Babesia in North America: An Update

Jonathan D Dear, DVM, MAS, DACVIM (SAIM)[1] and Adam Birkenheuer, DVM, PhD, DACVIM (SAIM)[2]
1. jddear@ucdavis.edu (corresponding author) – no financial or conflicts of interest to disclose
Department of Medicine and Epidemiology
School of Veterinary Medicine
University of California, Davis
One Shields Avenue
Davis, CA 95616

2. ajbirken@ncsu.edu – Co-Director of the Vector Borne Disease Diagnostic Laboratory at North Carolina State University (no financial compensation) and consulting and continuing education provided for Idexx Laboratories Inc, Boehringer-Ingelheim and Merck.
Department of Clinical Sciences
College of Veterinary Medicine
North Carolina State University
1060 William Moore Drive
Raleigh, NC 27607

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Key points:

- Canine babesiosis is caused by five unique species of *Babesia* in North America.
- Clinical signs can vary from subclinical or mild to severe and life-threatening
- Fever, lymph node enlargement and splenomegaly are the most common physical examination findings. Thrombocytopenia and anemia are the most common clinicopathologic findings.
- While tick vectors are known or suspected for several species other routes of transmission include transplacental, blood transfusion and direct transmission via dog bites.

Synopsis

Canine babesiosis results from infection of one of five identified protozoal species in the United States (*Babesia conradae, Babesia sp. "coco", Babesia gibsoni, Babesia vogeli* and *Babesia vulpes*). They are part of the Apicomplexa family of protozoa and are obligate intra-erythrocytic parasites. Domestic and wild canids are suspected of being intermediate hosts. This updated chapter aims to provide practical guidance about the clinical manifestations of disease, treatment options and outcomes. Additionally, we hope to provide some clarity about the taxonomy and nomenclature of these organisms as they have undergone multiple changes since their initial

discovery. The scope of this review includes *Babesia* species encountered in North America. The reader is encouraged to seek out other resources for information about *Babesia canis, Babesia rossi* and feline babesiosis.

Introduction

Demystifying Canine *Babesia*

Since their discovery, members of the genus *Babesia* have been referred to as bacteria (*Micrococcus*), parasitic fungi (*Coniothecium stilesiarum*) and various names referring to protozoa (*Piroplasma canis*, *Pyrosoma bigeminum* var *canis* etc). Formal changes in eukaryotic nomenclature should be proposed through the International Commission on Zoological Nomenclature (ICZN). Unfortunately, the majority of proposed name changes for canine *Babesia* species have not followed ICZN guidelines or submitted to the organization.The resulting shifting nomenclature of these organisms reflects the challenges clinician scientists have had, and continue to have, categorizing and understanding the complex biology of this genus.

The first scientific mention of the organisms was in 1888 by Victor Babes who described the cytologic appearance of a parasite in the peripheral blood of cattle as “round and bright,…….about 0.5 µm in the middle it is divided by a light line in two parts, others in 4 by a second transverse line.”[1] Initially it wasn’t clear whether the organisms he was describing caused disease. In 1893 the physician and veterinarian duo, Drs. Theobald Smith and Frederick Kilbourne, identified *Pyrosoma bigeminum* as the cause of Texas fever and, in doing so, postulated that it was a tick vectored disease (the first ever identified).[2,3] We now know that *Babesia bigeminum* and *Babesia bovis* are the organisms that that cause this disease of cattle.

Around the turn of the 20th century *Piroplasma canis* and *Pyrosoma bigeminum* var *canis* were described as a cause of anemia in dogs in Europe, Asia and Africa. However, the genus *Babesia* wasn’t used to describe disease in dogs until 1918. Research occurring into the 1930s highlighted differences in vector ecology of canine *Babesia* despite them having similar cytopathologic appearance. During this period canine *Babesia* tended to be identified cytologically by their relative size, with large (2.5-5 µm) *Babesia* being considered *B. canis* and small organisms (1-2.5 µm) being named *B. gibsoni.* Differences in clinical disease manifestations and geographic distributions also informed speciation efforts.

Work in the late 1980’s by Uilenberg et al. and Zahler et al. defined characteristics of three ‘large’ *Babesia* and suggested that there were three subspecies, *B. canis subsp canis, rossi* and *vogeli.*[4,5]

Meanwhile, things were just starting to get confusing in the world of small *Babesia*. *Babesia gibsoni* was initially described in India in 1910 and historically, until molecular identification nearly 100 years later all small

babesia were called *B gibsoni*.[6] In 1991 Conrad et al. documented what was suspected to be *B. gibsoni* in dogs in southern California.[7] Then in 1999, Birkenheuer et al. described a series of dogs in North Carolina that were infected with *B. gibsoni*. Both of these reports were beyond the suspected geographic range of that protozoa.

As time marched on and molecular techniques facilitated phylogenetics, two major shifts occurred: the large *Babesia* subspecies in the *B. canis* group are now considered to represent 3 distinct species; *B. canis, B. rossi* and *B. vogeli*. Additionally, DNA sequencing determined that the *B. gibsoni* strains from North America and Asia were genetically divergent. In addition, the North American strains are comprised of 3 distinct species of small piroplasma, *B. conradae*, *B. gibsoni* and *B. vulpes*.[8,9] *B. conradae* was previously referred to as *B. gibsoni* and the 'California isolate' and *B. vulpes*[10] was referred to as *B. microti*-like, the 'Spanish isolate' and *Theileria annae* .[11,12]

Babesia Basics

Though we continue to learn about the ecologic and molecular differences that unite and separate *Babesia* species, there are certain key characteristics that likely apply to all species. *Babesia* undergo an asexual reproductive cycle in the dog, which serves as an intermediate host. Sexual reproduction occurs in the definitive host, which is suspected to be ticks for most *Babesia* species. Using the strictest criteria for genus designation, *Babesia* are considered to be obligate erythrocyte parasites, and species capable of infecting monocytes in addition to red blood cells are typically given an alternate genus designation, such as *Theileria* or *Cytauxzoon*. Thus, because it may infect both mononuclear cells and red blood cells, *B. vulpes* may, at some point in the future, no longer be considered to be a babesial organism.

When *Babesia* organisms are transmitted to a dog, they travel through connective tissue to reach capillary beds where they are able to infect red blood cells and undergo the aforementioned asexual reproduction. During this time *Babesia* can split via binary fission appearing in pairs, or as intraerythrocytic inclusions with varied morphology. (**Figure 1**).

Following infection, there is a spectrum of disease that develops in the dog that varies depending on theinfecting species, the immune response and the inoculated parasite burden. Clinical signs can range from subclinical to mild to to severe. In dogs with less severe infection, splenomegaly, lymphadenomegaly, lethargy and anorexia may be the only clinical signs detected. Severe disease manifestations can include systemic inflammatory disease, shock and death.

Several species of *Babesia* appear to be geographically constrained, which is likely related to the distribution of the corresponding resivoir hosts and tick

vectors (**Figure 3**). As with all infectious diseases, it is important to consider travel history in patients with clinical and laboaratory findings consistent with babesiosis in both endemic and non-endemic areas.[13]

Table 1. Species name, former names, references in which new names were proposed, and known or suspected tick vectors for *Babesia* species that infect dogs in North America.

	FORMER NOMENCLATURE	PRIOR REFERENCES	TICK VECTOR
BABESIA SP. 'COCO'	Unnamed Large *Babesia*	Birkenheuer 2004, Sikorski 2010	*Amblyomma Americanum* (suspected)
BABESIA CONRADAE	'California isolate' 'California genotype'		Unknown
	B. gibsoni	Conrad 1991, Wozniak 1997, Yamane 1993, Zahler 2000, Macintire 2002 Birkenheuer 2003, 2005	
BABESIA GIBSONI	'Asian genotype'	Birkenheuer 2003, 2004, 2005	*Haemaphysalis longicornis** *Haemaphysalis bisponosa* *Haemaphysalis hystricus* *(jongejan 2018)*
BABESIA VOGELI	*B. canis*	Zahler 1998, Freeman 1994	*Rhipicephalus sanguineus*
	B. canis vogeli	Solano-Gallego 2008, Carli 2009, Uilenberg 1989	
BABESIA VULPES	*B. microti*-like	Birkenheuer 2010, Garrett 2022	Unknown in US
	'Spanish isolate'	Garcia 2006, Yeagley 2009	
	Theileria annae	Baneth 2015, Dixit 2010, Camacho 2003	

*No documented cases of tick transmission in North America

Transmission

Tranmission can occur through tick bite, dog fight, blood transfusion or transplacentally.

Tick Transmission

Ticks are likely the definitive hosts for all *Babesia* species. Competent tick vectors have been identified for many (**Table 1**). In Asia, *Haemaphysalis longicornis* (the longhorn tick), *Haemaphysalis bisponosa* and *Haemaphysalis hystricus* have been implicated as the major vectors for *B. gibsoni*. [14,15] Historically, these ticks have not been found in North America. However, *H. longicornis* was recently documentedin New Jersey and subsequently reported in 9 states between 2017-2018. Other means of transmission are more important in the United States as detailed below. .[16,17] *Rhipicephalus sanguineus* (the brown dog tick) is thought to be the major vector of *B. vogeli* which accounts for its worldwide distribution, including North America.

The tick vectors and reservoir hosts for *B. 'coco'*, *B. conradae*, and *B. vulpes* have not been definitively determined. However, *Amblyomma americanum* is suspected to be the likely tick vector for *B. coco* based on the geographical congruence of vector and diseasedistribution .[18] Early studies evaluated *Rh. sanguineous* and *Dermacentor* spp. ticks as potential vectors for *B. conradae*, but transmission did not occur in these experimental settings.[14] A recent survey of ticks in the United States detected *B. conradae* DNA in 2 *Dermacentor albipictus* ticks found on cats, but evidence for competence of transmission cannot be assumed.[19] Using PCR, a study of California coyotes found the overall prevalence of *B. conradae* to be 4.3%. Whether coyotes serve as reservoir or incidental hosts for this organism is not known.[20] In Spain, *Ixodes hexagonus* is thought to be the primary vector of *B. vulpes.* This tick has not been documented in North America. Other *Ixodes* spp. may be involved in the transmission of this species of *Babesia*.[21]

Transfusion

Though there are only a few published reports of blood transfusion leading to transmission of *Babesia* species to naïve recipients in the literature,[22,23] experimental infections are induced by injection of infected blood.[24,25] Consequently, it is seems reasonable that routine screening of donors should include PCR testing for *Babesia*.[26] Moreover, blood bank personnel should be aware that some PCR assays designed to detect *B. gibsoni* and *B. vogeli* do not detect *B.' coco', B. conradae*, and *B. vulpes*. Thus familiarity with the sensitivity and specificity of PCR assays for all relevant *Babesia* species in North America is important in developing blood donor screening protocols.

Transmission via biting

Many *Babesia* species are suspected of being transmitted during dog fights. For instance, *B. gibsoni* lacks a competent tick vector in most of North America. It was likely introduced by imported dogs from Asia used for dogfighting. Several studies continue to document a greater prevalence in American pit bull terriers and related breeds (hereafter referred to as American pit bull terriers – APBT) and dogs rescued from dog fighting operations in both North America and Asia.[27-30] Likewise, studies have documented *B. conradae* infections in dogs used for coyote hunting. A history of aggressive interactions with coyotes was found to be a risk factor associated with infection in one study.[31,32] *Babesia vulpes* has also been linked to a history of dog fighting.[33]

Transplacental transmission

Transplacental transmission has been definitively documented or suspected for most canine *Babesia*. Though direct infection via a shared environmental source (i.e., a tick vector or other mechanism of infection) cannot be definitely excluded, 7 of 12 dogs infected with *B. conradae* in one case series of naturally infected dogs descended from a single bitch.[34] In another study, experimental infection during pregnancy led to transmission *in utero* of the entire litter; 1 puppy was stillborn and the remaining 4 puppies died shortly after birth.[35]

Diagnosis, Treatment and Prevention

General Diagnostic Principles:

In general, there are 3 methods to diagnose *Babesia* infections in dogs: blood smear cytology, serology and PCR. Each method has its own strengths and limitations. Cytology is inexpensive and can be performed within the clinic, however identification of intraerythrocytic piroplasma on a blood smear can be hampered by very low levels of parasites circulating in blood and inadequate staining. Wright stain is superior to rapid in-clinic staining but is often not available in small animal practices. PCR is sensitive, however like cytology, a negative test can occur in actively infected patients due to low numbers of circulating organisms. In addition, some PCR assays that target certain species do not detect all species of *Babesia*. Serologic testing is available for several of the more commonly diagnosed *Babesia* species and can be less expensive that PCR testing. Unfortunately, the lag in antibody response makes serology less helpful for diagnosing acute disease. Additionally, the degree of serologic cross-reactivity between betweenspecies of *Babesia* is not clear. Clinicians should contact individual laboratories to determine the sensitivity and specificity of the assays used. Generally speaking, when possible, combining PCR with serology can increase overall cinical sensitivity.

General Treatment Principles

A number of anti-protozoal medications have been used to treat dogs with babesiosis. Details of treatment trials are discussed in the context of the individual species below, but there are some common strategies for treatment that are discussed here. *Babesia vogeli* seems to be the parasite most responsive to treatment and is generally cleared after 2 doses of imidocarb (**Table 2**). Pretreatment with anticholinergics helps to prevent side effects. Resolution of clinical signs occurs in response to treatment with the combination therapy of atovaquone and azithromycin for infection with the small *Babesia* (*B. conradae, B. gibsoni* and *B. vulpes* - **Table 2**), but, there is a concern that infection with *B. gibsoni* and *B. vulpes* might persist despite clinical improvement. Persistence of *B. conradae* following treatment appears to occur less frequently. Not enough is known about *B. 'coco'* to make evidence based recommendations for its treatment.

Following treatment, dogs should be retested by PCR at 60 and 90 days to help verify remission. This is recommended even in the absence of an initial negative PCR since dogs can remain seropositive for months to years following successful treatment. Dogs respond clinically within a few days and become PCR-negative very quickly with treatment (e.g., as soon as 5 day) but disease can relapse after treatments. Clinicopathologic abnormalities begin to resolve within days to weeks but some, including hyperglobulinemia and proteinuria, might take several months to resolve. If a dog fails to clinically respond within 7-10 days, testing for coinfection or concurrent disease is recommended.

Atovaquone is a hydroxynapthoquinone antiprotozoal medication that inhibits electron transport in parasite mitochondria. Two commercial formulations exist; Mepron® (GlaxoSmithKline, Brentford, UK) and Malarone® (GlaxoSmithKline, Brentford, UK). Both formulations should be administered with a fatty meal to promote absorption. In the past Mepron® has been recommended because Malarone® contains proguanil and may result in more frequent adverse effects including vomiting and anorexia.This has raised a concern that subtherapeutic drug levels secondary to vomiting. The resulting lack of absorption might facilitate the development of cytochrome B mutations that convey resistance to atovaquone.[36] In spite of this concern, a recent case series evaluating the combination of Malarone® azithromycin and artusenate, showed promising results for the treatment of *B. gibsoni.* Gastrointestinal side effects such as vomiting and diarrhea were not reported to occur following treatment in this trial. Interestingly, artusenate is a derivative of artemisinin, which is a compound extracted from a Chinese herb with antiprotozoal properties.

Treating splenectomized dogs infected with *Babesia* poses a particular challenge as the spleen plays a central role in disease premunition. For dogs that develop clinical disease after splenectomy, the 'kitchen sink' approach,

along with the use of alternating therapies can minimize clinical disease. In splenectomized dogs that have failed to clear the infection with atovaquone and azithromycin, one author (AB) has had success using a combination of atovaquone and azithromycin, imidocarb, artemisin, clindamycin, doxycycline and metronidazole. Anti-emetics are typically used pre-emptively in these cases. For dogs that have not been splenectomized, a 90-day course of clindamycin, doxycycline and metronidazole with or without artemisin has led to clinical improvement in dogs infected with *B. gibsoni* that fail to respond to atovaquone and azithromycin.

Table 2. Medications reported for use in the treatment of canine babesiosis in North America. Drug efficacy varies with infecting species and drugs are used in specific combinations. See text for details.

MEDICATION	DOSE	ROUTE	FREQUENCY	DURATION
ARTUSENATE[A]	12.5 mg/kg	PO	Q 24h	10 days
ATOVAQUONE[A]	13.3 mg/kg	PO	Q 8h	10 days
AZITHROMYCIN[A]	10 mg/kg	PO	Q 24 h	10 days
CLINDAMYCIN[B]	25 mg/kg	PO	Q 12 h	90 days
DOXYCYCLINE[B]	5 mg/kg	PO	Q 12 h	90 days
DIMINAZENE ACETURATE	3.5 mg/kg	IM	Once	N/A
IMIDOCARB DIPROPRIONATE	6.6 mg/kg	IM or SQ	Twice	14 days apart
METRONIDAZOLE[B]	10 mg/kg	PO	Q 12 h	90 days

A. Atovaquone and azithromycin are always used in combination for treatment of *B. conradae*, *B. gibsoni, B. vulpes* and, potentially, *Babesia spp* 'Coco'. Artusenate was added to this comination in one study and appeared well tolerated. B. Clindamycin, doxycycline and metronidazole have been used in combination for treatment of dogs with *B. gibsoni* that don't respond to atovaquone and azithromycin.

General Principles for Prevention

As transmission can occur via tick bite, dog bite, blood transfusion or transplacentally, there is not a 'one-size-fits-all' approach to disease prevention. It seems reasonable to support the use of acaricidal medications for the prevention of *B. vogeli* and *B. coco,* as tick transmission is probably the primary method of transmission of these infections. However, prevention of infection with *B. conradae, B. gibsoni* and *B. vulpes* may not be

accomplished using this same strategy as infection largely occurs via dog bites or transplacentally and more rarely via blood transfusions. Given this reality, intact or pregnant APBT should be screened for *B. gibsoni* and *B. vulpes* routinely, as should all blood donor dogs in North America.

Species specific information

Babesia vogeli

Babesia vogeli has been described by some as being the 'cosmopolitan' *Babesia* given its worldwide distribution.[37] In North America, infections in domestic dogs tend to correlate with heaviest burdens of its tick vector, *Rh. Sanguineus* (**Figure 3)**. As one of the least virulent of the large *Babesia* species, most immunocompetent dogs infected with *B. vogeli* seem to have subclinical infections.[38] However, young dogs or dogs that are immunocompromised (e.g., dogs that are splenectomized, coinfected with *E. canis*, or receiving chemotherapy or other immunosuppressive medications) tend to develop clinical disease and are at greater risk of death from infection.[25,39-41]

Infected dogs tend to present with nonspecific clinical signs such as anorexia and lethargy. Fever is the most common physical examination finding.[25,40] When affected, moderate to marked thrombocytopenia is the most common clinicopathologic distubance.[25] Immune-mediated hemolysis appears to be common in dogs with *B. vogeli* as anti-erythrocyte IgG has been detected in many infected dogs, though immunocompetent dogs might not develop anemia.[42]

In a small case series of 11 dogs infected with *B. vogeli*, 3 died or were euthanized, presumably from consequences related to their primary disease. In this report there was a single dog with chronic kidney disease along with severe and diffuse membranoproliferative glomerulonephritis likely resulting from babesiosis given the dogs's young age (7 months).[40] Given this finding, it seems likely that chronic *B. vogeli* infection might result in protein-losing nephropathy similar to that seen in dogs with other *Babesia* infections.

Definitive diagnosis of *B. vogeli* can be achieved by a PCR assay using whole blood. PCR assays that detect *B. gibsoni* are widely available at commercial laboratories and academic centers. Though cytologic identification of erythrocyte piroplasms is specific for babesiosis, like with other babesial parasites it is impossible to speciate the organism on morphology alone. Furthermore, the sensitivity of microscopy is limited by the typically low parasitemia (often < 1% of erythrocytes) even in severely affected dogs (**Figure 2**).[39] Serologic testing (IFA) is available and can be used in the diagnosis of *B. vogeli*, though PCR is more specific. Combining PCR with

serologic testing, using acute and convalescent serologic testing and repeat testing using PCR may increase sensitivity.

Babesia vogeli can be treated with imidocarb with a favorable prognosis. It is believed that the parasite is eliminated after two doses administered 14 days apart.[39,40] That said, controlled clinical trials using PCR for follow-up testing are have not been performed. Given that they share the same tick vector, dogs with *B. vogeli* infections should also be screened for *Ehrlichia canis* as concurrent infections can result in more severe clinical disease.

Babesia gibsoni

Babesia gibsoni is the most common *Babesia* species infecting North American dogs. In one study the organism accounted for 79% of *Babesia* positive samples submitted to a commercial laboratory.[43] *Babesia gibsoni* has been diagnosed across North America (**Figure 3**). The majority of infected dogs are APBT. American Pit Bull Terrier Type dogs accounted for approximately 75% of the positive cases in samples submitted to one university laboratory.[36]

Although the majority of dogs diagnosed with *B. giboni* are APBT, approximately 25% of positive dogs belong to other breeds. In infected dogs from breeds other than APBT, there has been an association between infection and history of dog bite, particularly bites from APBT.[28,44] Given that most dogs in North America infected with *B. gibsoni* have a history of a dog bite, coinfections with haemotropic *Mycoplasma* spp. and *B. vulpes*, are relatively common.
Although not often described, transfusion associated infections do occur and in the authors' opinion are probably more common than reported.[22]

Most dogs that are infected with *B. gibsoni* have mild to moderate disease. The most common clinical and laboratory findings are pale mucous membranes, splenomegaly, thrombocytopenia and hemolytic regenerative anemia. Splenomegaly can be occasionally detected on abdominal palpation or diagnostic imaging. The splenomegaly can be generalized or associated with benign splenic masses.[45] Therefore *B. gibsoni* infection should be ruled out and/or treated in all APBT dogs prior to any non-emergent splenectomy.

Thrombocytopenia can be mild to severe. As is observed canine infection with other *Babesia* species found in North America, petechiation and ecchymosis rarely or never occur, even when thrombocytopenia is severe.[46] The anemia can also vary from mild to severe and tends to be regenerative, though nonregenerative anemia can occur early in acute disease. Hyperbilirubinemia seems to occur more frequently with *B. gibsoni* infections that other species.[46] Hemolysis can occur because of oxidative damage or anti-erythrocyte antibody targeting.[47] Consequently, testing for *B. gibsoni* should be considered for all dogs suspected of having immune-mediated

hemolytic anemia prior to immunosuppressive therapy, especially in at risk breeds.[48]

Hyperglobulinemia and protein-losing nephropathy have also been reported as a consequence of *B. gibsoni* infection. Proteinuria does not develop frequently and may resolve with treatment of the infection.[45]

Identifying characteristic intraerythrocytic organisms with cytologic examination of blood smears can confirm the clinical suspicion of babesiosis in a typical breed with compatible clinical signs. However cytology is insensitive and cannot allow differentiation among species of small *Babesia* as they appear morphologically identical (**Figure 2**). Likewise, seroreactivity supports a diagnosis but is not definitive due to serologic cross-reactivity with other species and that without documenting seroconversion, seroreactivity indicates exposure but not necessarily active infection. PCR is the only way to definitely identify the species and confirm active infection. PCR assays that target *B. gibsoni* are available through most veterinary reference laboratories. Combining PCR with serology and repeat testing of the same or additional samples using PCR increases diagnostic sensitivity.[49]

Combination therapy with atovaquone and azithromycin has been considered the optimal treatment protocol to induce clinical remission. However, as with all *Babesia* sp. infections splenectomy post-treatment followed by sub-inoculation of blood into naïve splenectomized dogs would be required to determine if infection is truly cleared (**Table 2**).[50] Combination therapy with atovaquone (Malarone®), azithromycin and artusenate seems to result in remission based on long-term PCR monitoring similar to atovaquone (Mepron®) and azithromycin alone.[51] Controlled studies directly comparing Mepron® and azithromcyin to Malarone®, azithromycin and artusenate are indicated.

In dogs with infections resistant to atovaquone, long-term therapy using a combination of clindamycin, doxycycline and metronidazole improves clinical health and, in some dogs may result in clearance of infection based on long term PCR monitoring.[52] Unfortunately, no controlled trials have been performed and evidence based therapeutic protocols using these drugs are not available. Treatment with imidocarb and diminazene aceturate can reduce clinical signs and laboratory abnormalities but does not clear the parasite.[50,53] Lumefantrine has been studied in vitro for its potential synergism with artemisinin-related compounds, but there are no published reports documenting its efficacy and limited clinical observations suggest that it, too, cannot clear infection.[54]

Babesia vulpes

As mentioned at the beginning of this chapter, *B. vulpes* has been referred to by many names (*B. microti*-like, *Theileria annae, Babesia* Spanish dog isolate

and *B. gibsoni* in some early studies) and it will likely be renamed again. *Babesia microti,* the type species for the clade of organisms to which *B. vulpe*s belongs, has a lifecycle that involves both an erythrocytic and a monocytic phase in vertebrate hosts, differentiating it from 'true' *Babesia*. *Babesia vulpes* has been detected in dogs in Europe and the Eastern United States (**Figure 3**) and was the third most frequently identified canine *Babesia* in one North American veterinary diagnostic laboratory.[43] In Spain *Ixodes hexagonus* is suspected of being its main vector, though its vector in North America has not been determined. Transmission has been reported to occur frequently in dogs used in fighting and APBT accounted for 92% of PCR-positive dogs in one study.[33] Foxes are likely the reservoir host of this parasite. Both red and grey foxes are infected in North America with a prevalence between 25-40%.[55] Other wildlife such as the American river otter can be infected and might also play a role in the epidemiology of this disease.[56]

Clinical disease in dogs infected with *B. vulpes* resembles infection with other small species of *Babesia* with splenomegaly, asplenia from previous splenectomy, or bite wounds reported in many dogs. Clinicopathologic evaluation often reveals moderate to marked anemia (typically regenerative), mild to marked thrombocytopenia and hyperglobulinemia.[33] Coinfection with *B. gibsoni* is common. Azotemia and proteinuria have been reported in dogs infected with *B. vulpes*, but whether infection caused the renal abnormalities is not fully known.[33]

As is the case for other small *Babesia*, microscopic evaluation of a blood smear can facilitate diagnosis, however, cytologic examination cannot differentiate between related species (**Figure 2**). There is no serologic assay designed to detect *B. vulpes* specific antibodies. The amount of serologic cross-reactivity that occurs when using assays designed to detect antibodies that target other *Babesia* species is has not been thoroughly studied but some cross-reactivity has been documented.[33] PCR is the only way to definitively diagnose infection, however it is important to note that not all *Babesia* spp. PCR assays will detect this species and identify it as *B. vulpes*.

Combination therapy with atovaquone and azithromycin has been used to treat dogs with *B. vulpes* infections, but no long-term, controlled trials have been performed to explore their efficacy.[33] There are no published reports of alternative therapies for *B. vulpes* infections. Coinfections with *B. gibsoni* and haemotropic *Mycoplasma* are common.[33,57]

Babesia conradae

As one of the small parasites originally identified as *B. gibsoni*, much of the early work documenting the epidemiology and pathophysiology of *B. conradae* is published under it's former species designation. Most of these

sentinel studies originated from Dr. Patricia Conrad's laboratory at University of California Davis, for whom this species was subsequently named.[7,14,24]

Most dogs diagnosed with *B. conradae* are from the Central Valley or Southern part of California.[32,34,58] There is one of coyote hunting dogs in Oklahoma that have been infected with this species.[31] It's unclear whether these dogs were infected in Oklahoma or whether they were imported from or traveled to California as a result of interstate trading.

Infected dogs tend to develop acute disease which typically presents as lethargy and anorexia. On physical examination dogs often have pale mucous membranes, an elevated body temperature, splenomegaly, and, in the case of coyote hunting dogs, evidence of wounds.[59] Complete blood count tends to reveal mild to marked regenerative hemolytic anemia and moderate thrombocytopenia along with leukopenia characterized by neutropenia.[7,32] Infected dogs also tend to have low serum albumin and high serum globulin concentrations. Although the magnitude of thrombocytopenia is typically not low enough to cause spontaneous bleeding, some dogs with untreated *B. conradae* infections have bleeding diatheses. Because the magnitude of thrombocytopenia is typically not low enough to cause spontaneous hemorrhage, additional mechanisms may contribute to disordered hemostasis (**Figure 4**).

Diagnosing infection is best accomplished using species specific PCR. Some assays targeting the Genus *Babesia* will not detect this organism.[32] Blood smears can reveal intraerythrocytic parasites, but as for other *Babesia* species, the sensitivity of microscopy is usually hampered by low levels of parasitemia. Typically less than 2-3% of erythrocytes are infected, even with severe clinical disease (**Figure 2**). As with other *Babesia* infections, it is impossible to determine the infecting species by visual inspection alone. An indirect fluorescent antibody test has been developed but is not commercially available.[60]

The combination of atovaquone and azithromycin effectively clears infection, which is defined as a negative PCR test of peripheral blood 60 and 90 days post treatment along with resolution of clinical and laboratory abnormalities.[31,34,61] Dogs infected with *B. conradae* do not appear to relapse with disease as can be observed with *B.* gibsoni infections. Although, the author has encountered dogs effectively treated with combination therapy that test positive years later, in all cases, re-infection was suspected based on a high incidence of recurrence in dogs with continued aggressive interactions with coyotes. Imidocarb and dimininazene aceturate appear to be ineffective in clearing the organism.[7] Coinfections with haemotropic *Mycoplasma* are common in dogs with wildlife contact.The combination of atovaquone and azithromycin appears to clear infection with '*Candidatus* Mycoplasma haematoparvum' but not *Mycoplasma haemocanis*.[61]

Babesia sp. 'Coco'

Babesia 'coco' was initially described in a case report of a dog ('Coco') with multicentric lymphoma who developed clinical signs of babesiosis but sequencing of the involved piroplasm was inconsistent with known species.[62] Subsequently, this species has been detected in dogs with clinical babesiosis from the Mid-Atlantic, Southeastern and Southcentral United States (**Figure 3**). It appears that immuncompromised dogs are more susceptible to this disease as all 7 dogs in a small case series had identifiable cause of immunocompromise (splenectomy in 6 dogs).[63]

The prevalence of *B. 'coco'* is unknown though a review of *Babesia* positive samples submitted to the North Carolina State University Vector Borne Disease Diagnotic Laboratory documented approximately the same prevalence as *B. vogeli* (0.17% of dogs tested).[33]

When clinical signs are present, fever may be the only abnormal physical examination finding. Petechiation and ecchymosis are absent, even when severe thrombocytopenia is present. Thrombocytopenia and mild anemia, which can be regenerative or nonregenerative are the most common hematologic findings.[63]

Although published reports of ill dogs infected with *B. 'coco'* have identifiable exogenous or endogenous causes of immunocompromise (splenectomy or chemotherapy), recent information suggests that approximately 25% of positive cases do not. (AB unpublished data) *Babesia 'coco'* has been suspected as the cause of fever of unknown origin in some unpublished observations, but further study is needed to characterize the role of the protozoa in these cases.

As with other species of *Babesia*, microscopic evaluation of blood smears can facilitate the diagnosis of *B. 'coco'*, but visual evaluation cannot determine species (**Figure 2**). Serologic testing is not currently commercially available and cross-reactivity with other species is inconsistent. PCR is the only method of establishing definitive diagnosis, though negative PCR does not rule out infection in cases of low parasitemia of if an appropriate species-specific assay is not used.

Optimal treatment has not been established. Infections appear to respond to administration of either imidocarb or combination atovaquone and azithromycin (**Table 2**).[63] Anecdotally, it appears that dogs are more likely to become PCR negative with the latter approach.

Prevention

Though there is evidence that many *Babesia* spp. are transmitted through tick bites, there is a paucity of data evaluating acaricide use as a strategy in

the prevention of canine babesiosis in North America. The majority of studies evaluating the ability of acaracides to reduce the risk of transmission have used a European *Babesia* species, *Babesia canis* (*canis*) as the pathogen. Studies evaluating the natural tick-vectors of the canine *Babesia* species in North America and whether acaracides reduce the risk of their transmission are needed. While acaracide use is important, the reality is that many North American dogs are infected via alternate routes such as vertical transmission and biting.

Human Health Implications

Canine babesiosis is not a zoonotic disease. While several *Babesia* species that infect humans such as *Babesia microti* and *Babesia duncani* have wildlife reservoirs, transmission of these protozoa likely requires a tick vector. In addition, infection with the organisms that infect people have not been documented in canine patients.

Summary

Canine babesiosis in North America is caused by one of five idenfied *Babesia* species and results in multisystemic disease. *Babesia* are intracellular parasites of erythrocytes and can be transmitted transplacentally, via tick bite, blood transfusion or aggressive interactions. Clinical signs include lethargy, anorexia and depression while physical exam findings include pallor and splenomegaly. Clinicopathologic findings associated with babesiosis include hemolytic anemia, thrombocytopenia, hyperglobulinemia and proteinuria. Diagnosis can achieved by evaluation of blood smear, serology or PCR, but only PCR allows for speciation. Treatment can be challenging and dogs might relapse following cessation of therapy (especially those undergoing immunosuppression such as chemotherapy or splenectomy). More randomized, controlled studies are needed to assess best practices for treatment.

Clinics Care Points

- Babesiosis should be considered in dogs with thrombocytopenia, anemia, hyperglobulinemia, splenomegaly, proteinuria or azotemia.
- Diagnostics should include blood smear examination, PCR assays that can detect all relevant *Babesia* spp. and serology.
- In high risk breeds (APBT or Greyhounds) or dogs with high risk of infection (tick exposure, dog bites or blood transfusions from high risk breeds) consider empirical treatment.
- To determine if therapy has been successful, monitoring for resolution of laboratory abnormalities, and performing PCR to document at least

2 consecutive negative tests approximately 60 and 90 days post-treatment is recommended.

- Antibody titers can remain positive for months to years after treatment, and are therefore less useful for determining whether infection has been cleared.

Figure 1. An illustration from the seminal publication *Investigations into the nature, causation, and prevention of Texas or southern cattle fever* demonstrating different cytologic appearances of *Babesia* organisms (d) in bovine blood. (Public domain https://collections.nlm.nih.gov/bookviewer?PID=nlm:nlmuid-62350480R-bk). The original figure legend reads: … "*a* represents modified red corpuscles, *b* a leukocyte, *c* a hematoblast, and *d* the parasites. Note the variation in the size of the red corpuscles. The parasites are mainly in pairs, they vary in size and form, and perhaps represent stages of degeneration." Modified red corpuscles refer to reticulocytes and hematoblasts refer to rubricytes.

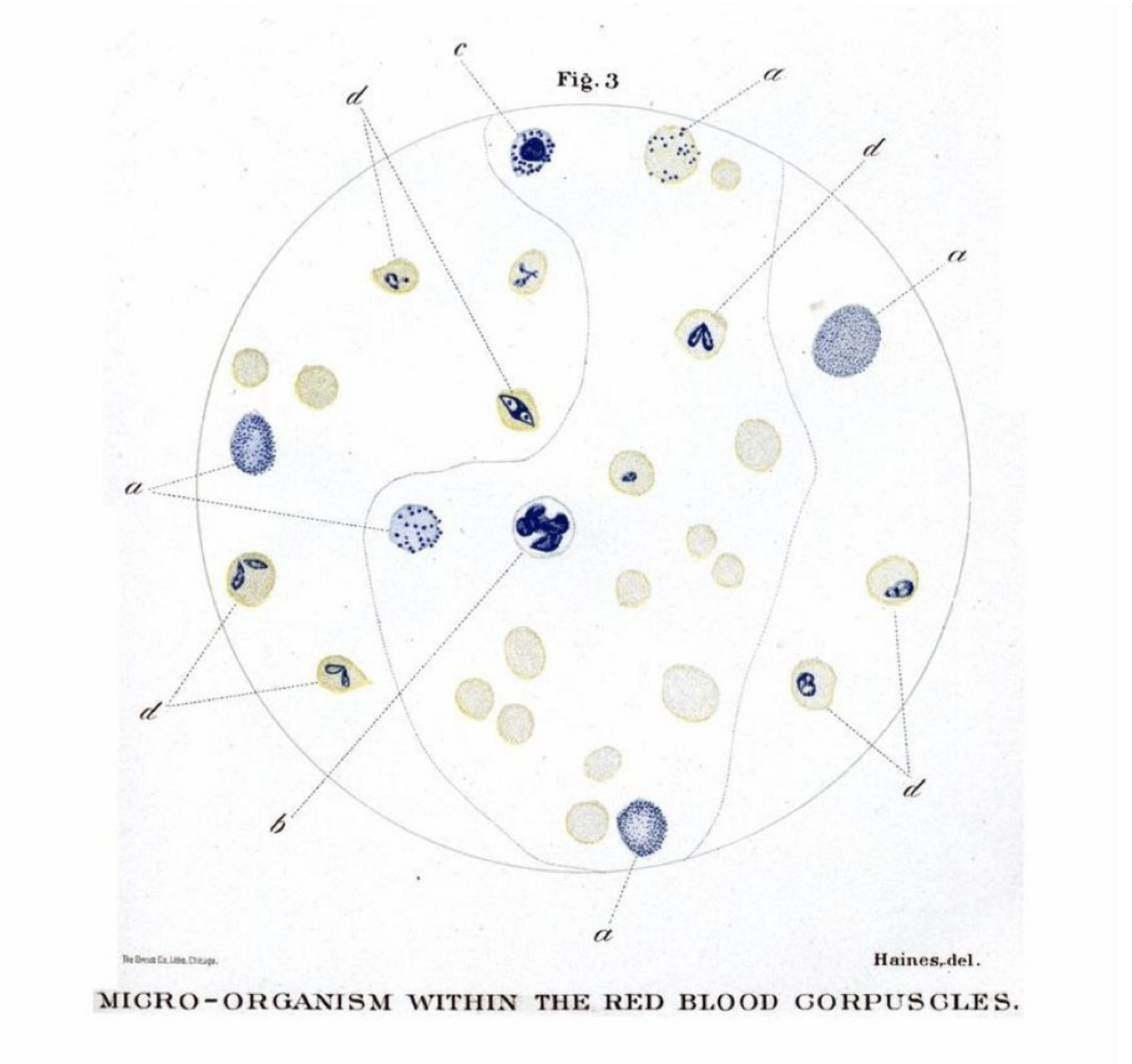
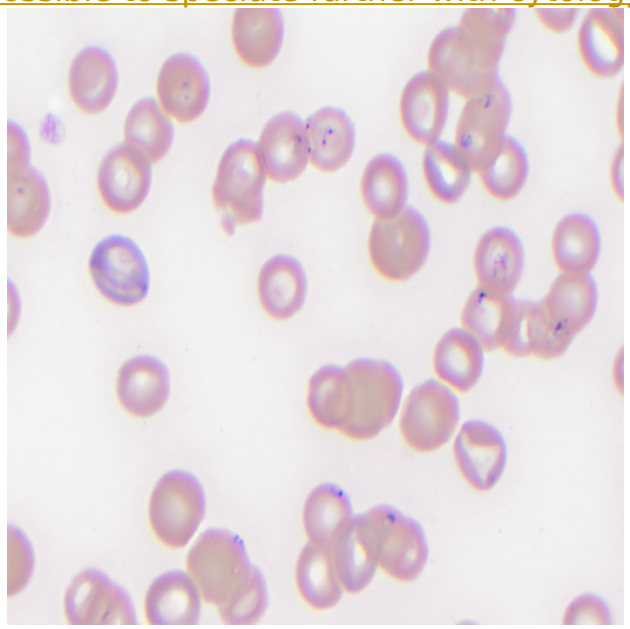
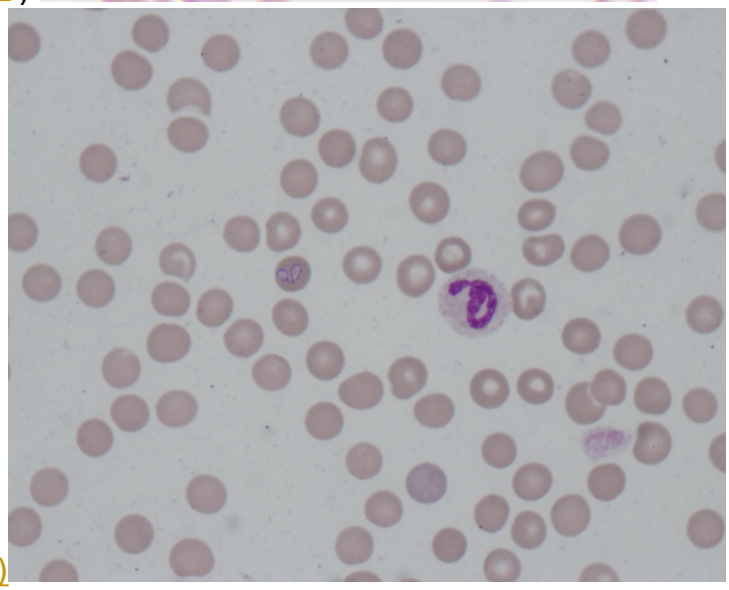


Figure 2. Representative blood smears showing small (A – *B. conradae*) and large (B – *B. vogeli*) *Babesia* spp. in canine erythrocytes. Though blood smear can often help to differentiate between large or small *Babesia*, it is impossible to speciate further with cytology alone.

AB)

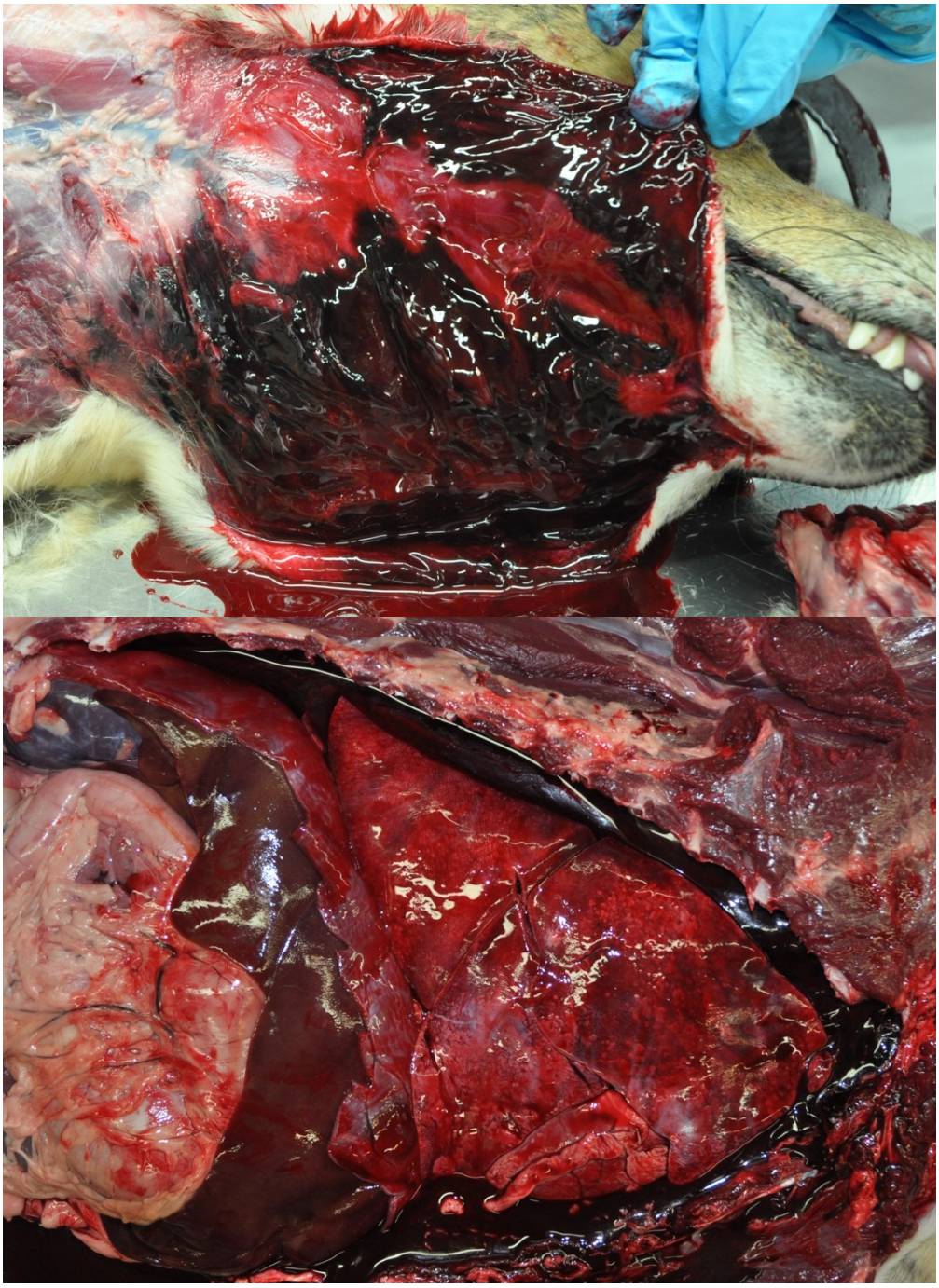




B)

Figure 3. Map of the United States showing the location of dogs that were diagnosed with *B. sp 'Coco'* (green square), *B. gibsoni* (red dot), *B. vogeli* (blue star) and *B. vulpes* (black triangle).
Coco : 11 NC, 8 VA, 3 NJ, 1 NY, 3 OK, 2 GA, 2 MD, 2 MO, 2 TN and 2 AL, 1 CO, 1 DE, 1 FL, 1 KS, 1 PA, 1 SC and 1 WI
Gibsoni : Alabama
(n = 1), Colorado (n = 1), Connecticut (n = 2), Florida (n = 7),
Georgia (n = 1), Illinois (n = 3), Kansas (n = 3), Kentucky (n = 1),
Louisiana (n = 3), Massachusetts (n = 3), Maryland (n = 3), Michigan (n = 3),
Minnesota (n = 1), Missouri (n = 3), Mississippi (n = 5), North
Carolina (n = 20), New Jersey (n = 1), Nevada (n = 2), New York
(n = 65), Ohio (n = 19), Oklahoma (n = 1), Oregon (n = 1), Pennsylvania
(n = 4), South Carolina (n = 3), South Dakota (n = 1), Tennessee
(n = 2), Texas (n = 6), Virginia (n = 6), Wisconsin (n = 2), and Canada
(n = 1).
Vogeli : NC, VA, NJ, OK, GA, MD, MO, TN, AL, CO, DE, FL, KS, PA, SC and WI
Vulpes :

Figure 4. Necropsy images from a 3 year-old male Greyhound mix that was diagnosed with *B. conradae* but died suddenly prior to treatment. Post mortem findings included multisystemic (including mandibular – A) and cavitary (B) hemorrhage despite only a moderate thrombocytopenia (122,000/µL). Images courtesy of the UC Davis VMTH Anatomic Pathology Service.



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