Tick-borne Ehrlichiae and Rickettsiae of Dogs (2-May-2003)

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Introduction

Over the past several decades, tick-borne diseases caused by obligate intracellular bacteria have emerged as important threats to mammals worldwide, and have gained notoriety because of growing concern that changing climate conditions may favor vector-borne disease transmission. Canids not only become clinically affected with specific ehrlichiae and rickettsiae, but may also serve as reservoir hosts for organisms that cause disease in humans. While this article will focus on a handful of the species in the genera *Ehrlichia* and *Rickettsia* of most clinical importance to canids (Table 1), a large number of species exist, many of which have only recently emerged, and others that may still be unrecognized.

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Canine Ehrlichiae

While the ehrlichiae were formerly considered host specific, we now realize that many species in the genus *Ehrlichia* infect canids, including *E. canis*, *E. chaffeensis* and *E. ewingii*. *Ehrlichia canis*, the agent of classic canine monocytic ehrlichiosis (CME; historically referred to as canine tropical pancytopenia), is the most commonly recognized tick-borne ehrlichial organism that infects canids. *Ehrlichia chaffeensis*, a species closely related to *E. canis*, has also been associated with CME, and is more commonly known as the agent of human monocytic ehrlichiosis. *Ehrlichia (Cowdria) ruminantium*, the agent of heartwater was recently detected in dogs with symptoms suggestive of canine ehrlichiosis in South Africa [1]. In addition to the currently recognized ehrlichiae, two organisms, *Anaplasma phagocytophilum*, formerly known as three separate ehrlichiae: *Ehrlichia equi*, the agent of human granulocytic ehrlichiosis (HGE), *E. phagocytophilum*, and *Anaplasma platys*, formerly known as *Ehrlichia platys*, will continue to be considered important canine ehrlichiae for the purposes of this chapter.

In general, the *Ehrlichia* spp. can be distinguished by the type of cells they invade, as suggested by the name of the disease that each organism causes. Both *E. canis* and *E. chaffeensis*, which cause CME, invade mononuclear cells (Fig. 1a and Fig. 1b). *A. platys*, the agent of canine cyclic thrombocytopenia (CCT) invades platelets. The agents of canine granulocytic ehrlichiosis (CGE), *A. phagocytophilum* and *E. ewingii*, which also cause human ehrlichiosis, invade neutrophils (Fig. 1c and
Endothelial cells are the target cells for *E. ruminantium*.

**Figure 1a.** Intracellular morulae of *Ehrlichia canis*, one of the agents of canine monocytic ehrlichiosis, in monocytes. With permission of the University of Georgia. - To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 1b.** Intracellular morulae of *E. chaffeensis*, one of the agents of canine monocytic ehrlichiosis, in monocytes. With permission of the Center for Disease Control, Atlanta, GA. - To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 1c.** Intracellular morulae of *A. phagocytophilum*, one of the agents of canine granulocytic ehrlichiosis, in neutrophils. With permission of the Center for Disease Control, Atlanta, GA. - To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 1d.** Intracellular morulae of *E. ewingii*, one of the agents of canine granulocytic ehrlichiosis, in neutrophils. With permission from Yabsley MJ, Varela AS, Tate CM, et al., [33]. - To view this image in full size go to the IVIS website at www.ivis.org.

*Neorickettsia risticii* (originally known as *Ehrlichia risticii*), the agent of Potomac horse fever, has been identified by culture isolation, and morphological, antigenic and molecular characteristics from dogs presenting with signs of ehrlichiosis but lacking seroreactivity to *E. canis* or *E. sennetsu* [2]. Because this organism is transmitted by trematodes, and the distribution and significance of this pathogen in dogs has not been fully established, this species will not be mentioned further.

**Canine Rickettsiae**

Organisms in the genus *Rickettsia* exist worldwide and most fall into either the spotted fever group or the typhus group. Species that are transmitted by ticks fall within the spotted fever group, whereas species in the typhus group are usually transmitted by fleas or lice. The primary species that causes disease in dogs is also infectious to humans, *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever, so named because it was first described in humans from Montana and Idaho in the late 19th century. Like *E. ruminantium*, *R. rickettsii* invades endothelial cells in the vertebrate host (Fig. 2). Unlike *E. canis*, which is recognized worldwide, *Rickettsia rickettsii* occurs only in North and South America and is replaced by its sister species, *R. conorii*, the cause of Mediterranean spotted fever or Boutonneuse fever in humans, in the Eastern Hemisphere. Other tick-transmitted *Rickettsia* spp. that infect dogs are generally nonpathogenic. For example, dogs experimentally infected with *R. montana* show no clinical signs, and dogs are considered reservoir hosts, lacking clinical signs, for *R. conorii* [3,4]. *Coxiella burnetti*, the rickettsial organism causing Q fever, is a significant public health threat in five continents and of veterinary importance to livestock, but is not of clinical importance to dogs. Consequently, because of the significance of *R. rickettsii* in dogs and the lack of information recognizing other pathogenic *Rickettsia* spp. of dogs, this article will focus only on *R. rickettsii* as the canine rickettsia of importance.

**Figure 2.** *Rickettsia rickettsii* within tick hemolymph cells. With permission of the Center for Disease Control, Atlanta, GA. - To view this image in full size go to the IVIS website at www.ivis.org.
Tick Transmission
Second only to mosquitoes, ticks are infamous for vectoring pathogens of medical and veterinary importance [5]. Ticks in the family Ixodidae are responsible for transmission of the canine ehrlichiae and rickettsiae, and the organisms are generally specific to one or a few tick species. Although the distribution of canine tick-borne disease is usually a reflection of the distribution of ticks, in modern times of increased travel, the movement of infected dogs into regions where the diseases are not endemic has greatly broadened the geographical distribution of canine tick-borne diseases.

*Rhipicephalus sanguineus* (brown dog tick) (Fig. 3a) and *Amblyomma americanum* (lone star tick) (Fig. 3b) transmit the agents of CME, *E. canis* and *E. chaffeensis*, respectively. *Ehrlichia canis* can be passed transstadially through *R. sanguineus* to infect dogs, which serve as the reservoir host for *E. canis* and the vertebrate host for all stages of *R. sanguineus*. Transovarial transmission does not appear to play a significant role in the maintenance of *E. canis* [6]. White-tailed deer and *A. americanum* are the primary vertebrate reservoir and tick host of *E. chaffeensis*, respectively, and transstadial transmission of this pathogen has been observed [7]. Although *A. americanum* has been implicated as the primary vector of *E. ewingii*, molecular evidence of *E. ewingii* has also been detected in *Dermacentor variabilis* (American dog tick) and *R. sanguineus* [8,9]. Other agents of granulocytic ehrlichiosis are transmitted by *Ixodes* spp. throughout the world, including *Ixodes scapularis* (black-legged tick) (Fig. 3c) and *I. pacificus* in the United States, and *I. ricinus* in Europe. The tick vector responsible for transmission of *A. platys* is still unknown, but experimental studies suggest that *Rhipicephalus sanguineus* is not a suitable vector [10].

In the Western Hemisphere, *Dermacentor andersoni* (Rocky Mountain wood tick) in the western United States and several areas of Canada, and *D. variabilis* (American dog tick) primarily in the midwestern and eastern United States and southern Canada, vector *R. rickettsii* (Fig. 4a and Fig. 4b). Maintenance of *R. rickettsii* occurs through a cycle between *Dermacentor* spp. and rodents or small mammals, including dogs. Both transovarial and transstadial passage of *R. rickettsii* occurs.
Transmission of canine ehrlichiae and rickettsiae occurs through the bite of the infected ticks, however, the process of transmission for many of the pathogens has not been studied in depth. A general scenario, as has been described for \textit{R. rickettsii}, may be present in canine ehrlichiae and other rickettsiae. Upon acquisition, \textit{R. rickettsii} organisms replicate in the midgut and subsequently penetrate through the gut epithelium to disseminate throughout the tick’s body and invade virtually all tissues, including the salivary glands [11]. Initiation of feeding on the vertebrate host stimulates replication of the pathogen; transmission occurs via the saliva. Numerous components, such as vasodilators, anticoagulant factors, and immunosuppressants, have been described in tick saliva that facilitate blood feeding and, perhaps inadvertently, the entrance of disease agents. Among these salivary components are prostaglandins in \textit{A. americanum}, and anticoagulants in \textit{I. ricinus} and \textit{D. andersoni} [12-14]. The characterization and importance of these factors has become of increasing interest because of the potential to use them in vaccines.

**Clinical Signs**

**Canine Ehrlichiae** - Canine ehrlichiae are generally present with multisystemic signs that may vary slightly depending on the \textit{Ehrlichia} species involved. Three stages of disease have been described in dogs experimentally infected with \textit{E. canis}, but may not be easy to distinguish in naturally infected dogs [15]. After transmission of \textit{E. canis}, there is a 8 - 20 day incubation period in dogs preceding the start of a 2 - 4 week acute phase. Clinical signs observed in the acute phase include fever, anorexia, weight loss, dyspnea, lethargy, depression, lymphadenopathy, and edema of the limbs or scrotum. Central nervous system signs have been observed due to inflammation or bleeding into the meninges, and are more common in the acute phase of the disease. Thrombocytopenia and/or leukopenia are generally present during the first 10 - 20 days after infection, but bleeding disorders are not often seen at this stage. Anemia may also occur. The subclinical phase follows resolution of acute signs and usually ranges from 40 - 120 days, though this phase may last for years, during which time the infected dog appears clinically healthy. Laboratory findings during subclinical infection often only include mild thrombocytopenia and a positive antibody titer; slightly decreased packed cell volume or leukocytes and hypergammaglobulinemia may also be observed [16].

In immunocompetent dogs \textit{E. canis} may be cleared, otherwise the chronic phase begins. Clinical signs during this stage vary from nonexistent to mild disease, but may be severe in certain breeds such as German shepherds. Weight loss and hematologic changes, including pancytopenia, are commonly observed. During the chronic stage, ocular manifestations may develop, including anterior uveitis and retinal disease, and may progress to blindness.

Dogs experimentally infected with \textit{E. chaffeensis} appear to have only mild signs limited to fever; thrombocytopenia has not been observed [17]. Both \textit{E. ewingii} and \textit{A. phagocytophilum} cause granulocytic ehrlichiosis in dogs and produce similar clinical signs. Fever, anorexia, and thrombocytopenia are often observed, and other hematologic changes similar to CME may occur, but unlike CME, a common finding in canine granulocytic ehrlichiosis is polyarthritis [15]. Polyarthritis typically involves joint swelling and pain, lameness of one or multiple limbs, and stiffness, with a reluctance to rise, and is often accompanied by neutrophilic inflammation.

Few clinical signs are associated with infection by \textit{E. platys}. Dogs with CCT present with mild fever and clinical signs due to low platelet counts, such as petechiae and ecchymoses. Cyclic rickettsemia and thrombocytopenia give CCT its name. Uveitis has been reported in one case [18]. Fever, weight loss and anorexia have been described in dogs infected with more pathogenic strains outside of the USA [19].

**Canine Rickettsiae** - \textit{Rickettsia rickettsii} is the most pathogenic of the spotted fever group rickettsiae. Dogs infected with \textit{R. rickettsii} have a rapid and severe disease course and will show clinical signs nearly identical to those in the acute phase of CME. Fever occurs 4 - 5 days after the tick bite, petechiae and ecchymoses develop on mucous membranes due to decreased platelet numbers, and clinical signs progress to edema and eventually multisystemic organ disorders. Leukopenia may be
present early in the disease and change to a leukocytosis later.

Although very similar to the canine ehrlichiae, RMSF has a few distinguishing clinical characteristics. The duration of illness with *R. rickettsia* is much shorter, generally lasting about 2 weeks, occurs seasonally in the warmer months of the year (March through October), is more rapid in onset, and lacks a chronic phase [20,21]. The latter is due to the development of protective immunity, which explains why RMSF most often affects younger (≤ 3 years old) dogs [21].

**Diagnosis**

**Canine Ehrlichiae** - Canine ehrlichiosis is diagnosed using a combination of clinical signs and laboratory findings including serology, hematologic abnormalities and changes in serum chemistry. Thrombocytopenia is the most common hematologic change that supports a diagnosis of ehrlichiosis, while many dogs also have nonregenerative anemia and leukopenia. Various serum chemistry abnormalities may assist with diagnosis of canine ehrlichiosis and most frequently include hyperproteinemia, resulting from hyperglobulinemia, hypoalbuminemia and increased alanine aminotransferase and alkaline phosphatase activity.

The indirect fluorescent antibody (IFA) test is generally used for detection of antibodies in canine cases of ehrlichiosis and is considered the "gold standard" serologic assay for diagnosis of CME. Other serologic assays have also been evaluated. For example, the MAP2 ELISA was shown to be 97% accurate in comparison to the IFA, and has the added advantage of being quantitative, although it is a cumbersome test to perform [22]. Rapid tests, while attractive for practical purposes, still do not compare to the IFA in specificity or sensitivity and interpretation of any of these tests must be made with caution because of serologic cross-reactivity between the monocytic *Ehrlichia* spp. and *E. ewingii*. There is no serologic cross-reactivity between the monocytic *Ehrlichia* spp. and *A. platys*. Cut-off titers for positive serologic assays will depend on the laboratory. The recent identification of two recombinant proteins, rP28 and rP43, as candidates for specific serodiagnosis of *E. canis*, may solve the limitations of IFAs due to cross-reactivity [23].

Visualization of morulae in the respective cell types provides a definitive diagnosis and allows for differentiating between the monocytic and granulocytic ehrlichia. Although this process is time-consuming and can be unrewarding, the sensitivity of the technique is improved by evaluating up to 1000 oil immersions field from prepared buffy coats or lymph node smears [24]. The use of species-specific PCR assays to detect DNA from ehrlichial organisms is also used to diagnose infection with specific *Ehrlichia* spp.

**Canine Rickettsiae** - Like the canine ehrlichiae, diagnosis of RMSF is based on a combination of clinical presentation, including clinical signs and laboratory findings, serology, and molecular detection, if available. The indirect fluorescent antibody test is also considered the standard assay to measure antibody titers. Acute and convalescent (≥ 3 weeks apart) serum samples are preferred, with evidence of recent infection defined as a four-fold or greater increase in convalescent IgM titer. Single IgM titers ≥ 64 in addition to suggestive clinical signs also supports a diagnosis of RMSF [21]. Another diagnostic tool that has proven useful is direct fluorescent antibody (FA) staining of organisms in tissue biopsies and for confirmation of infection postmortem [25].

Culture of both canine ehrlichiae and rickettsiae, due to the long time period required for isolation, is impractical as a normal diagnostic tool and is limited to use in research laboratories.

**Pathology**

**Canine Ehrlichiae** - After entering the canine host through the bite of the tick vector, ehrlichial organisms travel through the circulation, invade cells and disseminate to various tissues, especially tissues rich in mononuclear cells, such as the lymph nodes and spleen, in the case of acute CME. Once in tissues, they continue to invade, persist, and replicate in cells. Circulating infected cells may induce vasculitis and subsequent intravascular coagulation which, in combination with an altered cell-mediated immunity, result in the destruction, consumption and sequestration of platelets. Similar destruction of leukocytes and erythrocytes in combination with decreased erythrocyte production may cause clinical leukopenia and anemia, respectively. During the subclinical phase, thrombocytopenia, leukopenia, and anemia may continue. Hyperglobulinemia may be observed in the chronic stages, and is not related to serum antibody levels. Also during chronic infection, function of the bone marrow is impaired, although the mechanisms for suppression are not completely understood. The pathogenesis of polyarthritis, observed more often with infection by granulocytic ehrlichiae, arises from hemarthrosis and immune complex deposition into the joints.
Canine Rickettsiae - Upon transmission to the vertebrate host, rickettsiae become distributed via the circulation and invade endothelial cells of venules where they replicate, resulting in damage to the cells and subsequent vasculitis. The coagulation process is activated, followed by progressive necrotizing vasculitis and increased vascular permeability. Extravasation of blood cells and plasma from leaky vessels results in edema, hemorrhage, hypotension and ultimately shock. The effect of these processes is multisystemic, with edema in the CNS causing neurologic signs, pulmonary edema causing respiratory distress, ocular damage due to inflammatory cell infiltrates and retinal petechiae, and vascular obstruction leading to tissue loss and organ damage. In severe cases, decreased tissue perfusion due to hypotension may lead to acute renal failure and inflammation of the myocardium may be life-threatening.

Treatment and Prevention
Canine Ehrlichiae - While tetracycline and oxytetracycline have traditionally been used to treat canine ehrlichiosis, they have been replaced by the modern tetracycline class antimicrobials doxycycline and minocycline as the drugs of choice. Dosages vary, but both doxycycline and minocycline are generally administered at 10 mg/kg every 12 or 24 hours [26,27]. The Infectious Disease Study Group of the ACVIM recommends a doxycycline protocol of 10 mg/kg every 24 hours for 28 days to treat canine ehrlichiosis [27]. Other antimicrobials that have been used to treat canine ehrlichiosis are chloramphenicol, amicarbalide, and imidocarb dipropionate [26,27]. Enrofloxacin was found to be ineffective against canine ehrlichiosis [28]. Dogs in the acute stage of CME usually respond to treatment within 24 - 48 hours and have a favorable prognosis, but some develop chronic infections that are difficult to treat and become reservoirs of infection.

Canine Rickettsiae - Treatment of canine rickettsiae is similar to that of canine ehrlichiae. Currently, doxycycline (prescribed at 10 - 20 mg/kg every 12 hours for 7 days or 5 mg/kg every 12 hours for 14 days) is considered the drug of choice to treat RMSF in dogs [15,29]. Tetracycline, the antirickettsial first called upon to treat RMSF, is still used, but is contraindicated in young (< 6 month old) dogs due to dental staining. When administered, tetracycline is given over 7 - 14 days at a dose of 22 mg/kg every 8 hours [29]. Chloramphenicol is also an effective treatment. While the fluoroquinolones, enrofloxacin and trovafloxacin, have been shown to be efficacious in experimental trials with canine RMSF, their use in natural infections has not been fully evaluated [30]. The prognosis for dogs with RMSF will depend greatly on the how far the infection has progressed when chemotherapeutics are initiated as well as the effectiveness of ancillary supportive therapy. Dehydration, anemia, blood coagulation disorders, and secondary manifestations such as organ failure must be diagnosed efficiently and treated concurrently during drug therapy. At present, no vaccines are available for the prevention of either canine ehrlichiosis or RMSF.

Vector Control
Perhaps the most important step towards control of canine ehrlichiosis and rickettsiosis is prevention of transmission through the use of an effective prophylactic acaricide. Breaking the life cycle of the tick vector at the level of the canine host will eliminate the source of numerous pathogenic agents, in addition to ehrlichiae and rickettsiae, that infect dogs, and may decrease the risk of transmission to humans for those tick vectors with broad host ranges. Common acaricides such as amitraz, fipronil, and pyrethrins, when used according to the manufacturer’s instructions, are effective. In a study conducted during 2000 in CME endemic areas of West and Eastern Africa, the use of fipronil on kennel dogs was found to significantly reduce the seroprevalence of CME in treated dogs compared to untreated dogs due to elimination of the tick vector, *R. sanguineus* [31]. Selamectin, a recently approved semi-synthetic avermectin, is labeled for the control of *D. variabilis*, the vector of Rocky Mountain spotted fever. By targeting the vector, the life cycle and consequently transmission of ehrlichiae and rickettsiae will be interrupted. Because many of the marketed endectocides are designed to control several parasites, they are an appealing approach to the prevention of tick-borne diseases. Their use, in combination with environmental control and chemotherapeutics, are essential to the overall control of tick-borne ehrlichiae and rickettsiae that infect canids, as well as those that are zoonotic.

References


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