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EHRlichIA AND ANAPLAsMA INFECTIONS **Gad Baneth, DVM. Ph.D., Dipl. ECVCP** **Rehovot, Israel**

INTRODUCTION

Ehrlichioses and anaplasmoses are infections caused by bacterial organisms of the genera *Ehrlichia* and *Anaplasma* that typically target certain host blood cells. *Ehrlichia canis* and *E. chaffeensis* infect monocytes, *Anaplasma phagocytophilum* and *E. ewingii* infect granulocytes, and *A. platys* infects platelets. *Ehrlichia* and *Anaplasma* species are obligatory intracellular bacteria that are transmitted by ticks. The significance of these diseases has been highlighted since the discovery of the human ehrlichioses caused by *E. chaffeensis*, *E. ewingii*, and *A. phagocytophilum*.

EHRlichIA CANIS

E. canis, the etiologic agent of canine monocytic ehrlichiosis, has been recognized worldwide as an important canine infectious agent. *E. canis* infection has been reported from Africa, Asia, America, and Europe^{1,2,3}. Autochthonous (non-imported) cases of *E. canis* in Europe have been reported mostly from Spain, Portugal, Southern France, Italy, the Balkans, Turkey and Greece. *Ehrlichia canis* morulae found in monocytes and macrophages are a "microcolony" of bacteria surrounded by a membranous vacuole. Morulae may contain 100 or more ehrlichiae resembling elementary bodies of chlamydiae. *E. canis* is transmitted by the three-host tick *Rhipicephalus sanguineus*. The pathogenesis of the disease involves an incubation period of 8-20 days, followed by 3 consecutive phases: an acute phase which lasts 1-4 weeks, a subclinical phase which may last from months to years, and a chronic phase. Not all infected dogs develop the chronic severe form of the disease and the conditions that lead to the development of this stage are unknown.

The clinical presentation of the disease caused by *E. canis* may vary, and the clinical signs most frequently reported are depression, lethargy, anorexia, fever, lymphadenomegaly, splenomegaly and hemorrhages (mainly petechiae, ecchymoses and epistaxis). Ocular manifestations of canine ehrlichiosis include anterior uveitis, keratoconjunctivitis, hyphema, glaucoma, chorioretinitis and retinal detachment. Polyarthritides and polymyositis have also been described in *E. canis* infection^{1,2,3}. The neurological abnormalities found in canine ehrlichiosis are associated with vasculitis, meningoencephalitis, and lymphocytic infiltration of the central and peripheral nervous system or hemorrhages. *E. canis* infection has been termed by some clinicians as the "silent killer". It is often inapparent during the early and sub-clinical stages of infection and when the disease is diagnosed in the chronic stage, it may be too late to save the canine patient, as treatment may not be helpful in reversing the severe pancytopenia and immune mediated phenomena associated with this disease.

Laboratory abnormalities in canine monocytic ehrlichiosis include hematologic and serum biochemistry changes. Thrombocytopenia is the most frequent hematological abnormality occurring in more than 90% of cases. Anemia, usually non-regenerative normocytic and normochromic, is another common finding in this disease. In addition, mild to severe leucopenia is a frequent abnormality. Hyperglobulinemia, hypoalbuminemia and mild elevation of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities are frequently reported in ehrlichiosis^{1,2,3}. Dogs in the chronic severe stage of the disease may develop severe pancytopenia as their bone marrow becomes hypocellular. The prognosis of these chronically ill dogs is grave.

Immune-mediated responses play a major role in the pathogenesis of *E. canis* infection. Anti-platelets antibodies (APA) have been demonstrated less than a week after experimental *E. canis* infection of dogs. Platelet aggregation abnormalities, anti-nuclear antibodies (ANA), RBC autoagglutination with positive coombs' test, and circulating immune-complexes have been shown in infected dogs and are associated with the disease process.

The decrease in platelets during canine ehrlichiosis is probably a result of several mechanisms. These mechanisms include increased consumption with vascular endothelial changes, platelet sequestration and pooling in the spleen, thrombophagocytosis with immunological destruction, a decrease in the half life time of circulating platelets possibly due to opsonization with antibodies, and production impairment due to bone marrow destruction and hypocellularity. In addition to the decrease in circulating

platelet number, platelets dysfunction (thrombocytopenia) has also been implicating as an additional factor contributing to lack of platelet functionality in canine monocytic ehrlichiosis.

Co-infections with hemoparasites or other infectious agents are often detected in conjunction with canine ehrlichiosis. *Hepatozoon canis* and *Babesia canis vogeli* are transmitted by the same vector tick, *R. sanguineus*. In addition, *Leishmania infantum* is another common co-infecting protozoal pathogen whose vector, phlebotomine sand flies are often found in the same sub-tropical climate conditions and ecological niches, as *R. sanguineus* ticks transmitting *E. canis* infection.

The laboratory diagnosis of *E. canis* infection includes evaluation of the hemogram and serum biochemistry panel. The detection of morulae in monocytes in stained blood smears is rare and can not serve as a main diagnostic option. Detection of the presence of *E. canis* DNA by the polymerase chain reaction (PCR) is highly sensitive and specific and has become the most useful diagnostic test for the confirmation of canine ehrlichiosis. Several conventional and real-time PCR protocols have been described for *E. canis* and the assay can be performed on blood or tissue including the spleen and bone marrow^{1,2,3,4}.

Anti-*E. canis* antibodies persist long after recovery from the disease. Serum antibodies are thought not to be protective or play an important role in eliminating this intracellular infection. Serology is indicative of exposure to *E. canis* and may often be helpful in ruling out progressive infection. Antibodies may not be detectable during the early stage of infection. However, seropositive dogs with previous exposure to the pathogen may also present due to other urgent disease conditions. Several commercial "in house" test kits are available for *E. canis* infection in addition to the laboratory indirect fluorescent antibody test (IFAT) which is often considered the golden standard for serology. Some serologic cross-reactivity between different *Ehrlichia* species may occur. Anti-*E. canis* antibodies have been reported to cross-react with *E. chaffeensis*, *A. phagocytophilum* & *E. ewingii* but not with *A. platys*.

E. canis is susceptible to tetracyclines and doxycycline is most widely used for treatment of infection^{1,2,3,4}. Doxycycline is very efficient in clearing rickettsemia in acute cases of *E. canis* infection. Clinical recovery is noticed within 48-72 hours, yet treatment should be commenced for 3 weeks, as some dogs may remain carriers when shorter treatments are applied. Treatment with the injectable drug imidocarb dipropionate has been shown to be ineffective in totally eliminating *E. canis* in some studies. However, it is often used in combination with doxycycline when *Babesia* co-infection is suspected. The control of tick infestation by topical treatment with acaricidal and environmental eradication of ticks is recommended for the prevention of *E. canis* infection. No commercial vaccine against *E. canis* infection is currently available.

ANAPLASMA PHAGOCYTOPHILUM

Anaplasma phagocytophilum infects a broader spectrum of hosts in comparison to *E. canis*, including humans. Natural infection has been reported from numerous hosts including: rodents, horses, canines, camelids, domestic cats, domestic and wildlife ruminants, and people^{5,6}. Unlike *E. canis* infection where the domestic dog is the main reservoir host, dogs and also humans are considered to be accidental hosts of this infection which circulates in sylvatic cycles in different habitats. There are several strains of *A. phagocytophilum* with distinct genetic characteristics based on the nucleotide sequences of major surface protein genes and apparent different host tropism and virulence. Canine granulocytic ehrlichiosis caused by *A. phagocytophilum* is transmitted by the ticks *Ixodes ricinus* (Europe), *I. pacificus* (western USA), *I. scapularis* (Northeastern USA and upper Midwest), *I. persulcatus* and *Dermacentor silvarum* (Asia). In general, these tick species tend to be prevalent in cooler climate regions unlike *R. sanguineus* which transmits *E. canis* mostly in tropical and sub-tropical climates.

A. phagocytophilum is transmitted transtadially between the tick's life stages. Infection of the mammalian hosts takes place at least 36-48 hours after the tick attachment to the host's skin. The bacterium binds to neutrophil membrane receptors and undergoes endocytosis avoiding destruction by the phagolysosomal mechanism and inhibiting neutrophil's intracellular pathogen destruction mechanisms. *A. phagocytophilum* is able to manipulate and modify neutrophil function by decreasing cell motility and adherence to endothelial cells, and limiting its migration through endothelial barriers. It is also able to delay neutrophil apoptosis facilitating pathogen multiplication for longer than the host cell normally survives⁶.

A. phagocytophilum infection of dogs is usually an acute disease that does not tend to manifest as a chronic illness unlike canine monocytic ehrlichiosis caused by *E. canis*. The disease is considered to be self-limiting in dogs and fatalities have not been reported to date in case reports. The major clinical signs

caused by *A. phagocytophilum* are fever, lethargy, weakness, anorexia, depression, polyarthritis, limb edema and neurological signs. More rarely, GI signs and bleeding tendencies have been reported including melena, epistaxis and petechiae. Thrombocytopenia is reported in about 90% of the dogs with clinical *A. phagocytophilum* infection. Mild non-regenerative anemia and leucopenia have also been reported in infected dogs and the most common serum biochemistry abnormalities include hyperglobulinemia, hypoalbuminemia and increased alkaline phosphatase activity^{5,6}.

The diagnosis of canine *A. phagocytophilum* infection is confirmed by the detection of morulae in blood neutrophils or in other body fluids. Blood morulae were detected in the majority of the cases described in published case series in contrast to the rare detection of morulae in *E. canis* infection. PCR and serology by IFA showing seroconversion are used as ancillary diagnostic techniques with PCR confirming infection. Antibodies may persist for a year or more following infection and are therefore indicative mostly of exposure to the pathogen.

Canine *A. phagocytophilum* infection is treated with doxycycline at 5 mg/kg PO q 12 hr for 14 days. Prevention of infection is carried out by topical and environmental acaricidal treatment and by screening for ticks on dogs and removing them from the skin early before they manage to transfer the pathogen, e.g. no later than 36 hrs after attachment^{5,6}.

OTHER EHRLICHIA AND ANAPLASMA SPP. THAT INFECT DOGS

A. platys belongs to the *A. phagocytophilum*-genogroup. It infects platelets and causes a disease commonly recognized as infectious canine cyclic thrombocytopenia. The presumed natural vector of *A. platys* is the tick *R. sanguineus*. In the United States the infection is considered to be subclinical, and is manifested mainly by bacteremia followed by episodes of cyclic thrombocytopenia. A clinical disease was described in the Mediterranean basin and was characterized by anorexia, lethargy, depression, fever, weight loss, lymphadenomegaly, petechiae and ecchymoses, thrombocytopenia and anemia. These clinical signs resemble those caused by *E. canis* however with decreased severity.

Ehrlichia chaffeensis is transmitted by the tick *Amblyomma americanum* and infects monocytes. It is the cause of human monocytic ehrlichiosis. Puppies experimentally infected with this organism have shown fever and no other signs. One report of three dogs naturally infected with *E. chaffeensis* documented more serious clinical signs including vomiting, epistaxis, lymphadenomegaly and uveitis. These manifestations are considered indistinguishable from ehrlichiosis caused by *E. canis*.

E. ewingii causes canine granulocytic ehrlichiosis. It infects humans as well as dogs. It is transmitted by the tick *A. americanum* and infects granulocytes. Natural and experimental infection of dogs with *E. ewingii* may cause polyarthritis characterized by lameness, joint swelling, stiff gait, and thrombocytopenia.

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