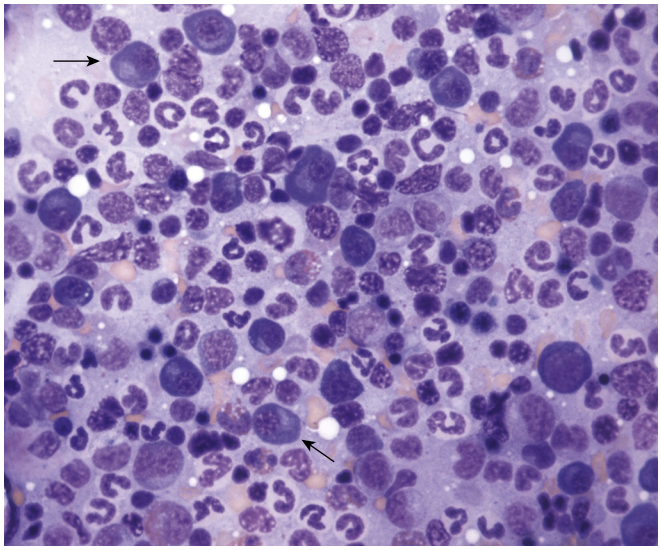

FIGURE 28-4 Bone marrow plasmacytosis in an 8-year-old female spayed Labrador retriever with canine monocytic ehrlichiosis. Serum globulin was 12.1 g/dL (reference range, 2.3-4.4 g/dL), and serum protein electrophoresis revealed a polyclonal gammopathy. Plasma cells have a clock-faced nucleus and a clear area adjacent to the nucleus; two plasma cells are identified with arrows. Mild to moderate megakaryocyte hyperplasia, mild erythroid hypoplasia, and mild mature granular lymphocytosis were also present. Romanowsky stain.

Serologic Diagnosis

Most often, the diagnosis of CME is made using serology, which may be performed using indirect immunofluorescent antibody (IFA) testing, ELISA technology, or Western blotting. Using IFA testing, which is considered the gold standard, antibodies can be detected between 7 and 28 days after initial infection. Dogs with acute ehrlichiosis may have false-negative test results if sufficient time has not elapsed for antibody production to occur. PCR assays may be helpful for diagnosis in this situation. A positive initial serum antibody titer may reflect previous exposure, and not necessarily ehrlichial disease. Retesting should be performed 2 to 3 weeks later to demonstrate seroconversion, and results of serology should be interpreted in light of a dog's clinical signs and the results of testing for other potential causes of the dog's illness. Dogs with chronic *E. canis* infection frequently have extremely high IFA titers, sometimes greater than 1:600,000, and these antibodies may persist in the face of treatment, suggesting persistence of the organism.[22] Seroconversion does not generally occur in dogs with chronic disease, although antibody titers may decline in some dogs with treatment. High titers do not correlate with the severity of hyperglobulinemia, disease in general, or duration of illness. Because of variability of reporting between laboratories, there is no standard "cutoff" titer that is used to separate positive and negative results. Serologic cross-reactivity to other *Ehrlichia* species occurs, which includes *E. ewingii* and especially *E. chaffeensis*. Cross-reactivity to *A. phagocytophilum* antigens can occur to a lesser extent. In areas where other rickettsial agents are endemic, Western blotting has been used in an attempt to confirm that positive antibody titers on IFA are truly to *E. canis* antigens. However, Western blotting is laborious to perform and interpret and not routinely available, and when *E. canis* antigens are the target used, it may be difficult to distinguish between *E. canis* and *E. chaffeensis* infection.[37] As a result, Western blotting has predominantly been used on a research basis.

TABLE 28-2

Diagnostic Assays Available for Ehrlichiosis in Dogs and Cats

Assay	Specimen Type	Target	Performance
Cell culture	Whole blood	*Ehrlichia canis*; *Ehrlichia ewingii* cannot be cultured	Not widely offered or utilized for routine diagnostic purposes. Requires several weeks' incubation.
Morula detection	Whole blood, buffy coat smears, body fluids, tissue aspirates	*E. canis* or *E. ewingii* morulae	Low sensitivity (especially for chronic *E. canis* infection). *E. canis* morulae cannot be distinguished from those of *E. chaffeensis*, and *E. ewingii* morulae cannot be differentiated from those of *A. phagocytophilum*. Morulae may be confused with platelets, cytoplasmic granules, phagocytized nuclear material, and lymphoglandular bodies.
IFA serology	Serum	Antibodies to *E. canis*	Acute and convalescent serology is required for diagnosis of acute infection, because initial results may be negative in dogs with acute disease, and positive results can reflect previous exposure rather than active infection. Dogs with chronic infection generally do not seroconvert. Cross-reactivity occurs to other ehrlichial species and occasionally to *Anaplasma* spp.
ELISA serology	Serum	Antibodies to *E. canis* or *E. ewingii* antigens	Rapid, inexpensive, can be performed as an in-practice test. Similar limitations as for IFA. Lack of quantitation limits ability to document seroconversion.
Western immunoblotting	Serum	Antibodies to specific *E. canis* or *E. ewingii* antigens	Technically difficult; primarily used on a research basis to identify serologic responses to specific ehrlichial species. May be difficult to distinguish antibody responses to *E. canis* and *E. ewingii*.
PCR	Whole blood; spleen, lymph node or bone marrow aspirates; buffy coat or tissue specimens	*E. canis*, *E. muris*, or *E. ewingii* DNA	Confirms active infection. Sensitivity and specificity may vary depending on assay design and specimen type. Sensitivity for diagnosis of chronic CME may be low. Assays that specifically detect *E. muris* are not widely available on a commercial basis.

CME, Canine monocytic ehrlichiosis; IFA, immunofluorescent antibody.

A variety of ELISA assays have been developed for detection of antibodies to *E. canis*. A point-of-care lateral-flow ELISA device for the simultaneous detection of canine heartworm antigen, antibodies to *E. canis* or *E. ewingii*, antibodies to *Borrelia burgdorferi*, and antibodies to *Anaplasma* spp. in canine serum, plasma, or whole blood has been marketed for use in companion animals (SNAP 4Dx Plus, IDEXX Laboratories, Westbrook, ME), which includes recombinant surface proteins of *E. canis* and *E. ewingii* on a single spot. According to the manufacturer, when IFA and Western blotting were used as the gold standard, the sensitivity and specificity of the *E. canis* antigen for detection of *E. canis* antibodies in 104 samples positive for *E. canis* antibodies and 236 samples negative for *E. canis* antibodies was found to be 96.2% and 100%, respectively. Other point-of-care ELISA assays for detection of *E. canis* antibodies have also been developed. A silicon disc-based assay is available in the United States through Antech Diagnostic laboratories that detects *Dirofilaria immitis* antigen and antibodies to *E. canis*, *Anaplasma* spp., and *B. burgdorferi* (Accuplex 4, Antech Diagnostics, Irvine, CA). The performance of this assay for diagnosis of ehrlichiosis has not been thoroughly investigated at the time of writing. The incidental finding of *E. canis* seroreactivity in dogs screened using these assays for heartworm antigenemia should prompt performance of a thorough physical examination and basic laboratory testing (CBC, chemistry panel, and urinalysis) to evaluate for thrombocytopenia, hyperglobulinemia, and proteinuria. When sick dogs test positive, quantitative serology should be performed so that a titer can be obtained as a baseline for acute and convalescent serologic testing or, for dogs suspected to have chronic CME, to evaluate for titers of very high magnitude that might be consistent with a dysregulated immune response to the organism.

Molecular Diagnosis Using the Polymerase Chain Reaction

Whole-blood PCR assays for *E. canis* DNA is more sensitive for early diagnosis of CME than IFA or ELISA in dogs with acute disease. PCR assays are widely available for routine diagnosis of *E. canis* infection. Several laboratories offer panels that include PCR assays for a variety of different vector-borne pathogens. The results of these assays should be interpreted in light of a dog's history, clinical signs, and the results of appropriate serologic assays; the last should be performed to support the results of PCR testing. PCR assays for *E. canis* may be performed on

blood, lymph node aspirates, splenic aspirates, or bone marrow. Convalescent IFA or ELISA testing is much more sensitive than PCR assays for diagnosis of chronic CME.[22,38,39] The sensitivity of PCR assays for diagnosis of CME when performed on bone marrow in dogs with chronic ehrlichiosis can range from 25% to 68%, depending on the laboratory.[22] The use of PCR assays in the absence of serology is currently not suitable for screening potential blood donors for infection. PCR assays may be useful to confirm infection in the first week of illness, when serologic assays are often negative. Depending on the assay used, when positive, PCR can also be used to confirm the *Ehrlichia* species involved.

Blood Culture

E. canis can be cultured in certain cell lines, such as DH82 cells. This is time-consuming and generally performed only on a research basis.

Pathologic Findings

Gross pathologic findings in CME include widespread petechial and ecchymotic hemorrhages, generalized pallor, edema, lymphadenopathy, and splenomegaly.[40,41] Ascites may also be present. On histopathology, lymphoid and plasma cell hyperplasia, lymphoplasmacytic infiltrates, and vasculitis may be present in numerous organs, such as the brain, eye, spinal cord, spleen, liver, kidneys, lymph nodes, bone marrow, and lungs. Histiocytic infiltrates may be found in lymph nodes. With chronicity, the proportion of plasma cells increases. Non-suppurative meningitis and perivascular cuffing within the CNS can also occur.[40-42] Glomerulonephritis may be evident,[40] although dogs with acute CME have had interstitial nephritis and electron microscopic abnormalities that are more consistent with minimal-change glomerulonephritis, with fusion of podocyte processes.[30] Little information is available in regard to the prevalence and type(s) of glomerulonephritis that develop in dogs with chronic CME.

Treatment and Prognosis

Antimicrobial Treatment

The treatment of choice for CME is doxycycline (10 mg/kg PO q24h) (Table 28-3). It was the consensus of the ACVIM Infectious Disease Study Group that dogs and cats should be treated for a minimum of 28 days.[28] Mixed results have been obtained in studies that have evaluated the efficacy of doxycycline for treatment of *E. canis* infection. One study suggested that acute infection can be eliminated after treatment for just 16 days.[43] Another study that used ticks to infect dogs showed a failure of doxycycline, when given for 14 days, to eliminate the organism from subclinically infected dogs.[44] In another study, dogs with acute and subclinical infections became negative by PCR assay on blood after 28 days of doxycycline treatment, but dogs with chronic infections remained intermittently positive. However, ticks still became PCR positive after they fed on treated dogs, regardless of the stage of disease when treatment was initiated.[45]

Whether or not persistence of infection occurs, most dogs with acute disease show clinical improvement within 24 to 48 hours. Dogs with severe chronic disease may not respond to therapy, or cytopenias may resolve over a period of several months. Strong risk factors for mortality in one study were severe leukopenia (WBC <930 cells/μL), severe anemia (HCT <11.5%), hypokalemia (<3.7 mmol/L), and prolonged APTT (>18.25 s).[46] Platelet counts generally improve and normalize by 2 weeks following institution of therapy. After treatment, titers can decline and become negative in 6 to 9 months. Some dogs retain high titers for several years. Treatment for these dogs should be based on resolution of platelet counts and improvement of hyperglobulinemia, although hyperglobulinemia can resolve over several months after treatment is discontinued. The use of PCR assays on splenic aspirates could be considered to determine if persistent infection is present in these dogs, but whether ongoing treatment with doxycycline changes the outcome for these dogs is unknown. Platelet counts should be reassessed 1 and 3 months after discontinuation of therapy, because of the potential for relapse or reinfection. Other causes of illness (especially other vector-borne diseases such as babesiosis or bartonellosis) should also be considered in dogs that fail to respond to treatment.

TABLE 28-3

Antimicrobial Drug Doses Used for Treatment of Ehrlichiosis in Dogs and Cats

Drug	Dose	Route	Interval (hours)	Minimum Duration (days)
Doxycycline	5 mg/kg	PO, IV	12*	*E. canis* 28 days; *E. ewingii* 14 days
Oxytetracycline	7.5-10 mg/kg	IV	12	Until gastrointestinal signs abate, and then change to oral doxycycline as above

*Or 10 mg/kg PO q24h.

Other drugs used to treat CME with variable success are chloramphenicol, imidocarb dipropionate, and enrofloxacin.[47-50] *Ehrlichia canis* appears to have intrinsic gyrase-mediated resistance to fluoroquinolones,[51] so although their use can be associated with clinical improvement, it is not recommended. The antiprotozoal drug imidocarb dipropionate appeared efficacious for treatment of *E. canis* infection in some studies but not others.[48,49]

At this time, treatment of seroreactive but otherwise healthy dogs that have normal routine bloodwork results is controversial, because it is unknown if treatment changes outcome for these dogs and has the potential to lead to antimicrobial resistance or adverse effects of drug therapy.

Supportive Care

For dogs with CME that are dehydrated or anemic, IV fluids or blood products may be required. Use of erythropoietin and granulocyte colony-stimulating factor together with prednisone was associated with treatment success in a dog with severe chronic ehrlichiosis in one case report.[52] Desmopressin acetate (DDAVP) (1 mcg/kg SC q24h) for 3 days appeared to be efficacious to treat bleeding disorders in a few dogs with CME,[33] and treatment resulted in reduction of the BMBT and APTT. If thrombocytopenia fails to resolve with

doxycycline administration, a short course (up to a week) of therapy with immunosuppressive doses of glucocorticoids could be considered in addition to ongoing therapy with doxycycline.

Immunity and Vaccination

A vaccine for *E. canis* infection is currently not available, but in one study vaccination of dogs with an attenuated strain dramatically reduced disease in dogs challenged with a virulent strain of *E. canis*.[53] The immune response to *E. canis* infection is not well understood. Because *E. canis* is an intracellular pathogen, cell-mediated immune responses are required for pathogen elimination. In one study, dogs with acute infection developed CD8+ lymphocytosis that subsided after several weeks, despite organism persistence.[27] Cytokine studies suggest that the immune response may vary with the strain of *E. canis* involved.[54,55] Genetic factors are also likely to be important determinants of the immune response and outcome of infection.

Prevention

Avoidance of tick-infested areas and routine inspection of dogs for ticks after outdoor activities can help to prevent ehrlichiosis. Early removal of ticks may help to reduce transmission, because of the 24- to 36-hour delay that occurs between tick attachment and feeding. Clients should be instructed to remove ticks properly and avoid handling ticks with bare hands or crushing them, to prevent exposure to infected hemolymph. A variety of devices are available to assist tick removal, which are placed around the area where the mouthparts enter the skin to avoid crushing or squeezing the tick and leaving the mouthparts behind. Fine-tipped tweezers can be used to grasp the tick as close to the skin as possible, followed by steady retraction to remove the tick. The bite wound should then be thoroughly cleaned with a suitable antiseptic solution (such as iodine or chlorhexidine-based antiseptics) or soap and water. Ticks can be disposed in alcohol or tested for vector-borne pathogens using PCR assays.

Topical ectoparasiticides with activity against ticks also prevent tick-borne infectious diseases. Examples of canine ectoparasiticides with activity against ticks include those that contain amitraz, fipronil, pyrethroids (permethrin, etofenprox, pyrethrin, deltamethrin, flumethrin), and selamectin. In one field study, the use of monthly permethrin effectively prevented infection with *E. canis* by kenneled dogs.[56] Products that contain pyrethroids and amitraz have the most potent activity against ticks. Permethrin, deltamethrin, and amitraz cannot be used in cats, and use of these products should be avoided on dogs that co-habitate with cats. Flumethrin collars are available for use on cats (and on dogs) because unlike the other pyrethroids, flumethrin does not require hepatic glucaronidation for metabolism.[57] Products that contain amitraz, a monoamine oxidase inhibitor, should not be used in dogs treated with selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, and pet owners that are receiving SSRIs should also use alternative preventatives for their dogs. Preventatives should be applied consistently at the recommended interval for optimum activity. No preventatives completely protect dogs or cats from tick attachment, especially where tick infestation rates are high. Unfortunately, acaricide-resistant strains of *R. sanguineus* have been reported as a result of indiscriminate use of these drugs.[58]

Low dose doxycycline (6.6 mg/kg q24h PO) has also been used to prevent infection in dogs residing in kennels in which E. canis infection is a problem. Resistance to doxycycline remains a theoretical concern in this situation.

Because of chronic, subclinical infection, blood donor dogs should be screened with serology (or serology and PCR assays) for evidence of *E. canis* exposure or infection. All seropositive dogs should be excluded as donors. Transport of chronically infected dogs to non-endemic regions has the potential to introduce the infection or new strains of *E. canis* to these regions if the appropriate tick vectors are present.

Public Health Aspects

E. canis DNA has been detected in some human patients with clinical signs of human monocytic ehrlichiosis,[59] suggesting that *E. canis* might be a cause of monocytic ehrlichiosis in people. Appropriate precautions should be taken to prevent transmission when handling engorged ticks as well as blood and tissue specimens from infected dogs, and care should be taken to prevent needle-stick injuries.

Ehrlichia ewingii Infection

Etiology and Epidemiology

Ehrlichia ewingii is an unculturable bacterium that causes granulocytic ehrlichiosis in humans and in dogs. It was first recognized in dogs[2] and occurs in North America and more recently has been detected in dogs from Africa and Brazil.[60,61] Infection occurs in the south-central and southeastern parts of the United States, which reflects the distribution of the primary tick vector, *Amblyomma americanum*. Transmission within the tick is transstadial. More than 40% of dogs from an endemic area in Oklahoma and Arkansas were seropositive.[62] In another large study the overall seroprevalence was 14.5% in the central states (Oklahoma, Arkansas, Missouri, and Kansas) and 5.9% in the southeast.[63] In one study, most cases occurred from May through July,[64] but in another, cases occurred throughout the year.[65] In the study from the south-central United States, dogs were more likely to test positive with a PCR assay in August.[62] *E. ewingii* is maintained in white-tailed deer.[66] The DNA of *E. ewingii* has also been found in *Dermacentor variabilis* and *R. sanguineus* ticks,[67] but *A. americanum* is the only proven vector.

Clinical Features

Signs and Their Pathogenesis

In contrast to *E. canis* infection, *E. ewingii* infection appears to cause only acute disease; a chronic phase of disease has not been described. Like *A. phagocytophilum*, *E. ewingii* replicates in neutrophils and delays neutrophil apoptosis, which prolongs the life span of the host cell.[68]

Dogs with *E. ewingii* infection may show no signs, or fever, lethargy, anorexia, and neutrophilic polyarthritis may occur. After experimental infection, signs develop after an incubation period of 3 to 4 weeks.[69-71] Vomiting and diarrhea occur uncommonly. Neurologic signs have been described in some naturally infected dogs,[65] but the possibility of concurrent infections with

other pathogens such as *Rickettsia rickettsii* was not ruled out in these dogs. *E. ewingii* can be found in apparently healthy dogs, so dogs may act as a reservoir for infection.

Physical Examination Findings

On physical examination, dogs with *E. ewingii* infection may have evidence of lethargy, fever, lameness, reluctance to move, a stiff gait, joint effusion, and pain on joint palpation. Reported neurologic signs include anisocoria, tremors, and a head tilt.[65]

Diagnosis

Laboratory Abnormalities

Common laboratory findings in dogs with *E. ewingii* infection are nonregenerative anemia and thrombocytopenia.[65,72] Reactive lymphocytosis can also occur.[65] The biochemistry panel may be unremarkable or show mild nonspecific abnormalities. Synovial fluid analysis reveals neutrophilic polyarthritis. Morulae may be detected within granulocytes in the peripheral blood or synovial fluid, but are indistinguishable from those of *A. phagocytophilum*. Nevertheless, the finding of morulae in granulocytes in *E. ewingii* endemic areas and where *A. phagocytophilum* infection is uncommon or absent is strongly suggestive of *E. ewingii* infection. In experimental infections, morulae are visible in the peripheral blood of some dogs before the onset of clinical signs, around 2 to 3 weeks postinfection.[71]

Microbiologic Tests

Serologic Diagnosis

Serologic diagnosis of *E. ewingii* infection is currently limited to a point-of-care ELISA assay that detects the presence of antibodies to a specific peptide of *E. ewingii* (SNAP 4Dx Plus, IDEXX Laboratories, Westbrook, ME). Because this peptide is combined on a spot with a peptide that detects antibodies to *E. canis*, the antibody response to these pathogens cannot be distinguished from one another. Cross-reactions may also occur to other ehrlichial species such as *E. chaffeensis*. Antibodies appear approximately 1 month after infection.[71] Dogs with acute illness may initially test negative with this assay, and positive test results may reflect the presence of antibodies from previous exposure or subclinical infection. A change from a negative to a positive result over a 2- to 4-week time period may help support a diagnosis of *E. ewingii* infection in endemic areas.

Molecular Diagnosis Using the Polymerase Chain Reaction

Whole-blood PCR assays are available for specific diagnosis of *E. ewingii* infection through some veterinary diagnostic laboratories. The sensitivity of these assays may vary between laboratories. Currently *E. ewingii*–specific PCR assays are the only means to confirm active infection with *E. ewingii* (as opposed to infection with *A. phagocytophilum* or other *Ehrlichia* species). In experimental studies, PCR becomes positive as early as 4 days after inoculation.[71]

Treatment and Prognosis

Antimicrobial Treatment

For *E. ewingii* infection, treatment with doxycycline results in rapid (within 24 to 48 hours) clinical improvement. Treatment for 2 to 4 weeks may be sufficient to eliminate infection. Subclinically infected dogs that test positive with whole-blood PCR assays may spontaneously clear infection within weeks to months. Treatment may not be required for these dogs.

Prevention

Prevention of *E. ewingii* infection involves avoidance of tick exposure and use of tick preventatives (see previously). Early tick removal also has the potential to reduce transmission. Potential blood donor dogs could be screened for infection with *E. ewingii*–specific ELISA and PCR assays. PCR assays alone (i.e., without serology) should not be used to screen blood donors.

Public Health Aspects

Human infection with *E. ewingii* has been rarely described in endemic regions of the United States.[73] Affected people have had headache, fever, and thrombocytopenia, with or without leukopenia, and responded to doxycycline treatment. Most were receiving immunosuppressive drug therapy. Although direct dog-to-human transmission does not occur, blood from affected dogs should be handled with caution.

Ehrlichia chaffeensis Infection

Ehrlichia chaffeensis causes human monocytic ehrlichiosis in North America, an emerging disease that is characterized in human patients by fever, headache, myalgia, thrombocytopenia and leukopenia, and elevations in hepatic transaminases.[74] Gastrointestinal signs, neurologic involvement, and a toxic shock–like syndrome also occur in some infected people. In the United States, it occurs primarily in the south-central, southeastern, and mid-Atlantic states, which reflects the distribution of *A. americanum* and the concurrent presence of white-tailed deer, which are reservoirs for the organism (as for *E. ewingii*). Evidence of *E. chaffeensis* DNA has also been found in humans, dogs, other animal species (including cats[18]), and ticks in Africa, Israel, Central and South America, and Asia. The organism is transmitted transstadially within the tick,[75] which feeds aggressively on humans. In naturally infected dogs, *E. chaffeensis* infection has been associated with clinical signs of lymphadenopathy, anterior uveitis, and epistaxis,[76] but the clinical implications of this infection for dogs are still unclear. Dogs maintain high antibody titers and are PCR positive for months after infection, which supports a possible role of the dog as a reservoir.[77]

Ehrlichia muris-like Infection

Ehrlichia muris was first described in a wild mouse from Japan in the mid-1990s[78,79] and *E. muris* DNA was detected in a febrile person in Russia for the first time in 2008.[80] In 2009, the DNA with strong homology to that of *Ehrlichia muris* was detected in four humans from Minnesota and Wisconsin.[80] These individuals had clinical signs and laboratory abnormalities that resembled those of granulocytic anaplasmosis (see Chapter 29), were seronegative to *A. phagocytophilum*, and showed variable seroreactivity to *E. chaffeensis*. Two of the individuals were solid-organ transplant recipients and the other two people were apparently immunocompetent. Morulae were not seen, but in mice *E. muris* forms morulae in monocytes.[78] In 2012, DNA with homology to *E. muris* was detected in an

ill dog from northern Minnesota that was seronegative for *E. canis* and seroreactive to *A. phagocytophilum*,[4] although the extent to which this organism contributed to the dog's clinical signs was unclear. Because of the geographic distribution of infection, *Ixodes scapularis* is the suspected tick vector (see Chapter 29). *E. muris* DNA has been detected in *I. scapularis* ticks from northern Wisconsin.[81] The extent to which *E. muris* causes disease in dogs and humans in the United States and other countries requires further investigation but it should be considered as a possible cause of unexplained febrile illness in dogs, whether or not they have evidence of antibodies to *E. canis*.

CASE EXAMPLE

Signalment: "Ditto" a 13-year old male neutered border collie mix from Dixon, CA

History: Ditto was brought to the University of California, Davis, because of a 4-day history of lethargy and inappetence. Ditto had been drinking and there was no vomiting or diarrhea. There was no history of exposure to ticks, toxins, or trauma. Ditto was mainly confined to the backyard and did not have access to standing water. The dog had been adopted in Guam and brought to the United States several years ago. There was no other significant travel history. Ditto's diet consisted of a commercial senior dry dog food.

Current Medications: Prednisolone (0.45 mg/kg PO q48h) for allergic skin disease; this had been administered over the preceding 10 days.

Physical Examination:

Body Weight: 22.3 kg

General: Quiet, reluctant to stand. T = 102.2°F (39.0°C), HR = 152 beats/min, panting, mucous membranes pale pink, CRT <2 s. Approximately 5% to 7% dehydrated.

Integument: A sparse hair coat was present; there were no other clinically significant abnormalities. No ectoparasites were noted.

Eyes, Ears, Nose, and Throat: Severe dental calculus was present.

Musculoskeletal: Body condition score was 6/9. The dog would stand but was reluctant to walk.

Cardiovascular and Respiratory Systems: No clinically significant findings were present.

Gastrointestinal and Urogenital Systems: Cranial organomegaly was noted on abdominal palpation. Rectal examination revealed no significant abnormalities.

Lymph Nodes: All peripheral lymph nodes were normal sized.

Laboratory Findings:

CBC:

HCT 28.0% (40%-55%)
MCV 72.4 fL (65-75 fL)
MCHC 30.4 g/dL (33-36 g/dL)
Reticulocyte count 7600 cells/µL (7000-65,000 cells/µL)
Nucleated RBC 1/100 WBC
WBC 19,600 cells/µL (6000-13,000 cells/µL)
Neutrophils 15,876 cells/µL (3000-10,500 cells/µL)
Lymphocytes 392 cells/µL (1000-4000 cells/µL)
Monocytes 1568 cells/µL (150-1200 cells/µL)
Eosinophils 392 cells/µL (0-1500 cells/µL)
Basophils 392 cells/µL (0-50 cells/µL)
Platelets 127,000 platelets/µL (150,000-400,000 platelets/µL)
MPV 20.5 fL (7-13 fL).

Serum Chemistry Profile:

Sodium 141 mmol/L (145-154 mmol/L)
Potassium 4.2 mmol/L (3.6-5.3 mmol/L)
Chloride 105 mmol/L (108-118 mmol/L)
Bicarbonate 18 mmol/L (16-26 mmol/L)
Phosphorus 6.1 mg/dL (3.0-6.2 mg/dL)
Calcium 8.6 mg/dL (9.7-11.5 mg/dl)
BUN 55 mg/dL (5-21 mg/dL)
Creatinine 1.9 mg/dL (0.3-1.2 mg/dL)
Glucose 66 mg/dL (64-123 mg/dL)
Total protein 6.1 g/dL (5.4-7.6 g/dL)
Albumin 2.2 g/dL (3.0-4.4 g/dL)
Globulin 3.9 g/dL (1.8-3.9 g/dL)
ALT 49 U/L (19-67 U/L)
AST 153 U/L (19-42 U/L)
ALP 98 U/L (21-170 U/L)
Creatine kinase 341 U/L (51-399 U/L)
Gamma GT 4 U/L (0-6 U/L)
Cholesterol 282 mg/dL (135-361 mg/dL)
Total bilirubin 0.2 mg/dL (0-0.2 mg/dL)
Magnesium 2.6 mg/dL (1.5-2.6 mg/dL).

Urinalysis: SGr 1.018; pH 7.0, protein 75 mg/dL, no bilirubin, no glucose, hemoprotein 25 erythrocytes/µL, 0-2 WBC/HPF, 0-2 RBC/HPF, rare granular casts

Urine Protein: Creatinine Ratio: 19.3 (reference range, <1)

Plasma Antithrombin: 64% (reference range, 80%-120%)

Imaging Findings:

Thoracic Radiographs: The right cranial mainstem bronchus was slightly widened throughout its visible length and bronchiectasis was suspected. The cardiovascular and remaining pulmonary structures were within normal limits for the age of the patient.

Abdominal Radiographs: An ill-defined soft tissue structure was present that displaced the caudal border of the stomach cranially. The cecum was not well visualized. The liver extended past the costochondral arches with mildly rounded borders, which was interpreted as mild hepatomegaly. The soft tissue structure was thought to represent an enlarged lymph node, pancreas, or spleen.

Abdominal Ultrasound: The liver had normal parenchymal architecture. The gallbladder contained hyperechoic sludge. The spleen was enlarged with rounded edges and a heterogenous, more hypoechoic parenchyma. Both kidneys showed a somewhat thickened cortex with a good distinction between cortex and medulla. The left renal pelvis was mildly dilated, and the papilla was blunted. There were several cystic lesions within the abdomen that measured up to 3.6 cm × 5.4 cm. Septa were visualized within the cystic lesions. The cystic lesions appeared to arise from lymph nodes.

Cytologic Findings: Cytology of ultrasound-guided lymph node aspirate: There was a moderate amorphous basophilic proteinaceous background with low numbers of nucleated cells and erythrocytes. Nucleated cells were composed primarily of a mixed lymphocyte population with lower numbers of macrophages and nondegenerate. neutrophils.

Microbiologic Testing: 4Dx SNAP test serology (IDEXX Laboratories, ME): Positive for antibodies to *Ehrlichia canis*. Weakly positive for antibodies to *Anaplasma* species. Negative for antibodies to *Borrelia burgdorferi* and antigen of *Dirofilaria immitis*.

Vector-borne disease serology (IFA): Positive for antibodies to *Ehrlichia canis* at 1:163,840 and *Anaplasma phagocytophilum* at 1:2560. Negative for antibody to *Rickettsia rickettsii* at <1:40.

Vector-borne real-time PCR panel (whole blood): Positive for *Ehrlichia canis* DNA. Negative for *Anaplasma phagocytophilum, Anaplasma platys, Bartonella* spp., *Rickettsia* spp., and *Borrelia burgdorferi* DNA.

Diagnosis: Canine monocytic ehrlichiosis, characterized by thrombocytopenia, abdominal lymphadenopathy, and protein-losing nephropathy.

Treatment: Ditto was initially treated with IV crystalloids (lactated Ringer's solution supplemented with 20 mEq/L KCl), famotidine (0.5 mg/kg IV q12h), and ampicillin (20 mg/kg IV q8h). The hematocrit dropped to 18%, and 1 unit of packed RBC was administered, which was followed by a transfusion reaction, characterized by hemoglobinuria, vomiting, and icterus. Systolic blood pressure remained within normal limits throughout hospitalization. When the results of serology for *E. canis* were obtained, antimicrobial drug treatment was changed to doxycycline (5 mg/kg PO q12h). Enalapril (0.25 mg/kg PO q24h) and aspirin (0.5 mg/kg PO q24h) were used to manage the protein-losing nephropathy. Two days after initiation of doxycycline treatment, the dog's attitude and appetite improved. At discharge (day 9 of hospitalization), the hematocrit was 26.6%, reticulocytes 150,000 cells/μL, platelet count 496,000/μL, BUN 31 mg/dL, creatinine 1.5 mg/dL, albumin 1.8 g/dL, globulin 5 g/dL, and urine protein:creatinine ratio was 4. At a recheck examination 1 week after discharge, the hematocrit was stable, the platelet count was 635,000 platelets/μL, BUN 41 mg/dL, creatinine 1.3 mg/dL, and albumin 2.2 mg/dL. A kidney biopsy was not performed. Ditto was subsequently managed for protein-losing nephropathy with enalapril and a reduced protein diet for nearly 2 years, at which time hematocrit was 38.9%, platelet count 561,000 platelets/μL, BUN 27, creatinine 1.2 mg/dL, albumin 3.2 g/dL, globulin 3.6 g/dL, and the urine protein:creatinine ratio was 1.6. The spleen had returned to a sonographically normal appearance, and the cystic lymph nodes were markedly reduced in size. Additional serology or follow-up PCR was not performed. He was subsequently lost to follow-up.

Comments: In this dog, active infection with *E. canis* was confirmed through the use of whole-blood PCR assay. Where the dog became infected with *E. canis* was unclear, but infection was most likely acquired many years earlier in Guam, where ticks are abundant and *R. sanguineus* and *E. canis* are present. It is also unclear whether the glucocorticoid treatment played any role in reactivation of chronic infection. The improvement in hematologic and biochemical parameters in association with doxycycline treatment supported a role for *E. canis* in the disease. Hyperglobulinemia may have been initially masked by renal protein loss. In this case, treatment was continued for several months at the owner's request while laboratory parameters and ultrasound findings showed signs of progressive improvement. The seropositivity to *A. phagocytophilum* may have reflected previous exposure to this organism, *Anaplasma platys,* or serologic cross-reactivity between antibodies to *E. canis* and the *A. phagocytophilum* antigen used in the test kit.

SUGGESTED READINGS

Komnenou AA, Mylonakis ME, Kouti V, et al. Ocular manifestations of natural canine monocytic ehrlichiosis (*Ehrlichia canis*): a retrospective study of 90 cases. Vet Ophthalmol. 2007;10:137-142.

Mylonakis ME, Koutinas AF, Breitschwerdt EB, et al. Chronic canine ehrlichiosis (*Ehrlichia canis*): a retrospective study of 19 natural cases. J Am Anim Hosp Assoc. 2004;40:174-184.

REFERENCES

1. Donatien A, Lestoquard F. Existence en Algerie d'une *Rickettsia* du chien. Bull Soc Pathol Exot. 1935;28:418-419.
2. Anderson BE, Greene CE, Jones DC, et al. *Ehrlichia ewingii* sp. nov., the etiologic agent of canine granulocytic ehrlichiosis. Int J Syst Bacteriol. 1992;42:299-302.
3. Anderson BE, Dawson JE, Jones DC, et al. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. J Clin Microbiol. 1991;29:2838-2842.
4. Hegarty BC, Maggi RG, Koskinen P, et al. *Ehrlichia muris* infection in a dog from Minnesota. J Vet Intern Med. 2012;26:1217-1220.
5. Mavromatis K, Doyle CK, Lykidis A, et al. The genome of the obligately intracellular bacterium *Ehrlichia canis* reveals themes of complex membrane structure and immune evasion strategies. J Bacteriol. 2006;188:4015-4023.
6. Dunning Hotopp JC, Lin M, Madupu R, et al. Comparative genomics of emerging human ehrlichiosis agents. PLoS Genet. 2006;2:e21.
7. Felsheim RF, Kurtti TJ, Munderloh UG. Genome sequence of the endosymbiont *Rickettsia peacockii* and comparison with virulent *Rickettsia rickettsii*: identification of virulence factors. PLoS One. 2009;4:e8361.
8. Harrus S, Perlman-Avrahami A, Mumcuoglu KY, et al. Molecular detection of *Ehrlichia canis*, *Anaplasma bovis*, *Anaplasma platys*, *Candidatus* Midichloria mitochondrii and *Babesia canis vogeli* in ticks from Israel. Clin Microbiol Infect. 2011;17:459-463.
9. Masala G, Chisu V, Foxi C, et al. First detection of *Ehrlichia canis* in *Rhipicephalus bursa* ticks in Sardinia, Italy. Ticks Tick Borne Dis. 2012;3:396-397.
10. Wielinga PR, Gaasenbeek C, Fonville M, et al. Longitudinal analysis of tick densities and *Borrelia*, *Anaplasma*, and *Ehrlichia* infections of *Ixodes ricinus* ticks in different habitat areas in The Netherlands. Appl Environ Microbiol. 2006;72:7594-7601.
11. Satta G, Chisu V, Cabras P, et al. Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia. Italy. J Med Microbiol. 2011;60:63-68.
12. Johnson EM, Ewing SA, Barker RW, et al. Experimental transmission of *Ehrlichia canis* (Rickettsiales: Ehrlichieae) by *Dermacentor variabilis* (Acari: Ixodidae). Vet Parasitol. 1998;74:277-288.
13. Beugnet F, Marie JL. Emerging arthropod-borne diseases of companion animals in Europe. Vet Parasitol. 2009;163:298-305.
14. Groves MG, Dennis GL, Amyx HL, et al. Transmission of *Ehrlichia canis* to dogs by ticks (*Rhipicephalus sanguineus*). Am J Vet Res. 1975;36:937-940.

15. Harrus S, Kass PH, Klement E, et al. Canine monocytic ehrlichiosis: a retrospective study of 100 cases, and an epidemiological investigation of prognostic indicators for the disease. Vet Rec. 1997;141:360-363.
16. Breitschwerdt EB, Abrams-Ogg AC, Lappin MR, et al. Molecular evidence supporting *Ehrlichia canis*-like infection in cats. J Vet Intern Med. 2002;16:642-649.
17. de Oliveira LS, Mourao LC, Oliveira KA, et al. Molecular detection of *Ehrlichia canis* in cats in Brazil. Clin Microbiol Infect. 2009;15(suppl 2):53-54.
18. Braga Mdo S, André MR, Freschi CR, et al. Molecular and serological detection of *Ehrlichia* spp. in cats on São Luís Island, Maranhão. Brazil. Rev Bras Parasitol Vet. 2012;21:37-41.
19. Harrus S, Waner T, Keysary A, et al. Investigation of splenic functions in canine monocytic ehrlichiosis. Vet Immunol Immunopathol. 1998;62:15-27.
20. Brandao LP, Hasegawa MY, Hagiwara MK, et al. Platelet aggregation studies in acute experimental canine ehrlichiosis. Vet Clin Pathol. 2006;35:78-81.
21. Cortese L, Terrazzano G, Piantedosi D, et al. Prevalence of antiplatelet antibodies in dogs naturally co-infected by *Leishmania infantum* and *Ehrlichia canis*. Vet J. 2011;188:118-121.
22. Mylonakis ME, Koutinas AF, Breitschwerdt EB, et al. Chronic canine ehrlichiosis (*Ehrlichia canis*): a retrospective study of 19 natural cases. J Am Anim Hosp Assoc. 2004;40:174-184.
23. Gould DJ, Murphy K, Rudorf H, et al. Canine monocytic ehrlichiosis presenting as acute blindness 36 months after importation into the UK. J Small Anim Pract. 2000;41:263-265.
24. Komnenou AA, Mylonakis ME, Kouti V, et al. Ocular manifestations of natural canine monocytic ehrlichiosis (*Ehrlichia canis*): a retrospective study of 90 cases. Vet Ophthalmol. 2007;10:137-142.
25. Buoro IBJ, Kanui TI, Atwell RB, et al. Polymyositis associated with *Ehrlichia canis* infection in two dogs. J Small Anim Pract. 1990:31.
26. Dubey JP, Pimenta AL, Abboud LC, et al. Dermatitis in a dog associated with an unidentified *Toxoplasma gondii*-like parasite. Vet Parasitol. 2003;116:51-59.
27. Hess PR, English RV, Hegarty BC, et al. Experimental *Ehrlichia canis* infection in the dog does not cause immunosuppression. Vet Immunol Immunopathol. 2006;109:117-125.
28. Neer TM, Breitschwerdt EB, Greene RT, et al. Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. American College of Veterinary Internal Medicine. J Vet Intern Med. 2002;16:309-315.
29. Leiva M, Naranjo C, Pena MT. Ocular signs of canine monocytic ehrlichiosis: a retrospective study in dogs from Barcelona, Spain. Vet Ophthalmol. 2005;8:387-393.
30. Codner EC, Caceci T, Saunders GK, et al. Investigation of glomerular lesions in dogs with acute experimentally induced *Ehrlichia canis* infection. Am J Vet Res. 1992;53:2286-2291.
31. Codner EC, Maslin WR. Investigation of renal protein loss in dogs with acute experimentally induced *Ehrlichia canis* infection. Am J Vet Res. 1992;53:294-299.
32. Cortese L, Pelagalli A, Piantedosi D, et al. Platelet aggregation and haemostatic response in dogs naturally co-infected by *Leishmania infantum* and *Ehrlichia canis*. J Vet Med A Physiol Pathol Clin Med. 2006;53:546-548.
33. Giudice E, Giannetto C, Gianesella M. Effect of desmopressin on immune-mediated haemorrhagic disorders due to canine monocytic ehrlichiosis: a preliminary study. J Vet Pharmacol Ther. 2010;33:610-614.
34. Meinkoth JH, Hoover JP, Cowell RL, et al. Ehrlichiosis in a dog with seizures and nonregenerative anemia. J Am Vet Med Assoc. 1989;195:1754-1755.
35. Mylonakis ME, Koutinas AF, Leontides LS. Bone marrow mastocytosis in dogs with myelosuppressive monocytic ehrlichiosis (*Ehrlichia canis*): a retrospective study. Vet Clin Pathol. 2006;35:311-314.
36. Mylonakis ME, Day MJ, Siarkou V, et al. Absence of myelofibrosis in dogs with myelosuppression induced by *Ehrlichia canis* infection. J Comp Pathol. 2010;142:328-331.
37. Rikihisa Y, Ewing SA, Fox JC. Western immunoblot analysis of *Ehrlichia chaffeensis*, *E. canis*, or *E. ewingii* infections in dogs and humans. J Clin Microbiol. 1994;32:2107-2112.
38. Seaman RL, Kania SA, Hegarty BC, et al. Comparison of results for serologic testing and a polymerase chain reaction assay to determine the prevalence of stray dogs in eastern Tennessee seropositive to *Ehrlichia canis*. Am J Vet Res. 2004;65:1200-1203.
39. Gal A, Loeb E, Yisaschar-Mekuzas Y, et al. Detection of *Ehrlichia canis* by PCR in different tissues obtained during necropsy from dogs surveyed for naturally occurring canine monocytic ehrlichiosis. Vet J. 2008;175:212-217.
40. de Castro MB, Machado RZ, de Aquino LP, et al. Experimental acute canine monocytic ehrlichiosis: clinicopathological and immunopathological findings. Vet Parasitol. 2004;119:73-86.
41. Hildebrandt PK, Huxsoll DL, Walker JS, et al. Pathology of canine ehrlichiosis (tropical canine pancytopenia). Am J Vet Res. 1973;34:1309-1320.
42. Reardon MJ, Pierce KR. Acute experimental canine ehrlichiosis. I. Sequential reaction of the hemic and lymphoreticular systems. Vet Pathol. 1981;18:48-61.
43. Harrus S, Kenny M, Miara L, et al. Comparison of simultaneous splenic sample PCR with blood sample PCR for diagnosis and treatment of experimental *Ehrlichia canis* infection. Antimicrob Agents Chemother. 2004;48:4488-4490.
44. Schaefer JJ, Needham GR, Bremer WG, et al. Tick acquisition of *Ehrlichia canis* from dogs treated with doxycycline hyclate. Antimicrob Agents Chemother. 2007;51:3394-3396.
45. McClure JC, Crothers ML, Schaefer JJ, et al. Efficacy of a doxycycline treatment regimen initiated during three different phases of experimental ehrlichiosis. Antimicrob Agents Chemother. 2010;54:5012-5020.
46. Shipov A, Klement E, Reuveni-Tager L, et al. Prognostic indicators for canine monocytic ehrlichiosis. Vet Parasitol. 2008;153:131-138.
47. Harrus S, Waner T, Neer TM. *Ehrlichia and Anaplasma* infections. In: Greene CE, ed. Infectious Diseases of the Dog and Cat. 4th ed. St. Louis, MO: Elsevier Saunders; 2012:227-238.
48. Matthewman LA, Kelly PJ, Brouqui P, et al. Further evidence for the efficacy of imidocarb dipropionate in the treatment of *Ehrlichia canis* infection. J S Afr Vet Assoc. 1994;65:104-107.
49. Eddlestone SM, Neer TM, Gaunt SD, et al. Failure of imidocarb dipropionate to clear experimentally induced *Ehrlichia canis* infection in dogs. J Vet Intern Med. 2006;20:840-844.
50. 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;13:501-504.
51. Maurin M, Abergel C, Raoult D. DNA gyrase-mediated natural resistance to fluoroquinolones in *Ehrlichia* spp. Antimicrob Agents Chemother. 2001;45:2098-2105.
52. Aroch I, Harrus S. The use of hemopoietic growth factors: recombinant human granulocyte colony stimulating factor and recombinant human erythropoietin in severe pancytopenia due to canine monocytic ehrlichiosis. Israel J Vet Med. 2001;56:65-69.
53. Rudoler N, Baneth G, Eyal O, et al. Evaluation of an attenuated strain of *Ehrlichia canis* as a vaccine for canine monocytic ehrlichiosis. Vaccine. 2012;31:226-233.
54. Tajima T, Rikihisa Y. Cytokine responses in dogs infected with *Ehrlichia canis* Oklahoma strain. Ann N Y Acad Sci. 2005;1063:429-432.
55. Unver A, Huang H, Rikihisa Y. Cytokine gene expression by peripheral blood leukocytes in dogs experimentally infected with a new virulent strain of *Ehrlichia canis*. Ann N Y Acad Sci. 2006;1078:482-486.
56. Otranto D, Paradies P, Testini G, et al. Application of 10% imidacloprid/50% permethrin to prevent *Ehrlichia canis* exposure in dogs under natural conditions. Vet Parasitol. 2008;153:320-328.

57. Stanneck D, Rass J, Radeloff I, et al. Evaluation of the long-term efficacy and safety of an imidacloprid 10%/flumethrin 4.5% polymer matrix collar (Seresto®) in dogs and cats naturally infested with fleas and/or ticks in multicentre clinical field studies in Europe. Parasit Vectors. 2012;5:66.
58. Dantas-Torres F. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. Vet Parasitol. 2008;152:173-185.
59. Perez M, Bodor M, Zhang C, et al. Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. Ann N Y Acad Sci. 2006;1078:110-117.
60. Ndip LM, Ndip RN, Esemu SN, et al. Ehrlichial infection in Cameroonian canines by *Ehrlichia canis* and *Ehrlichia ewingii*. Vet Microbiol. 2005;111:59-66.
61. Oliveira LS, Oliveira KA, Mourao LC, et al. First report of *Ehrlichia ewingii* detected by molecular investigation in dogs from Brazil. Clin Microbiol Infect. 2009;15(suppl 2):55-56.
62. Little SE, O'Connor TP, Hempstead J, et al. *Ehrlichia ewingii* infection and exposure rates in dogs from the south central United States. Vet Parasitol. 2010;172:355-360.
63. Beall MJ, Alleman AR, Breitschwerdt EB, et al. Seroprevalence of *Ehrlichia canis, Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs from North America. Parasit Vectors. 2012;5:29.
64. Liddell AM, Stockham SL, Scott MA, et al. Predominance of *Ehrlichia ewingii* in Missouri dogs. J Clin Microbiol. 2003;41:4617-4622.
65. Goodman RA, Hawkins EC, Olby NJ, et al. Molecular identification of *Ehrlichia ewingii* infection in dogs: 15 cases (1997-2001). J Am Vet Med Assoc. 2003;222:1102-1107.
66. Paddock CD, Yabsley MJ. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. Curr Top Microbiol Immunol. 2007;315:289-324.
67. Murphy GL, Ewing SA, Whitworth LC, et al. A molecular and serologic survey of *Ehrlichia canis, E. chaffeensis*, and *E. ewingii* in dogs and ticks from Oklahoma. Vet Parasitol. 1998;79:325-339.
68. Xiong Q, Bao W, Ge Y, et al. *Ehrlichia ewingii* infection delays spontaneous neutrophil apoptosis through stabilization of mitochondria. J Infect Dis. 2008;197:1110-1118.
69. Stockham SL, Tyler JW, Schmidt DA, et al. Experimental transmission of granulocytic ehrlichial organisms in dogs. Vet Clin Pathol. 1990;19:99-104.
70. Anziani OS, Ewing SA, Barker RW. Experimental transmission of a granulocytic form of the tribe Ehrlichieae by *Dermacentor variabilis* and *Amblyomma americanum* to dogs. Am J Vet Res. 1990;51:929-931.
71. Yabsley MJ, Adams DS, O'Connor TP, et al. Experimental primary and secondary infections of domestic dogs with *Ehrlichia ewingii*. Vet Microbiol. 2011;150:315-321.
72. Goldman EE, Breitschwerdt EB, Grindem CB, et al. Granulocytic ehrlichiosis in dogs from North Carolina and Virginia. J Vet Intern Med. 1998;12:61-70.
73. Buller RS, Arens M, Hmiel SP, et al. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. N Engl J Med. 1999;341:148-155.
74. Dumler JS, Madigan JE, Pusterla N, et al. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin Infect Dis. 2007;45(suppl 1):S45-S51.
75. Long SW, Zhang X, Zhang J, et al. Evaluation of transovarial transmission and transmissibility of *Ehrlichia chaffeensis* (Rickettsiales: Anaplasmataceae) in *Amblyomma americanum* (Acari: Ixodidae). J Med Entomol. 2003;40:1000-1004.
76. 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.
77. Zhang XF, Zhang JZ, Long SW, et al. Experimental *Ehrlichia chaffeensis* infection in beagles. J Med Microbiol. 2003;52:1021-1026.
78. Kawahara M, Suto C, Rikihisa Y, et al. Characterization of ehrlichial organisms isolated from a wild mouse. J Clin Microbiol. 1993;31:89-96.
79. Wen B, Rikihisa Y, Mott J, et al. *Ehrlichia muris* sp. nov., identified on the basis of 16S rRNA base sequences and serological, morphological, and biological characteristics. J Bacteriol. 1995;45:250-254.
80. Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. N Engl J Med. 2011;365:422-429.
81. Telford III SR, Goethert HK, Cunningham JA. Prevalence of *Ehrlichia muris* in Wisconsin deer ticks collected during the mid 1990s. Open Microbiol J. 2011;5:18-20.