CHAPTER 30

Rocky Mountain Spotted Fever

Linda Kidd and Edward B. Breitschwerdt

Overview of Rocky Mountain Spotted Fever

First Described: *R. rickettsii* was first described in Montana, United States, 1909[1] (by Ricketts)

Cause: *Rickettsia rickettsii*, a gram-negative, obligately intracellular bacteria

Affected Hosts: Humans and dogs

Geographic Distribution: North, Central, and South America

Primary Mode of Transmission: Ticks (*Dermacentor* spp., *Rhipicephalus sanguineus, Amblyomma* spp.). Most affected dogs lack a history of a tick bite.

Major Clinical Signs: Rocky Mountain spotted fever (RMSF) is an *acute,* generally febrile illness. Clinical signs are consistent with a vasculitis because *R. rickettsii* infects endothelial cells. Major clinical signs include fever, vomiting, ocular signs, lymphadenomegaly, splenomegaly, peripheral edema, cutaneous hyperemia and necrosis, polyarthritis, and neurologic signs. Signs may be complicated by simultaneous infection with other tick-borne pathogens.

Differential Diagnoses: Infection with other tick-borne agents such as *A. phagocytophilum, Ehrlichia* spp., *Bartonella* spp., *Babesia* spp., and *Borrelia burgdorferi* should be considered. Other causes of severe systemic decompensation and vasculitis, such as sepsis and SIRS due to other causes may mimic RMSF. Leptospirosis and other causes of vasculitis and thrombocytopenia may also mimic RMSF. Other differential diagnoses for the neurologic signs seen in RMSF must also be considered. Appropriate antimicrobial treatment with doxycycline must begin before the diagnosis is confirmed by laboratory testing. Misdiagnosis and delayed or inappropriate antimicrobial drug therapy increase morbidity and mortality.

Human Health Significance: Owners and their physicians should be contacted whenever RMSF is diagnosed in any canine patient, because illness in dogs can coincide with or precede illness in humans.

Etiologic Agent

Rocky Mountain spotted fever (RMSF) is caused by *Rickettsia rickettsii,* an obligately intracellular bacteria in the alphaproteobacteria (genus *Rickettsia,* family Rickettsiaceae, order Rickettsiales).[2] The terminology used to describe rickettsial disease is confusing and inconsistent due to multiple changes in the taxonomic classification of organisms in recent years.[3] The terms "rickettsial disease," "rickettsioses," and even the term "*Rickettsia*" have been used to refer to several obligately intracellular organisms including *Rickettsia, Bartonella, Ehrlichia, Anaplasma, Coxiella,* and *Neorickettsia*. Previously, all of these organisms belonged to the order Rickettsiales and most were in the family Rickettsiaceae, based on their fastidious or intracellular nature and other characteristics. Therefore, collectively members of these diverse genera were referred to as "rickettsial organisms."[4,5] Recent advances in molecular biologic techniques have resulted in the reclassification of several of these organisms. Many have been moved out of the family Rickettsiaceae, and others have been moved out of the order Rickettsiales. Now the order Rickettsiales includes only two families, the family Anaplasmataceae, which contains the genera *Anaplasma, Ehrlichia, Wolbachia,* and *Neorickettsia*; and the family Rickettsieaceae, which includes the genera *Rickettsia* and *Orientia*.[2,3,5] Currently, "rickettsial" refers to diseases caused by organisms in the genera *Anaplasma, Ehrlichia, Rickettsia, Neorickettsia,* and *Orientia,* "rickettsioses" refers to diseases caused by organisms in the family Rickettsiaceae (*Rickettsia* and *Orientia*), and the term "*Rickettsia*" refers specifically to members of the genus *Rickettsia*.[3-5]

The genus *Rickettsia* is currently divided into the spotted fever group (SFG) and the typhus group based on phenotypic and, more recently, genotypic characteristics.[3,5] Some of the phenotypic characteristics that have historically been used to group the organisms include the types of vectors that transmit them and the pathophysiologic manifestations of the disease.[3] The SFG rickettsial species are transmitted by arthropod (primarily tick) vectors and infect endothelial cells in mammalian hosts.[5] The two most pathogenic and well-studied SFG rickettsiae are *Rickettsia rickettsii,* the cause of RMSF in the Western Hemisphere, and *Rickettsia conorii,* the cause of Mediterranean spotted fever (MSF) in other areas of the world. The first case of RMSF was described in the late 1800s and the first case of MSF was described in the 1920s.[6] Therefore, these organisms and their associated diseases are the most well characterized among the SFG rickettsiae.[3] Currently, there are 20 or more species of SFG rickettsiae.[3,6] Some species appear to be nonpathogenic endosymbionts of ticks.[6] However, some SFG rickettsiae previously thought to be nonpathogenic, such as *R. parkerii* and *R. massiliae,* have recently been associated with disease in people.[7-9] Previously thought to have rather limited geographic boundaries, many SFG rickettsiae have also been detected in expanding geographic locales around the world.[6] For example, RMSF, originally described in the Bitterroot Valley of Montana, was subsequently found to be a frequent tick-transmitted infection in the eastern United States, but only recently has transmission via the brown dog tick been documented in the southwestern

United States.[10,11] Increasing travel and the effects of climate change on tick populations and habitat are thought to be in part responsible for this phenomenon.[12,13]

Epidemiology

Hosts, Life Cycle, and Transmission

Dogs are sentinels for SFG rickettsioses in people. Both *R. rickettsii* and *R. conorii* infect and cause disease in dogs.[14-20] *Rickettsia rickettsii* infection in dogs can occur before, or coincide with, outbreaks of RMSF in people in the same household or community.[21-24] Several serosurveys in endemic areas have shown an increased risk of MSF in people who live near dogs that are seropositive for SFG rickettsiae.[25-27] Other SFG rickettsial species likely infect dogs, but the extent to which they cause clinical disease has not yet been established. Species implicated in natural infection in dogs include *R. massiliae, R. japonica,* and *R. australis*.[28-31]

Young and purebred dogs are overrepresented in some but not other studies.[17,18] Although some studies suggest male dogs are at increased risk, no sex predilection has been definitively documented.[17,18,32] Severe disease has been reported in English springer spaniels with phosphofructokinase (PFK) deficiency and German shepherd dogs.[18,32] Although antibodies to SFG rickettsiae can be detected in cats that live in endemic areas, the ability of SFG rickettsiae to actively infect and cause disease in cats has not been well characterized.[33] Similarly, the ability of typhus group *Rickettsia* to cause disease both in dogs and cats has also not been well characterized and will not be discussed here.

Rickettsia rickettsii is transmitted by several hard (ixodid) ticks including *Dermacentor variabilis* (the American dog tick) (Figure 30-1, *A*), *Dermacentor andersoni* (the Rocky Mountain wood tick) (Figure 30-1, *B*), *Amblyomma americanum, Amblyomma cajennense, Amblyomma aureolatum,* and *Rhipicephalus sanguineus*.[34-38] *Amblyomma cajennense* and to a lesser extent, *A. aureolatum* transmit the rickettsia in South America. Ticks that transmit *R. rickettsii* feed once during each life stage (Figure 30-2). Both transstadial and transovarial transmission occurs in *Dermacentor* spp. infected with *R. rickettsii,* so these ticks serve as a reservoir of infection.[39,40] *Dermacentor* spp. and *Amblyomma* spp. are three-host ticks. Infection is transmitted among mammalian hosts and ticks when the tick feeds on different hosts during each stage of molting. The sylvatic cycle for *Dermacentor* ticks involves small mammals, such as chipmunks, pine voles, mice, and ground squirrels. After organisms are ingested by the tick, SFG rickettsiae initially replicate in the epithelial cells of the tick midgut, enter the hemolymph and hemocytes, and then multiply in tissues.[40] Once in the salivary glands, the organism is transmitted to a naïve mammalian host on feeding. Transmission can occur within hours of attachment. However, this may be prolonged (up to 48 hours) when changes in virulence occur under conditions such as starvation of the tick. These "dormant" rickettsiae undergo a process called reactivation after the tick begins feeding.[41] Therefore, rapid tick removal can decrease the risk of transmission in many, but not all, circumstances.

Rhipicephalus sanguineus is a one-host tick, with dogs being the preferred host.[42] *Rh. sanguineus* can adapt to hot environments and commonly resides in walls of housing structures in close proximity to humans.[42] It occasionally feeds on humans, especially when ambient temperatures are high.[12] The role of this tick as a reservoir for *R. rickettsii* in nature has yet to be elucidated.

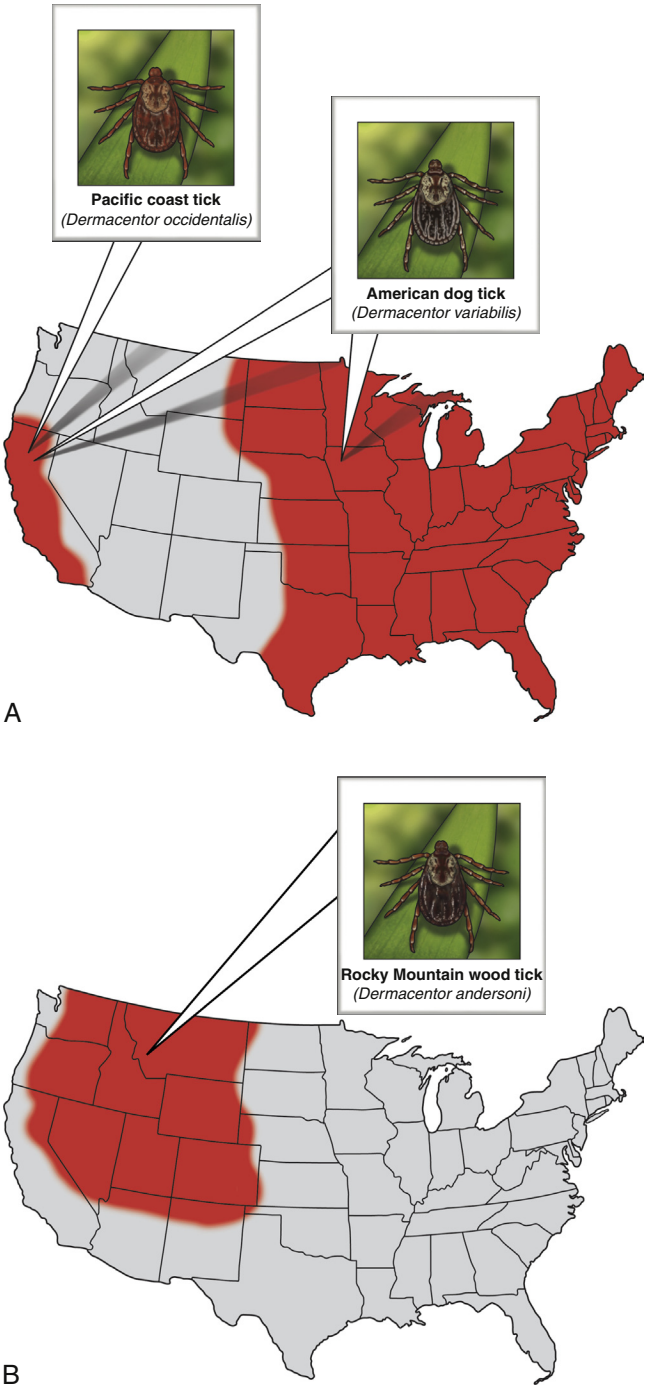

FIGURE 30-1 **A,** Distribution of *Dermacentor variabilis* and *Dermacentor occidentalis* ticks in the United States. **B,** Distribution of *Dermacentor andersoni* ticks in the United States.

The role of dogs as a natural reservoir of infection for *R. rickettsii* is also unknown.[39] Dogs are thought to act as incidental hosts when *Dermacentor* ticks are the vectors, because ticks rarely acquire the organism from rickettsemic dogs.[43] In contrast, all stages of *Rh. sanguineus* acquired infection at a high rate from experimentally infected dogs in one study, but this may have been related to the virulence of the rickettsial strain used.[38] A relatively high infection rate with SFG rickettsiae in naturally infected *Rh. sanguineus* has also been described.[37,44] Therefore, dogs may play a role in maintenance of a reservoir

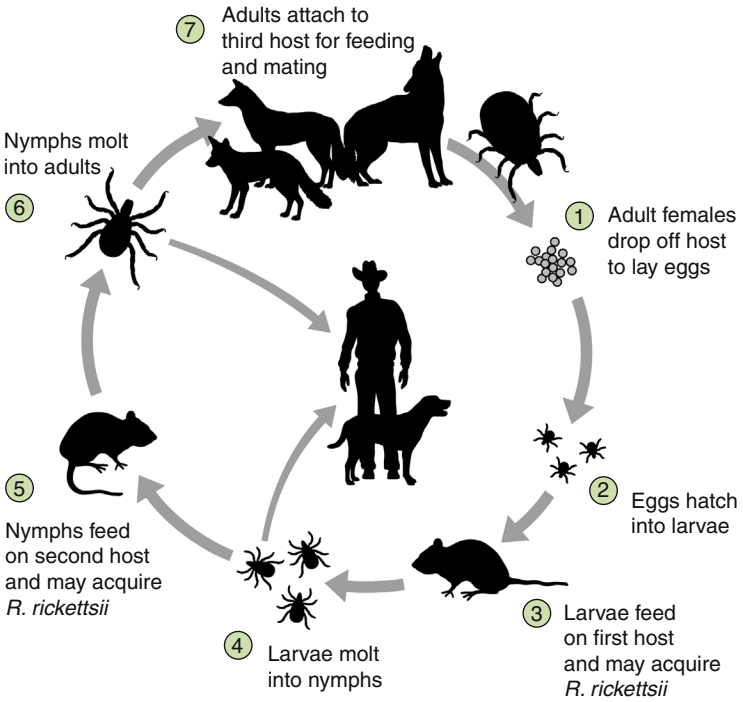

FIGURE 30-2 Transmission cycle of *Rickettsia rickettsii* in tick populations, people, and dogs. After adult female ticks lay eggs and they hatch (1 and 2), *Dermacentor* spp. ticks acquire *R. rickettsii* upon feeding as larvae or nymphs on rodents (3); they then transmit during subsequent feeding as nymphs or adults (4 and 6). Adult ticks feed on a variety of canid species, such as foxes and possibly wolves and coyotes. In addition, *R. rickettsii* infection can be maintained transovarially in *Dermacentor* ticks, so some larvae that hatch from the egg mass already harbor an infection. (Redrawn from Nicholson WL, Allen KE, McQuiston JH, et al. The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol 2010;26[4]:205-212.)

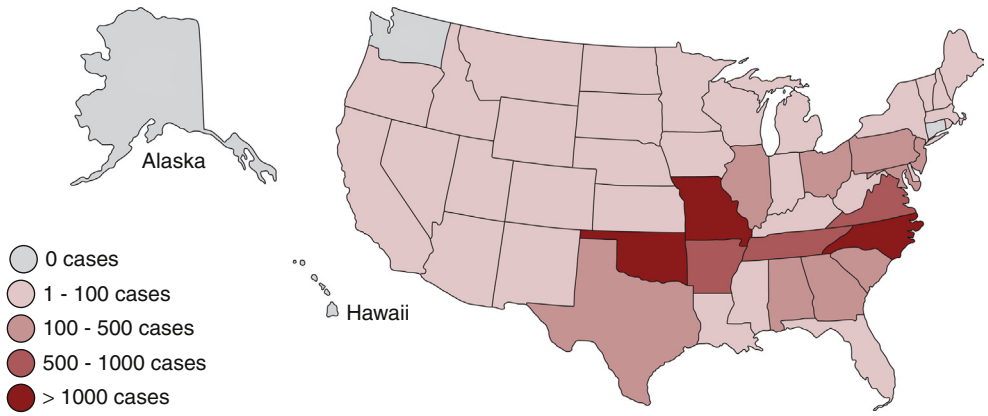

FIGURE 30-3 Geographic distribution of the incidence of spotted fever group rickettsioses in people in the United States, 2005-2009. (Compiled from data from the Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Reports.)

of *R. rickettsii* infection in endemic foci where *Rh. sanguineus* is the primary vector.

Direct transmission of *R. rickettsii* to people has been described in laboratory settings through aerosolization. Direct transmission from blood or other contaminated biologic products also has the potential to occur.

Incidence and Geographic Distribution

Rocky Mountain spotted fever occurs in North, Central, and South America (where it is known as Brazilian spotted fever). In North America, most cases of RMSF occur in the southeastern and south central states (Figure 30-3). Disease distribution in the United States primarily follows the distribution of the primary vectors, *D. variabilis* and *D. andersoni*, although the importance of *D. variabilis* as vectors in endemic areas has recently been questioned.[45] Although disease can occur any time of year, most cases of canine and human RMSF are reported from April through October, months of peak tick activity.[17,46,47] Dogs that live outdoors, particularly those with access to shrubs and high grass, are at increased risk.[17]

In recent years the incidence of RMSF in people has increased.[47] Some of the increase may be due to misdiagnosis of disease caused by species of SFG rickettsiae other than *R. rickettsii*.[48,49] A similar increase in seroprevalence rates has been documented in dogs suspected to have a tick-borne infection in the United States.[50] The geographic distribution of RMSF in the United States has also increased beyond the distribution of *D. variabilis* and *D. andersoni*. *Rhipicephalus sanguineus*

is the main vector for *R. rickettsii* in some parts of the Western Hemisphere, including Mexico and now the southwestern United States. In South America, *Amblyomma* species are the main tick vector. Tick species other than *D. variabilis, D. andersoni,* and *Rh. sanguineus* are also infected with *R. rickettsii* in the United States.[44] For example, *A. americanum* transmitted RMSF to a person in North Carolina.[36] An outbreak in Mexicali, Mexico, was attributed to *R. rickettsii* transmitted by *Rh. sanguineus* ticks associated with dogs.[51] This prompted ongoing surveillance efforts in ticks, dogs, and people in southern California, a nonendemic area for RMSF. Thus far, no *R. rickettsii* has been found in *Rh. sanguineus* ticks from dogs residing in animal shelters located just across the U.S. border from the outbreak.[52,53] This may be due to subtle differences in microenvironment or other factors that affect regional infection rates in ticks.[54]

Clinical Features

Signs and Their Pathogenesis

The endotheliotropism associated with these bacteria results in the characteristic clinical sign of infection, which is disseminated vasculitis. In people, cutaneous macules, papules, and petechiation that occur in association with vasculitis form a rash that looks like "spots," hence the name Rocky Mountain spotted fever.[6] The clinical signs, time course of illness, and response to therapy for RMSF in dogs are very similar, if not identical, to those associated with RMSF in people.[17-20,34] However, cutaneous lesions are not always present in dogs or people ("Rocky Mountain spotless fever"). In people, spotted fever rickettsioses caused by organisms other than *R. rickettsii* are often associated with eschar (an area of cutaneous necrosis) formation.[2,13] Clinicians should keep in mind that dogs from nonendemic areas that have classic signs of RMSF, or those that present with atypical clinical signs, may be infected with *R. rickettsii* or other SFG rickettsial species.

Once the organism is inoculated, it spreads through lymphatics or directly into the bloodstream to the small capillaries. *R. rickettsii* primarily infects endothelial cells, although smooth muscles and monocytes may also be infected.[2,55] The bacterial outer membrane protein A (OmpA) and outer membrane protein B (OmpB) are important for attachment, adhesion, and virulence.[56,57] These proteins are also responsible for differences in serotype, and antibodies to OmpB confer immunity to infection in experimental settings. SFG rickettsiae enter cells by inducing phagocytosis and are released into the cytoplasm through the action of enzymes such as phospholipase D and hemolysin C.[57] The bacteria live in the cytoplasm and the nucleus, deriving nutrients and energy from the host cell.[2,56] They spread from cell to cell by inducing actin to polymerize, which pushes the bacteria directly into adjacent cells.[57,58] This helps them evade the immune response and to disseminate without rupturing the cell. They are also released into circulation when they exit the luminal surface of the cell membrane or when endothelial cell death or detachment occurs.[57] Spotted fever group rickettsiae activate the transcription factor NFκB, which inhibits apoptosis and fosters further growth of the organism.[56,57]

Damage to endothelial cells leads to vasculitis and an increase in microvascular permeability. Mechanisms of cellular damage include oxidative injury through the production of reactive oxygen species, cellular necrosis, and induction of endothelial apoptosis by CD8+ T cells.[55-57,59] Activation of PFK may be important in maintaining vascular integrity and energy metabolism in endothelial cells under hypoxic or oxidative stress,[60,61] which may explain the predisposition of English springer spaniels with PFK deficiency to severe disease. Vasculitis associated with *R. rickettsii* infection manifests as disordered primary hemostasis, tissue edema, hypovolemia, and microthrombosis.

Increased vascular permeability and the associated edema and hypovolemia results from disruption of adherens junctions, endothelial cell death, expression of inflammatory cytokines such as Il1-β, IFN-γ, and TNF-α, and induction of COX-2 with subsequent prostaglandin production.[55] Microthrombosis results from altered platelet adherence to endothelium, increased tissue factor expression, increased plasminogen activator inhibitor, and the release of von Willebrand's factor.[55]

Low numbers of organisms circulate in blood for approximately 13 days after infection, which includes the time that clinical signs are observed.[19,62,63] Organisms are free and also contained within circulating endothelial cells, which are thought to be released from the vessels because of decreased adhesion after rickettsial invasion.[57] Thus, RMSF is an *acute* disease. Chronic infection has not been documented in dogs or people. Co-infection with other vector-borne agents is common and should be considered if the clinical signs are atypical, if there is an incomplete response to doxycycline therapy, or if clinical signs have been present for a week before the time of evaluation.[17,64]

Because of variation in the extent and severity of vascular injury among dogs, a range of signs can occur, and importantly, disease manifestations are initially mild and nonspecific.[15,17,18] Often, there is no known history of a tick bite. Therefore, the clinician (physician and veterinarian) must have a high index of suspicion in order to correctly diagnose and treat this disease. This is very important because a delay in diagnosis and appropriate antimicrobial therapy dramatically increases morbidity and mortality in people and in dogs.[17,46] A "One Health" approach to the management of canine and human RMSF is clearly logical. Lethargy and anorexia are common and may be the only clinical signs. Vomiting and diarrhea occur frequently in dogs and people with RMSF. Melena may be observed, as may a variety of CNS abnormalities including vestibular disease and seizures.[17,18,20] Dramatic and rapid weight loss has been described.[20]

Physical Examination Findings

Fever is present in approximately 80% of naturally infected dogs. Ocular signs are also frequently observed and may include a mucopurulent discharge, scleral and conjunctival injection and hemorrhage, conjunctivitis, uveitis, retinal hemorrhage, and retinitis (Figure 30-4). Lymphadenomegaly and splenomegaly also occur. Respiratory abnormalities include nasal discharge, epistaxis, and tachypnea. Mucocutaneous and cutaneous abnormalities include petechiae, ecchymosis, peripheral edema (which can be localized over a joint, the prepuce, or the mammary chain), hyperemia, and necrosis. Gangrenous necrosis can be so severe as to require reconstructive surgery after successful treatment of the acute febrile illness.[15-18,20,65,66] Orchitis and scrotal edema, hyperemia, and epididymal pain are common in intact male dogs and should prompt consideration of RMSF when present. Generalized myalgia and arthralgia can be observed. Arrhythmias may also be detected. CNS abnormalities can be focal or generalized and include paraparesis, tetraparesis, ataxia, hyperesthesia, ataxia, central or peripheral vestibular signs, stupor, seizures, and/or coma. Neurologic signs are more common in dogs with a high *R. rickettsii* antibody

titer, which suggests a longer duration of illness or a delay in diagnosis.[17] Residual neurologic deficits may occur after infection in severely affected individuals. Microvascular hemorrhage, thrombosis, hypotension, oliguric renal failure, cardiovascular collapse, and coma can occur terminally.

Diagnosis

A combination of diagnostic testing is often necessary to confirm infection by SFG rickettsiae. Active infection is confirmed in a patient with compatible acute clinical signs and demonstration of the organism using PCR assays or immunohistochemistry (IHC), or documentation of seroconversion (Figure 30-5). Importantly, a high index of suspicion based on clinical signs is necessary, because treatment must be instituted before the results of diagnostic tests (including rapid PCR assays) confirm infection.[17,20] Co-infection with other vector-borne disease agents should be considered in patients who fail to respond rapidly to treatment (within the first 24 to 48 hours after initiation of doxycycline treatment).[64] Because direct inoculation into blood or aerosolization can cause infection, all specimens should be handled with care and marked clearly as biohazards. Needle-stick injuries, contact with cuts in the skin, and aerosolization of rickettsemic blood should be avoided.

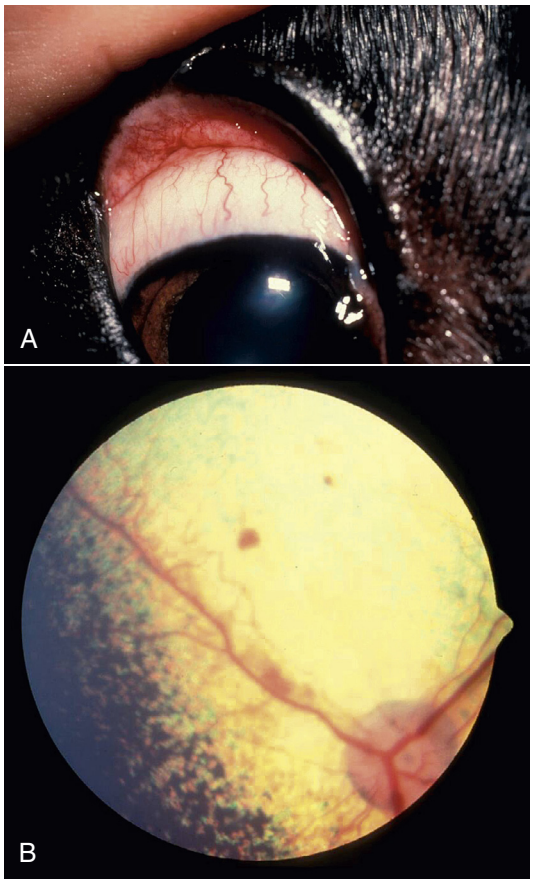

FIGURE 30-4 Ocular complications in dogs with RMSF. **A,** Conjunctival hyperemia and scleral injection. **B,** Retinal hemorrhages.

Laboratory Abnormalities

Complete Blood Count

Thrombocytopenia is common in RMSF and occurs due to vasculitis and immune-mediated platelet destruction.[67] However, it does not occur in all dogs with RMSF.[17,20] The white blood cell count may initially decrease and then tends to increase with duration of illness.[18-20] Neutrophils may have toxic change.[17] Despite the acute nature of this severe illness, a nonregenerative anemia may be present and persist until the dog is treated appropriately and begins to recover.[15,17,20]

Serum Biochemical Tests

Serum biochemical abnormalities can include hypoalbuminemia (due to vasculitis or protein-losing nephropathy), increased ALP activity, hyponatremia, and mild hyperbilirubinemia.[15,17,20,65] Hyponatremia has been associated with the syndrome of inappropriate antidiuretic hormone secretion (SIADH) in people with RMSF.

Urinalysis

Urinalysis results in dogs with RMSF are variable and may include proteinuria, hematuria and bilirubinuria, and pyuria. Granular casts can be observed.[17,18]

Coagulation Profile

Coagulation abnormalities include prolonged APTT and increased serum fibrinogen concentration. Less commonly, prolonged PT and increased fibrin degradation products are observed. Although a prothrombotic state can occur during fulminant disease, disseminated intravascular coagulation is uncommon.[15,17]

Body Fluid Cytology

Cytologic examination of aspirates from enlarged lymph nodes in dogs with RMSF is consistent with reactive lymphoid hyperplasia.[17,18] Arthrocentesis may reveal neutrophilic

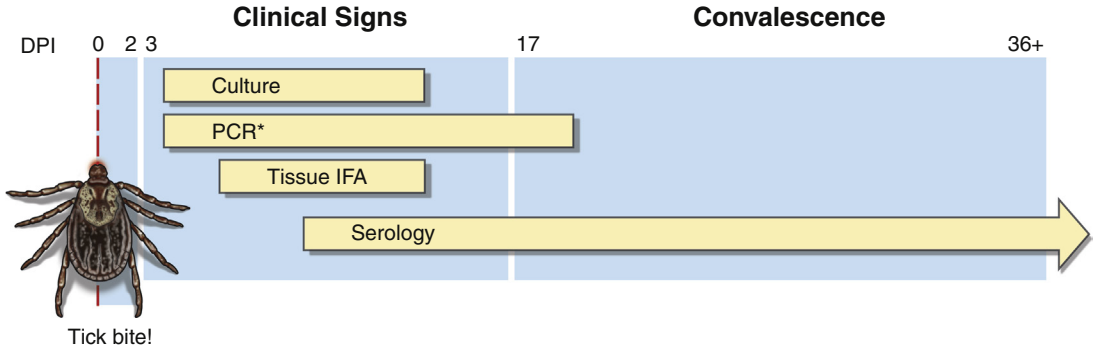

FIGURE 30-5 Timing in relation to day postinfection (DPI) that clinical signs and diagnostic tests for *Rickettsia rickettsii* may be positive in an infected dog[16,62,63,70,76] Further studies are needed to determine exactly how DNA can be detected in peripheral blood after infection.[62,63]

polyarthritis.[17,18] Cerebrospinal fluid analysis in dogs with neurologic signs may reveal a mixed cellular, neutrophilic, or lymphocytic pleocytosis.[17,18,20,68]

Diagnostic Imaging

Thoracic radiographs in dogs with RMSF may show an unstructured interstitial pattern.[18,69] Testicular ultrasound findings may be consistent with orchitis in intact males.[66]

Microbiologic Tests

Diagnostic assays available for RMSF in dogs are shown in Table 30-1. Because of the pathogenesis of the infection, laboratory diagnostics (including serology and PCR assays) may not indicate infection is present at the time that clinical signs manifest (see Figure 30-5). In addition, most diagnostic tests do not differentiate among species of SFG rickettsiae.

Culture

Because of the obligately intracellular and extremely pathogenic nature of *R. rickettsii,* culture is difficult to perform and processing of specimens for isolation is limited to BSL 3 facilities (see Table 1-4). Therefore, culture is not commonly used for routine diagnosis. Currently, *R. rickettsii* is listed as a Category A Bioterrorism agent, so all isolates must be reported to the Centers for Disease Control and Prevention or destroyed immediately.

Serologic Diagnosis

Indirect microimmunofluorescence (MIF) assays that detect IgM and IgG antibodies are most commonly used to document seroconversion in dogs infected with *R. rickettsii*.[19] Because of a lack of specificity, assays that detect IgM alone cannot be used to accurately diagnose acute RMSF.[70] There is extensive serologic cross-reactivity among SFG rickettsiae, and thus a positive titer only indicates exposure to a SFG rickettsial species. In general, the infecting species is presumed based on geographic locale, so RMSF is the presumptive diagnosis in dogs that seroconvert in the southeastern United States. However, other species of SFG rickettsiae may also be present and induce disease.[28,71] Exposure to nonpathogenic SFG rickettsiae, some of which are common endosymbionts in ticks, may be a common cause of positive titers, particularly low and persistent titers, to *R. rickettsii* in healthy people and dogs.[49,72] Serologic cross-reactivity with SFG rickettsiae also may occur in dogs and people infected with *Bartonella henselae*.[73] In addition, previous infection with *R. rickettsii* may result in persistent antibody titers.[70] Thus, infection with *R. rickettsii* cannot be definitively diagnosed based on a single positive titer. However, a single high titer in the context of acute and compatible clinical signs and an appropriate response to therapy suggests infection.[17] Titers are commonly negative early in the course of infection, because clinical signs often occur before seroconversion in naturally and experimentally infected dogs.[20,70,74] Thus, acute and convalescent serology (run in the same laboratory and ideally in the same batch) and documentation of a fourfold change in titer is necessary to confirm acute SFG rickettsiae infection. The convalescent serum specimen should be drawn 2 to 3 weeks after the acute specimen.

TABLE 30-1

Diagnostic Assays Available for Rocky Mountain Spotted Fever in Dogs

Assay	Specimen Type	Target	Performance
Indirect microimmunofluorescence assay (MIF)	Serum	IgM and IgG antibodies against *R. rickettsii*	Demonstration of a fourfold change in titer (seroconversion) is very sensitive. False-negative results are common during the acute phase of infection. A positive antibody response may reflect prior exposure to a nonpathogenic SFG rickettsia, or long-lived circulating antibody from previous infection with SFG rickettsiae. Cross-reactivity to other SFG rickettsiae and to *B. henselae* occurs.
PCR	Whole blood	Gene target varies with testing laboratories	In vitro sensitivity may approach 100%; however, absolute (clinical) sensitivity is lower, approximately 60%, because organisms circulate in blood in low numbers and transiently during the acute phase of infection. Sensitivity is further reduced by antimicrobial drug treatment. Specificity approaches 100%, particularly if the laboratory sequences all PCR amplicons. PCR testing for other tick-borne organisms that cause similar clinical signs is available as PCR panels from some testing laboratories. Only a few PCR assays differentiate among *Rickettsia* species. Negative PCR assay results do not rule out infection.
Histopathology with direct IFA or Gimenez staining	Cutaneous biopsy (inguinal or flank targeting areas with lesions) or necropsy specimens	Characteristic perivascular inflammation and vasculitis with necrosis and presence of the organism	Direct IFA sensitivity is approximately 80% during acute infection. Biopsy of a petechial or ecchymotic lesion may increase sensitivity. False negatives occur if organisms are absent from the lesion because of random chance, timing of specimen collection, or prior antimicrobial treatment. Does not differentiate among species of SFG *Rickettsia*.

Molecular Diagnosis Using Polymerase Chain Reaction

PCR testing of whole blood can be used to confirm infection in seronegative dogs during the acute phase of disease.[62,63,74] A sensitive PCR assay that detects and differentiates among SFG rickettsiae that infect dog blood has been described, and a variety of PCR assays are available through commercial veterinary diagnostic laboratories.[74] However, because *R. rickettsii* circulates in blood in low numbers and only transiently, the sensitivity of any diagnostic assay to detect infection in the bloodstream is limited. One real-time PCR assay, which could detect as few as five copies of organism DNA, had a sensitivity of only 53% for detection of *R. rickettsii* in acute samples from naturally infected dogs with signs of RMSF.[74] The sensitivity of another *R. rickettsii* PCR assay for detection of *R. rickettsii* in experimentally infected dogs ranged from 33% to 100% depending on the day of sampling.[63] Furthermore, false negatives can occur after initiation of appropriate antimicrobial treatment. Therefore, although a positive PCR assay result confirms infection, a negative result does not exclude the diagnosis. Some PCR assays can differentiate among infecting *Rickettsia* species, whereas others only amplify conserved rickettsial DNA targets.[62,74] Clinicians should consult with laboratories to determine the in vitro (analytical) and in vivo (clinical) sensitivity of a PCR assay and its ability to differentiate among infecting *Rickettsia* species.

Pathologic Findings

Gross Pathologic Findings

Gross pathologic findings in dogs with RMSF are consistent with vasculitis and may include edema, particularly of the ears, muzzle, and scrotum, and ecchymosis, petechiae, and/or focal hemorrhages of the skin, mucous membranes, and viscera (including the brain). Lymphadenomegaly and splenomegaly are also common findings.[15,16,20,22]

Histopathologic Findings

Microscopically, the predominant lesion is vasculitis. Neutrophils, monocytes, and/or lymphoreticular cells predominate in the inflammatory lesions. The inflammation may surround and invade large small and medium-size vessels of multiple organs and tissues and is frequently accompanied by focal necrosis and hemorrhage. Meningoencephalitis, splenitis, myocardial necrosis, glomerulonephritis, and renal vasculitis have been described.[15,20,22]

Direct IFA can be used to detect organisms in skin and other organs. Identification of abundant SFG rickettsiae within and around small to medium-size vessels and vascular endothelial cells can confirm infection.[20] In experimentally infected dogs, the sensitivity of IHC was 78.3%, and organisms could be visualized between days 7 and 12 of infection (days 3 to 8 of fever).[75] In that study, specimens collected from areas other than the central focus of vasculitis were negative. However, in naturally infected dogs, the sensitivity was 80% for skin specimens from the inguinal region. Half of those specimens lacked gross lesions.[18] Thus, the diagnostic sensitivity of IHC is most likely enhanced by obtaining lesional biopsies, but may not be decreased when a lesion is not biopsied. The sensitivity of direct IHC is decreased by antibiotic therapy. IHC does not differentiate between SFG rickettsial species. Gimenez stain can also be used to visualize rickettsial organisms in tissues or tissue culture isolation attempts, but this stain is not rickettsia specific and does not allow for differentiation among species.

Treatment

Antimicrobial Treatment

Appropriate antibiotic therapy must be immediately instituted based on clinical suspicion, and before diagnostic tests confirm infection. Inappropriate or delayed antibiotic therapy may increase morbidity and mortality.[17,46] Some antimicrobial drugs such as trimethoprim sulfonamides may actually worsen disease progression in human patients.[46] Doxycycline is the treatment of choice (Table 30-2). It effectively eliminates infection and is active against *A. phagocytophilum, Ehrlichia* spp., and *B. burgdorferi,* which may be present in co-infections or cause disease that resembles RMSF. Seven days of treatment is adequate in most cases. Treatment a few days past defervescence is recommended. A longer course of treatment is recommended for dogs that are co-infected with *Ehrlichia* spp. or *B. burgdorferi*. Chloramphenicol was effective in experimentally infected dogs.[76] However, it may be less effective than doxycycline for treating RMSF in people and has less activity in vitro against *Ehrlichia chaffeensis* and *A. phagocytophilum*.[46] Enrofloxacin was effective for treatment of RMSF in experimentally infected dogs.[76] However, enrofloxacin is not effective for treatment of *E. canis* infections.[77] Use of parenteral antimicrobial drugs may be necessary in severely debilitated or vomiting patients.

Supportive Care

Many dogs require hospitalization.[20] Aggressive supportive care for complications such as thrombosis, CNS deficits, and gastrointestinal signs may be necessary. Because of the loss of vascular integrity, fluids should be administered with caution, and colloids may be warranted in some cases. The clinician should avoid exacerbation of interstitial edema, which can contribute to cerebral edema and death. The use of glucocorticoids in dogs with RMSF is controversial. Antiinflammatory and immunosuppressive doses did not affect overall outcome experimentally infected dogs, but rickettsemia was prolonged in dogs concurrently treated with immunosuppressive doses of glucocorticoids and doxycycline.[78] Antiinflammatory doses of glucocorticoids have been used in dogs with severe CNS manifestations and may be necessary topically or systemically for treatment of ocular abnormalities.[68,79]

Prognosis

The response to appropriate antibiotic therapy in dogs with RMSF is rapid (24 to 48 hours). Co-infection with

TABLE 30-2

Antimicrobials Used to Treat Rocky Mountain Spotted Fever in Dogs

Drug	Dose (mg/kg)	Route	Interval (hours)	Duration (days)
Doxycycline	5	PO, IV	12	7 to 14
Chloramphenicol*	30	PO, IV	8	7 to 14
Enrofloxacin	3† to 5	PO, IV	12	7 to 14

*May cause aplastic anemia in humans (wear gloves); may be less effective than doxycycline.
†Dose used in experimentally infected dogs.[76] Caution required in young animals (see Chapter 8).

B. burgdorferi, Ehrlichia spp., *Babesia* spp., and *Bartonella* spp. should be considered in dogs with severe or prolonged clinical signs or dogs that fail to respond to doxycycline. Residual CNS and other deficits may occur in severely affected patients.[17] The prognosis is good to excellent if the disease is diagnosed and treated with appropriate antibiotics and supportive care early in the course of illness.[17,68]

Immunity and Vaccination

Cell-mediated and innate immunity is important in the clearance of rickettsial infections. CD4+ and CD8+ cells, along with macrophages and dendritic cells, are believed to be sources of inflammatory cytokines such as IFN-γ, TNF-α, Il-1β, and RANTES (CCL5). These cytokines increase production of nitric oxide synthetase and hydrogen peroxide by the endothelial cell, which helps to eliminate the organisms.[56,57] The endothelial cells themselves also produce cytokines such as IL-6, Il-8, and MCP-1 (CCL2) that recruit immune cells and combat infection. Antibodies are not thought to be important initially for clearance of infection because they form after the fulminant stages of disease.[57] However, antibodies are long-lived and together with cell-mediated immunity may prevent subsequent infection. Immunity to reinfection is thought to be lifelong in people, and experimentally infected dogs did not develop illness following rechallenge at 6 months and 3 years after the initial infection. Vaccination is not available for dogs or people currently, but may be in the future.[80]

Prevention

Avoidance of tick-infested areas and routine inspection of dogs for ticks after outdoor activities can help to prevent RMSF. The reader is referred to Chapter 28 for information on tick prevention and safe tick removal. Safe tick removal is especially critical when exposure to *R. rickettsii* is a possibility. In cases where *Rh. sanguineus* is the vector, environmental control of ticks is particularly important.

Public Health Aspects

People living near dogs and with dogs in endemic areas are at increased risk for acquiring RMSF. This is likely due to increased human contact with ticks through interaction with tick-infested dogs. Also, because of transovarial transmission, dogs and people can independently acquire the infection from different ticks questing in the same environment. Because dogs have higher exposure to ticks than their human counterparts, they serve as excellent environmental sentinels for RMSF.[21,22,24,71] In the United States, the diagnosis of SFG rickettsioses in humans is notifiable to public health authorities. Some counties in certain states also require reporting for dogs, particularly during suspected outbreaks.[81] From a public health standpoint, it is important for veterinarians to confirm a diagnosis of RMSF in a dog whenever possible, and to warn the family of the increased risk of acquiring a *R. rickettsii*–infected tick in the same location as the dog acquired the disease. Veterinarians must communicate with owners and physicians that infection in a dog may precede tick-transmitted infection to owners or neighbors. Clients should be instructed to remove ticks properly. Education of owners with regard to the importance of tick control and prevention for both people and pets and the environment is critical. Novel and well-characterized species of SFG rickettsiae are important causes of emerging infectious disease in humans. Furthermore, *R. rickettsii* is considered a potential bioterrorist agent.[80] Because dogs are sentinels for infection, veterinarians play an important role in detecting, defining, and preventing illness in their canine patients and their human companions.

CASE EXAMPLES

Rocky Mountain Spotted Fever in a Dog

Signalment: A 5-year-old MC Australian shepherd from North Carolina

History: The dog was examined by the North Carolina State University veterinary emergency service on April 11 because of recent onset of lethargy, fever, tachypnea, and a stiff gait when walking. Historically the dog had been healthy, had received routine vaccinations, heartworm preventive, and a flea acaricide, and ran approximately 3 miles each evening with the owner, who was a veterinarian. On the day of presentation and several hours after the evening run, the dog became lethargic, refused to eat dog food or treats, and seemed painful upon manipulation. The owner noticed that the dog's heart rate, which was normally 50 to 60 beats/min, was 120 beats/min at rest. Rectal temperature at home just prior to presentation was 104.4°F (40.2°C). Three days before the dog was evaluated, the owner had removed a non-engorged tick from the dog.

Physical Examination: Physical examination findings included a body weight of 21.3 kg, 3%-5% dehydration, pain score of 1 out of 4, rectal temperature of 103.7°F (39.8°C), HR 130 beats/min, tachypnea (42 breaths/min), and a normal CRT. The body condition score was 5/9. The dog stood in a hunched position and had a stiff gait, but localizing pain was not elicited on palpation of the spine, joints, or long bones. There was no nasal or ocular discharge, no petechiae or rash, and no obvious swelling or joint effusion. Popliteal lymph nodes were prominent, but there were no other abnormalities noted on physical examination. Systolic blood pressure was 153 mm Hg, and diastolic and mean blood pressures were 93 mm Hg and 111 mm Hg, respectively.

Laboratory Findings: The hematocrit was 42%, total plasma protein was 6.2 mg/dL, and the platelet count was 120,000/μL. A serum biochemical profile showed only hypoglobulinemia (1.7 g/dL).

Microbiologic Testing: A SNAP 4Dx (IDEXX Laboratories) in-house ELISA assay was negative. Indirect IFA assays for *Babesia canis, Bartonella henselae, Bartonella vinsonii* subsp. *berkhoffii,* and *Ehrlichia canis* were negative (no detectable antibodies at a 1:16 screening dilution). The dog was seroreactive to *Rickettsia rickettsii* antigens at a titer of 1:64. A PCR panel capable of detecting *Anaplasma* spp., *Babesia*

spp., *Ehrlichia* spp., and *Rickettsia* spp. was negative on whole blood. A convalescent *R. rickettsii* antibody titer was 1:256, which supported a diagnosis of SFG rickettsiosis, most likely RMSF.

Treatment: Because of the acute onset of lethargy and fever, the history of recent tick attachment, the documentation of thrombocytopenia, and the relatively high frequency of RMSF in dogs and people in North Carolina, doxycycline (4.8 mg/kg PO q12h) was prescribed for 3 weeks before the results of microbiologic testing became available. The dog responded rapidly after doxycycline treatment, with appetite and behaviors normalizing within 24 to 48 hours after the first dose.

Comment: Although the clinical signs in this dog were relatively nonspecific, the clinical presentation was very typical of RMSF. Because *R. rickettsii* causes generalized vascular injury resulting in increased vascular permeability, fluid and protein leakage out of the intravascular space, and an acute neutrophilic inflammatory response throughout tissue sites within the body, pain that appears to shift in location (back pain, joint pain, abdominal pain, neck pain) is a typical disease manifestation. Also, the hunched appearance reported in this dog is commonly observed in dogs with RMSF. As this dog was owned by veterinarian and a tick had been removed a few days earlier, a clinical diagnosis and appropriate treatment were initiated earlier in the course of illness than is typical of most dogs with RMSF. The rapid initiation of doxycycline may have blunted the humoral antibody response and decreased the convalescent antibody titer to a level that is lower than expected in most cases of RMSF in dogs. It is important to note that PCR assay is insensitive in the early stages of RMSF, as there are often inadequate numbers of organisms in the blood to achieve successful amplification of rickettsial DNA. A very rapid response to doxycycline is expected, unless there has been a delay in diagnosis, accompanied by the onset of neurologic abnormalities. Dogs that develop neurologic complications experience a more prolonged recovery and can have residual neurologic deficits.

Rocky Mountain Spotted Fever in a Human Patient

On May 9, a 61-year-old man, who resided on a farm in Wake County, North Carolina, removed an embedded tick from the hairy portion of his right armpit. Although duration of attachment was unknown, it was likely that the tick was acquired 2 to 3 days earlier while pulling weeds from a hay field. Using a tick identification key, an experienced research technician classified the tick as a male *Amblyomma americanum* and stored the tick in a vial containing alcohol. On May 16, while working on the farm, the patient experienced mild nausea after drinking water and became transiently dizzy. The next morning chills developed, and by the mid-afternoon the patient became febrile (101.2°F [38.4°C]), developed muscle pain, a mild headache and remained in bed until the next morning. These symptoms became progressively more severe during the day and the tick attachment site had developed into an erythematous, circular lesion with induration and a necrotic center, consistent with a rickettsial eschar. Due to the history of tick attachment and fever (maximum temperature 102.9°F [39.4°C]), a spotted fever rickettsiosis, such as RMSF, or neutrophilic or monocytic ehrlichiosis, caused by *Ehrlichia ewingii* and *Ehrlichia chaffeensis*, respectively, was suspected. Neutropenia (2553 cells/μL) was accompanied by a left shift (5% band neutrophils) and thrombocytopenia (148,000/μL). Doxycycline was dispensed with instructions to take 100 mg q12h for 7 days. That evening, after sleeping for 6 hours, the patient ate a small quantity of food, after which he became severely nauseated and fainted, and impact with the floor was associated with a severe blow to the back of the head. The next day (May 19) a maculopapular rash appeared that predominantly involved the inferior portions of the arms and legs. Throughout the day the distribution of the rash spread and the severity progressed from barely visible to obvious over most of the body. At no time did the rash involve the palms or plantar surface of the feet. Fever resolved within 36 hours after the initiation of doxycycline, and the rash began to fade gradually after 48 hours of treatment. Within a week after starting doxycycline, the patient was experiencing no symptoms and had no sequelae as a result of the infection or the fall. A repeat CBC (May 25) identified a normal neutrophil count (4378 cells/μL), no band neutrophils, and a normal platelet count (320,000/μL). Acute-phase serum was collected and stored until the convalescent sample was obtained. *Rickettsia* and *Ehrlichia* spp. PCR was performed immediately. PCR amplicons, obtained from the patient's blood and from the tick, were sequenced, confirming the presence of *R. rickettsii* DNA. PCR for *Ehrlichia* spp. DNA was negative from both the tick and the patient. Subsequently, seroconversion to *R. rickettsii* antigens was identified (1:64 acute titer, convalescent titer 1:512 after 4 weeks). The patient did not seroconvert to *Ehrlichia* spp. antigens.

Comment: Historically, transmission of *R. rickettsii* in North America was attributed solely to *Dermacentor variabilis* in the eastern United States and to *Dermacentor andersoni* in the western United States. However, between 2002 and 2004, researchers at the Centers for Disease Control and Prevention documented *Rh. sanguineus* transmission of *R. rickettsii* to people residing in Arizona. Although this is most likely an infrequent occurrence, this patient was infected with *R. rickettsii* by *A. americanum*. It is important to note the similarities between the historical, physical examination and laboratory findings for the dog discussed previously and the human patient. In several published case reports, dogs develop RMSF before a family member contracts the infection from a tick. Thus, it is important for veterinarians to recognize and confirm a diagnosis of RMSF in dogs and to educate the client as to the risk of tick exposure within their local environment.

SUGGESTED READINGS

Gasser AM, Birkenheuer AJ, Breitschwerdt EB. Canine Rocky Mountain spotted fever: a retrospective study of 30 cases. J Am Anim Hosp Assoc. 2001;37(1):41-48.

Elchos BN, Goddard J. Implications of presumptive fatal Rocky Mountain spotted fever in two dogs and their owner. J Am Vet Med Assoc. 2003;223(10):1450-1452:1433.

Nicholson WL, Allen KE, McQuiston JH, et al. The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol. 2010;26(4):205-212.

REFERENCES

1. Ricketts HE. Some aspects of Rocky Mountain spotted fever as shown by recent investigations, 1909. Rev Infect Dis. 1991;13(6):1227-1240.
2. Bechah Y, Capo C, Mege JL, Raoult D. Rickettsial diseases: from *Rickettsia*-arthropod relationships to pathophysiology and animal models. Future Microbiol. 2008;3(2):223-236.
3. Raoult D, Fournier PE, Eremeeva M, et al. Naming of Rickettsiae and rickettsial diseases. Ann N Y Acad Sci. 2005;1063:1-12.
4. Jensenius M, Davis X, von Sonnenburg F, et al. Multicenter GeoSentinel analysis of rickettsial diseases in international travelers, 1996-2008. Emerg Infect Dis. 2009;15(11):1791-1798.
5. Fournier PE, Raoult D. Current knowledge on phylogeny and taxonomy of *Rickettsia* spp. Ann N Y Acad Sci. 2009;1166:1-11.
6. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev. 2005;18(4):719-756.
7. Goddard J. Historical and recent evidence for close relationships among *Rickettsia parkeri, R. conorii, R. africae, and R. sibirica*: implications for rickettsial taxonomy. J Vector Ecol. 2009;34(2):238-242.
8. Garcia-Garcia JC, Portillo A, Nunez MJ, et al. A patient from Argentina infected with *Rickettsia massiliae*. Am J Trop Med Hyg. 2010;82(4):691-692.
9. Cragun WC, Bartlett BL, Ellis MW, et al. The expanding spectrum of eschar-associated rickettsioses in the United States. Arch Dermatol. 2010;146(6):641-648.
10. Nicholson WL, Paddock CD, Demma L, et al. Rocky Mountain spotted fever in Arizona: documentation of heavy environmental infestations of *Rhipicephalus sanguineus* at an endemic site. Ann N Y Acad Sci. 2006;1078:338-341.
11. Demma LJ, Eremeeva M, Nicholson WL, et al. An outbreak of Rocky Mountain spotted fever associated with a novel tick vector, *Rhipicephalus sanguineus*, in Arizona, 2004: preliminary report. Ann N Y Acad Sci. 2006;1078:342-343.
12. Parola P, Socolovschi C, Jeanjean L, et al. Warmer weather linked to tick attack and emergence of severe rickettsioses. PLoS Negl Trop Dis. 2008;2(11):e338.
13. Parola P, Labruna MB, Raoult D. Tick-borne rickettsioses in America: unanswered questions and emerging diseases. Curr Infect Dis Rep. 2009;11(1):40-50.
14. Solano-Gallego L, Kidd L, Trotta M, et al. Febrile illness associated with *Rickettsia conorii* infection in dogs from Sicily. Emerg Infect Dis. 2006;12(12):1985-1988.
15. Keenan KP, Buhles Jr WC, Huxsoll DL, et al. Studies on the pathogenesis of *Rickettsia rickettsii* in the dog: clinical and clinicopathologic changes of experimental infection. Am J Vet Res. 1977;38(6):851-856.
16. Keenan KP, Buhles Jr WC, Huxsoll DL, et al. Pathogenesis of infection with *Rickettsia rickettsii* in the dog: a disease model for Rocky Mountain spotted fever. J Infect Dis. 1977;135(6):911-917.
17. Gasser AM, Birkenheuer AJ, Breitschwerdt EB. Canine Rocky Mountain spotted fever: a retrospective study of 30 cases. J Am Anim Hosp Assoc. 2001;37(1):41-48.
18. Greene CE, Burgdorfer W, Cavagnolo R, et al. Rocky Mountain spotted fever in dogs and its differentiation from canine ehrlichiosis. J Am Vet Med Assoc. 1985;186(5):465-472.
19. Breitschwerdt EB, Walker DH, Levy MG, et al. Clinical, hematologic, and humoral immune response in female dogs inoculated with *Rickettsia rickettsii* and *Rickettsia montana*. Am J Vet Res. 1988;49(1):70-76.
20. Breitschwerdt EB, Meuten DJ, Walker DH, et al. Canine Rocky Mountain spotted fever: a kennel epizootic. Am J Vet Res. 1985;46(10):2124-2128.
21. Elchos BN, Goddard J. Implications of presumptive fatal Rocky Mountain spotted fever in two dogs and their owner. J Am Vet Med Assoc. 2003;223(10):1450-1452:1433.
22. Paddock CD, Brenner O, Vaid C, et al. Short report: concurrent Rocky Mountain spotted fever in a dog and its owner. Am J Trop Med Hyg. 2002;66(2):197-199.
23. Demma LJ, Traeger M, Blau D, et al. Serologic evidence for exposure to *Rickettsia rickettsii* in eastern Arizona and recent emergence of Rocky Mountain spotted fever in this region. Vector Borne Zoonotic Dis. 2006;6(4):423-429.
24. Nicholson WL, Gordon R, Demma LJ. Spotted fever group rickettsial infection in dogs from eastern Arizona: how long has it been there? Ann N Y Acad Sci. 2006;1078:519-522.
25. Raoult D, Toga B, Dunan S, et al. Mediterranean spotted fever in the South of France; serosurvey of dogs. Trop Geogr Med. 1985;37(3):258-260.
26. Mannelli A, Mandola ML, Pedri P, et al. Associations between dogs that were serologically positive for *Rickettsia conorii* relative to the residences of two human cases of Mediterranean spotted fever in Piemonte (Italy). Prev Vet Med. Jul 30 2003;60(1):13-26.
27. Segura-Porta F, Diestre-Ortin G, Ortuno-Romero A, et al. Prevalence of antibodies to spotted fever group rickettsiae in human beings and dogs from an endemic area of Mediterranean spotted fever in Catalonia, Spain. Eur J Epidemiol. 1998;14(4):395-398.
28. Beeler E, Abramowicz KF, Zambrano ML, et al. A focus of dogs and *Rickettsia massiliae*–infected *Rhipicephalus sanguineus* in California. Am J Trop Med Hyg. 2011;84(2):244-249.
29. Sexton DJ, Banks J, Graves S, et al. Prevalence of antibodies to spotted fever group rickettsiae in dogs from southeastern Australia. Am J Trop Med Hyg. 1991;45(2):243-248.
30. Satoh H, Tsuneki A, Inokuma H, et al. Seroprevalence of antibodies against spotted fever group *Rickettsia* among dogs and humans in Okinawa, Japan. Microbiol Immunol. 2001;45(1):85-87.
31. Inokuma H, Matsuda H, Sakamoto L, et al. Evaluation of *Rickettsia japonica* pathogenesis and reservoir potential in dogs by experimental inoculation and epidemiologic survey. Clin Vaccine Immunol. 2011;18(1):161-166.
32. Greene CE, Breitschwerdt EB. Rocky Mountain spotted fever, murine typhus-like disease, rickettsialpox, typhus, and Q fever. In: Greene CE, ed. Infectious Diseases of the Dog and Cat. 6th ed. Philadelphia, PA: Saunders Elsevier; 2006:232-241.
33. Bayliss DB, Morris AK, Horta MC, et al. Prevalence of *Rickettsia* species antibodies and *Rickettsia* species DNA in the blood of cats with and without fever. J Feline Med Surg. 2009;11(4):266-270.
34. Warner RD, Marsh WW. Rocky Mountain spotted fever. J Am Vet Med Assoc. 2002;221(10):1413-1417.
35. Guedes E, Leite RC, Prata MC, et al. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Mem Inst Oswaldo Cruz. 2005;100(8):841-845.
36. Breitschwerdt EB, Hegarty BC, Maggi RG, Lantos PM, Aslett DM, Bradley JM. *Rickettsia rickettsii* Transmission by a Lone Star Tick, North Carolina. Emerg Infect Dis. 2011;17(5):873-875.
37. Demma LJ, Traeger MS, Nicholson WL, et al. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. N Engl J Med. 2005;353(6):587-594.
38. Piranda EM, Faccini JL, Pinter A, et al. Experimental infection of *Rhipicephalus sanguineus* ticks with the bacterium *Rickettsia rickettsii*, using experimentally infected dogs. Vector Borne Zoonotic Dis. 2011;11(1):29-36.

39. Chen LF, Sexton DJ. What's new in Rocky Mountain spotted fever? Infect Dis Clin North Am. 2008;22(3):415-432:vii-viii.
40. Dantas-Torres F. Rocky Mountain spotted fever. Lancet Infect Dis. 2007;7(11):724-732.
41. Kidd LB. Transmission times and prevention of tick-borne diseases in dogs. Compend Contin Educ Pract Vet. 2003;25(10):742-751.
42. Dantas-Torres F. Biology and ecology of the brown dog tick. Rhipicephalus sanguineus. Parasit Vectors. 2010;3:26.
43. Norment BR, Burgdorfer W. Susceptibility and reservoir potential of the dog to spotted fever-group rickettsiae. Am J Vet Res. 1984;45(9):1706-1710.
44. Wikswo ME, Hu R, Metzger ME, Eremeeva ME. Detection of *Rickettsia rickettsii* and *Bartonella henselae* in *Rhipicephalus sanguineus* ticks from California. J Med Entomol. 2007;44(1):158-162.
45. Stromdahl EY, Jiang J, Vince M, Richards AL. Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* removed from humans, with comments on the role of other human-biting ticks associated with spotted fever group Rickettsiae in the United States. Vector Borne Zoonotic Dis. 2011;11(7):969-977.
46. Chapman AS, Bakken JS, Folk SM, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis—United States: a practical guide for physicians and other health-care and public health professionals. MMWR Recomm Rep. 2006;55(RR-4):1-27.
47. Centers for Disease Control and Prevention. Rocky Mountain Spotted Fever, Statistics and Epidemiology. http://www.cdc.gov/rmsf/stats:Last accessed May 27, 2012.
48. Moncayo AC, Cohen SB, Fritzen CM, et al. Absence of *Rickettsia rickettsii* and occurrence of other spotted fever group rickettsiae in ticks from Tennessee. Am J Trop Med Hyg. 2010;83(3):653-657.
49. Raoult D, Parola P. Rocky Mountain spotted fever in the USA: a benign disease or a common diagnostic error? Lancet Infect Dis. 2008;8(10):587-589.
50. Diniz PPVP, unpublished data. 2012.
51. Eremeeva ME, Zambrano ML, Anaya L, et al. *Rickettsia rickettsii* in *Rhipicephalus* ticks, Mexicali. Mexico. J Med Entomol. 2011;48(2):418-421.
52. Tinoco-Gracia L, Quiroz-Romero H, Quintero-Martinez MT, et al. Prevalence of *Rhipicephalus sanguineus* ticks on dogs in a region on the Mexico-USA border. Vet Rec. 2009;164(2):59-61.
53. Fritz CL, Kriner P, Garcia D, et al. Tick infestation and spotted fever group *Rickettsia* in shelter dogs, California, 2009. Zoonoses Public Health. 2012;59(1):4-7.
54. Telford SR. Status of the "East Side hypothesis" (transovarial interference) 25 years later. Ann N Y Acad Sci. 2009;1166:144-150.
55. Sahni SK, Rydkina E. Host-cell interactions with pathogenic *Rickettsia* species. Future Microbiol. 2009;4(3):323-339.
56. Walker DH, Valbuena GA, Olano JP. Pathogenic mechanisms of diseases caused by *Rickettsia*. Ann N Y Acad Sci. 2003;990:1-11.
57. Walker DH. Rickettsiae and rickettsial infections: the current state of knowledge. Clin Infect Dis. 2007;45(suppl 1):S39-S44.
58. Balraj P, Renesto P, Raoult D. Advances in rickettsia pathogenicity. Ann N Y Acad Sci. 2009;1166:94-105.
59. Eremeeva ME, Liang Z, Paddock C, et al. *Rickettsia rickettsii* infection in the pine vole, *Microtus pinetorum*: kinetics of infection and quantitation of antioxidant enzyme gene expression by RT-PCR. Ann N Y Acad Sci. 2003;990:468-473.
60. Cummiskey JM, Simon LM, Theodore J, Ryan US, Robin ED. Bioenergetic alterations in cultivated pulmonary artery and aortic endothelial cells exposed to normoxia and hypoxia. Exp Lung Res. 1981;2(3):155-163.
61. Asahina T, Kashiwagi A, Nishio Y, et al. Impaired activation of glucose oxidation and NADPH supply in human endothelial cells exposed to H_2O_2 in high-glucose medium. Diabetes. 1995;44(5):520-526.
62. Breitschwerdt EB, Papich MG, Hegarty BC, et al. Efficacy of doxycycline, azithromycin, or trovafloxacin for treatment of experimental Rocky Mountain spotted fever in dogs. Antimicrob Agents Chemother. 1999;43(4):813-821.
63. Piranda EM, Faccini JL, Pinter A, et al. Experimental infection of dogs with a Brazilian strain of *Rickettsia rickettsii*: clinical and laboratory findings. Mem Inst Oswaldo Cruz. 2008;103(7):696-701.
64. Kordick SK, Breitschwerdt EB, Hegarty BC, et al. Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. J Clin Microbiol. 1999;37(8):2631-2638.
65. Greene CE. Infectious Diseases of the Dog and Cat. 3rd ed. St. Louis, MO: Saunders Elsevier; 2006.
66. Ober CP, Spaulding K, Breitschwerdt EB, et al. Orchitis in two dogs with Rocky Mountain spotted fever. Vet Radiol Ultrasound. 2004;45(5):458-465.
67. Grindem CB, Breitschwerdt EB, Perkins PC, et al. Platelet-associated immunoglobulin (antiplatelet antibody) in canine Rocky Mountain spotted fever and ehrlichiosis. J Am Anim Hosp Assoc. 1999;35(1):56-61.
68. Mikszewski JS, Vite CH. Central nervous system dysfunction associated with Rocky Mountain spotted fever infection in five dogs. J Am Anim Hosp Assoc. 2005;41(4):259-266.
69. Drost WT, Berry CR, Breitschwerdt EB, Davidson MG. Thoracic radiographic findings in dogs infected with *Rickettsia rickettsii*. Vet Radiol Ultrasound. 1997;38(4):260-266.
70. Breitschwerdt EB, Levy MG, Davidson MG, et al. Kinetics of IgM and IgG responses to experimental and naturally acquired *Rickettsia rickettsii* infection in dogs. Am J Vet Res. 1990;51(8):1312-1316.
71. Nicholson WL, Allen KE, McQuiston JH, et al. The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol. 2010;26(4):205-212.
72. Breitschwerdt EB, Moncol DJ, Corbett WT, et al. Antibodies to spotted fever-group rickettsiae in dogs in North Carolina. Am J Vet Res. 1987;48(10):1436-1440.
73. Solano-Gallego L, Bradley J, Hegarty B, et al. *Bartonella henselae* IgG antibodies are prevalent in dogs from southeastern USA. Vet Res. 2004;35(5):585-595.
74. Kidd L, Maggi R, Diniz PP, et al. Evaluation of conventional and real-time PCR assays for detection and differentiation of spotted fever group *Rickettsia* in dog blood. Vet Microbiol. 2008;129(3-4):294-303.
75. Davidson MG, Breitschwerdt EB, Walker DH, et al. Identification of rickettsiae in cutaneous biopsy specimens from dogs with experimental Rocky Mountain spotted fever. J Vet Intern Med. 1989;3(1):8-11.
76. Breitschwerdt EB, Davidson MG, Aucoin DP, et al. Efficacy of chloramphenicol, enrofloxacin, and tetracycline for treatment of experimental Rocky Mountain spotted fever in dogs. Antimicrob Agents Chemother. 1991;35(11):2375-2381.
77. Neer TM, Eddlestone SM, Gaunt SD, Corstvet RE. Efficacy of enrofloxacin for the treatment of experimentally induced *Ehrlichia canis* infection. J Vet Intern Med. 1999;13(5):501-504.
78. Breitschwerdt EB, Davidson MG, Hegarty BC, et al. Prednisolone at anti-inflammatory or immunosuppressive dosages in conjunction with doxycycline does not potentiate the severity of *Rickettsia rickettsii* infection in dogs. Antimicrob Agents Chemother. 1997;41(1):141-147.
79. Low RM, Holm JL. Canine Rocky Mountain spotted fever. Compend Contin Educ Pract Vet. 2005;27(7):530-538.
80. Walker DH. The realities of biodefense vaccines against *Rickettsia*. Vaccine. 2009;27(suppl 4):D52-D55.
81. Bonilla D. Tick borne disease surveillance: spotted fever group *Rickettsia*. Calif Dept Public Health Vector Borne Dis Bull. 2009;3(2):4.