

## Canine Ehrlichiosis: Update

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### Overview

*Ehrlichia* species are tick-transmitted, obligate intracellular bacteria that can cause granulocytic or monocytic ehrlichiosis. *Ehrlichia* species that have been detected in the blood and tissues of clinically ill dogs in North America include *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and Panola Mountain *Ehrlichia* species (Table 1). Clinicopathologic abnormalities reported in dogs with ehrlichiosis vary depending on the species of *Ehrlichia*, strain variances and the immune or health status of the dog. The course of disease may present as subclinical, acute, chronic or even result in death (Table 1). *E. canis* and *E. ewingii* are the most prevalent and frequently described Ehrlichia infections in dogs.

### *E. canis*:

Transmitted by *Rhipicephalus sanguineus*, *E. canis* is found world-wide. Within North America, the highest seroprevalence rates have been reported in the Southern U. S.<sup>2,12</sup> *E. canis* typically infects canine mononuclear cells. Canine monocytic ehrlichiosis (CME) is characterized by 3 stages: acute, subclinical and chronic. Following an incubation period of 1-3 weeks, infected dogs may remain subclinical or present with nonspecific signs including fever, lethargy, lymphadenopathy, splenomegaly, lameness, edema, bleeding disorders and mucopurulent ocular discharge. Less commonly reported nonspecific signs include vomiting, diarrhea, coughing and dyspnea. Bleeding disorders can include epistaxis, petechiae, ecchymoses, gingival bleeding and melena. Ocular abnormalities identified in *E. canis* infected dogs have included anterior uveitis, corneal opacity, retinal hemorrhage, hyphema, chorioretinal lesions and tortuous retinal vessels.<sup>8</sup> Following an acute phase (2-4 weeks), clinical signs may resolve without treatment and the dog could remain subclinically infected indefinitely or naturally clear the pathogen. Some dogs, however, will go on to develop chronic CME. It is still not clear why some dogs progress to the chronic phase, but possible reasons include co-infections, *E. canis* strain virulence, or the immune status of the dog. Some reports suggest a defective cell-mediated immune response may have a significant role in determining the course of disease.<sup>10</sup> German Shepherd dogs, Siberian Huskies and Belgium Malinoise develop the chronic form more commonly than other breeds. Dogs with chronic CME typically become bicytopenic or pancytopenic, developing anemia, thrombocytopenia and neutropenia due to *E. canis*-suppression of hematopoietic stem cells.<sup>8</sup> Dogs with acute CME can transiently develop a milder bicytopenia or pancytopenia. Bone marrow cytology or histology in dogs with chronic CME (myelosuppression) will have a marked reduction in hematopoietic tissue.<sup>8</sup> Lymphopenia or lymphocytosis may also be present.<sup>17</sup> For dogs that survive chronic CME, it can take up to 6 – 8 months for cytopenias to fully resolve once *E. canis* has been cleared after treatment. Common symptoms of chronic CME include fever, anorexia, weight loss, depression, edema and more severe bleeding disorders. Secondary infections may occur due to neutropenia. Sequela of chronic CME may include arthritis, renal failure, interstitial pneumonia or polymyositis.<sup>8</sup> Death can occur from hemorrhages or secondary infections. Neurological signs, likely due to meningitis or cerebral hemorrhage, have occasionally been reported in acute and chronic CME including ataxia, seizures, cranial nerve deficits hyperesthesia, vestibular disease and paresis.<sup>8</sup>

### *E. chaffeensis*:

Transmitted by *Amblyomma americanum*, *E. chaffeensis* infects mononuclear cells and rarely causes clinical disease in naturally infected dogs. Fever and lethargy, along with mild thrombocytopenia and monocytosis has been reported.<sup>1,14,20</sup>

### *E. ewingii*:

Transmitted by *Amblyomma americanum*, *E. ewingii* is the most seroprevalent canine *Ehrlichia* spp. in North America, predominantly in the Southern and Midwestern United States.<sup>2,12</sup> It infects granulocytes, and

clinicopathologic abnormalities reported in dogs with canine granulocytic ehrlichiosis include fever, lameness, neurological abnormalities, lymphadenomegaly, peripheral edema, thrombocytopenia, leukopenia, and neutrophilic polyarthritis.<sup>3, 5, 19</sup> Conversely, several of these studies also reported naturally infected dogs with no clinical abnormalities. A recent study reported that dogs experimentally infected with *E. ewingii* after exposure to wild tick species in Oklahoma could be persistently PCR+ for up to two years and not develop overt clinical disease.<sup>15</sup> Persistent infection may occur when *Ehrlichia* spp. evade the host immune response through various mechanisms, including suppression of apoptosis and modulation of chemokine and cytokine response.<sup>18</sup> Differences in clinical presentation in dogs infected with *E. ewingii* could be due to the immune status or health of the dog, the duration of infection or strain variances. It is possible *E. ewingii* may contribute to co-morbidities or function as a precipitating factor in a spectrum of clinical syndromes in dogs. A recent retrospective analysis of 32 dogs naturally infected with *E. ewingii*, without other vector-borne disease co-infections, identified limb and joint pain as the most frequently reported clinical finding, followed by heart murmur, gastrointestinal distress and lymphadenopathy.<sup>11</sup> Notable hematological and biochemical abnormalities included abnormal lymphocytosis, neutrophilia, anemia, elevated ALP and ALT, elevated creatinine, and hypoalbuminemia. Urinalysis abnormalities included proteinuria. Common diagnoses in this group of *E. ewingii* infected dogs included kidney disease and IMHA.

#### ***E. muris*:**

Little is known about *E. muris* infections in dogs. One case report describes an Anaplasma seropositive, *E. muris* PCR (+) dog that had been exposed to ticks, and presented with a fever, decreased activity, stiff gait and mild thrombocytopenia.<sup>6</sup> All Ehrlichia serology tests were negative (SNAP 4Dx and *E. canis* IFA). The dog was *E. muris* PCR (-) following treatment with doxycycline. *E. muris* is likely transmitted by *Ixodes scapularis*.

#### **Panola Mountain *Ehrlichia* species (PME):**

Little is known about PME infections in dogs. PME was first identified by PCR in a goat experimentally infested with *A. americanum* ticks collected from Panola Mountain State Park, Georgia. One case report describes a PME PCR (+) dog with hepatomegaly, elevated ALT and ALP (the dog had a history of chronic hepatobiliary disease), atypical lymphocytosis, clonal T-cell expansion and mild thrombocytopenia.<sup>13</sup> A population of abnormal lymphocytes in the liver and lymph node consistent with lymphoma was identified and flow cytometry revealed CD3+ cells, consistent with T-cell lymphoma. PARR testing of a liver aspirate documented clonal T-cell expansion. *E. canis* IFA results were positive at 1:1028 but negative for Ehrlichia by SNAP 4Dx Plus. The dog became PCR (-) and resolution of the thrombocytopenia and lymphocytosis occurred 1 week after starting doxycycline treatment, but the ALP and ALT remained elevated. Clinical signs of lymphoma resolved after doxycycline treatment, and subsequent CBCs remained normal over the next 6 months. A year following this case report, a second dog with lymphocytosis and clonal T-cell expansion was diagnosed by PCR with PME and *E. ewingii*. This dog had been referred to a veterinary oncologist for “chronic leukemia” and atypical lymphocytosis; it was mildly thrombocytopenic, *E. canis* IFA seropositive at 1:650, and positive for Ehrlichia by SNAP 4Dx Plus. Clinical signs resolved upon treatment with doxycycline. PME is transmitted by *Amblyomma americanum* and *A. manulatum*.

#### **Ehrlichia Diagnostics:**

Cytological Examination: Microscopic examination of Giemsa stained blood smears to identify the presence of morulae in mononuclear cells and neutrophils can strengthen a diagnosis of ehrlichiosis, but it is not sensitive and may only detect ~10% of positive cases. In addition, documentation of morulae cannot be used to determine the *Ehrlichia* spp.; however, you may be able to narrow down the species based on cell tropism (*E. canis* or *E. chaffeensis* commonly infect monocytes, while *E. ewingii* or *A. phagocytophilum* commonly infect neutrophils).

Indirect fluorescent antibody: *E. canis* IFA detects antibodies reactive to whole-cell *E. canis* antigens. Antibodies to *E. chaffeensis*, *E. ewingii* and PME may cross-react with *E. canis*, thus IFA should not be used to speciate an Ehrlichia infection. A two-fold increase in serial dilutions between acute and convalescent serum samples, collected approximately 4 weeks apart, can confirm an Ehrlichia infection. A decline in *E. canis* IFA titers is variable after treatment and not recommend for monitoring treatment efficacy. Some dogs will remain *E. canis* IFA positive for up to a year after apparent elimination of the *E. canis* infection.

**SNAP® 4Dx Plus:** A point-of-care, enzyme-linked immunosorbent assay (ELISA) detects antibodies to peptide-antigens from *E. canis* and *E. ewingii*. A positive result is not quantitative and represents exposure or potential infection. Dogs can remain positive for years after apparent elimination of the Ehrlichia infection. While a dog previously positive by SNAP 4DX Plus may not be currently infected, it is important to remember that there is no evidence supporting long-lasting, protective immunity, thus dogs can be re-infected or recrudescence can occur after the initial infection. SNAP 4DX Plus Ehrlichia positive dogs without clinical signs should be tested for Ehrlichia infection by PCR or a CBC examined for any evidence of current infection.

**Ehrlichia PCR:** Infection by *Ehrlichia* spp. is strongly supported when Ehrlichia DNA is detected in blood or tissue samples. The best tissue sample to use in a PCR assay to detect *Ehrlichia* spp. DNA is whole blood, but other tissues such as bone marrow, lymph node, spleen, and liver can be submitted. Positive PCRs have even been obtained using serum samples containing residual cellular material. PCR is highly sensitive and can detect infections prior to seroconversion. Many diagnostic laboratories utilize primers designed to amplify a region of DNA shared by all pathogens in the Rickettsiales order (*Ehrlichia*, *Anaplasma*, and *Neorickettsia* genus), which may be more likely to detect a wider range of *Ehrlichia* spp.; subsequently, species-specific PCR assays, DNA sequencing, or both can be used to determine specific organisms. PCR assays vary between diagnostic laboratories based on gene targets, sensitivity and specificity. Due to low pathogen loads and PCR assay limitations, the clinician should consider that a negative PCR result does not always indicate the absence of an infection but instead signifies pathogen DNA was not amplified from a particular sample.

**Organism cultivation:** Infection by *Ehrlichia* spp. can be confirmed by cell culture. It is not routinely performed to diagnose *Ehrlichia* spp. in a clinical setting; rather, it is more commonly used in the context of research studies. Furthermore, not all *Ehrlichia* spp. can be grown in cell culture.

### **Ehrlichia Treatment:**

Largely based on experimental infection studies, as well as empirical data and clinical experience with naturally infected dogs, the recommended treatment for Ehrlichia infections is doxycycline at 5 mg/kg every 12 hours or 10 mg/kg every 24 hours for 30 days.<sup>4</sup> Differences in treatment response with doxycycline have been reported in some experimentally infected dogs<sup>7</sup> and dogs with natural infections in different parts of the world. This may be due, in part, to experimental inoculation methods (ticks versus infected blood inoculum), strain differences or unknown co-infections with other VBDs, which contribute to adverse treatment outcomes. There is little evidence to support the use of other tetracyclines to treat canine ehrlichiosis; however, anecdotal evidence and clinical experience has shown that minocycline may be used at 5 mg/kg every 12 hours or 10 mg/kg every 24 hours for 30 days. Rifampicin at 15 mg/kg every 12 hours for 7 days used to treat 2 dogs experimentally infected with *E. canis* produced PCR (-) results.<sup>14a</sup> However, another study showed rifampicin at 10 mg/kg every 24 hours for 3 weeks successfully alleviated clinical signs of CME in 5 dogs experimentally infected with *E. canis*, but 3 dogs remained *E. canis* PCR (+).<sup>16</sup> Rifampicin was effective at clearing a human *E. chaffeensis* infection.<sup>1</sup> Imidocarb dipropionate is not an effective treatment to clear Ehrlichia infections, although in some cases may help alleviate clinical signs.

If a dog presents with lameness or joint pain, a short course of an anti-inflammatory dose of glucocorticoids (prednisone or prednisolone) may be administered.

Clinical response to doxycycline usually occurs between 24 and 48 hours for acute ehrlichiosis. When clinical signs persist after treatment with doxycycline, look for other causes, including different infectious agents, immune-mediated disease or neoplasia. See Table 2 for additional treatment and monitoring recommendations.

**Table 1.** *Ehrlichia* species that have been detected in the blood and tissues of clinically ill dogs in North America, including reported clinical signs, blood abnormalities, primary tick vector and US location. Subclinical infections have also been reported for *E. canis*, *E. chaffeensis* and *E. ewingii*.

<i>Ehrlichia</i> sp.	Cell Tropism	Commonly Reported Clinical Signs	Reported Hematological Abnormalities	Primary Tick Vector	Geographic Distribution in U. S.
<i>E. canis</i>	monocytes	Fever, lethargy, lameness, ocular signs (uveitis), bleeding disorders, neurologic signs	Thrombocytopenia, nonregenerative anemia, leukopenia, lymphocytosis, or abnormal lymphocytosis <sup>c</sup> , hyperglobulinemia (polyclonal gammopathy, rarely monoclonal), hypoalbuminemia, pancytopenia, elevated ALP and ALT	<i>Rhipicephalus sanguineus</i>	Nationwide
<i>E. chaffeensis</i>	monocytes	Fever, lethargy	Mild thrombocytopenia, monocytosis	<i>Amblyomma americanum</i>	Central, Southeast, MidAtlantic
<i>E. ewingii</i>	neutrophils	Fever, lethargy, lameness, polyarthropathy, neurologic signs	Thrombocytopenia, anemia, leukopenia, monocytosis, abnormal lymphocytosis <sup>c</sup> , hyperglobulinemia, elevated ALP and ALT	<i>Amblyomma americanum</i>	Central, Southeast, MidAtlantic
<i>E. muris</i> <sup>a</sup>	unknown	Fever, lethargy, lameness, polyarthropathy	Thrombocytopenia	<i>Ixodes scapularis</i>	Upper Midwest, Northeast
Panola Mnt. <i>Ehrlichia</i> sp. <sup>b</sup>	unknown	Asymptomatic	Thrombocytopenia, abnormal lymphocytosis <sup>c</sup>	<i>Amblyomma americanum</i> and <i>A. manulatum</i>	Central, Southeast, MidAtlantic

<sup>a</sup> based on 1 case report <sup>6</sup>

<sup>b</sup> based on 1 case report <sup>13</sup> and 1 case consult

<sup>c</sup> abnormal lymphocytes are defined as small to intermediate in size with increased amounts of pale cytoplasm, not blastic in appearance, which can be seen secondary to reactive processes such as chronic inflammation or infectious causes.

**Table 2.** Treatment and monitoring guidelines for *Ehrlichia* infection/exposure.

Ehrlichia Serology	Ehrlichia PCR	Clinical Signs Present	GUIDELINES
SNAP®4DXplus (+/-)	(+)	Yes or No	<ul style="list-style-type: none"> <li>• Treat: doxycycline (5-10 mg/kg) BID for 30 days</li> <li>• Discuss tick prevention</li> <li>• Run PCR panel to look for co-infections</li> </ul>
SNAP®4DXplus (+)	(-) or ND	Yes	<ul style="list-style-type: none"> <li>• If clinical signs do not resolve after treatment, run PCR for Ehrl sp. and other VBDs to identify treatment failure or the presence of co-infections</li> <li>• SNAP®4DX Plus may remain positive for years, even with pathogen clearance. Do not use to monitor treatment response.</li> </ul>
	(-) or ND	No	<ul style="list-style-type: none"> <li>• Run PCR (Ehrl or VBD panel) and/or perform BW and UA</li> <li>• PCR (+) and/or BW or UA abnormal → treat w/ doxycycline (5-10 mg/kg) BID for 30 days</li> <li>• PCR (-) and BW wnl → treat or don't treat.</li> <li>• Discuss tick prevention</li> <li>• If the dog remains SNAP positive at annual screenings, repeat BW/UA and/or PCR. Reinfection is always possible.</li> </ul>
<i>E. canis</i> IFA (+)	(+)	Yes or No	<ul style="list-style-type: none"> <li>• Treat: doxycycline (5-10 mg/kg) BID for 30 days</li> <li>• Discuss tick prevention</li> </ul>
	(-) or ND	Yes	<ul style="list-style-type: none"> <li>• Run PCR panel to look for co-infections</li> <li>• If clinical signs do not resolve after treatment, run PCR for Ehrl sp. and other VBDs to identify treatment failure or the presence of co-infections</li> <li>• Decline in IFA titers will be variable and is not a reliable indicator of treatment success</li> </ul>
	(-) or ND	No	<ul style="list-style-type: none"> <li>• Run PCR (Ehrl or VBD panel) and/or perform BW</li> <li>• PCR (+) and/or BW abnormal → treat w/ doxycycline (5-10 mg/kg) BID for 30 days</li> <li>• PCR (-) and BW wnl → treat (if high titers <math>\geq 1:256</math>) or don't treat (but follow up with convalescent titers)</li> <li>• Discuss tick prevention</li> <li>• Decline in IFA titers will be variable and is not a reliable indicator of treatment success</li> </ul>
SNAP®4DXplus (+) <i>E. canis</i> IFA (-)			<ul style="list-style-type: none"> <li>• Likely <i>E. ewingii</i> exposure or infection</li> <li>• see action above for SNAP®4DXplus (+) scenarios</li> </ul>

<p><i>E. canis</i> IFA (+) (suspect chronic CME)</p>	<p>(+/-) or ND</p>	<p>Yes – suspect chronic CME with bi- or pancytopenia</p>	<ul style="list-style-type: none"> <li>• Treat: doxycycline (5-10 mg/kg) BID for 30 – 44 days</li> <li>• Supportive treatment (crystalloids, +/- blood transfusion)</li> <li>• Monitor CBCs; neutropenia should start to improve first. It can take 6-8 months for abnormalities to resolve.</li>   <li>• <u>No fever</u>: moderate-to-severe (neutrophil count &lt;1,000/<math>\mu</math>l) and persistent neutropenia (&gt;2weeks), consider prophylactic antibiotics fluoroquinolones (e.g. enrofloxacin, 10 mg/kg, orally, SID) in addition to doxycycline, until the neutrophil count exceeds 1,000/<math>\mu</math>l; recommend home confinement and periodic temperature measurement (avoid sulfonamides, chloramphenicol, and penicillin) (based on reference <sup>8</sup>)</li> <li>• <u>Fever</u>: antibiotic selection should be based on culture and sensitivity testing (e.g. blood and/or urine culture) or a combination of an intravenous fluoroquinolone and a <math>\beta</math>-lactame (e.g. cefazolin, 30 mg/kg, TID, iv or im with hospitalization). If fever does not abate within 2 days, add metronidazole (15 mg/kg, TID, iv) to strengthen anaerobic bacteria spectrum. (based on reference <sup>8</sup>)</li> </ul>
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BW = blood work; ND = not done; SID = every 24 hours; BID = every 12 hours; TID = every 8 hours; UA = urinalysis; VBD = vector-borne disease; wnl = with in normal limits  
SNAP<sup>®</sup>4DXplus made by IDEXX Laboratories, Inc., Westbrook, Maine

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