SPOTLIGHT ON

Ehrlichia ewingii

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In many parts of the United States, infection of dogs with the pathogen *Ehrlichia ewingii* is far more common than infection with the better-known *Ehrlichia canis*. Although these two ehrlichial pathogens share a phylogenetic similarity, there are many important differences, including tick vectors, host-cell tropisms, disease manifestations, geographic restrictions, and zoonotic potential. In fact, *E. ewingii* produces morulae in granulocytes rather than monocytes, is transmitted by a highly prevalent environmental tick (*Amblyomma americanum*, the lone star tick) as compared with the localized kennel tick (*Rhipicephalus sanguineus*, the brown dog tick), and often causes acute lameness or polyarthritis as a primary component of the dog's clinical presentation.

It is important for veterinarians practicing in *E. ewingii*-endemic regions of the south central and southeastern United States to be familiar with tick transmission, disease consequences, diagnosis, treatment, and the zoonotic implications that are particular to *E. ewingii*.

Transmission and geographic distribution

The only proven competent vector for the transmission of canine granulocytic ehrlichiosis is *Amblyomma americanum*, the lone star tick. Although *E. ewingii* infection has also been documented in dogs from both South America and Africa, the tick species responsible for natural transmission in those regions remains to be determined.[1,2] Transstadial transmission in the lone star tick assures that ticks can be infected during all three stages of the life cycle (larva, nymph, and adult) and remain competent to transmit *E. ewingii* at their next blood meal.[3,4]

White-tailed deer, the major host species for *A. americanum*, apparently serve as the major reservoir for *E. ewingii*. Not surprisingly, *E. ewingii* infections occur most often in the southeastern and south central United States where both white-tailed deer and lone star ticks are plentiful (*Figure 1*).[5-8] In fact, dogs are more likely to be seropositive for *E. ewingii* than for either *E. canis* or *Ehrlichia chaffeensis* in Missouri, Oklahoma, Arkansas, Kansas, Georgia, Alabama, Mississippi, Tennessee, Florida, Virginia, and New Jersey.[9] The average seroprevalence for *E. ewingii* is 8.5% in these states.[9] Lone star ticks are aggressive ticks that commonly feed on dogs, people, and numerous wildlife species.[10,11] As a result, serologic evidence supporting exposure to *E. ewingii* is very common in *A. americanum*-endemic states. Antibodies against *E. ewingii* have been detected in 26% to 44.8% of randomly sampled dogs in Oklahoma, Missouri, and Arkansas, with 9% to 20% testing positive for *E. ewingii* DNA by polymerase chain reaction (PCR).[9,12,13]

Canine granulocytic ehrlichiosis caused by *E. ewingii* occurs most often in the southeastern and south central United States where both white-tailed deer and lone star ticks are plentiful.

Pathogenesis and clinical disease

Ehrlichia ewingii is a small, obligate intracellular bacterium, and, following the bite of an infected tick, it invades granulocytes forming membrane-bound, intracytoplasmic colonies of organisms known as morulae.[14] The time required from tick attachment to pathogen transmission is unknown. Morulae can be observed within granulocytes in as little as 12 days after experimental inoculation.[3,15,16]

Clinical illness associated with canine granulocytic ehrlichiosis is most often an acute febrile condition associated with musculoskeletal signs. Reluctance to stand or walk, lameness, a stiff or stilted gait, and joint effusion are common findings in *E. ewingii*-infected dogs and may be quite severe.[12,17-19] Lethargy, anorexia, and central nervous system signs (*e.g.*, head tilt, tremors, and anisocoria) may also be present.[19,20] Rarely reported findings have included hemorrhage, weight loss, organomegaly, uveitis, pruritus, vomiting, and diarrhea.[12,19-21] Onset of clinical signs generally occurs within 7 to 14 days following infection.

It is quite likely that many dogs infected with *E. ewingii* either remain clinically healthy or develop a brief, self-limiting illness, as has been reported in experimentally infected dogs.[15,16] The often mild pathogenicity following infection is further supported by the high seropositive rates among dogs lacking historical evidence of clinical disease attributable to canine granulocytic ehrlichiosis, and the lack of reported mortality due to *E. ewingii* infection.[3,9,15] Immunosuppression may exacerbate or potentiate disease manifestations in infected dogs, as it apparently does in *E. ewingii*-infected people.[22-24] Experimental infection with *E. ewingii* has been more successfully attained in dogs receiving either cyclophosphamide or glucocorticoids.[3,16,25] Similarly, co-infection with other tick-transmitted pathogens may worsen disease manifestations in infected dogs.[26]

Evidence to date suggests that canine and human granulocytic ehrlichiosis causes only an acute illness in dogs and people. This association is in contrast to the pathogenesis of canine monocytic ehrlichiosis, caused by *E. canis*, where the acute illness typically resolves spontane-

ously, after which the dog enters a period of subclinical infection, followed in some cases by the development of chronic disease manifestations that can be quite challenging to treat.[27-29] Due to the acute nature of canine granulocytic ehrlichiosis, disease manifestations have only been identified when vector lone star ticks are active, predominantly during the late spring and summer.[19,30] At the University of Missouri Veterinary Medical Teaching Hospital, from 32 dogs with granulocytic morulae and clinical illness compatible with *E. ewingii infection*, only two were identified outside of the months May to August (in September and November) (Cohn, L: Unpublished data). Despite the fact that illness documented to result from *E. ewingii* infection is acute in nature, actual infections can be persistent.[15,19] It is possible that chronic *E. ewingii* infections may contribute to as-of-yet unrecognized disease manifestations or pathophysiologic consequences.

FIGURE 1. This map shows the distribution of *Amblyomma americanum* (the lone star tick; *inset*) in the United States.

Clinicopathologic findings and diagnosis

As with most ehrlichial infections, thrombocytopenia is the most consistent clinicopathologic abnormality associated with *E. ewingii* infection.[3,15,18-20] Nevertheless, a normal platelet count does not rule out the possibility of active infection, and thrombocytopenia has been found to be cyclical in some experimentally infected dogs over a period of weeks.[15] Occasionally, mild anemia and reactive lymphocytes are identified.[12,19] Similar to other rickettsial infections, short-lived leukopenia was recognized during the early acute phase of experimental infection in dogs.[15] Serum biochemical abnormalities are mild and nonspecific but may include increases in hepatic enzyme concentrations, hyperglobulinemia, hypokalemia, and hyperphosphatemia or hypophosphatemia.[15,20,21] Proteinuria, which has been associated with both *E. canis* and *Anaplasma phagocytophilum* infections, has thus far not been identified as a component of canine granulocytic ehrlichiosis.[31,32] Arthrocentesis from joints of affected dogs with polyarthritis reveals neutrophilic inflammation.[19] On occasion, morulae are identified in granulocytes from peripheral blood (*Figure 2*), cerebrospinal fluid, joint fluid, prostatic fluid (Cohn L.: Personal observation), or potentially other bodily fluids.[18-20] Unfortunately, observation of morulae is neither sensitive nor specific; microscopic differentiation of *A. phagocytophilum* from *E. ewingii* morulae is impossible, therefore PCR confirmation of the infecting organism is recommended, particularly when morulae are found in dogs in non-endemic regions.

A presumptive clinical diagnosis can be made based on compatible signs in dogs from endemic regions during the spring or summer. However, there is substantial overlap in the clinical presentation for dogs infected with other tick-borne pathogens, including *Rickettsia rickettsii* and *Bartonella* species. Examination of a peripheral blood smear to identify thrombocytopenia and allow visual inspection of granulocytes for morulae is often the only diagnostic test performed before treatment is initiated. Culture of the pathogen from the blood has thus far been unsuccessful in a research setting, so disease diagnosis via pathogen isolation is not possible. Instead, identification of *E. ewingii* nucleic acid via PCR has been used. PCR testing not only detects *E. ewingii*, but also can be used to distinguish

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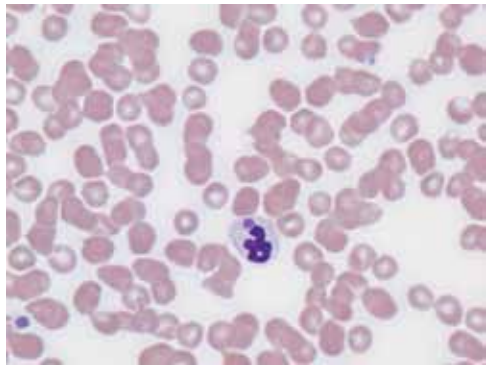

FIGURE 2. A morula in a neutrophil. These morulae caused by infection with either *Ehrlichia ewingii* or *Anaplasma phagocytophilum* appear identical. While microscopic recognition of morulae is extremely useful, it is an insensitive diagnostic tool for disease confirmation. *(Courtesy of Dr. Linda Berent, University of Missouri Veterinary Medical Diagnostic Laboratory.)*

Ehrlichia ewingii infection in dogs

CLINICAL SIGNS

- Clinical signs include fever, musculoskeletal signs (reluctance to stand or walk, lameness, stiff or stilted gait, joint effusion), lethargy, anorexia, and central nervous system signs (head tilt, tremors, anisocoria)
- Signs may be worse if there is co-infection with other tick-transmitted pathogens
- Some *E. ewingii*-infected dogs remain clinically healthy; some develop brief, self-limiting illness

LABORATORY ABNORMALITIES

- *E. ewingii* infects predominantly neutrophils (granulocytes) forming membrane-bound colonies of organisms known as morulae that are sometimes identified on review of a stained blood smear
- Thrombocytopenia is the most consistent laboratory finding, but normal platelet count does not rule out active infection

DIAGNOSTIC PROCEDURES

Serologic Testing

- *E. canis* IFA — May detect cross-reactive antibodies from a dog infected with *E. ewingii*.
- ELISA (SNAP® 4Dx® Plus Test) — Uses species-specific peptides for detection of antibodies to *E. canis*, *E. ewingii*, and *E. chaffeensis;* indicates exposure to one or a combination of *Ehrlichia* species
- Lack of antibody response during acute illness does not rule out infection; retest in 2 to 3 weeks

PCR Testing

- Can identify active *E. ewingii* infection; PCR detects *E. ewingii*, and can distinguish it from related *Ehrlichia* species and *Anaplasma phagocytophilum*

this pathogen from related *Ehrlichia* species and from *A. phagocytophilum*. In addition, PCR testing may be helpful in identifying those dogs with subclinical, persistent *E. ewingii* infections.[12,19,22,33]

Serologic tests are often used in epidemiologic studies of ehrlichial prevalence, and can also be used as a tool when diagnosing canine ehrlichiosis. Depending on the route of exposure, experimentally infected dogs develop *E. ewingii* antibodies between 7 and 21 days after infection and remain seroreactive for at least 10 months.[15] As with any acute infection, the absence of an antibody response during the acute illness does not rule out infection. If an initial serologic test finding is negative, convalescent titers acquired 2 to 3 weeks later are required to confirm a clinical diagnosis through seroconversion or to rule out exposure to *E. ewingii*. Seronegative infection has not been reported. Just as importantly, because antibody titers persist for months after an acute, tick-transmitted infection, positive documentation of antibody titers against *E. ewingii* can only confirm prior exposure, but does not confirm disease causation or active infection at the time of sample collection. Based upon experimental studies, some dogs may be persistently infected, yet PCR negative, potentially because the organism is sequestered outside of the vasculature or persists in the blood at levels undetectable by PCR.

Multiple serologic methodologies are available for antibody detection, including *E. canis* immunofluorescence assay (IFA) and ELISA (SNAP® 4Dx® Plus Test — IDEXX Laboratories, Inc.). Detection of cross-reactive antibodies from dogs infected with *E. ewingii* on *E. canis* IFA can be variable; on the other hand, antibodies to *E. ewingii* are not serologically cross-reactive with *A. phagocytophilum* by any serologic methodology.[18,19,21,33-35] The *E. canis* peptides in the commercially available, in-clinic SNAP® 3Dx® Test do not detect *E. ewingii* antibodies in most infected dogs, whereas these peptides do cross-react with *E. chaffeensis* antibodies.[34,36] For the SNAP® 4Dx® Plus Test kit, addition of an *E. ewingii* peptide allows, for the first time, serologic detection of *E. ewingii*-specific antibodies. Thus, a single "positive blue dot" on a SNAP 4Dx Plus Test will reflect serologic evidence of exposure to *E. canis*, *E. ewingii*, *E. chaffeensis*, or any combination of these three *Ehrlichia* species. Importantly, serologic testing can be used not only to diagnose a specific infection, but can also be used to rule out infections with other pathogens that might cause similar clinical signs or pathologic abnormalities (*e.g.*, anaplasmosis, bartonellosis, Lyme disease, canine monocytic ehrlichiosis, Rocky Mountain spotted fever) or to characterize atypical or more severe disease manifestation that occurs during co-infections.[37,38]

Treatment and prevention

Treatment should **not** be withheld pending disease confirmation from dogs with clinical signs compatible with canine granulocytic ehrlichiosis in endemic regions. Although mortality has not been reported, dogs with illness attributable to canine granulocytic ehrlichiosis often seem to be in marked pain. Fortunately, the clinical and hematologic manifestations of illness usually respond rapidly, within 24 to 48 hours after treatment with tetracycline or doxycycline begins. Although a 14-day course of oral doxycycline (5 mg/kg every 12 hours or 10 mg/kg once daily) is thought to clear *E. ewingii*, *E. canis* is generally treated for 28 days.[19,20] Therefore, unless granulocytic morulae are observed or serologic or PCR evidence confirms the ehrlichial species responsible for infection is *E. ewingii*, the longer course of therapy is justified. Supportive care, including analgesia for polyarthropathy, may be necessary. Clinical, hematologic, and PCR evidence suggests that dogs may clear *E. ewingii* spontaneously within

weeks to several months, even if treatment is withheld.[15,16]

Healthy dogs that are seroreactive to any *Ehrlichia* species do not require immediate treatment. Considerations for diagnostic evaluation and the potential initiation of treatment will become even more important as veterinarians use screening tests that recognize antibodies to any of these three important ehrlichial species (*E. ewingii*, *E. canis*, and *E. chaffeensis*). Healthy dogs with a positive serologic response to any *Ehrlichia* species antigen might have been infected without evidence of obvious clinical illness, might have recovered spontaneously from a mild clinical infection, or might be chronically infected. In each case, further diagnostic

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Acute onset of canine granulocytic ehrlichiosis in a young dog

On May 31, 2010, a 2-year-old 27-kg (59.4-lb) neutered male mixed-breed dog was presented to the University of Missouri Veterinary Medical Teaching Hospital's Community Practice clinic for progressive lethargy and difficulty walking of about 4 days' duration. The morning of presentation, the dog refused to get up to go outside. It was up-to-date on its core vaccinations (*i.e.*, rabies, DA2LPP) and received monthly heartworm preventive medication but no routine ectopic parasite control therapy.

On arrival at the clinic, the dog had to be brought into the building on a gurney. It would stand with reluctance and would walk a short distance with coaxing but appeared ataxic. On examination, the temperature was 39.5 C (103.2 F), the heart rate was 130 beats/min., and the respiratory rate was 32 breaths/min. Joint effusion was identified in the right carpus and stifle. It was difficult to evaluate conscious proprioception since the dog was unwilling to stand.

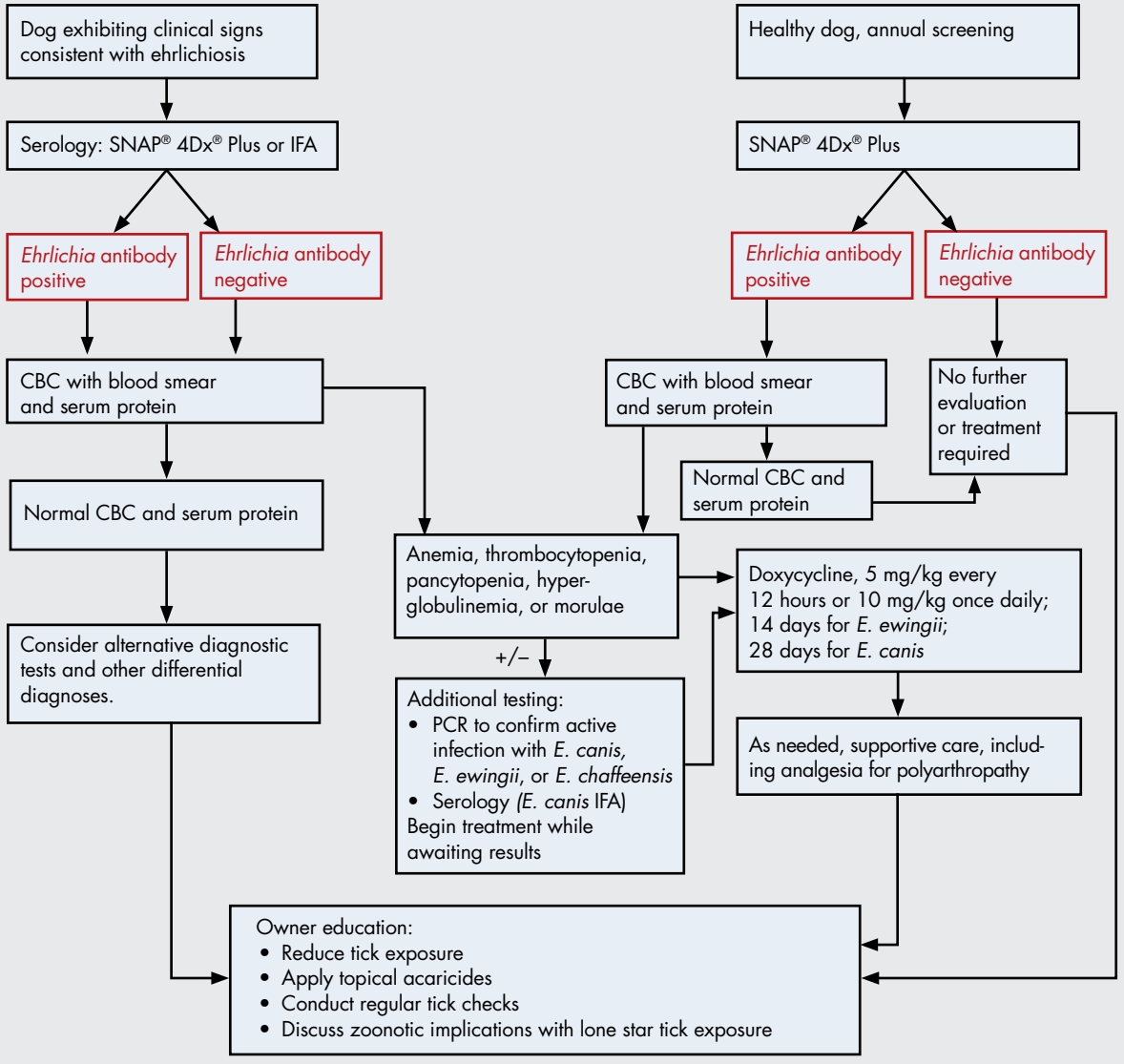
Initial evaluation included a complete blood count, serum biochemical profile, urinalysis, and a SNAP® 3Dx® Test (IDEXX Laboratories). The hematocrit was normal at 42% with total protein of 6.5, but the platelets were decreased at 80.2 x 10^3/µl. The white blood cell count was 8.8 x 10^3/µl with a mild lymphopenia of 0.880 x 10^3/µl. On blood smear examination, platelet clumps were not identified, but several granulocytic inclusions compatible with *Ehrlichia ewingii* or *Anaplasma phagocytophilum* were seen. No abnormalities were identified on the serum biochemical profile. The urine specific gravity was well concentrated at 1.067 with a 1+ protein and an inactive urine sediment. The SNAP 3Dx Test result was negative for antibodies to *Dirofilaria immitis*, *Borrelia burgdorferi*, and *Ehrlichia canis*.

Neither the morulae nor clinical presentation can be used to distinguish between canine granulocytic ehrlichiosis and canine anaplasmosis. However, because Missouri is considered a highly endemic region for *E. ewingii* but not *A. phagocytophilum*, a presumptive diagnosis of canine granulocytic ehrlichiosis was made. With the introduction of the SNAP® 4Dx® Plus Test,

it is now possible to detect antibodies to *E. ewingii*. In this case, however, antibody results may still have been negative even had the new SNAP 4DX Plus Test been used given that this dog had an acute onset illness and might not have yet seroconverted.

Treatment was initiated with doxycycline at a dose of 10 mg/kg once daily by mouth. The dog was placed on intravenous fluids at twice maintenance rates; buprenorphine was given for analgesia (0.02 mg/kg subcutaneously every 8 hours). By the next morning, the temperature had normalized, and the dog was willing to stand and walk and eat canned dog food. Fluids were discontinued, and the dog was discharged from the hospital with instructions to continue a 14-day course of doxycycline. Additionally, 50-mg tramadol tablets (x10) were prescribed with instructions to administer 1 to 2 tablets by mouth twice daily as needed for pain control. Telephone follow-up about 2 weeks after discharge revealed that the dog's clinical improvement continued, with a return to clinical normalcy within 2 days of hospital discharge. The owner had discontinued tramadol after the first dose. Clinical signs did not recur after discontinuation of treatment. The owner did not return the dog for a recommended repeat complete blood count to re-evaluate the platelet count.

Ehrlichia infection: Treatment and prevention strategies



investigation is warranted, beginning with a complete blood count and blood smear evaluation. For healthy dogs with a normal platelet count and the absence of leukocytic morulae, anemia, or hyperglobulinemia, no further evaluation or treatment may be required. If morulae are identified in neutrophils, monocytes, or (rarely) other circulating cell types or a dog is found to have thrombocytopenia, anemia, or hyperglobulinemia, PCR is recommended to confirm active infection and treatment is warranted. Alternatively, some veterinarians will simply opt to treat seroreactive dogs with a 28-day course of doxycycline, which is adequate to treat either an acute infection with *E. ewingii* or a chronic infection with *E. canis*.[39,40]

There are no vaccines for canine granulocytic ehrlichiosis or for any canine or human ehrlichial infection, so prevention consists of reducing exposure to tick bites. Year-round topical acaricides are recommended for all dogs in tick-endemic regions.[41] (This is in accordance with the Companion Animal Parasite Council's recommendations.) Rapid manual removal of ticks may be helpful, but the length of time necessary for transmission of *E. ewingii* following vector tick attachment is unknown.

Public health considerations

Human infection with *E. ewingii* occurs most often in immunocompromised people, and reported cases are relatively uncommon.[22,24,42,43] *Ehrlichia chaffeensis* causes a more severe and potentially life-

threatening illness in humans (monocytic ehrlichiosis), is carried by the same tick vector as is *E. ewingii*, and can also infect dogs.[5,9,10,33,44-46] Although experimental infection of dogs with *E. chaffeensis* has produced only clinically inapparent infection or mild thrombocytopenia, co-infection with other pathogens or concurrent immunosuppression might result in more severe disease manifestations.[33,45,46] Certainly, both human granulocytic and human monocytic ehrlichiosis can result in serious morbidity and, in the case of *E. chaffeensis* infection, mortality.[47,48] While there are no reports of direct dog-to-human transmission of either *E. ewingii* or *E. chaffeensis*, identification of an infected pet would suggest that the pet's owner may also come into contact with ticks carrying these pathogens. In endemic regions, veterinarians should educate their clientele about the importance of tick control and the possibility of human ehrlichial infections. Veterinarians play a critically important role in the national public health infrastructure relative to the prevention of human tick-borne illness.

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Ehrlichia ewingii Q&A

What is *Ehrlichia ewingii*?

Ehrlichia ewingii is a tick-transmitted member of the alpha Proteobacteria that can induce intra-neutrophilic infection in dogs and acute febrile illness in people.

What are the clinical presentations of the disease that it causes?

Clinical signs can be highly variable among dogs. Following tick-transmission of *E. ewingii*, clinical signs will most often develop within 7 to 14 days. Clinical manifestations include fever, lethargy, lameness, polyarthropathy, and neurologic manifestations including ataxia, paresis, proprioceptive deficits, and vestibular dysfunction.

How is *Ehrlichia ewingii* different from other species of *Ehrlichia*, and what problems does that present?

In North America, dogs can be infected with *E. ewingii*, *Ehrlichia canis*, and *Ehrlichia chaffeensis*. Although the clinical presentations associated with infection with each *Ehrlichia* species can be similar with overlapping disease manifestations, *E. ewingii* appears to be somewhat unique in its ability to cause a neutrophilic polyarthritis. Historically the world literature relative to canine ehrlichial disease is biased toward *E. canis*; only recently, with the advent of PCR testing, have clinicians been able to definitively determine which *Ehrlichia* species is causing disease in their patients.

What role does the lone star tick play as a vector of *Ehrlichia ewingii*, and what other diseases (particularly human diseases) does it carry?

Although the most prevalent tick species to infest cats, dogs, people, and many wild animal species in the southern and central United States, *Amblyomma americanum* (the lone star tick) has been the least studied as a vector for canine and human disease. It is important to realize that the distribution of this tick species continues to move northward, with increasing sightings as far north as New York and Maine.

A. americanum transmits *E. ewingii* and *E. chaffeensis* to dogs and people and *Cytauxazoon felis* to domestic and wild cats. The lone star tick also carries *Borrelia lonestari* and *Rickettsia amblyommii*, but the pathogenic potential for each of these organisms for dogs and people remains unclear.

What do you do with a dog that tests positive for *Ehrlichia ewingii*?

With the advent of the SNAP® 4Dx® Plus Test (IDEXX Laboratories), veterinarians practicing in regions of the country with high numbers of *A. americanum* should anticipate an increase in the number of *Ehrlichia* positive SNAP results they see in both healthy and sick dogs. With the addition of an *E. ewingii*-specific peptide to the SNAP platform, antibodies to all three *Ehrlichia* species will now be detectable. Because the SNAP platform is designed as a screening test, it is important to realize that antibody levels may not be detectable during the acute phase of infection (*i.e.*, the first 7 to 14 days following tick transmission). However, the SNAP Test can be used to confirm seroconversion following an acute infection in dogs. Optimally, when a dog tests positive for *Ehrlichia* antibodies using the SNAP 4Dx Plus Test, the veterinarian should recommend a complete blood count and a serum protein measurement. If abnormalities are noted (anemia, thrombocytopenia, neutropenia, pancytopenia, hyperglobulinemia) a PCR test can be performed to confirm active infection with an *Ehrlichia* species or the dog can be treated with doxycycline (5 mg/kg every 12 hours or 10 mg/kg once daily for 4 weeks). Western blot testing is not available for *E. ewingii* testing because the organism has never been cultured. Immunofluorescence assay (IFA) testing is less specific to differentiate among species, but can be used to detect and quantify antibodies or to confirm seroconversion, if acute and convalescent sera are tested. To document seroconversion by SNAP Test or IFA, an acute serum sample should be collected and the dog should be retested in about 3 weeks. Seroconversion requires a fourfold increase in the IFA titer or a change from a negative to positive *Ehrlichia* SNAP Test result.

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