Ehrlichiosis, Anaplasmosis and other Vector Borne Diseases You May Not Be Thinking About
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Canine Monocytic Ehrlichiosis

*Ehrlichia canis*

The common etiologic agent of canine monocytic ehrlichiosis, has been recognized worldwide as an important canine infectious agent. First identified in the 1930s it is one of the most studied of canine tick-borne diseases. Despite that, there is still much debate and unknown surrounding the practical aspects of the disease. *Ehrlichia canis* is present in much of the world including Africa, Asia, America, and Europe (mostly around the Mediterranean). Vertebrate hosts include many different canine species, the domestic dog is considered the definitive host along with foxes and jackals. The bacteria are transmitted by the brown dog tick (*Rhipicephalus sanguineus*). We are only beginning to realize the potential role of co-infection in tick borne diseases in dogs. Co-infection with *A. platys* may, for example, play an important role in many cases of monocytic ehrlichiosis.

**Pathogenesis**

Virulence – It is unclear why some dogs die from acute or chronic ehrlichiosis while others are minimally affected. Factors thought to play a role include tick or bacterial burden, immune response and overall health status, strain of the organism, co-infection and dog breed. German shepherd dogs and greyhounds are thought to be more severely affected. The bacteria are transmitted from the tick during the blood meal through the salivary glands. Following infection and a 1-3 week incubation period clinical ehrlichiosis may develop. Classically this infection is divided into 3 stages (may or may not all be apparent in the individual dog)

Acute disease – fever, anorexia, lethargy, lymphadenopathy, splenomegaly, petechiation and epistaxis resulting from acute thrombocytopenia are thought to be most common. Polyarthritis, polymyositis, uveitis, glomerulonephritis and neurological manifestations may also occur. This stage typically lasts 2-4 weeks.

Subclinical phase – Untreated dogs or possibly even some treated ones may enter this phase. Although overt clinical disease is not recognized, mild to moderate thrombocytopenia still exists. During this stage the dogs are still likely to harbor organism, mostly in the spleen.

Chronic phase – Pancytopenia as a result of bone marrow suppression is the hallmark of this stage. Severely affected dogs carry a grave prognosis at this point. Immune mediated reactions likely play a major role in many of the common manifestations of the disease including the thrombocytopenia, anemia, polyarthritis, uveitis, glomerulonephritis and meningitis.

**Diagnosis**

Laboratory Findings

Thrombocytopenia and a mild to moderate anemia are the most frequent hematological abnormality occurring in more than 90% of cases. CBC abnormalities may also include a moderate to severe leukopenia. *Ehrlichia canis* morulae can occasionally be found in monocytes and macrophages during acute disease. These are of bacteria surrounded by a membranous vacuole Blood chemistry typically reveals an increased globulin to albumin ratio and possibly mild elevations in liver enzymes. As mentioned above, in the chronic stage a severe pancytopenia may develop as a result of bone marrow hypoplasia.
The diagnosis of *E. canis* infection includes:

1. Appropriate history (travel, tick exposure etc..) and physical examination.
2. Cytology. The detection of morulae in monocytes in stained blood smears is rare though.
3. Serology. Anti-*E. canis* antibodies can be detected in dogs infected with this pathogen and persist long after recovery from the disease. Thus they are difficult sometimes to interpret. Typical findings of history, PE, and CBC abnormalities should be combined to serologic results when examining a sick dog suspected of suffering from *E. canis* infection. When screening healthy dogs – a follow up CBC is warranted to make treatment decisions. The in house ELISA kits - SNAP® 4Dx® (IDEXX Laboratories, Westbrook, Maine) is a very sensitive method of measuring antibodies as are reference laboratory IFAs. Serum antibodies are thought not to be protective or play an important role in eliminating this intracellular infection.
4. PCR. Detection of the presence of *E. canis* DNA by the polymerase chain reaction (PCR) is highly sensitive and specific and has become a popular assay in research of this disease although it is too early to say exactly what a negative PCR means when used in clinical diagnosis.

**Treatment**

Ehrlichial organisms are susceptible to tetracyclines, and doxycycline is most widely used for treatment of infection. Doxycycline can be used at 10mg/kg/day for 3 weeks. If this medication cannot be used then fluoroquinolones can be considered. In the acute phase response to therapy is rapid. Immunosuppression should also be considered if immune mediated conditions are apparent especially in the case of chronic disease with bone marrow suppression. Alternatives to prednisone could include azathioprine, cyclosporine or mycophenalate.

Treatment of non-clinical dogs is recommended if CBC abnormalities are present.

**E. chaffeensis**

Only mild clinical signs of fever have been repeatedly associated with this organism in experimental infections in dogs. Immunocompromised dogs may be more likely to be clinically affected. If suspected the treatment is identical to *E. Canis*.

**What organisms cause Anaplasmosis?**

Canine anaplasmosis is an infectious disease caused by one of 2 organisms currently known as *Anaplasma phagocytophilum* and *Anaplasma platys*. These are gram-negative, obligate, intracellular bacteria. They belong to the order *Rickettsiales*, which also include members of the genera *Ehrlichia*, *Neorickettsia* and others. They are likely to be more familiar to some under their previous classification as *Ehrlichia* organisms. In the past few years a major reclassification was done.¹ These organisms were reclassified to *Anaplasma*. More interestingly, though, 3 separate *Ehrlichia* organisms or syndromes previously known as *E. equi*, *E. phagocytophila* and the previously unnamed agent causing Human Granulocytic Ehrlichiosis (HGE) were found to be one and the same! So – Those equine, canine and human diseases are all caused by what we used to call *E. Equi*, and now call *A. phagocytophilum*. Similarly *E. platys* became *A. platys*.

**ANAPLASMA PHAGOCYTOPHILUM**

**Epidemiology**
Since the 1980s sporadic reports or experimental infections have been of this organism have been documented in dogs. A single case report describing meningitis in a dog was published in 1994. Larger serological studies were first performed in Oklahoma in 1989 and in the northern midwest in 1996. The distribution of A. phagocytophilum is worldwide with many reports from Asia, the midle east, and Europe. In the US the organism is found mostly in the northeast, midwest and northern california and oregon coast, a remarkably similar geographic distribution to the of Lyme disease. The A. phagocytophilum organism affects humans as well as the there is considerable overlap between areas with a high prevalence of canine exposure to that of the human population. In the United States there appears to be a seasonal distribution of clinical canine cases. This may coincide with the emergence of the tick vectors in spring and early summer (May and June), and then again the fall (September).

**Vector and other hosts**

Similar to *Borrelia burgdorferi*, the causative agent of Lyme disease, A. phagocytophilum, is transmitted by the dear tick. *Ixodes scapularis* is the primary vector in the upper Midwestern and northeastern US and *I. pacificus* in the west. *I. ricinus* is thought to be the primary vector in Europe and the United Kingdom. The organism can infect a wide range of mammalian hosts including dogs, cats, horses, ruminants, humans and many wildlife species as intermediate hosts. Definitive hosts are thought to be small rodents and the white-tailed deer. The transmission from the tick occurs during the blood meal. The transition into the tick salivary glans and eventual infection of the dog is thought to require at least 24 hours of feeding. Co-infection of ticks and subsequently of dogs with A. phagocytophilum together with other organisms like *B. burgdorferi* is likely to be a huge part of this story. The thought of multiple infectious agents causing a combined disease is possibly the most important challenge we face today in infectious disease medicine.

**Clinical Disease in Dogs:**

Despite the importance of this organism, surprisingly little has been studies regarding the pathogenesis and clinical course of the disease it causes in dogs. Historically is was thought that infection with this organism induces acute disease with no chronic consequences. New evidence suggests that this is possibly not the case. Based on the few available experimental infection studies there does appear to be acute, bacteremic phase of infection. In this stage most dogs may demonstrate vague clinical signs of illness including fever, lethargy, malaise, anorexia and general muscle pain resulting in reluctance to move. Some dogs exhibit joint pain and lameness resulting from inflammatory polyarthritis. These signs are remarkably similar to acute Lyme disease, and because of the very high prevalence of co-infection it is often impossible to know what exactly is caused by whom. Even more confusing is the fact that many cases attributed to Lyme disease in the literature and in every day practice, were not even tested for Anaplasmosis. Other clinical findings include gastrointestinal disease such as vomiting, diarrhea or both, or respiratory signs such as coughing and labored breathing. CNS disease (meningitis) can also occur resulting in seizure activity, ataxia or neurological deficits. In humans this is thought to occur most often when a co-infection of Anaplasmosis and Lyme disease exist, possibly with the *Anaplasma* have a permissive effect on the CNS penetration by the *Borrelia*!
Unlike Lyme disease, we may see some laboratory abnormalities associated with Anaplasmosis. The most commonly noted abnormality in clinical cases is thrombocytopenia.\textsuperscript{7,8,10} Lymphopenia early in the infection may also occur though lymphocytosis appears to be more common later. Other CBC changes have also been documented including a mild anemia. The most important finding on a blood smear, though, from a diagnostic standpoint, early in the disease process are morulae within the neutrophils. These are actually membrane-bound vacuoles of intracytoplasmic organisms, seen most often within a few days of the development of clinical signs.

**Diagnosis**

1. **Cytology** – The identification of morulae with neutrophils on a blood smear. This is the most accurate way of diagnosing the disease early on, at first presentation. At that time serology may still be negative.
2. **Serology** – Serology is the most commonly used method for diagnosing exposure this is done by indirect FA testing and recently with the new, in-house ELISA test SNAP® 4Dx® (IDEXX Laboratories, Westbrook, Maine). This test is extremely sensitive but will only become positive 8 days after infection according to a recent study.\textsuperscript{6} the test will also remain positive well after the clinical disease has resolved, or can be positive in exposed non-clinical dogs. The test is very specific for anaplasma organisms but does not differentiate between A. phagocytophilum and A. platys.
3. **PCR** - Several commercial laboratories offer PCR analysis of peripheral blood for detection of A. phagocytophilum. A positive result is a sign of active infection, but a negative result does not rule out exposure or infection as it appears that infected animals can be only intermittently bacteremic, especially during antibiotic therapy.

**Treatment and Prognosis**

*Anaplasma* organisms, like *Borrelia* and *Ehrlichia* spp. Can be best treated with Doxycycline. The “anti-Lyme” dose of 10mg/kg/day either SID or divided BID appears to be a reasonable choice although clinical studies have not been done to optimize the dose. It is unclear whether the organism is completely cleared following such therapy. In the case when doxycycline cannot be given a fluoroquinolone is probably the next best choice. Penicillins, though effective against Lyme, have not been shown to be beneficial when treating anaplasmosis. The prognosis for acute infection appears to be good with appropriate therapy, although some new evidence may suggest a chronically infected state.

**Anaplasma platys**

*Anaplasma platys* is the causative agent of infectious cyclic thrombocytopenia in dogs. It was formerly known as *E. platys*. It is found mostly in the southern part of the United States as well as in South America, Europe and Asia. It is unknown exactly how this organism is transmitted although ticks appear to be very likely. Co-infections also appear to be common especially with *Ehrlichia Canis*. As these organisms directly infect platelets the hallmark of this infection is thrombocytopenia and resulting petechiations and surface bleeding, epistaxis and lymphadenomegaly can occur. The diagnosis is very similar to *A. phagocytophilum* and includes visualization or morulae in platelets, serology and PCR (not yet widely available). The 4Dx
mentioned above will be positive in a dog exposed to *A. platys*. Treatment with doxycycline as described above appears to be effective.

**What else is there we should be talking about?**

In recent years we have become more and more aware of the vast number of emergent infectious diseases of humans, companion animals and livestock. Many of these diseases are vector borne. Such vectors include sand flies, fleas, lice, ear mites, ticks, and potentially other insect species. Even more sobering is the notion that many of these vectors are likely carrying and transmitting more than 1 species of infectious disease and understanding the pathogenesis and clinical signs of each individual disease, even if we could, would probably not be enough to understand the dynamics of co-infection. This talk will concentrate on a few emergent vector borne infectious diseases in dogs, which, in reality, are probably just the tip of the iceberg of what our pet population is truly exposed to every day in the outdoors.

**Canine Babesiosis**

*Babesia* spp. are protozoan parasites that belong to the order of *Piroplasmida*, within the phylum *Apicomplexa*. Within the *Piroplasmida; Babesia* organisms are often called piroplasms – a term for those organisms that utilize mammalian red cells in their life cycle. *Theileria* is the other genera besides *Babesia* that are included in the piroplasms. Until recently only two types of *Babesia* parasites were thought to occur in dogs. They were conveniently divided into the “large” *Babesia canis* and the smaller *Babesia gibsoni*. In the late 1980s *B. canis* was divided into 3 subspecies *B. canis canis, B. canis rossi* and *B. canis vogeli*. So we have 3 separate antigenically and molecularly distinct large babesias and recently a 4th large Babesia was recognized in North Carolina. *Babesia canis* is endemic in Europe, southern Africa, Asia, and the Americas. In the United States, the most common strain is still considered to be *B. canis vogeli*. This is also the least pathogenic one of the three. Thus, unlike in Africa and much of Europe most dogs in the US infected with *Babesia* do not show obvious clinical signs. Immunocompromised, young or heavily parasitized dogs can develop hemolytic anemia and thrombocytopenia. One amazing part of the US *Babesia* story is the very high percentage (46%) of racing greyhounds in Florida that have been exposed to the parasite. Many of the same dogs are also positive for *E. Canis*, possibly even infected by the same tick.

*Babesia gibsoni* was known to occur mostly in Asia and Europe. But in the last 5 years a large number of dogs in the US have been shown to be positive – the vast majority of them American Pit Bull Terriers or American Staffordshire Terriers. Whether this represents a breed predisposition or has to do with the life style of these dogs is unclear.

*Babesia* organisms are transmitted to dogs by ticks during feeding. Similar to other diseases (Lyme) this occurs towards the end of the 2-3 day blood meal. The tick suspected to be the vector in the US is the dog tick *Rhipicephalus sanguineus*. The same tick that carries *E. Canis* and possibly other infectious diseases as well.

The diagnosis of Babesiosis is based on:

1. Cytology - Direct visualization of the organism under light microscopy. This is a relatively insensitive method of diagnosis especially during low levels of parasitemia.
2. **Serology** – This mode of diagnosis is especially useful in chronic carriers because of low levels of parasitemia. The IFA is the most commonly used test. There is a large degree of cross reactivity between species such that positive serology should be followed up by PCR to differentiate *Babesia spp*.

3. **PCR** – PCR of whole blood samples is a sensitive and specific way of identifying and speciating dogs with active *Babesia* infection.

**Treatment**

The following treatment options will be discussed:

1. **Imidocarb dipropionate** is the only approved drug for treating Babesiosis in the US. Side effects of imidocarb include pain at the injection site, salivation, lacrimation, gastrointestinal signs, and tremors. Pre-treatment with atropine (0.04 mg/kg SC) may prevent these cholinergic side effects. This drug has been shown to be very effective against *B. canis*, but not as helpful against *B. gibsoni*. It is unclear if non-clinical dogs with positive titers or PCR results should be treated (such as greyhounds with *B. canis*) in the absence of biochemical abnormalities. It is also unclear whether the parasites are consistently cleared with the therapy.

2. **Biminazene aceturate** – not available in the US

3. **Antibiotics**: Clindamycin, azithromycin and metronidazol may have a role especially in treating *B. gibsoni* infections.

4. **Supportive care** in clinical cases.

5. **Immunosuppression** in cases with suspected secondary IMHA or ITP is controversial as it may exacerbate the parasitemia.

**Bartonellosis**

One of the most amazing aspects of this emergent infectious disease in dogs is the surprisingly high prevalence it is seen in association with a wide variety of disease states. And although a cause and effect relationship is often hard to prove between an infectious agent and clinical signs, it is clear that this is an important organism that we need to be aware of and to learn more about. In this lecture a brief review of the disease states that have been associated with *Bartonella spp.* in dogs will be given. The most important *Bartonella spp.* known in dogs today is, *Bartonella vinsonii* (*berkhoffii*). It has been associated with many disease states in naturally infected dogs including: endocarditis, myocarditis, cardiac arrhythmias, granulomatous rhinitis, and granulomatous lymphadenopathy.

**Diagnosis**

The diagnosis of Bartonellosis includes screening serology and confirmatory PCR. PCR of tissue is also a sensitive and specific mode of diagnosis.

**Treatment**

Although prospective clinical trials are lacking azithromycin attains very high intracellular antimicrobial concentrations, and appears to be today’s drug of choice for the treatment of *B. vinsonii* (*berkhoffii*) in dogs. Post treatment serologic testing for *B. vinsonii* (*berkhoffii*) antibodies is recommended to support therapeutic elimination of the infection.