ABSTRACT: Erythrocyte parasites should be considered in the differential diagnosis of hemolytic anemia in dogs. In the United States, both canine haemobartonellosis and babesiosis typically cause subclinical disease. However, dogs that are immunocompromised by concurrent disease or immunosuppressive therapy or that are splenectomized are more likely to develop clinical signs. Because both diseases can be transmitted via ticks or contaminated blood transfusion, ectoparasite control and screening of blood donors are important for prevention.

Haemobartonellosis and babesiosis are tick-borne parasitic diseases of canine erythrocytes. Although these diseases typically cause mild to subclinical signs in dogs residing in the United States, both can cause hemolytic anemia. The Haemobartonella and Babesia strains found in the United States are Haemobartonella canis, Babesia canis vogeli, and Babesia gibsoni. This article reviews the pathogenesis, clinical findings, diagnosis, treatment, and prevention of Haemobartonella and Babesia infection.

HAEMOBARTONELLOSIS

Pathogenesis

Haemobartonella canis, a gram-negative, non–acid-fast, epicellular rickettsial parasite found in dogs, is classified under the order Rickettsiales in the family Anaplasmataceae, although recent studies suggest that Haemobartonella may be more closely related to Mycoplasma. H. canis contains both DNA and RNA and replicates through binary fission. Ticks and contaminated blood transfusions are the main modes of transmission. In dogs experimentally inoculated intravenously (IV), the prepatent period ranged from a few days to more than 2 weeks. H. canis infection is primarily associated with hemolytic anemia.

The brown dog tick Rhipicephalus sanguineus is the predominate tick vector. Both transstadial (larva-nymph-adult) and transovarial transmissions of H. canis occur in the tick. R. sanguineus can survive as a one-host tick (i.e., can remain on one animal or one species for its entire life cycle) or can use different species during its three life stages. Thus environmental infestation can perpetuate the tick’s life cycle, causing recurrent or persistent infection in a household or kennel. Since
**R. sanguineus** is a potential vector of many diseases, dogs with *H. canis* infection should be screened for other tick-borne parasites (e.g., *Ehrlichia canis*, *Babesia canis*, *Rickettsia rickettsii*, *Hepatozoon canis*).

**Clinical Findings**
Generally, clinical signs of haemobartonellosis are seen in splenectomized dogs or those with concurrent immunosuppressive disease (e.g., parasitic, viral, or bacterial infection or neoplasia) or receiving immunosuppressive therapy.\(^1,2\) Even in these dogs, clinical signs are typically mild, with few organisms found on blood smears. However, some dogs can become heavily parasitized and acutely anemic. Parasitemia can be episodic, resulting in varying degrees of illness. Recurrent parasitemia can increase the likelihood of developing clinical signs.

Clinical signs result from anemia and may include lethargy and pale mucous membranes. The anemia can vary in severity and rate of onset, depending on duration and severity of parasitemia; is usually regenerative; and can have an immune-mediated component, causing spherocytosis or a positive Coombs’ tests.\(^1,2\) If the disease is diagnosed early, the anemia may be nonregenerative, as it typically takes 3 to 7 days for a maximum regenerative response from the bone marrow. Thrombocytopenia may occur in some dogs.\(^1\) Biochemical changes are generally mild and are secondary to anemia and associated hypoxia. Hyperbilirubinemia is uncommon, although marked bilirubinuria can occur.\(^1,2\)

**Diagnosis**
Diagnosis is based on finding *H. canis* on a blood smear. When viewed microscopically, *H. canis* may appear in chains or as individual organisms across the surface of the erythrocyte*\(^1,3\)* (Figure 1). When viewed by electron microscopy, indentations or grooves are visible in the erythrocyte membrane, where *H. canis* has altered the surface (Figure 2). Because *H. canis* organisms can detach themselves from erythrocytes in stored blood, blood smears should be made immediately after a sample is collected. Parasitized erythrocytes may become trapped in capillary beds, and thus smears of blood taken from the quick of toenails or a nick at the ear margin may offer the greatest likelihood of obtaining a positive sample.

**Treatment**
Supportive care and parasiticidal management are involved in treating dogs with haemobartonellosis. Supportive care includes cross-matched blood transfusions or administration of synthetic hemoglobin products (Oxyglobin \(^*\), Biopure, Cambridge, Massachusetts) to restore blood volume, improve oxygen delivery to the tissue, and allow time for specific parasiticidal therapy to work. Fluid and nutritional support may also be necessary, depending on the severity of the disease. In addition, if the dog is not splenectomized or receiving immunosuppressive therapy, an underlying immunosuppressive disorder should be suspected and appropriate evaluation undertaken, including a minimum database (complete blood count, biochemical profile, urinalysis), radiographs of the thorax and abdomen, and serologic testing for other infectious diseases.

Specific drug treatments directed at resolving *H. canis* infection have been described, including oral tetracycline (20 to 22 mg/kg three times daily for 21 days) and oral or IV chloramphenicol (20 to 22 mg/kg two or three times daily for 9 to 21 days).\(^1,3\) Tetracycline (or a tetracycline derivative) is the mainstay of therapy and...
should be the first choice, with chloramphenicol treatment a possible alternative in dogs that do not respond to tetracycline treatment. Chloramphenicol was used successfully in one experimentally infected dog. Because antibiotic therapy may not completely eliminate *H. canis* organisms, clinical signs may recur if underlying immunosuppressive disease develops or is not reversible. Glucocorticoids (e.g., 1 mg/kg oral prednisone twice daily and gradually tapered once the hematocrit normalizes and is stable) may be effective if *H. canis* infection is associated with immune-mediated hemolysis, although care must be taken when administering a glucocorticoid to potentially immunosuppressed patients.

The efficacy of other drugs, such as enrofloxacin or imidocarb, in dogs with haemobartonellosis has not been studied. Enrofloxacin has been advocated as treatment of other tick-borne infections, including those caused by *E. canis* and *R. ricettfsii*; however, a recent report refuted claims that enrofloxacin is efficacious in the treatment of *E. canis* infection.5 Imidocarb is only approved for use in dogs with *B. canis* and *E. canis* infections.6

**Prevention**

Prevention of *H. canis* infection is a primary concern in splenectomized or immunocompromised dogs. Since ticks are the main mode of natural transmission, ectoparasite control is imperative and should include application of products containing fipronil or selamectin or use of shampoos or dips marketed for tick control. Owners should be advised to monitor indoor and outdoor environments for signs of the brown dog tick. In addition, blood donors should be screened before their blood is used for transfusions, and routine annual screening and tick prevention should be standard practice for these dogs.3,4

**BABESIOSIS**

**Pathogenesis**

Babesiosis is caused by tick-borne intracellular hemoprotozoan parasites.3 Babesial organisms are classified under the class Sporozoa in the order Piroplasmida and in the family Babesiidae. In the United States, both *B. canis* and *B. gibsoni* have been reported to cause natural disease in dogs.7,8 In addition, *B. canis* infection has been diagnosed in dogs in southern Europe; Africa; Asia; and North, Central, and South America.7,8 In the United States, *B. canis* primarily affects dogs in the Gulf Coast region and southern states and is the most common babesial organism found.7 Tick vectors that can transmit *B. canis* under natural conditions include *R. sanguineus*, *Dermacentor reticularus*, *Dermacentor marginatus*, and *Haemaphysalis leachi*.3,8,9 *B. gibsoni* infection is found primarily in northern Africa and the Far East but also occurs in the southwestern United States.7 A recent report documented an increased incidence of *B. gibsoni* in North Carolina.10 These dogs exhibited mild to severe anemia and thrombocytopenia.10 The specific tick vector for *B. gibsoni* in the United States is not known, although in other countries it is reportedly transmitted by *R. sanguineus*, *Haemaphysalis bispinosa*, and *Haemaphysalis longicornis*.8–10

The tick must feed on the dog for 2 to 3 days before transmission occurs.7,8 Once babesial organisms enter the bloodstream, they are endocytized into the erythrocyte, where they replicate by binary fission. Organisms then leave the erythrocyte to infect additional erythrocytes. Both transstadial and transovarial transmissions can occur in ticks.7

*Babesia canis* organisms are large and piriform in shape (2.4 × 5.0 µm) and usually occur in pairs within infected erythrocytes (Figure 3) but have been seen in groups of eight or more.5,7,9 Three strains have been
identified: B. canis vogeli, B. canis canis, and B. canis rossi.7–9 B. canis vogeli, the least pathogenic of the three, is the one reported in the United States.7–9 B. canis canis is of intermediate pathogenicity and is found mainly in Europe and Asia.7–8 B. canis rossi is highly pathogenic and is the main babesial organism found in southern Africa.7–8 B. gibsoni is smaller and more pleomorphic (1.0 × 3.2 µm) than B. canis7,8,10 and can appear as a signet (Figure 4), rod, or cocci shape, with usually only one organism infecting an erythrocyte.3,10

**Clinical Findings**

Because of its mode of transmission, there is an increased incidence of babesiosis in the summer. Greyhounds have a higher incidence of B. canis infection than other breeds, which may be related to breed susceptibility or environmental influences.7,8 In the United States, most infected dogs are young, possibly because of immaturity of the immune system.7,8 Subclinical disease is most common, although clinical disease may become apparent if a dog develops concurrent immunosuppressive disease or requires immunosuppressive therapy. Splenectomized dogs are more severely affected.8 Transplacental transmission of B. canis and B. gibsoni infection is suspected, as infected dams have given birth to “fading puppies” with babesiosis.7,10

The prepatent period for babesiosis is 10 to 21 days,3,9 followed by a transient parasitemia and subsequent secondary parasitemia.7 In dogs, hemolytic anemia is the hallmark sign and can be intravascular, extravascular, or both. The anemia results in hypoxia and tissue damage, which causes release of inflammatory mediators and vascular endothelial damage.3,7

Depending on disease severity, one of two syndromes may occur: hemolytic anemia or hypotensive shock with multiorgan failure.7,11 Some authors have classified B. canis infections as uncomplicated (anemia of varying severity) or complicated (anemia progressing to multiorgan failure) or have categorized infection on chronicity of the disease (i.e., hyperacute, acute, chronic, and subclinical).3,7,8 The hyperacute form is characterized by shock and multiorgan failure; death usually occurs within 24 hours of the onset of clinical signs.7,8,11 This form is rare in the United States but can occur in infected puppies. The acute form of babesiosis may be more common in dogs with B. gibsoni infection than with B. canis infection and usually presents as fever, lethargy, and anemia.7 In the United States, the chronic form of babesiosis has not been identified in dogs with B. canis infection but may be seen with B. gibsoni infection.7,8 The chronic form usually involves intermittent fever, lethargy, and weight loss.7,8 Fortunately, the least pathogenic B. canis strain (B. canis vogeli) is the most common in the United States and causes only subclinical disease in most infected dogs.8 The B. canis rossi strain more commonly induces hyperacute and acute disease leading to hypotensive shock. Clinical signs are not solely related to the degree of parasitemia but also to the body’s immune response to the infection. Dual infections (e.g., with E. canis) can increase morbidity and mortality.8

Babesiosis can cause anemia by several mechanisms, and the anemia is typically macrocytic, hypochromic, and regenerative. The parasitemia increases osmotic fragility of erythrocytes, decreasing their lifespan.7,8,11 The reticuloendothelial system is activated, causing erythropagocytosis.11 Secondary immune-mediated hemolysis occurs as the result of parasite antigens on the erythrocyte or parasite-induced erythrocyte membrane damage and possible exposure of membrane-associated antigens.7–9,11 Oxidative damage may also occur.7 As a result of parasite damage, hemoglobin in the remaining erythrocytes may be less effective in delivering oxygen to the tissues, exacerbating hypoxia.4 Sludging of parasitized erythrocytes and sequestration of infected cells within the spleen may contribute to anemia and hypoxia.7,8,11

Leukocyte changes are inconsistent. Low-grade thrombocytopenia is a fairly consistent finding (especially in dogs with B. gibsoni infection) but is rarely severe enough to cause spontaneous hemorrhage.7,8,11 Biochemical abnormalities depend on the severity of the anemia, leading to variable tissue hypoxia.8 Hyperbilirubinemia is rare with B. gibsoni infection, although it may occur in dogs with acute B. canis infections.7,8

The syndrome characterized by hypotensive shock and multiorgan failure can occur in dogs with severe anemia and both direct and indirect parasite damage to multiple organs and thus would be rare in dogs in the United States. Complications associated with the hypotensive shock syndrome include disseminated intravascular coagulation (DIC), acute renal failure (ARF), hepatopathy, red biliary syndrome, central nervous system signs, acute respiratory distress syndrome (ARDS), and multiorgan dysfunction syndrome (MODS).11

Disseminated intravascular coagulation may occur secondary to vascular stasis, endothelial damage related to parasitized erythrocytes and hemolysis, or acidosis or may result from release of inflammatory mediators secondary to tissue damage and hypoxia.7,11 Thrombocytopenia can be secondar y to consumption from DIC or immune-mediated destruction.7

Acute renal failure may occur from hypotension and hypovolemic shock leading to ischemic renal injury, and hemoglobin released when erythrocytes are lysed can be nephrotoxic.8,11 Cellular casts from tubular dam-
age may accumulate and obstruct renal tubules, adding to the ongoing damage. ARF may be more common in older dogs, suggesting the contribution of preexisting renal disease to ARF.

Central nervous system derangement can occur in dogs infected with *B. canis*, including altered mentation, muscle tremors, ataxia, nystagmus, anisocoria, seizures, or coma. Sludging of parasitized erythrocytes attributable to increased erythrocyte stickiness and increased cell rigidity can cause vascular stasis and cerebral ischemia. Hemorrhage secondary to concurrent DIC, thrombocytopenia, or both may add to these signs. Hypoxia resulting from hypovolemia, hypotension, and hemoconcentration secondary to red biliary syndrome may predispose to central nervous system damage; dogs that survive typically do not have permanent damage.

Red biliary syndrome, unique to *B. canis* infection, is a paradoxical syndrome of hemoconcentration with hemoglobinemia and hemoglobinuria. This causes a normal to elevated hematocrit in the face of hemolysis. Serum total protein concentration remains normal, indicating that the hemoconcentration is not the result of dehydration. It is believed that plasma is translocated from the intravascular to extravascular space secondary to increased capillary permeability.

Hepatocellular injury in dogs infected with *H. canis* may occur secondary to DIC, hemorrhage, hypoxia, and hypotension as well as direct damage to the reticuloendothelial system. Histopathologically, centrilobular congestion and necrosis (secondary to hypoxia) may occur. Since icterus is uncommon in dogs with babesiosis, hyperbilirubinemia warrants consideration of hepatic involvement or damage.

Pulmonary edema secondary to increased capillary permeability can lead to ARDS. Widespread hypoxia, inflammatory mediator release, and tissue damage can culminate in MODS.

**Diagnosis**

Diagnosis is generally made from blood smear evaluation. Babesial organisms may be easiest to see on Giemsa-stained smears. Infected erythrocytes tend to be at the periphery of the smear. A centrifugation technique has been used to optimize identification of parasitized cells on a blood smear. With this technique, the infected erythrocytes tend to locate in the upper layer of the centrifuged sample and thus this layer is used to make the blood smear.

Serologic assays (e.g., immunofluorescent antibody [IFA], enzyme-linked immunosorbent assay, polymerase chain reaction) have also been used. IFA is the most widely used assay but is expensive and has low sensitivity. Cross-reactivity occurs between *B. canis* and *B. gibsoni* organisms on the IFA. Titters of 1:40 or less are considered negative, titters of 1:80 or higher generally occur in *B. canis* infections, and titters greater than 1:320 in dogs with appropriate clinical signs are typically recommended before a diagnosis of *B. gibsoni* infection can be made. Because an antibody response requires approximately 8 to 10 days, paired titters may be useful. Treatment should not be withheld if the disease is highly suspected in dogs with an initial negative serologic result.

**Treatment**

Treatment includes supportive care and parasiticidal therapy. Supportive care includes administration of blood or synthetic hemoglobin products to avoid continued hypoxia. Intravascular volume should be supported with crystalloids and/or colloids, which may help treat DIC by maintaining microvascular perfusion. Supplemental oxygen may benefit some patients.

Three specific agents are currently recommended for treating babesiosis. Imidocarb dipropionate acts directly on the parasite’s DNA, causing unwinding and denaturation. Because of the possibility of cholinergic side effects (i.e., salivation, urination, defecation, vomiting) related to the anticholinesterase activity of the drug, pretreatment with subcutaneous (SC) atropine (0.05 mg/kg) is recommended. In an early study, a single dose of imidocarb was effective in treating 95.8% of dogs infected with *B. canis*; none had detectable parasites at 2 weeks, and only 3.3% (10 of 304) had parasitemia evident at 6 weeks. Current dose recommendations are 5.0 to 6.6 mg/kg IM or SC, repeated in 2 weeks to treat clinical disease. A one-time IM dose of 7.5 mg/kg has been administered to greyhounds in an attempt to eliminate the carrier state (in asymptomatic dogs) and eradicate the infection in clinical cases. Imidocarb may have a prophylactic effect lasting 4 to 15 weeks and is the drug of choice in dogs with dual infections of *B. canis* and *E. canis*. However, imidocarb is less effective in treating *B. gibsoni* infection.

A second compound, diminazene, is not currently available in the United States except in research facilities. The drug binds to and inhibits parasite DNA synthesis. Diminazene has been effective in treating both *B. canis* and *B. gibsoni* infections. The standard one-time IM dose is 3.5 mg/kg. Side effects include pain at the injection site; gastrointestinal upset; and neurologic signs (e.g., behavioral changes, vestibular signs, paresis, coma), which can lead to death. Because of the side effects associated with this drug, it has not been recommended as initial therapy in dogs with the hyperacute form of babesiosis. To eliminate the infection, diminazene may need to be followed by imidocarb.
A third treatment option is trypan blue (1% solution), which blocks the C3b receptor on the erythrocyte membrane and parasite, preventing the parasite from entering the erythrocyte and thereby suppressing parasitemia. This drug has a high therapeutic index and can be used initially in severely affected animals (single dose of 10 mg/kg IV), which may decrease parasitemia and allow stabilization of the patient but does not eliminate the parasite. Therefore, trypan blue must be followed by either imidocarb or diminazene therapy. Trypan blue is only reported to be effective in treating *B. canis* infections, not *B. gibsoni*.

The use of steroids is controversial, although they may be beneficial if immune-mediated anemia or thrombocytopenia is present or to treat shock. If steroids are used, they should be administered with caution since immunosuppressive therapy can worsen parasitemia. Tetracycline antibiotics have not been successful as treatment of babesiosis. Clindamycin and metronidazole have both been used but have had inconsistent responses.

No specific therapy has proven consistently effective in eliminating *B. gibsoni* infections, although imidocarb and diminazene therapy can lessen the severity of disease. There is one report of successful treatment of *B. gibsoni* infection by administering SC phenamide (7.5 to 10 mg/kg once daily for 2 days).

Prophylactic use of oral doxycycline (5 to 20 mg/kg/day) has been suggested in dogs at risk for *B. canis* infection. Treated dogs are not protected from developing disease; but if a dog becomes infected, the clinical signs are often less severe. Dogs receiving the higher dose did not exhibit clinical signs of babesiosis, although infection did occur (infected erythrocytes were found on blood smears). Depending on the perception of risk, prophylactic therapy may be appropriate.

**Prevention**

Prevention involves ectoparasite control to eliminate the tick vector and screening of blood donors. Para-site control of both animals and the environment is important because of the life cycle of the brown dog tick. If a potential blood donor is positive for *B. canis* or *B. gibsoni* organisms on a peripheral blood smear or serologic assay, the dog should be eliminated from the donor pool. If the donor’s blood smear is negative, a splenectomy can be performed to increase the likelihood of obtaining a positive result in subclinical carriers, as the spleen can no longer sequester parasitized erythrocytes (this approach is not recommended for
client-owned blood donors). These animals should then have blood smears screened daily for 2 weeks after splenectomy and periodically thereafter. If B. canis or B. gibsoni organisms are detected, the animal should be eliminated from the donor pool.

In France, a vaccine is being developed for B. canis canis infection. Efficacy against homologous strains is reported to be 70% to 100%. Although early reports show that the vaccine does not eliminate the disease, dogs receiving the vaccine tend to have less severe clinical signs. Vaccines may be useful when dealing with B. canis canis and B. canis rossi infections in which premunition (chronic subclinical disease) prevents the more fulminant and acute disease. In areas where these strains predominate, it may not be ideal to attempt eradication of the disease, as chronically infected dogs are more resistant to the development of clinical signs. Use of vaccination in the United States, where subclinical or mild disease is the most common form, may be of limited value.

Transmission of B. canis or B. gibsoni infection to humans has not been reported. However, infected dogs may serve as sentinels of other tick-borne diseases in humans.

REFERENCES


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ARTICLE #1 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. Choose the best answer to each of the following questions; then mark your answers on the test form inserted in Compendium.

1. The main clinical finding in immunocompromised dogs with haemobartonellosis is
   a. hemolytic anemia  d. lymphadenopathy.
   b. thrombocytopenia  c. hepatosplenomegaly.
   a. leukocytosis.

2. Diagnosis of canine haemobartonellosis is best based on
   a. lymph node cytology.
   b. serology.
   c. a blood smear made from capillary blood taken from the toenail or ear margin.
   d. a blood smear made from jugular venipuncture.

3. What is the mainstay of treatment for canine haemobartonellosis?
   a. steroids  d. imidocarb
   b. tetracycline  c. thiacetarsamide sodium

4. Prevention of both haemobartonellosis and babesiosis includes
a. monthly imidocarb.
b. ectoparasite control.
c. annual vaccinations.
d. screening of potential blood donors.
e. b and d

5. Which babesial organisms are found in the United States?
   a. *B. gibsoni*
   b. *B. canis rossi*
   c. *B. canis canis*
   d. *B. canis vogeli*
   e. a and d

6. In the United States, most dogs with babesiosis have
   a. severe anemia.
   b. subclinical disease.
   c. hypotensive shock with multiorgan failure.
   d. severe thrombocytopenia.

7. Treatment of babesiosis may include administration of
   a. tetracycline.
   b. imidocarb.
   c. diminazene.
   d. trypan blue only.
   e. b and c

8. Which breed has an increased incidence of babesiosis?
   a. German shepherd
   b. cocker spaniel
   c. greyhound
   d. Labrador retriever
   e. Siberian husky

9. Babesiosis is caused by an __________ tick-borne parasite, and haemobartonellosis is caused by an __________ tick-borne parasite.
   a. epicellular; epicellular
   b. epicellular; intracellular
   c. intracellular; epicellular
   d. intracellular; intracellular

10. Imidocarb is effective against and labeled for which of the following?
    a. *H. canis*
    b. *E. canis*
    c. *B. gibsoni*
    d. *B. canis*
    e. b and d