Anaplasma phagocytophilum

Anaplasma phagocytophilum is an intracellular, gram-negative bacterium in the family Rickettsiaceae. This organism resides in granulocytes of infected mammalian hosts. Although this agent can be transmitted by inoculation of infected blood, most infections are tick-transmitted. Ticks are capable of harboring and transmitting the disease for several months after they are infected, making them important reservoirs. This review discusses the clinical findings and laboratory diagnosis of agents responsible for most clinical cases of canine Anaplasmosis.

Etiology and Epidemiology

A. phagocytophilum can cause clinical disease in dogs, horses, cats and ruminants (primarily in European countries). Many wildlife species have been found to harbor the organism although white-tailed deer are thought to be the primary reservoir. Due to travel of people and pets, A. phagocytophilum infections may be encountered in many geographic locations in United States, Europe and Asia. However, highest prevalence is found in areas of heavy deer tick populations. In the US, highest seroprevalence in dogs is Northeastern US, upper Central US (Wisconsin and Minnesota) and on the West Coast (California). These areas seem to correlate well with the number of reported cases in humans as well. Golden retrievers and Labrador retrievers are breeds that are over-represented in at least 3 studies reviewing the incidence of anaplasmosis in dogs. It is unclear if this is the result of a genetic predisposition, breed popularity or risk of exposure. However, all breeds are susceptible to infection.

Transmission and Pathogenesis

A. phagocytophilum can infect people as well as dogs. However, most infections occur as a result of inoculation from a tick bite. It appears that direct transmission from a dog to a human is highly unlikely and to our knowledge has never been fully confirmed. A. phagocytophilum is transmitted by the deer tick (aka. black-legged tick). Two species of deer ticks are present in the US; *Ixodes scapularis* is found along the Eastern coast of the United States as far north as Maine, spreading westward to Iowa and in the south, from Florida to central Texas. *Ixodes pacificus* is found only on the western coast of the United States, primarily throughout Washington, Oregon and California. The abundance and distribution of the tick follows that of the white-tailed deer. Since transovarial transmission has not been demonstrated, ticks must first acquire the infection at the larval or nymph state from a mammalian reservoir host. Once the infected tick attaches to a susceptible dog, the organism is transmitted in the tick saliva. The exact length of time required to transmit the infection is not specifically known. However, it is suspected that prolonged tick attachment of at least 16-24 hours is required for A. phagocytophilum transmission. Once inoculated into the body, the organism invades granulocytes and multiplies in membrane bound vacuoles forming morulae that contain several bacteria. Organisms will be found in circulating granulocytes, granulocytes in synovial fluid and in various tissues throughout the body including liver and spleen.

Clinical Findings
Acute infections appear to be responsible for most clinical cases of canine granulocytic anaplasmosis (CGA). Chronic, subclinical infections have been well documented in experimentally infected animals. However, the ability for these chronic infections to manifest themselves clinically still remains unknown. Clinical findings for either infection are similar and in acute cases will develop one to three weeks after tick inoculation. Common clinical findings include lethargy, anorexia and lameness or reluctance to walk (associated with polyarthropathy). Less common findings include vomiting or diarrhea. Coughing or evidence of neurological disease (meningitis) have also been.

Fever is the most common physical exam finding. Pain or swelling in the multiple joints, particularly distal joints (carpus and stifles) is seen in animals with polyarthritis. Hemorrhage, epistaxis, melena and petechia have been occasionally reported. Lymphadenopathy (mild) and splenomegaly is observed in some animals and in those with neurological disease may have proprioception deficits or other signs of meningitis.

**Diagnosis**

The diagnosis is suspected in any dog with a febrile lameness (or other clinical findings listed above) that lives in or has recently traveled to an endemic area. Confirmation of infection requires either finding morulae in circulating neutrophils or synovial fluid, a positive in-clinic ELISA, such as SNAP 4Dx Plus (IDEXX Laboratories, Westbrook, ME), a positive serology from a reference laboratory or PCR analysis. Finding morulae in granulocytes would confirm the dog as having either *A. phagocytophilum* or *E. ewingii*. However, the ability to find the morulae is dependent on the stage of infection and skills of the clinician / technician. In peracute infections, antibody levels may not be detectable and in those cases PCR analysis is a more sensitive method for confirmation.

**Hematologic Findings and other Diagnostic tests**

Thrombocytopenia is the most frequently encountered laboratory abnormality and is seen in up to 90% of infected animals. A mild anemia may develop, particularly in dogs with evidence of hemorrhage. The leukogram can be quite variable. Initially dogs have a transient neutropenia which often returns to normal or develops into a neutrophilia. Lymphocyte counts are likewise variable and a reactive lymphocyte population is often visualized. Changes in the biochemical profile are nonspecific but elevations is liver enzymes (ALT and ALP) may be seen. A transient hypoalbuminemia has been seen in dogs acutely infected with *A. phagocytophilum*. Radiographs of the joints will typically reveal soft tissue swelling resulting from a nonerosive polyarthritis. In dogs with polyarthitis, the synovial fluid will contain increased numbers of leukocytes, most of which will be neutrophils. In some dogs, morulae can be identified within the cytoplasm of the neutrophils.

**Confirmation of Disease**

Buffy coat smears prepared from peripheral blood can increase the likelihood of being able to microscopically identify morulae within circulating granulocytes (See Figure Above). IFA tests are available for detection of antibodies to *A. phagocytophilum* in patient serum samples. A
reciprocal titer of > 64 with clinical signs is recommended to confirm a diagnosis. However, initial titers may be negative in acute cases. Therefore, ideally, a > 4 fold rise in titer between acute and convalescent serum samples taken 2 to 4 weeks apart is ideal for serological confirmation. The in-clinic ELISA, SNAP 4Dx Plus can also detect antibodies to A. phagocytophilum. This assay will also detect antibodies to A. platys in some animals infected with this agent. PCR analysis using peripheral blood can be performed at commercial labs and can detect and distinguish between A. phagocytophilum and E. ewingii. This method is much more sensitive in detecting organism than microscopic evaluation. However, animals that are chronically infected or subclinical carriers may be seropositive and PCR negative due to the low numbers of circulating organisms.

**Treatment and Prognosis**

Doxycycline is used as the treatment of choice and often results in rapid (24- 48 hours) resolution of clinical signs. However, the dose and length of therapy necessary to clear infection is not well established. As with other tick-borne pathogens, it is generally believed that, prolonged therapy initiated during the acute phase of infection is most effective in clearing the organism from the mammalian host. Under experimental conditions, doxycycline given at a dose of 10 mg/kg q 12 hrs for 28 days failed to clear chronically infected, subclinical career animals. Rifampin and levofloxacin have been shown to be effective in vitro, but to our knowledge has not been evaluated in vivo. Nonsteroidal anti-inflammatory drugs or anti-inflammatory doses of glucocorticoids can be judiciously used in dogs after a course in antimicrobial therapy has failed to relieve clinical signs of joint disease. Partial responders or animals that relapse after completion of doxycycline therapy should be tested for other vector-borne agents, particularly Bartonella spp. or Babesia spp. which are poorly responsive or nonresponsive to doxycycline.

**Borrelia burgdorferi**

**Etiology And Epidemiology**

*Borrelia burgdorferi* is a spirochete and the causative agent of Lyme disease in people, dogs and horses. The white-tailed deer are thought to be the primary reservoir although mice play an important role in the life cycle of the organism and disease transmission. Due to travel of people and pets, *B. burgdorferi* infections may be encountered in many geographic locations in United States, Europe and Asia. However, highest prevalence is found in areas of heavy deer tick populations. In the US, highest seroprevalence in dogs is Northeastern US, upper Central US (Wisconsin and Minnesota) and on the West Coast (California). These areas seem to correlate well with the number of reported cases in humans as well. Golden retrievers and Labrador retrievers are breeds that are frequently mentioned as at risk for developing chronic Lyme disease and severe, life-threatening forms such as protein losing glomerular disease. It is unclear if this predisposition is the result of a genetic predisposition, breed popularity or risk of exposure. However, all breeds are susceptible to infection.

**Transmission and Pathogenesis**
B. burgdorferi can infect people as well as dogs. However, most infections occur as a result of inoculation from a tick bite. Since this organism is rarely found in any body fluids or secretions, it would be very unlikely to encounter direct transmission from a dog to a human and to our knowledge has never been identified.

B. burgdorferi is transmitted by the deer tick (aka. black-legged tick). Two species of deer ticks are present in the US; Ixodes scapularis is found along the Eastern coast of the United States as far north as Maine, spreading westward to Iowa and in the south, from Florida to central Texas. Ixodes pacificus is found only on the western coast of the United States, primarily throughout Washington, Oregon and California. The abundance and distribution of the tick follows that of the white-tailed deer. Since transovarial transmission has not been demonstrated, ticks must first acquire the infection at the larval or nymph state from a mammalian reservoir host (often the mouse). Once the infected tick attaches to a susceptible dog or human, the organism is transmitted in the tick saliva. The exact length of time required to transmit the infection is difficult to predict due to the number of factors affecting transmission. However, it is suspected that prolonged tick attachment of at least 24 to 48 hours is required for B. burgdorferi transmission. Once inoculated into the body, the organism migrates through connective tissue and enters into joint capsules nearest tick attachment. There it induces cytokine production that results in a monoarthritis and polyarthritis.

**Clinical Findings**
The incubation period is often prolonged, anywhere between 2 to 6 months post-inoculation. If antimicrobial therapy is not initiated during these early stages of infection, chronic persistent infections can develop. Not all animals infected with B. burgdorferi develop clinical disease. Evidence of clinical disease is low, suspected to be only 5-10% of infected dogs. Seropositivity in dogs may be as high as 75% in endemic areas. In experimental infections, 75% of dogs had a 3-6 day episode of mono or polyarthritis of varied severity beginning at 2 mo. post-infection. These spontaneously recovered without therapeutic intervention. Animals then remained subclinically infected. In naturally infected, seropositive dogs, acute signs of fever, shifting leg lameness, anorexia, malaise and lymphadenomegaly have all been commonly reported. One of the earliest signs of clinical disease is often lameness (arthritis) in the limb where the tick bite occurred. Polyarthritis may eventually develop as the agent migrates to other joints or as the host immune response causes inflammatory disease at distant locations. If therapy is not initiated in this acute phase, the animals often spontaneously recover and develop subclinical infections. However, relapse of disease can occur causing the development of chronic Borreliosis. Manifestations include chronic polyarthritis, protein losing glomerular disease, heart block, and neurological disease. As in many tick-borne diseases, circulating immune-complexes produced by antibodies reactive to soluble antigens in the blood stream deposit in tissues where there is specialized vasculature. Such locations include the glomeruli, the synovial membranes and the meninges. For this reason, many dogs chronically infected with tick-borne pathogens develop glomerulonephritis, polyarthritis and meningitis.

**Diagnosis**
The diagnosis is suspected in any dog with a febrile lameness (or other clinical findings listed above) that lives in or has a history of travel to an endemic area. Confirmation of infection requires a positive in-clinic ELISA, such as SNAP 4Dx Plus (IDEXX Laboratories, Westbrook,
ME) or a positive serology (Western blot or ELISA) from a reference laboratory. Since the organisms rarely circulate in peripheral blood, PCR analysis is only useful in testing biopsy specimens from the joint or a skin rash associated with the tick bite.

**Hematologic Findings and other Diagnostic tests**
Borreliosis is not associated with any specific biochemical or hematologic abnormalities. However, synovial fluid analysis may be used to document inflammatory joint disease and urinalysis may be important in animals with glomerulonephritis and protein losing nephropathies. Changes in the biochemical profile in animals with glomerular disease include hyoalbuminemia and elevated BUN and creatinine. The microalbuminuria test may be useful in detecting development of early stage renal disease associated with infection. Radiographs of the joints will typically reveal soft tissue swelling resulting from a nonerosive polyarthritis. In dogs with polyarthritis, the synovial fluid will contain increased numbers of leukocytes, most of which will be neutrophils. Organisms will not be identified by light microscopy.

**Confirmation of Disease**
The in-clinic ELISA, SNAP 4Dx Plus can detect antibodies to *B. burgdorferi*. Because of the prolonged incubation period, most animals with clinical disease have positive antibody titers. This method is much more sensitive in detecting organism than microscopic evaluation. However, animals that are chronically infected or subclinical carriers may be seropositive and PCR negative due to the low numbers of circulating organisms.

**Treatment and Prognosis**
The prognosis is good for animals treated during the acute stage of infection, but guarded in animals with chronic Lyme disease. Animals that develop protein-losing glomerular disease have a poor long-term prognosis. Doxycycline is the first-line drug of choice. Azithromycin and third generation cephalosporins have been used to treat refractory cases in infected humans. Although resolution of clinical disease often occurs, antimicrobial therapy may not clear the organism from infected animals and relapse of clinical signs may occur. Nonsteroidal anti-inflammatory drugs or anti-inflammatory doses of glucocorticoids can be judiciously used in dogs after a course in antimicrobial therapy has failed to relieve clinical signs of joint disease. There is evidence that animals with chronic infections with *B. burgdorferi* are often not cleared of the organism even with appropriate antimicrobial therapy (doxycycline at 10 mg/kg, PO, BID for 30 days). The ability of this organism to form biofilm-like colonies and encysted colonies makes it difficult for antimicrobials or the host immune system to penetrate the structure and eliminate the organisms.