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Canine babesiosis

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Canine babesiosis is a protozoal, tickborne, hemolytic disease with worldwide distribution and global significance. Over 100 *Babesia* species have been identified, but only *Babesia canis* and *B gibsoni* have been shown to infect dogs [1]. Until recently, *Babesia* organisms infecting dogs were identified based on morphologic appearance. All large *Babesia* were designated *B canis*, whereas all small *Babesia* were thought to be *B gibsoni*. Molecular analysis and serologic surveys have shown these organisms to be much more prevalent and genotypically diverse than previously recognized. Genetic sequencing has revealed that there are at least three distinct subtypes of small *Babesia* affecting dogs [2–4]. In the United States, there are two clinically distinct strains of small *Babesia*. In California, a small *Babesia* closely related to *Theileria* infects a variety of dogs and causes relapsing parasitemia [2,5]. In the remainder of the United States, the classic Asian *B gibsoni* infects almost exclusively pit bull-type dogs and is often subclinical [6–8]. A third strain of small piroplasm has been identified recently in Europe and is most similar to a human and rodent pathogen, *B microti* [9].

Babesiosis is considered an emerging disease, as numerous cases are being reported in new areas throughout the United States and Europe. Transmission of canine babesiosis is facilitated by the international and interstate transportation of dogs and the availability of tick vectors. The presence of chronic subclinical carrier states, the inability to completely eradicate all infections, and transovarial transmission in the tick encourage the establishment of infected tick populations. Babesiosis also has gained attention as a persistent endemic disease in greyhound kennels [10,11]; a newly recognized problem in pit bulls [6–8], as a potential model for falciparum malaria in people [12,13], and as an emerging zoonosis [14].

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The organism

Babesia species (Fig. 1) (Fig. 2) generally are divided into large and small organisms. *B canis* is a larger piroplasm (3 to 5 μm) that is approximately twice the size of *B gibsoni* (0.5 to 2.5 μm) [2]. *B canis* organisms are pyriform and often occur in pairs. *B gibsoni* organisms appear in five to six different shapes and are frequently oval, often single, and may display a ring form [15]. Small *Babesia* are found occasionally as a maltase cross-form but are not reported pairs [5,7,16,17].

Babesia canis

Three distinct subspecies of *B canis* exist: *canis, rossi*, and *vogeli*. The subspecies demonstrate tremendous variation in clinical signs, geographic distribution, and infective tick vectors. Immune responses are fairly specific, and little cross-protection occurs among the different subspecies. Genetic analysis of rRNA sequences has revealed that *B canis canis* and *B canis vogeli* are most similar to each other, with 82% identity in nucleotide positions. *B canis rossi* is approximately 70% homologous to the other two subspecies and tends to cause the most severe disease [18]. In North Carolina, a fourth large canine piroplasm was identified in a Labrador retriever undergoing chemotherapy for lymphosarcoma [19]. This piroplasm was morphologically identical to *B canis*; however, polymerase chain reaction (PCR) and antibody tests for all known *Babesia* species were negative. The 18S rRNA gene fragments showed considerable differences to *B canis* and *B gibsoni*, and this organism probably represents a fourth species/subspecies of *Babesia* [19].

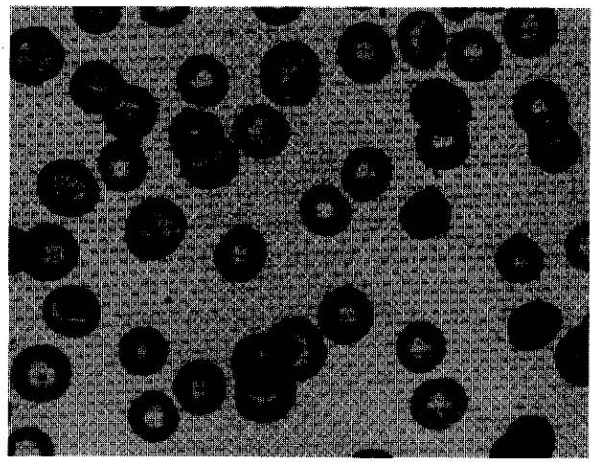

Fig. 1. *Babesia gibsoni* organisms are seen in the red blood cells of a dog.

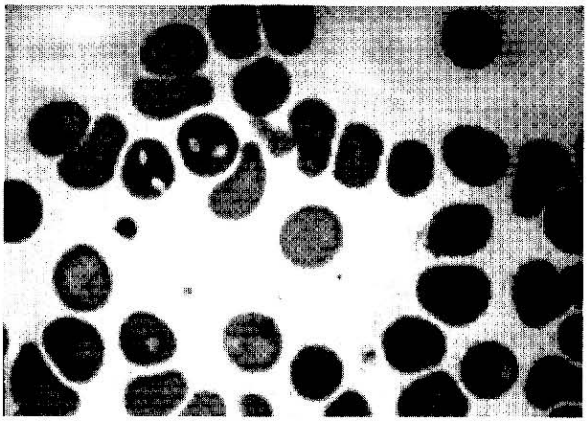

Fig. 2. *Babesia canis* usually appears as paired piriform organisms in canine red blood cells.

South African babesiosis is caused by *B canis rossi* and is transmitted by the *Haemophysalis* tick. This strain is very widespread and notoriously the most virulent. At the Onderstepoort Veterinary Academic Hospital (OVAH) in South Africa, 12% of sick patients were diagnosed with babesiosis. Approximately a third of patients were hospitalized, and fatalities were common, despite treatment [20].

In Europe and Asia, babesiosis is caused by *B c canis*, and *Dermacentor reticularis* is the tick vector. In endemic areas of France, up to 85% of over 500 dogs tested had antibodies to *Babesia*. A maximum of 14% displayed any clinical signs, however, and mortality was only 1.5% [21].When deaths occurred, they were attributed to hepatic and renal damage [21].

Babesia canis vogeli is transmitted by the brown dog tick, *R sanguineus*, and causes relatively mild disease in the United States and in tropical and subtropical areas. *B canis* first was reported in the United States in 1934 [22]. It is recognized throughout the United States, and the disease has been endemic in southeastern greyhound kennels for over 50 years. Babesiosis in greyhounds primarily causes anemic pups, which may be parasitemic but seronegative [10]. Vertical transmission is suspected, and *B canis* has been documented in pups under 2 days old [23]. The incubation period of natural *B canis* infection is approximately 10 to 21 days [24].

In a Mississippi kennel, 59% of greyhounds screened in the early 1980s had positive indirect fluorescent antibody (IFA) titers. Organisms were not evident on blood smears. The kennel routinely admitted numerous dogs without quarantine, and additional screening of dogs from source kennels in Mississippi, West Virginia, Oklahoma, Texas, and Florida revealed enzootic disease with seroprevalence rates of well over 50% [10]. More recently, 46% of almost 400 greyhounds of various ages screened in Florida were seropositive (greater than 1:80) for *B canis* antibodies [11]. In kennels with a history of anemic pups, 78.5% of the dogs were seropositive. In kennels

without a history of anemic pups, 23% were seropositive. None of the dogs currently racing were seropositive, suggesting reduced performance in infected dogs [11]. Babesiosis in greyhounds often is attributed to genetic susceptibility and environmental exposure to numerous ticks. Although most common in tick-infested kennels, *B canis* occasionally affects the general canine population also. In North Carolina, 3.8% of random source dogs were seropositive for *B canis* antibodies [25], and in California shelters, 13% of dogs were seropositive for *B canis* [26].

Babesia canis has the potential to become a more prevalent disease. The growing effort to rescue and adopt greyhounds may inadvertently be contributing to the perpetuation of a *B canis* reservoir in North America. In 1995, over 16,000 greyhounds were rescued and adopted [23]. It has been estimated that 20% to 60% of these dogs may be seropositive [23]. In reality, the probability of clinical disease in an adult greyhound dog is low, and significant spread is unlikely [23]. Age-related immunity is an important protective factor. *B canis* organisms isolated from anemic pups did not cause clinical disease, even in splenectomized adults [10]. Disease transmission is most likely to occur if infected greyhounds are used as blood donors or are kenneled with breeding animals and young puppies.

Babesia gibsoni

Unlike *B canis*, documented *B gibsoni* infection is relatively new in the United States. *B gibsoni* first was recognized in India in 1910 [27] and since has been reported in Asia, Northern and Eastern Africa, Brazil, and rarely in Europe [9,28]. In the United States and Europe, emergence of *B gibsoni* infection has been dramatic. In 1968, *B gibsoni* first was reported in the United States in a chronically infected dog from Malaysia [29]. In Malaysia, *B gibsoni* is endemic, and 18% of dogs demonstrated organisms on blood smear evaluation [30]. Dog fighting is legal in Malaysia, and the suspected international transport of dogs to and from this country may be an important source of small *Babesia* in the United States.

In 1979, small babesial infection was reported in a dog from Connecticut with no travel history [31]. Since that time, infections have been documented in dogs from California [5], North Carolina [7], Indiana [32], Oklahoma [33], and Alabama [6]. At the Vector-Borne Disease Testing Laboratory at the North Carolina State College of Veterinary Medicine, a PCR-based test has identified infected dogs in Arizona, California, Florida, Georgia, Kentucky, Maryland, Michigan, Minnesota, Mississippi, Missouri, New Jersey, New York, Ohio, Pennsylvania, South Carolina, Tennessee, Texas, Washington, Wisconsin, and Virginia (Birkenheuer et al, unpublished data) *B gibsoni* also has been reported occasionally in areas near military bases, where international working dogs are housed [23]. In Japan, *B gibsoni* is the most common species, except in Okinawa, where *B canis* is also prevalent [34]. Approximately 500 dogs are shipped annually from Okinawa to the

United States, and many of these dogs may be infected with *B canis* or *B gibsoni*.

Molecular analysis has led to a much broader understanding of the phylogenetic relationships and classification of the canine piroplasms. Historically, only one small *Babesia* was thought to infect dogs. Although morphologically identical, analysis of the 18S rRNA has shown that there are actually three distinct isolates of small *Babesia* [2]. Small *Babesia* infections now are attributed to the Asian isolate (*B gibsoni*), the Californian isolate, and a recently identified *B microti*-like organism in Europe. The Asian isolate is considered *Babesia* species *sensu stricto* and is identical to the strain identified in most of the United States, excluding California [2,4].

Babesia gibsoni organisms isolated from a dog in Oklahoma were sequenced and compared with the Californian small *Babesia* [33]. The Oklahoma isolate demonstrated 232 nucleotide differences in the 18S rRNA gene when compared with the Californian organism [33]. The Californian small *Babesia* is related closely to *Theileria* species and to *Babesia* isolates that infect wildlife and people in the Western United States [2]. *T equi* was most similar to the Californian strain with 92.7% identity [4]. The Californian organism showed only 88.2% sequence identity to *B gibsoni* [4]. There is also a suggestion, based on maximum-likelihood analysis, that the Californian piroplasm and *Cytauxzoon felis* are ancestral to the remaining *Theileria* and to the classic *B gibsoni* [35].

Babesia gibsoni appears to be less virulent than the Californian strain of small *Babesia*. Parasitemia, relapse rates, and overall mortality appear to be lower with *B gibsoni* infection in the southeast and Midwest. Breed differences also exist. *B gibsoni* affects almost exclusively pit bulls and American Staffordshire terriers in the United States. The disease has been reported only sporadically in other breeds. In North Carolina, a mixed breed dog with concurrent illness was naturally infected with *B gibsoni* [7], and in Indiana, a mixed-breed dog was infected with *B gibsoni* after being attacked by pit bulls and traveling to Florida [32]. The Californian strain has been reported in many breeds of dogs.

In 1994, a new small piroplasm was discovered in a German dog that presumably acquired the infection in Spain [9]. This organism appears to be related only distantly to *B gibsoni*. The organism shows the most similarity to *B microti*, *B rodhainii*, and *Theileria equi* [9]. Previous reports of small *Babesia* in Europe have been relatively scarce. The investigators who discovered the parasite have suggested the name *T annae* for this organism [9].

Over 150 dogs in northwest Spain have been identified with a small piroplasm (generally less than 2 μm) on blood smear analysis since the discovery of this organism. Ribosomal DNA analysis confirmed that the organism is 100% identical to *T annae* [36]. Parasitemia was subjectively rated as low in 149 dogs and moderate or severe in eight dogs. Ninety percent of dogs had a hematocrit less than 31%, and regeneration was

prominent. Leukocytosis was not common, and thrombocytopenia was present in about half of animals [36]. Marginal azotemia was very common. Approximately 10% of dogs had evidence of renal failure on serum chemistry analysis. Hepatic involvement appears minimal with *T annae*, and 75% of dogs had normal hepatic serum chemistry values. The Californian organism is reported to cause significant hepatic changes [37]. Elevations in creatine kinase (CK) were fairly common and may be a result of muscle damage seen with the sequestration of parasites.

Some *Babesia* species may be reclassified as *Theileria*. *Theileria* organisms differ from *Babesia* in that they have a pre-erythrocytic life stage in a lymphocyte, and they lack transovarial transmission in the tick [38]. The identification of a lymphocytic stage in *B equi* resulted in its reclassification as *T equi*. A lymphocyte stage also has been discovered recently in *B microti*, and it likely also will be reclassified as a *Theileria*.

The apparent incidence of *B gibsoni* has been rapidly increasing, since its first in the United States. The additional awareness among practitioners, pit bull and greyhound owners, and the contribution of sensitive PCR testing have led to an increase in the diagnosis of this new disease. More stringent regulations on the importation of dogs from endemic areas are needed to prevent further spread of this disease.

Pathogenesis

Dogs become infected with *Babesia* after the bite of an infective tick releases sporozoites into the circulation. Inside the host, the organisms attach to the red cell membrane and are engulfed by endocytosis [39]. The membrane disintegrates, and the parasites remain directly inside the cytoplasm. *Babesia* species undergo binary fission, resulting in merozoites. Ticks become infected with merozoites during feeding and may remain infective for several generations. Schizogony produces merozoites in the salivary glands, gastrointestinal (GI) cells, and oocytes of the tick. With *B canis*, ticks must feed for 2 to 3 days for transmission to be complete [40]. The incubation period of *B gibsoni* is reportedly 7 to 21 days, and for *B canis*, it is 10 to 21 days. Vertical transmission, blood-borne transmission, and possible transmission through bite wounds is suspected for *B gibsoni* and *B canis*. *B canis* has been documented in pups that were 36 hours old [23], and *B gibsoni* has been found in 3-day-old puppies and a dam [41].

The pathogenesis of canine babesiosis varies with the infective species and is proving more complex than initially recognized. Many of the pathogenic mechanisms are actually a result of host immune response to the organism rather than direct destruction of the erythrocyte by the parasite. Review of 662 cases of South African canine babesiosis suggested two basic disease mechanisms: hemolytic anemia and a hypotensive shock syndrome induced by inflammatory mediators. Disease in the severely anemic group was

attributed primarily to immune-mediated hemolytic anemia, and nonanemic babesiosis was attributed to an overwhelming immune response to the parasite.

Hemolytic anemia is a prominent feature of large and small babesiosis in dogs. Direct red blood cell (RBC) damage, intravascular hemolysis, and extravascular hemolysis are thought to occur [42,43]. Antierythrocyte antibodies, IgG-bound erythrocytes, erythrocyte oxidation, phagocytosis, osmotic fragility, and a hemolytic factor in serum have been implicated in *B gibsoni*-related RBC destruction [43–45].

In *B gibsoni* infections, parasitemia rarely exceeds 10%, yet severe anemia frequently develops [44,46]. In experimental *B gibsoni* infections, dogs experienced concurrent increases in parasitemia and decreases in hematocrit approximately 2 weeks postinfection. This may be caused by destruction by escaping parasites [44]. Spherocytes and autoagglutination were observed approximately 3 to 4 weeks postinfection, suggesting immune-mediated disease is also a significant factor. Anti-RBC IgG has been identified in *B gibsoni*-infected dogs with ELISA testing. The antibody levels are inversely related to the degree of anemia [47].

Complement dependent immune-mediated lysis and removal by the mononuclear phagocytic system are also important factors. Hemoglobinuria and diffuse erythrophagocytosis and hemosiderosis suggest intravascular and extravascular hemolysis [37]. It also has been suggested that a "hemolytic factor" is present in the serum of dogs infected with *B gibsoni* [46]. Sera from *B gibsoni*-infected dogs caused in vitro hemolysis of normal beagle RBCs. The relative activity of the hemolytic factor correlates well with the degree of parasitemia and anemia [48]. Soluble antigens also are thought to be involved in *B canis* anemia. Over 40% of complicated *B canis rossi* infections demonstrated autoagglutination [49]. Immune-mediated disease often causes continued hemolysis after babesiacidal treatment.

In many cases, the degree of tissue hypoxia is relatively severe for a given level of anemia [50]. A quantitative and qualitative deficit of hemoglobin can occur with *B canis* infections. The hemoglobin that remains in intact cells may function abnormally at the tissue level, especially under acidic and hypercapnic conditions [50]. Other *Babesia* and *Plasmodium* species are known to contain enzymes that cleave hemoglobin. Preliminary electrophoretic studies with *B canis* suggest similar enzymes may be present in canine infections [51]. It is not known whether these mechanisms exist for *B gibsoni*.

Canine babesiosis has several similarities to malarial infections and may prove to be a valuable model for studying human disease. In both diseases, a small subset of victims experience hemoconcentration, shock, neurologic signs, and multiple organ failure [13,52,53]. Nonanemic babesiosis is associated with an acute, overwhelming inflammatory response mediated by cytokines, nitric oxide, platelet activating factor, and eicosanoids. The

syndrome ("red babesia" "red biliary") is often peracute and associated with simultaneous massive hemolysis and increase in vascular permeability resulting in a hemoconcentrated state. Plasma, rather than a filtrate of plasma, is lost through the leaky endothelium [49]. Dogs with nonanemic babesiosis often have severe azotemia, electrolyte and acid–base disturbances, and minimal leukocyte responses or even leukopenia [13]. Leukopenia may be caused part by sequestration in the pulmonary vasculature. The severity of the inflammatory response may be so rapidly fatal that a significant leukocyte bone marrow response and the hemolytic state may not have time to develop.

Cerebral and cerebellar signs are encountered occasionally in complicated malarial and babesial infections [54]. Signs may include posterior paresis, muscle tremors, nystagmus, anisocoria, aggression, paddling, crying, and altered states of consciousness. In people, parasite-derived surface proteins serve as endothelial cell receptors. Parasites become sequestered in the microvasculature of the muscle and central nervous system (CNS) and concentrate the mediators of the inflammatory response in the areas where they lodge. Parasites also have been visualized in canine cerebral capillaries. Cerebellar signs may present acutely or may be delayed by several days to weeks in malaria and babesiosis [54].

Multiple organ dysfunctions may occur in severe cases of hematozoan infection [55]. Secondary lung injury is one of the most common findings. Pulmonary edema may result from increased endothelial permeability associated with systemic cytokine release, nitric oxide, free oxygen radicals, eicosanoids and platelet activating factor [49,56,57]. In malarial infections, approximately 3% to 10% of patients develop acute onset pulmonary edema relatively late in infection [58]. In dogs, acute respiratory distress syndrome (ARDS) is suspected when there is hypoxemia, acute dyspnea, poor response to diuretics, and diffuse infiltrates in the caudal lung fields. Cardiogenic overload and cardiac depression associated with acidemia should be ruled out. Evidence for cytokine-based disease has been documented in malarial infections. High levels of tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, IL-10, lymphokines, and neutrophil products have been found in malarial infections [49,59]. Nitric oxide also may play an important role in cytokine-mediated disease and TNF-mediated hypotension. Attempts to correlate levels of reactive nitric oxide metabolites with the severity of disease in canine babesiosis have been discouraging [60].

Acute renal failure is an uncommon but severe complication of virulent babesiosis and complicated malaria. In some malarial infections, intravascular hemolysis and gross hemoglobinuria ("blackwater fever") are found in association with renal failure. Historically, obstruction of renal tubules with hemoglobin was thought to cause anuria in malaria [61]. The role of hemoglobin in development of acute renal failure is debatable. Hypoxia and cytokine-mediated renal damage have been proposed more

recently as likely causes of renal damage [62,63]. In particular, TNF has been correlated with the development of renal failure [64].

In general, hemolytic anemia is the primary manifestation of *B gibsoni* infection. *B gibsoni* infection usually causes acute parasitemia followed by gradual decline in parasite numbers and development of a chronic asymptomatic carrier state. Carriers may have spontaneous relapses of parasitemia. Relapses also have been reported with corticosteroid administration, splenectomy, or stress [37,65]. Experimental infections with *B gibsoni* have helped define the histopathologic changes and mechanisms of disease of the Oklahoma/Asian strain [66] and the Californian small *Babesia* [37].

Dogs experimentally infected with the Californian isolate showed significant hepatic lesions. Findings included diffuse nonsuppurative periportal and centrilobular hepatitis and extramedullary hematopoiesis. Perivenular fibrosis, Kupffer cell hypertrophy, and erythrophagocytosis also were observed. An increased population of CD3 + lymphocytes were observed in the liver. Cells also showed an increased expression of ICAM-1, 2, 3 and VCAM 1, and LFA-3 ligands. Activated macrophages were seen within the central hepatic veins. Similar cells were seen in the closely related WA1 infection in hamsters and people. Renal lesions included IgM antibody deposition in inflamed arteries and glomeruli [37]. Infected dogs often maintain extremely high IFA titers, and chronic antigenic stimulation may lead to sequelae such as the membranoproliferative glomerulonephritis with IgM deposits that has been demonstrated in experimental infection with the Californian isolates [37].

Multifocal, segmental, necrotizing arteritis was seen in five of six dogs in the GI tract, mesentery, and skeletal muscle. Lymphadenopathy was seen in all dogs, mainly in the hepatic and peripancreatic nodes. Plasma cells were prominent in lymph nodes. Splenomegaly was noted in four of four dogs. Experimental infections in spleen intact animals resulted in less parasitemia but more severe anemia than in splenectomized dogs. This most likely is related to rapid removal by the mononuclear phagocytic system (MPS). All six dogs had antibody titers and eventually became Coomb's positive [37].

Experimental infection with the Oklahoma isolate produced milder signs of clinical disease. Five dogs (one splenectomized) were inoculated with organisms from one of two naturally infected dogs in Oklahoma. Parasitemia was relatively mild and first appeared 1 to 5 weeks after inoculation. At 4 to 6 weeks, parasitemia peaked at 1.9% to 6.0%. Parasitemia is reportedly higher in naturally occurring Californian infections, which reached parasitemias of 5% to 40% [5]. Clinical signs included lethargy, fever, and pale gums. All dogs were anemic and thrombocytopenic. The thrombocytopenia developed before and persisted longer than the parasitemia. [66]. Transient severe neutropenia also was noted in some dogs.

Clinical signs

The clinical signs of babesiosis can range from subclinical infections to a hyperacute fulminant fatal disease similar to complicated malarial infections. The clinical course of babesiosis is determined by the particular strain or subspecies of *Babesia*, the immune response of the host, the age of the host, the presence of concurrent infections, and previous exposure to the organism. Hyperacute, acute, chronic, and subclinical forms of the disease are known to exist.

Babesia canis

Babesia canis is capable of causing a wide range of clinical signs that may involve subclinical infection, anemia, thrombocytopenia, lethargy, anorexia, splenomegaly, hemoglobinuria, bilirubinuria, fever, and jaundice. Severe cases may be accompanied by acute renal failure, acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), hemoconcentration ("red biliary"), icterus, hepatopathy, and a neurologic syndrome referred to as cerebral babesiosis. Cerebral babesiosis and hemoconcentration are associated with high mortality rates [12]. Babesial shock can result from severe anemia or as a result of inflammatory mediators associated with multiple organ dysfunction syndromes and resembling septic shock [49]. Severe cases also may be complicated by lactic acidosis and acidemia. Rhabdomyolysis has been described recently as a complication of *B canis rossi* infection in two dogs [67]. One dog exhibited caramel colored urine, increased serum myoglobin, increased creatine kinase, and acute renal failure. A second dog exhibited muscle pain, tremors, cerebral babesiosis, pulmonary edema, and death. Muscle necrosis and hemorrhage were seen on histopathology [67]. Masseter muscle pain also has been reported. A disproportionate elevation in serum urea nitrogen (BUN) often is seen in complicated babesiosis and may be caused by increased muscle catabolism [67]. In human malaria, rhabdomyolysis is unusual, but asymptomatic muscle damage is very common. Biochemical evidence of muscle damage is also common in bovine babesiosis. Rarely reported complications of canine babesiosis include respiratory disease, diarrhea, vomiting, ascites, edema, periorbital edema, and hemorrhages.

Babesia gibsoni

Clinical signs of *B gibsoni* infection are similar to *B canis* and include fever, thrombocytopenia, regenerative anemia, splenomegaly, lymphadenopathy, and lethargy [66]. Thrombocytopenia is a prominent feature of small babesial infection and may occur before and after parasitemia [66]. Clinical icterus is uncommon. Mortality was one out of five in dogs experimentally infected with an Oklahoma strain of *B gibsoni* [66]. Two of nine naturally infected dogs in North Carolina died [7]. Six of 15

dogs naturally infected with the Californian strain died or were euthanized because of severe illness [5]. Subclinical *B gibsoni* was identified in over half of 33 pit bulls screened in Alabama [6]. Only 1 of 18 PCR-positive dogs displayed clinical signs [6]. The mean hematocrit was 31%, and the mean platelet count also was decreased at 154,000/μL with an increased mean platelet volume (MPV) [6].

Coinfection

In many cases, babesiosis is complicated with concurrent tickborne diseases or hemoparasitism. The role of *Ehrlichia* may be especially important as a source of immunosuppression. Experimental infection with *Ehrlichia canis* can precipitate clinical babesiosis in *B. canis* carrier dogs [68,69] Five of 15 dogs in Greece with babesiosis had another hemoparasitic infection (*Ehrlichia, Hemobartonella*, or *Leishmania*) [70]. In North Carolina Walker hounds, coinfection was prevalent. *E canis, B canis*, some *Rickettsia*, and possibly *Bartonella* are transmitted by *Rhipicephalus sanguineus* [71]. Coinfection with babesiosis and borreliosis (Lyme disease) also has been reported in people. The diseases share a common *Ixodes* tick vector, and coinfection may explain poor response to single-agent therapy.

Immune suppression may be induced by *B gibsoni* infection [72], resulting in an increased likelihood of concurrent infection and clinical relapse. Dogs with subclinical *B gibsoni* infection demonstrated suppressed lymphocyte blastogenesis. Dogs suffering from clinical relapses also had depressed lymphocyte blastogenesis and decreased production of antiparasite antibody [72].

Diagnosis/clinical pathology

Diagnosis of *B canis* and *B gibsoni* often is made by identifying organisms on blood smears. Organisms may be visualized better with Giemsa or Field stains than with quick stains [5,73]. Blood smears should be made immediately, since storage, even with refrigeration, may make organisms impossible to identify [73]. Diagnosis may be complicated, because clinically affected animals do not always have organisms visible on blood smears, and low levels of parasitemia are common even with patent infections. Examining buffy coat smears or blood smears made from capillary blood may increase parasite detection. In chronic or subclinical infections, organisms may be in such low numbers that it is difficult to find the organisms.

Percoll gradients also have been used in an attempt to improve diagnostic sensitivity [74]. Erythrocytes from Italian dogs with *B canis* were concentrated with discontinuous gradients of Percoll. Upper layers had higher parasitemia, because cells were larger and had lower specific weight [74]. Percoll gradient smears had greater than 134 times the concentration of

parasites than the center of a normal blood smear and greater than 30 times the number of parasites seen at the periphery of a blood smear.

Serologic testing of IFA titers often is used, but this may have limitations in endemic areas, suffer from cross reactivity, and be negative in very young animals or early in infection [7,8,75]. Antibodies usually take 8 to10 days to develop. ELISA and complement fixation tests are also available but are used less commonly. In a large survey of stray dogs and kenneled pit bulls in North Carolina, IFA titers for *B gibsoni*, the Californian isolate, and *B canis* were performed. Microscopic examination and PCR analysis also were performed in some of the dogs. Several dogs that were PCR positive did not have positive IFA titers, and in some cases the PCR result was not in agreement with the highest titer for a given species. The clinical significance of dogs that are seropositive but PCR and blood smear negative is problematic. It is possible that animals are infected persistently with levels of parasitemia that are below the detection of microscopic and PCR analysis [8].

A nested PCR to amplify *Babesia* small subunit ribosomal RNA is extremely sensitive and able to detect a parasitemia of 0.0001% [34]. A seminested PCR test offered through North Carolina State is also extremely sensitive, capable of detecting 50 organisms/mL, and it can differentiate *B gibsoni, B canis vogeli, B c rossi*, and *B c canis* [76]. Seminested PCR testing can detect parasitemia levels that are over 1000 times lower than the approximate 0.001% parasitemia detectable by light microscopy [76,77]. Diagnosis of canine babesiosis should be as specific as possible, since virulence, prognosis, and response to treatment are variable.

Clinical pathology

The severity of anemia is highly variable with babesial infections. Researchers found that 50% of dogs infected with *B canis rossi* were severely anemic (packed cell volume [PCV] less than 10) at presentation; 32% had moderate anemia, and 18% were nonanemic or polycythemic [13]. Disease in the severely anemic group was attributed primarily to immune-mediated hemolytic anemia, and approximately 90% of severely anemic dogs were Coombs positive [13].

Regeneration is usually proportional to the degree of anemia. Autoagglutination can be quite common and may occur in over 20% of dogs [49]. The leukocyte count is also extremely variable with babesiosis and can range from leukopenic to leukamoid [66]. Leukocytosis usually is associated with regenerative immune-mediated anemias.

Thrombocytopenia also is encountered frequently with babesial infections. The mechanism for the decrease in platelets is understood poorly. The MPV generally is increased, suggesting that there is normal bone marrow response to the use, sequestration, or destruction of platelets [6]. Local and systemic DIC, immune-mediated destruction, and sequestration of platelets in the spleen are possible mechanisms. *Babesia* infection triggers the host

coagulation system; fibrinogen degradation products (FDP's) are typically low, and a consumptive coagulopathy occurs at areas of local parasitism [78].

Biochemical evidence of hypoxic hepatic damage occurred in severely anemic *B canis rossi* infected dogs. Hepatic changes also were found in dogs infected with the Californian small piroplasm, but changes were rare with *T annae* [36]. Electrolytes tended to be normal, and leukocyte counts frequently were elevated in dogs with severe anemia [13,36]. Acute renal failure is reported to occur in less than 3% of *B canis* infections. Clinicopathologic data from small *Babesia* infections in Spain indicated that approximately 10% of parasitemic dogs had biochemical evidence of renal failure [36].

Therapy and immunity

Species of *Babesia* vary in their susceptibility to babesiacidal treatments. In general, small *Babesia* are considered more resistant to treatment. The development of a chronic carrier state is suspected for small *Babesia*, while most large *Babesia* infections are considered cleared with treatment. Some drug resistance also is seen with pathogenic South African strains. Several drugs have been used to treat babesiosis including: diminazene aceturate (Berenil), imidocarb dipropionate (Forray [65]; Imizol), trypan blue, phenamidine isethionate (Oxopirvedine) [68], and quinuronium sulfate [70]. Diminazene is not available in the United States, but it is probably the most commonly used drug worldwide. Diminazene is a diamidine derivative closely related to phenamidine isethionate and pentamidine isethionate(Lomidine) [23]. The drug is thought to interfere with aerobic glycolysis and inhibit DNA synthesis in the parasite [79]. A survey of practitioners in South Africa [68] indicated that drug resistance and relapses were suspected with diminazene treatment. Diminazene also may cause pain at the injection site, vomiting, neurologic signs, hypotension and parasympathomimetic effects [63]. Diminazene toxicity can cause depression, stupor, vocalization, ataxia, increased extensor tone, nystagmus, seizures, and possible death [63]. One dose of diminazene (3.5 mg/kg intramuscularly [IM]) is recommended. *B gibsoni* does not respond as well as *B canis* to treatment with diminazene.

Phenamidine isethionate is available in many countries for the treatment of canine *Babesia*. Pentamidine isethionate, a closely related drug, is approved in the United States as an orphan drug for the treatment of *Pneumocystis* pneumonia in people. The drug has shown efficacy against *B canis* and *B gibsoni* [80] but does not totally eliminate small babesial infections [38].

Imidocarb, also a diamidine, is the only drug approved for the treatment of *Babesia* in the United States. Imidocarb has direct effects on the nucleus and cytoplasm of the parasite. For treatment of *B canis* a single

dose of 6 mg/kg IM or subcutaneously may be effective for treating acute disease and eliminating the carrier state. *B gibsoni* infections typically are treated with a second injection repeated in 2 weeks. Possible adverse effects include pain at the injection site and parasympathomimetic signs. Salivation, depression, lacrimation, muscle tremor, restlessness, tachycardia, dyspnea and diarrhea may occur within 10 minutes of receiving the injection [63]. Shivering, periorbital edema, and fever have been reported 10 to 12 hours after injection [63]. Premedication with atropine often is given to reduce these adverse effects. Imidocarb toxicity can cause severe hepatic and renal damage, tachycardia, arrhythmias, lacrimation, diarrhea, and tremors [63].

Trypan blue is used frequently to treat dogs with severe shock caused by *B canis rossi* infection [68]. It may be able to block the entrance of the parasite into the RBC. Infections usually are not eliminated with trypan blue treatment, but parasitemia and clinical signs are reduced. Parasitemia often returns within 2 weeks. Adverse effects are minimal, and trypan blue has a much broader therapeutic index than other babesiacidal drugs. Trypan blue can be given as a 1% solution at a dose of 10 mg/kg intravenously (IV). Tissue irritation and bluish discoloration of the mucosa and urine may occur.

Ten of 15 dogs in Greece with *B canis* were treated with a 0.5% solution of quinuronium sulfate (two injections given subcutaneously 48 hours apart at a dose of 0.05 mL/kg) Clinical signs resolved within 48 hours of the first treatment [70]. The PCV may drop initially with treatment. This drop in hematocrit also is seen with treatment for malaria and may be related to parasite death or a lag phase before RBC destruction stops. More persistent declines in PCV after babesiacidal treatment may be caused by the development of immune-mediated destruction. Short-term therapy with prednisone may be indicated in some cases.

Chemotherapeutics are reportedly unable to completely eliminate *B gibsoni* infection. *Theileria* infections in cattle often are treated with hydroxynaphthoquinone derivatives like atovaquone (Mepron). Based on phylogenetic relationships, this also may be useful in Californian *B gibsoni*, human *B microti* infections, and other small *Babesia* species. Pravaquone has been suggested as a treatment for *B gibsoni* [15]. In hamster models, atovaquone used alone caused drug resistance but was effective when combined with azithromycin. Azithromycin/quinine, azithromycin/atovaquone and azithromycin/clindamycin/doxycycline were effective in human *B microti* infections that failed to respond to standard clindamycin/quinine therapy [81,82]. Preliminary studies using atovaquone and azithromycin in canine *B gibsoni* infections appear promising, and several treated dogs became PCR negative approximately 2 months after treatment [83].

Iron chelators are of potential benefit with cerebral babesiosis. Therapy with desferroxamine (DFO), an iron chelator, has proven beneficial in children with cerebral malaria [84]. Parasite clearance and recovery from

coma were faster in pediatric patients treated with an infusion of DFO than controls. Benefits are attributed to radical scavenging properties, possible enhancement of the TH1 immune response, and to reduced iron availability for parasites [84]. Oral iron chelators eventually may play a role in therapy of drug-resistant or relapsing strains of malaria. These agents have not been examined in dogs.

Other less frequently used drugs include amicarbalide, euflavine, and chloroquine. These drugs are less effective and have more severe adverse effects [63].

Immunity

Young dogs are more susceptible to babesiosis and frequently have more severe infections. [70]. At OVAH, 77% of dogs affected with *B canis* were less than 3 years old [85]. This is different from the situation with bovine babesiosis, where calves are more resistant than older animals [21]. A report of 70 naturally occurring cases of babesiosis in Nigeria found that greater than 70% were less than 1 year old [86]. Acute and hyperacute forms were seen in dogs as young as 4 weeks old [86]. In suckling pups, *B canis vogeli* is capable of causing severe disease that is manifested by anemia, marked thrombocytopenia, jaundice, renal failure, and death [10]. Identical strains subinoculated into splenectomized and nonsplenectomized adult dogs did not cause obvious disease. Breed predispositions are not defined strictly, but anecdotal information revealed that in South Africa, rottweilers, German shepherds, pit bulls, and border collies were over-represented [68]. Pit bulls also were over-represented among dogs that died of nonanemic *B canis rossi* infection [13]. The apparent susceptibility of pit bulls to large and small babesiosis is not understood. Genetic factors, environmental conditions, and vertical transmission likely are involved.

The development of immunity to babesial infection is characterized poorly and often questioned. Veterinarians in South Africa report that dogs treated with sterilizing drugs often relapse in the same season. Imidocarb and diminazene often are thought to clear *B canis* infection totally and may prevent the dog from developing protective immunity. Premunition is thought to be important in South Africa, where babesiosis is extremely virulent and widespread. Experimental studies have demonstrated that heterologous strains did not provide cross-protection. Antibody levels are thought to decline between 3 and 5 months after infection. In France, a first-generation vaccine has been available since 1984 [87]. The vaccine (Pirodog) is made from *B canis* cell culture supernatants with a saponin adjuvant. Efficacy is around 89% for *B c canis*. The vaccine is not cross-protective for the South African or other strains [87]. Vaccination reduces parasitemia but does not eliminate infection. The lack of cross-protection is probably partially responsible for vaccine failures in the field.

Human babesiosis

The first case of human babesiosis was described in 1968. Since then, hundreds of cases have been reported. Cases primarily occur along the East Coast and in the Great Lakes area. Virtually all of the zoonotic cases in the United States are caused by the rodent parasite *B microti*. In Europe, the bovine pathogen *B divergens* is responsible for zoonotic infections primarily in asplenic individuals with exposure to cattle [81]. Mortality rates for *B divergens* infections were approximately 38%, whereas mortality rates were only 5% for *B microti* infections in the United States [81]. *B microti* infections are typically treated with a combination of clindamycin and quinine [38]. In severe cases, erythrocyte exchange transfusion may be life-saving [81]. *B divergens* infections are more serious and are treated with IV clindamycin and oral quinine. In vitro studies showed efficacy with imidocarb and a combination of cotrimoxazole and pentamidine. Imidocarb is not approved for use in people but has been used successfully in two Irish patients [81].

A new species of small *Babesia* has been recognized on the West Coast [14]. In 1991, the pathogen, WA1, was isolated from a 41-year-old immunocompetent man in Washington with acute malarial-like symptoms. WA1 caused more serious and prolonged illness than previously reported human cases of *B microti*. Neighbors of the WA1 patient also showed serologic evidence of exposure. Since this discovery, seroreactivity has been found in many northern Californians. A similar situation exists with *B microti*, which often is accompanied by up to 17 silent infections for every positive case found [88]. Each year, more cases of WA1 are being diagnosed in Washington [14]. Two new strains, CA1 and MO1, also have been reported in primarily in asplenic individuals in California [14] and Missouri [89], respectively. Phylogenetic analysis revealed that the Californian small *Babesia* is the species most closely related to WA1.

Prevention

The best method of prevention in endemic areas is aggressive control of the tick vector. An effective topical acaricide combined with a flea/tick collar is usually very efficacious in preventing tick exposure. Owners should inspect their dogs daily for ticks. Prompt removal of ticks within 24 hours should prevent disease transmission, because the tick must be attached for 2 to 3 days to transmit the organism [40]. In kennels where puppies are being lost to disease, aggressive tick control measures should be instituted, including spraying the environment and treating animals. Because babesiosis can be transmitted vertically from dam to offspring, serologic testing should be performed to remove infected dogs from the breeding pool.

To avoid transmission through blood contamination, poor kennel practices such as sharing needles for vaccination or reusing surgical instruments for tail docking and ear cropping should be avoided. Dogs

with positive *Babesia* titers should never be used as blood donors. More stringent regulations concerning serologic testing, quarantine, and treatment of dogs entering the United States from endemic areas may prevent continued spread of the disease into this country. Dog fighting should be avoided, as it is also a potential method for spreading disease.

Treatment of asymptomatic dogs with subclinical infection is controversial, and it is not known how effective treatment is in eliminating the carrier state. Treatment with imidocarb followed by a negative PCR test in 2 months may help eliminate the reservoir of disease, however. Development of new vaccines, such as the French vaccination for *B canis*, is needed, because there is no cross-protection for the different *Babesia* organisms. As evidence mounts concerning the spread of this emerging tickborne disease to new areas, continued research is needed to determine better methods of prevention and treatment.

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