

DIAGNOSTIC DILEMMA. Anaplasmosis, ehrlichiosis, and Rocky Mountain spotted fever are tick-borne diseases commonly diagnosed in dogs; however, interpreting the results of diagnostic testing is challenging.

PARASITOLOGY

Tick-borne Rickettsial Infections of Dogs

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Rickettsial organisms are small, obligate intracellular bacteria in the order Rickettsiales. Two families—Anaplasmataceae and Rickettsiaceae—contain species that infect dogs. These pathogens are transmitted by a variety of tick vectors, maintained in wildlife and domestic reservoirs, and can cause clinical disease in humans, dogs, and other domestic animals. This article discusses the basic epidemiology, clinical presentation, diagnosis, and treatment of canine ehrlichiosis, anaplasmosis, and Rocky Mountain spotted fever (RMSF).

except Antarctica, but recent research has shown that *R. sanguineus* is made up of morphologically similar lineages that form a species complex, in which some lineages are more capable vectors for *E. canis* and other infective agents than others.² In the United States, dogs in southeastern states are most likely to be seropositive for *E. canis*, with approximately 2.3% (range, 0 to 6.3%) of dogs testing positive for species-specific antibodies to *E. canis*.^{3,4} Dogs infected with *E. chaffeensis* or *E. ewingii* are also most commonly found

CANINE EHRLICHIOSIS

Transmission and Prevalence

Ehrlichiosis is a rickettsial disease caused by infection with *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, or a combination of these and other tick-borne pathogens. *E. canis* is transmitted by *Rhipicephalus sanguineus*, the brown dog tick, while *E. chaffeensis* and *E. ewingii* are both transmitted by *Amblyomma americanum*, the lone star tick (TABLE 1).

Ehrlichiosis is an important disease of dogs, and in 2018, 2.94% of tested U.S. dogs had antibodies to an *Ehrlichia* species (FIGURE 1).¹ *E. canis* infects dogs worldwide, as *R. sanguineus* is found on every continent

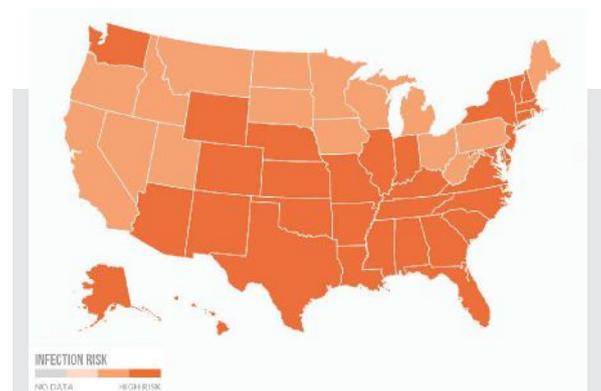


FIGURE 1. Ehrlichiosis prevalence across the United States in 2018. In 2018, 2.94% (165,819/5,643,290) of tested dogs were positive for an *Ehrlichia* species.

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**Table 1 Common Rickettsial Pathogens**

PATHOGEN	DISEASE	SPECIES AFFECTED	RESERVOIR	CELL INFECTED	PRIMARY TICK VECTOR
<i>Anaplasma phagocytophilum</i>	Human granulocytic anaplasmosis	Humans, dogs, cats, horses	Rodents, deer, birds	Neutrophil	<i>Ixodes scapularis</i> (blacklegged tick) <i>I. pacificus</i> (Western blacklegged tick)
<i>Anaplasma platys</i>	Canine thrombocytic anaplasmosis	Dogs	Dogs	Platelet	<i>Rhipicephalus sanguineus</i> (brown dog tick)
<i>Ehrlichia canis</i>	Canine monocytotropic ehrlichiosis	Dogs	Dogs	Monocyte	<i>Rhipicephalus sanguineus</i> (brown dog tick)
<i>Ehrlichia chaffeensis</i>	Human monocytotropic ehrlichiosis (HME)	Humans and dogs	White-tailed deer	Monocyte	<i>Amblyomma americanum</i> (lone star tick)
<i>Ehrlichia ewingii</i>	Canine granulocytic ehrlichiosis	Dogs	White-tailed deer, dogs	Neutrophil	<i>Amblyomma americanum</i> (lone star tick)
<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever	Humans and dogs	Rodents	Endothelial cell	<i>Dermacentor variabilis</i> (American dog tick) and <i>Dermacentor andersoni</i> (Rocky Mountain wood tick)*

*Recently, *R. sanguineus* and *A. americanum* have also been implicated in transmitting *R. rickettsii* to humans and/or dogs.

in southeastern states, but that distribution is expanding with the movement of *A. americanum* as far north as Maine.^{3,4} Several other *Ehrlichia* species, including an *Ehrlichia muris*-like agent and Panola Mountain *Ehrlichia*, have been reported sporadically in dogs, but their clinical significance is still unknown.

Clinical Signs

The clinical manifestations of ehrlichiosis vary according to *Ehrlichia* species and strain, host immune response and overall health, infectious dose of the pathogen, and co-infections acquired during tick feeding. Domestic dogs are the maintenance host for *E. canis* and all life stages of its tick vector, which can lead to large numbers of animals exposed to a high infectious dose of the bacteria.

Canine ehrlichiosis from *E. canis* infection is a febrile disease characterized by myalgia, splenomegaly, lymphadenopathy, and bleeding disorders caused by decreased platelet numbers and function.⁵ The acute infection occurs 2 to 4 weeks after transmission and can progress to a life-threatening bleeding diathesis (e.g., petechial hemorrhages, epistaxis, hematuria) and neurologic abnormalities (e.g., ataxia, head tilt, seizures) if not treated. This wide range of clinical signs appears to be related to the strain and dose of *E. canis* to which the dog is exposed, although some breeds, particularly German shepherds, appear to be more susceptible, pointing to other host factors.

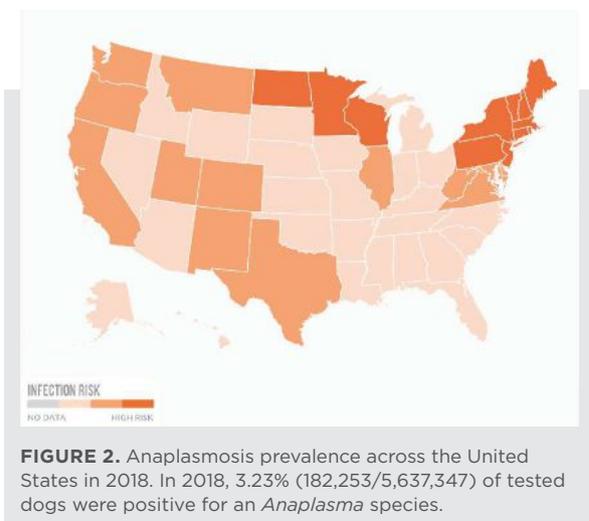
Clinical disease associated with *E. ewingii* and *E. chaffeensis* is typically much less severe than that seen with *E. canis* infection. The most common clinical signs associated with *E. ewingii* infection include lethargy, fever, lameness, lymphadenomegaly, peripheral edema, and thrombocytopenia, but disease can progress to more severe neurologic abnormalities. The lameness may appear to shift between legs as the neutrophilic polyarthritis waxes and wanes. *E. chaffeensis* is primarily a pathogen of humans, and only mild clinical signs have been reported in experimental infections of dogs.⁶

Regardless of disease severity, dogs appear to remain infected with *Ehrlichia* species long-term, especially if not treated appropriately. Therefore, dogs may have chronic, subclinical infections that can be comorbid with other disease processes.

CANINE ANAPLASMOSIS

Transmission and Prevalence

Infection with either *Anaplasma phagocytophilum* or *Anaplasma platys* can result in clinical disease in dogs. *A. phagocytophilum* is transmitted by *Ixodes scapularis*, and *I. pacificus*, the blacklegged and Western blacklegged ticks, respectively, and *A. platys* is transmitted by *R. sanguineus*. In 2018, 3.23% of tested U.S. dogs had antibodies to an *Anaplasma* species, with the highest prevalence in the Northeast and upper Midwest regions of the United States, associated



predominantly with *Ixodes* transmission of *A. phagocytophilum* (FIGURE 2).⁷ Similar to *E. canis*, transmission of *A. platys* appears to require the appropriate lineage of *R. sanguineus*, but it is commonly found in *R. sanguineus* in tropical areas. This accounts for cases of anaplasmosis and antibodies to an *Anaplasma* species in the southern United States; anaplasmosis may also be found in other U.S. regions when animals are transported from endemic areas.⁷

Clinical Signs

Clinical manifestations of anaplasmosis differ depending on the infecting species. Dogs infected with *A. phagocytophilum* often present with a fever and lameness due to a neutrophilic polyarthritis. Other pathologic changes consistent with a tick-borne infection, such as lymphadenopathy and splenomegaly, are also possible. Many serologically positive dogs remain clinically normal, but clinical signs may appear later in conjunction with an immunosuppressive event (e.g., course of steroids).⁸ The hallmark of *A. platys* infection is a waxing and waning thrombocytopenia caused by bacterial invasion and destruction of platelets. This can lead to a bleeding diathesis that is exacerbated by concurrent infection with other tick-borne diseases, such as *E. canis*.

CANINE ROCKY MOUNTAIN SPOTTED FEVER

Transmission

Of the rickettsial diseases of dogs, RMSF, caused by *Rickettsia rickettsii*, is the most life threatening. The

geographic distribution of cases is not as clearly defined as the other diseases discussed owing to a lack of nationwide surveillance, nonspecific diagnostic tests, and changing tick vectors.

Historically, *R. rickettsii* was known to be transmitted by *Dermacentor variabilis* and *Dermacentor andersoni* ticks in the United States. That changed in 2003 with a reported outbreak of RMSF associated with *R. sanguineus* on a reservation in Arizona.⁹ Several reports of *R. sanguineus* transmission of *R. rickettsii* in northern Mexico and the southwestern United States have since been published. Additionally, *A. americanum* has been shown to be a capable vector of *R. rickettsii*, and at least one human case of RMSF transmitted by a lone star tick has been reported.¹⁰ Both of these new tick vectors are worrisome, as they are often present in high numbers within their respective ranges, readily feed on dogs and humans, and are currently changing their geographic distribution.

Clinical Signs

Because *R. rickettsii* infects vascular endothelial cells, the resulting vasculitis can cause a range of clinical signs depending on where the infection develops and how disseminated it is. Dogs with RMSF typically present with a fever (102°F to 105°F) approximately 4 to 7 days after exposure. Widespread, abundant petechial hemorrhages on the mucous membranes or buccal cavity, macular to maculopapular skin rash, ocular or nasal discharge, and vascular injection of the sclera may also be seen.

Over time, dogs may begin to exhibit edema of the extremities, lymphadenomegaly, splenomegaly, and head or leg tremors.¹¹ One of the most common manifestations of late-stage disease is necrosis of the extremities, including the pinnae, feet, scrotum, and lips. Neurologic abnormalities (vestibular signs or altered mentation), myalgia, polyarthritis, and other signs of multiorgan failure are indicative of more severe, disseminated infection and carry a worse prognosis. Prompt diagnosis and initiation of treatment are key to preventing the progression of this disease.

DIAGNOSING AND DIFFERENTIATING RICKETTSIAL INFECTIONS IN DOGS

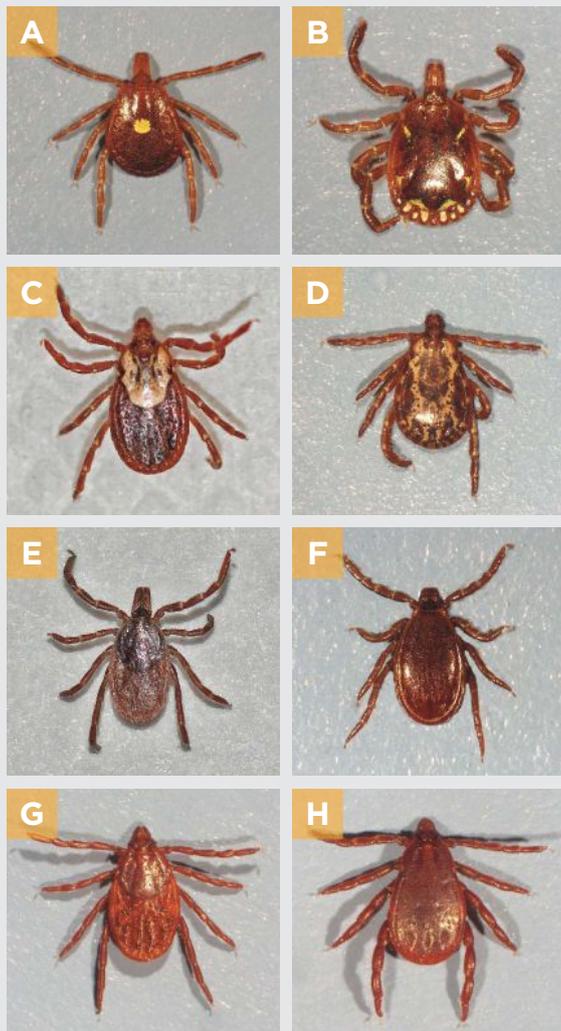
A variety of diagnostic tests can assist with accurate diagnosis of tick-borne rickettsial infections in dogs,



but interpreting their results can be challenging. Therefore, multiple diagnostic tests or treatment before definitive diagnosis may be necessary for dogs with severe clinical signs.

The hallmark clinicopathologic finding of these rickettsial infections is thrombocytopenia, but dogs may also have hypoalbuminemia, anemia, and hyperbilirubinemia. No complete blood count or serum chemistry abnormalities are definitively diagnostic.

Types of Ticks That Cause Rickettsial Infections



(A) *A. americanum* female. (B) *A. americanum* male. (C) *Dermacentor variabilis* female. (D) *Dermacentor variabilis* male. *D. andersoni* is morphologically similar. (E) *Ixodes scapularis* female. (F) *Ixodes scapularis* male. *I. pacificus* is morphologically identical. (G) *Rhipicephalus sanguineus* female. (H) *Rhipicephalus sanguineus* male.

Microscopy

Microscopic examination of peripheral blood smears can help diagnose *Ehrlichia* and *Anaplasma* infections in dogs. *E. canis* and *E. chaffeensis* most commonly infect monocytes, *E. ewingii* and *A. phagocytophilum* infect neutrophils, and *A. platys* infects platelets. After invading the cell, the bacteria begin to replicate within a vacuole, forming a morula, which can be observed on a stained blood smear as a basophilic, granular, intracytoplasmic structure. The morulae of *E. ewingii* and *A. phagocytophilum* are more numerous in circulating white blood cells than *E. canis* or *E. chaffeensis*, although all may pose a diagnostic challenge.

Visualizing *R. rickettsia* by microscopy is not considered an appropriate diagnostic test for RMSF because this organism infects vascular endothelial cells, and only low numbers of bacteria circulate in the bloodstream until very late in the disease process, when the vasculitis is profound.

Serology

In veterinary medicine, serology is the most common method used for diagnosing tick-borne rickettsial infections, either by indirect fluorescent antibody (IFA) assay, often used in reference or research laboratories, or enzyme-linked immunosorbent assay (ELISA), used in-clinic. Commercial, U.S. Department of Agriculture-approved ELISAs, such as the IDEXX® 4Dx® Plus (IDEXX Laboratories, idexx.com), AccuPlex® 4 (Antech Diagnostics, antechonline.com), and Vetscan® FLEX4 Rapid tests (Abaxis, abaxis.com) are all commonly used to detect antibodies to *Ehrlichia* and *Anaplasma* species. There are no in-clinic assays for *R. rickettsia*.

Both IFA and ELISA detect antibodies to the bacteria of interest; thus, they share similar drawbacks. First, these tests document that an animal has been exposed to the pathogen and mounted an immune response, but they do not distinguish if the animal is actively infected. Second, many of the acute clinical signs of rickettsial infections develop in the first 2 to 4 weeks after exposure, while the antibody response may take 3 to 5 weeks. Therefore, clinically ill, acutely infected patients may test negative on these assays.

Third, no commercially available tests can differentiate between species of the same genus (*E. canis* versus *E. ewingii* or *A. phagocytophilum* versus *A. platys*).^{12,13} There is a high level of cross-reactivity between the species; therefore, when interpreting a positive serologic

Doxycycline is the treatment of choice for all rickettsial infections of dogs.

result, veterinarians must take into account the tick exposure and regional tick-borne disease risk to make a diagnosis. Additionally, even the IFA used for detecting antibodies to *R. rickettsii* has a low specificity, likely detecting other tick-borne *Rickettsia* species of low or unknown pathogenicity in dogs. This complicates interpretation of results, as healthy dogs may have antibodies to a *Rickettsia* species without being infected with any rickettsial agent, let alone *R. rickettsii*.¹⁴

Finally, these antibodies can persist for at least a year and up to the life of the dog. This makes interpreting repeatedly positive tests in subsequent years even more challenging because the animal may have been re-exposed and infected or just have residual antibodies from the initial exposure. Overall, serologic assay results should be interpreted in conjunction with clinical signs, tick exposure, and results of other diagnostic tests.

Polymerase Chain Reaction

Molecular detection of rickettsial pathogens using polymerase chain reaction (PCR) to target species-specific nucleic acid sequences is now a commonly used diagnostic tool in reference laboratories. The specificity of these tests can allow veterinarians to screen specifically for the more pathogenic species described, especially in the face of conflicting antibody results.

PCR is the test method best suited for diagnosing an acutely ill patient with peak rickettsemia in the first 2 weeks of infection. Even within this window, however, the levels of bacteria in the blood can fluctuate, and positive results from whole blood samples most commonly coincide with peaks in fever.^{11,15} This is especially true for *R. rickettsia*, which infects vascular endothelial cells. Similar to microscopy, there often is not enough circulating organism in the blood to be detected by PCR; therefore, multiple tests on separate days are advised for definitive diagnosis.

Molecular diagnostics can also be helpful in monitoring response to treatment. Previously infected dogs without clinical signs that test negative by PCR for a rickettsial agent should be considered cleared, although recrudescence is possible.

Positive PCR results can be a very specific, definitive diagnosis, but negative PCR results must be interpreted with caution. Sample amount, sample quality, presence of PCR inhibitors, and low circulating pathogen levels can all affect the ability to amplify DNA. Therefore, in the face of a negative test result, other diagnostic techniques and clinical signs should be used to guide treatment for rickettsial diseases.

TREATMENT

While definitive diagnosis is an important step in good antimicrobial stewardship, delaying treatment can have grave consequences for rickettsemic patients, especially dogs with RMSF. Prompt initiation of antibacterial and supportive treatment for dogs with clinical signs consistent with a tick-borne rickettsial infection is the most prudent way to protect patients from progressing to a fatal, unrecoverable condition while waiting for results.

Doxycycline is the treatment of choice for all rickettsial infections of dogs. The most appropriate treatment regimen is 10 mg/kg q24h for 28 days.¹⁶ Recently, doxycycline shortages have made this drug prohibitively expensive, prompting researchers to test the efficacy of a similar compound, minocycline. A treatment regimen of minocycline at 10 mg/kg q12h for 28 days was as effective as the traditional doxycycline treatment regimen.¹⁷ Owing to its increased lipophilia, minocycline likely needs to be given at a higher dose than doxycycline, hence the twice a day dosing. Imidocarb, rifampin, and other tetracycline drugs have also been used to treat rickettsial infections but should be considered only when doxycycline or minocycline are unavailable.

Response to treatment is usually noted in the first few days, although owners should be reminded to give the full course. Stopping treatment early has been associated with recrudescence of infection. In severe cases, clinical signs may take longer to resolve, but dogs with disease lasting longer than 1 week after treatment should be evaluated for co-infections. Some animals may require parenteral fluids, a blood transfusion, or corticosteroids to get through the severe, acute phase of disease. A short course of corticosteroids (up to 7 days at 0.5 to 1 mg/kg/day) may be helpful in decreasing the



damage caused by inflammation (e.g., vasculitis, immune-mediated platelet destruction) but should be used in conjunction with antibiotic treatment and with caution, as these drugs have also been associated with recrudescence of latent rickettsial infections.

FOLLOW-UP AND PREVENTION

Treated animals can maintain detectable antibody levels for at least a year, and possibly for their entire life. A negative PCR result after treatment is an indicator that treatment was successful, but dogs can still be infected at levels below detectable limits and become clinically ill in the future.

The long-term antibodies are not protective, and dogs can be reinfected with other rickettsial agents or different strains of the same pathogen. Therefore, it is very important to keep all dogs on appropriate, effective tick prevention year round. No commercially available vaccine currently exists for any of the rickettsial agents, which makes acaricides the only barrier against infection. If owners notice a tick on their pet, they should promptly remove the tick using forceps, dispose of it properly, and contact their veterinarian if they notice any significant changes in the animal's health over the next 2 to 4 weeks.

CONCLUSION

These rickettsial pathogens are a significant medical concern to canine health. Changing tick distributions and nonspecific clinical signs can make identifying infected pets challenging, and the currently available diagnostic tests should be interpreted in light of the clinical presentation. Response to antibiotic therapy continues to be a necessary diagnostic option, especially for severely ill dogs. Based on the medical and diagnostic challenges, it is recommended that all dogs be on effective, year-round tick control. Client education and acaricide use are currently the only methods for preventing rickettsial infections in dogs. **TVP**

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Dr. Herrin received his DVM and PhD from Oklahoma State University Center for Veterinary Health Sciences. His primary research objectives are focused on the epidemiology and control of ticks and tick-borne diseases. Some of his recent interests are the epidemiology of Lyme borreliosis in humans and dogs in North America, evaluation of diagnostic assays for tick-borne diseases, and surveillance of ticks and tick-borne diseases of horses. Although his research focus is on ticks, Dr. Herrin enjoys educating about all parasites of veterinary importance through diagnostic service and teaching/outreach opportunities.