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Canine babesiosis – a never-ending story

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Introduction

When Bayer HealthCare, Animal Health Division, called for the 1st International CVBD® Symposium in 2006, it was the first and initial step to address the global threat of canine vector-borne diseases (CVBD®). This was based on the belief that vector-borne diseases of the dog should be treated as one topic and dealt with on a global level and in an interdisciplinary way. During the past years, CVBD® have become a global issue and have even sparked public interest. Many of the parasite-transmitted diseases affect humans as well as animals. The dog as man's best friend plays an important role – as dogs serve as host for some of the zoonotic pathogens and are greatly affected by them.

At the first symposium, the participants agreed to form the CVBD® World Forum. Besides gathering knowledge, the main task for this group of international experts has been to raise awareness for the specific regional risks of CVBD® and to foster preventative measures. For this reason, the CVBD® World Forum created a website (www.cvbd.org) to provide the veterinary practitioner with cutting-edge and clinically relevant scientific information on CVBD®.

In the CVBD® Digest, relevant findings from CVBD® symposia are presented periodically to veterinary practitioners. The Fourth International CVBD® Symposium has taken place, yet again reviving the intense discussion about the current trends and prospects. Canine babesiosis was one highlight during the recent symposium. Meanwhile, experts from all over the world increasingly recognize infection with *Babesia* spp. as one of the most important CVBD®. The latest data shed light on the role of fighting dogs in disease transmission, and how the suspected spread of tick vectors into moderate climate regions paved the way for the subsequent protozoan parasite infection. However, even in non-endemic regions veterinarians should be aware of the disease when dogs display fever, anemia or thrombocytopenia after returning from endemic regions.

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Canine babesiosis – a never-ending story

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Babesiosis is the oldest tick-borne disease reported in domestic animals and recently has attracted even more interest in the field of canine vector-borne diseases (CVBD). Due to emerging or newly identified pathogens, canine babesiosis still presents a significant challenge to the practitioner. This is especially true for diagnosis and therapy.
Besides new pathogen species, spreading of the disease to former non-endemic areas has been reported as a consequence of international travel and expansion of tick vector habitats. To keep up with this development, practitioners should be aware of a variety of clinical signs and the treatment procedure. Control and prevention can be supported by continuous use of ectoparasiticides that minimize blood-feeding by repelling and killing the tick vectors.

Babesiosis is a parasitic infection caused by hemotropic protozoa of the genus *Babesia* (Order: Piroplasmida). The pathogen was discovered at the end of the 19th century by the Romanian scientist Victor Babes, who was investigating febrile hemoglobinuria in cattle.[1] Only seven years after these initial descriptions, *B. canis* was first described in dogs in Italy.[2] The protozoan infects erythrocytes of wild and domestic animals worldwide and is transmitted by a variety of ticks, including the Brown Dog tick *(Rhipicephalus sanguineus)* and different members of the *Dermacentor* genus. More than 100 *Babesia* species are reported so far, infecting many mammalian and some avian species. *Babesia* spp. infections have probably been complicating the lives of humans and their domestic livestock since antiquity. In the biblical book Exodus 9:3, a plague of the cattle of the Egyptian Pharaoh Ramses II is described that could have been red water fever of cattle (caused by *B. bovis*), including hemoglobinuria as a prevalent sign.[3]

Two-host-life cycle and the Pit Bull paradox

Generally, *Babesia* spp. possesses two types of hosts: an invertebrate host (ixodid ticks) and a vertebrate host. In the tick vector, *Babesia* gamonts fuse to zygotes that later, after sporogony, develop to sporozoites within the salivary glands of the tick. The female tick may pass the pathogens to its offspring (transovarial transmission). Sporozoites are injected into the vertebrate host together with the saliva during blood feeding and directly infect red blood cells. In these cells, they develop into piroplasms. Multiplication usually results in two (sometimes four) daughter cells (merozoites), which leave the host cell and each enter another red cell. This continues until the death of the vertebrate host, or until the immune system of the host terminates the process.
Over the last 10 years, *Babesia gibsoni* (see below) infections have been reported in many countries outside of Asia, predominantly in American Pit Bull Terrier-type dogs, in the absence of tick vectors. There is now convincing evidence that these cases have arisen due to biting and fighting between infected and non-infected dogs.[4,5] The fighting dogs themselves are considered to be the reservoir of this *Babesia* species[6] and the massive injuries during fighting as well as the breed of dogs itself seem to foster this non-vectorial spreading. Due to the worldwide popularity of this breed and similar ones, it is speculated that *B. gibsoni* will eventually be reported from all countries where (usually illegal) dog fighting is practiced.[6] Nevertheless, the vast majority of *Babesia* spp. transmission worldwide is due to tick biting and blood feeding.

Canine babesiosis: *Babesia* or *Theileria*?

Historically, *Babesia* spp. in dogs were identified by their morphological appearance in erythrocytes of blood smears. All large forms (intraerythrocytic merozoite stage measures 3 to 5 µm, i.e. half or more of the diameter of a red blood cell, see Figure 1) were designated *B. canis* in former times, whereas all small forms (merozoites measuring 1 to 3 µm, i.e. less than half the diameter of an erythrocyte, see Figure 2) were thought to be *B. gibsoni. Babesia canis* was categorized into three subspecies *(B. canis canis, B. canis rossi, B. canis vogeli)* on the basis of cross-immunity, serological testing, vector specificity and molecular phylogeny.[7,8] These subspecies are now considered to be separate species.[9,10] Another, fourth 'large' *Babesia* sp. (yet unnamed) has been described recently in a number of dogs with clinical signs and hematological parameters consistent with babesiosis in North Carolina, USA (see Table 1).[11,12]

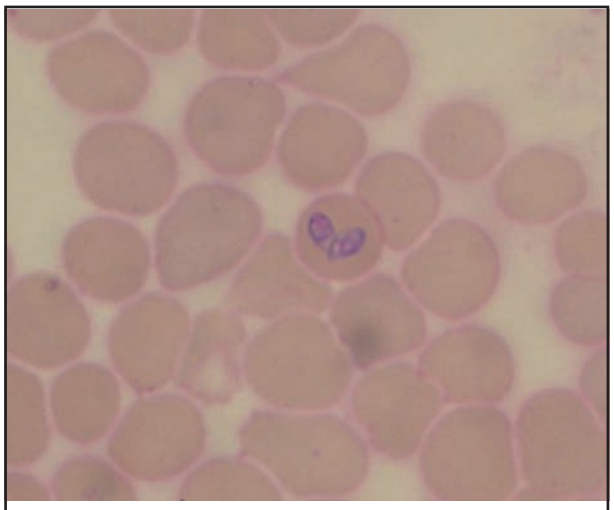

Fig. 1 *Babesia canis* within a red blood cell: note the proportion of the cell occupied by this large *Babesia* species. *With permission of P. Bourdeau, Nantes, France.*

The status of *B. gibsoni* as the only small *Babesia* species in dogs had to be abandoned in favor of three genetically and clinically distinct species causing canine diseases: *B. gibsoni, B. conradae* (reported in dogs in the western United States),[13,14] and a *B. microti*-like piroplasm (named *Theileria annae)*.[15,16] Recently, reclassification of *Babesia* species into *Theileria* species was carried out on the basis of differences in the life cycle.

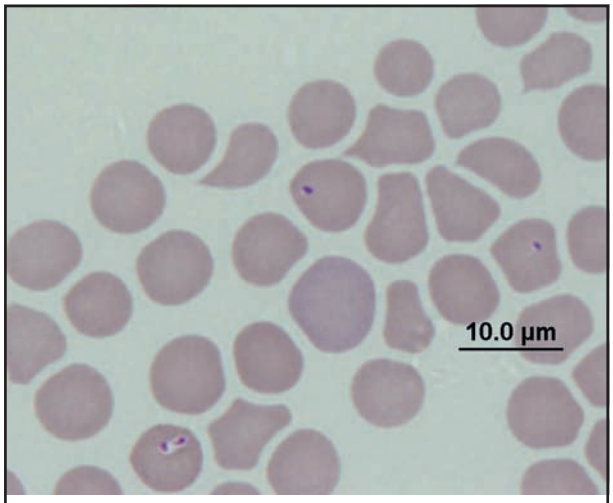

Fig. 2 *Babesia gibsoni* within red blood cells: note how the organism occupies markedly less than half of the diameter of the cell. *With permission of P. Irwin, Murdoch, Australia.*

Babesia, once injected into the host, enter directly into red blood cells. In contrast, *Theileria* sporozoites do not infect red blood cells but penetrate lymphocytes (or macrophages) where they develop into schizonts. *Theileria* merozoites enter red blood cells where they grow into piroplasms and multiply by budding into four daughter cells, forming tetrads, often in the shape of a Maltese cross. Further differences exist in the developmental cycle within the tick vector (transovarial transmission in *Babesia* spp. versus transstadial transmission in *Theileria* spp.). In summary, as soon as schizonts are seen, the parasite in question is not considered to be *Babesia* anymore; but whether it is a true *Theileria* also depends on the cycle in the tick vector – a very vague species classification.[17]

Three *Theileria* species have been isolated in the blood of a small number of dogs in Europe in recent years *(T. equi* and *T. annulata)*[18,19] and from 82 dogs in South Africa (unnamed *Theileria* sp. related to an isolate obtained from antelope)[20] (see Table 1). However, until more information is available, the competence of the dog as a host for these piroplasms is uncertain; the clinical correlation is unknown, and neither intra- nor extraerthrocytic stages have so far been visualized[6]. At least for *T. annae* infection, severe hemolysis and azotemia have been reported.[16]

Size	Species	Synonyms	Vector	Geographic distribution	Comments
Large	*Babesia vogeli*	*Babesia canis vogeli*	*Rhipicephalus sanguineus*	Wide range: tropical, subtropical and Mediterranean regions	
	Babesia canis	*Babesia canis canis*	*Dermacentor* spp.	Europe	
	Babesia rossi	*Babesia canis rossi*	*Haemaphysalis elliptica* (formerly *H. leachi)*	Sub-Saharan Africa, South Africa	
	Babesia sp.	Unnamed large *Babesia* sp., North Carolina isolate	Unknown	North Carolina, USA	
Small	*Babesia gibsoni*	*Babesia gibsoni* Asia strain	*Haemaphysalis longicornis*	Asia including Japan, sporadic occurrence worldwide	Outside Asia this infection is often associated with Pit Bull Terriers and other fighting dogs
	Babesia conradae	In original reports described as *B. gibsoni*	Unknown	Western USA, specifically California	
	Theileria annae	*Babesia microti*-like Spanish isolate/ piroplasm/agent	*Ixodes hexagonus* (putative)	Spain, Portugal	
	Theileria sp.	Unnamed *Theileria* sp., South African *Theileria* sp.	Unknown	South Africa	Molecular detection only
	Theileria annulata		Unknown	Africa, Europe, Asia	Molecular detection only
	Theileria equi	*Babesia equi*	Unknown	Africa, Europe, Asia	Molecular detection only

Tab. 1: Piroplasm species of domestic dogs (modified after P. Irwin (6))

Worldwide distribution

When assessing the distribution of the disease, Irwin considered it useful to categorize the regional prevalence and incidence at two levels: (i) regions where the specific parasite is well established (endemic) and clinically recognized and (ii) regions where sporadic autochthonous infections or cases associated with traveling dogs have been reported.[6] Nevertheless, practitioners working within regions of the second type should bear in mind that not only the disease may be imported by traveling dogs into their local region, but that, as long as the tick vector is abundant, an establishment of new disease foci is possible. The distribution of babesiosis is changing continuously, mainly driven by the spreading of the vectors within their ecological ranges (see Figure 5a/b). As previously mentioned, this distribution is further augmented by a non-vectorial dog-to-dog transmission in the case of *B. gibsoni*. A summary of the geographical distribution of the different *Babesia* species is listed in Table 1. *B. vogeli* with its ubiquitous vector the Brown Dog tick *(Rhipicephalus sanguineus)* is widely distributed throughout tropical and subtropical regions and is spreading into cooler latitudes. *B. canis* (sensu stricto), transmitted by *Dermacentor*

spp. (see Figure 3), has already preceded this development and is increasingly being discovered throughout central Europe. *B. rossi*, originally only recorded in South Africa, has extended its range to the Sub-Saharan zone (e.g., Nigeria[21] and Sudan[22]). The most important small *Babesia* species, *B. gibsoni*, was originally identified in a number of different southern, eastern and southeastern Asian countries. Meanwhile, it has been reported in many countries outside Asia in connection with American Pit Bull Terrier-type dogs. Geographical information on recently characterized small piroplasm species as well as the additional so far unnamed large *Babesia* species is scarce (see Table 1), as all these have been detected so far in fairly limited areas of distribution.

Fig. 3 *Dermacentor* spp. (female *D. reticulatus* shown here) are vectors for *Babesia canis*. *With permission of T. Naucke, Bonn, Germany*

Complex clinical signs due to species diversity

The severity of babesiosis in dogs ranges from subclinical infection through the development of mild anemia to broad organ failure and death. The critical determinant of this variable pathogenesis is the piroplasm species. Other factors such as age, immune status, concurrent infections or illness should also be considered. All species may cause pyrexia, anorexia, splenomegaly, anemia and thrombocytopenia.[6]
The most common clinical signs during *Babesia* spp. infections in dogs are hemolytic anemia (see Figure 4) and thrombocytopenia. Hemolytic anemia in the course of the infection can occur due to direct erythrocyte lysis by replication of intracellular parasites or intravascular and extravascular hemolysis.[23] The clinical signs and clinicopathological abnormalities vary in their severity according to species and host:

- *B. vogeli* is the least pathogenic of the three main large canine *Babesia* species. The infection causes subclinical to mild to moderate clinical disease.[10,24] In puppies, severe to fatal hemolytic anemia is common.[25,26] Adult diseased dogs often have a concomitant disease or show predisposing factors as splenectomy, immunocompromized conditions etc.
- Acute *B. canis* infection is characterized by a mild to severe disease, in which parasitemia is often low and does not necessarily correlate with the severity of clinical illness.[24] The main acute clinical signs are dehydration, lethargy, anorexia and fever. At initial clinical examination, the majority of dogs present with mild to severe thrombocytopenia, hyperfibrinogenemia, mild to moderate anemia, hemolysis and neutropenia.[25]
- *B. rossi* infection is the most severe infection of the three main large canine *Babesia* species. A large proportion of dogs develop complications, some of which (hemoconcentration, neurological signs, acute renal failure and pulmonary edema) require early aggressive and intensive treatment and carry a poor prognosis.[27]

The unnamed large *Babesia* sp. from North Carolina has been reported to be associated with nonspecific illness (lethargy and anorexia), pigmenturia and mild fever, especially in splenectomized dogs.[6]
Concerning the small *Babesia* species, *B. conradae* is considered to be more pathogenic than *B. gibsoni*, resulting in higher parasitemias and more severe anemia.[14] *T. annae* infection in Spain is associated with severe hemolysis and azotemia.[16]
The clinical consequences of chronic babesial infection are unclear. Even though most dogs seem to

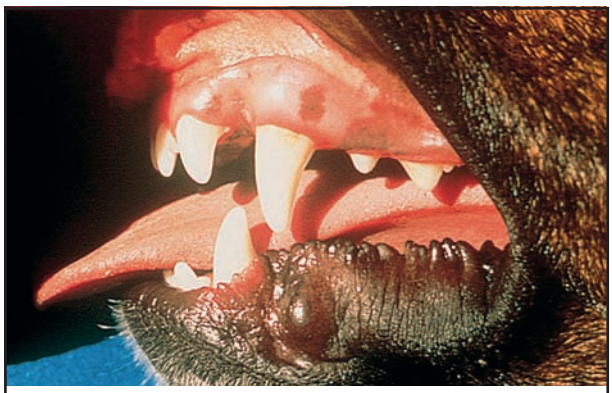

Fig. 4 Anemia, here shown in a dog, is one of the main presenting signs of acute babesiosis.

tolerate the state of premunity with few ill effects, a risk of developing immune-mediated complications and recrudescence of clinical disease in case of later immunocompromised conditions theoretically remains.[6]

A range of diagnostic tools

During acute infection, microscopy is reasonably sensitive at detecting intraerythrocytic parasites in Giemsa or Wright's stained blood smears and remains the simplest and most accessible diagnostic test.[6] Molecular diagnostic tools (e.g., PCR) have greatly increased the sensitivity and specificity of *Babesia* detection. However, low parasitemia can result in a false-negative PCR.

The diagnosis of chronically infected reservoir dogs remains a significant challenge due to a very low, often intermittent parasite burden.[6] A higher detection rate in chronically infected dogs might be achieved by testing on more than one occasion, but the alternative, complementary use of serology is nevertheless advisable.[28,29] Due to the fact that *B. gibsoni* can be transmitted without tick vectors, a history of biting should always be included in the anamnesis.

Concerning serology, immunofluorescent antibody testing (IFAT) has been the most widely supported serological diagnostic test for canine babesiosis over the last 30 years.[30,31] Besides the general limitation of serology to differentiate acute from chronic infections and cross-reactions, subjectivity and inadequacy of large-scale screening have been limiting factors of IFAT.[32] Additionally to IFAT, research has been conducted in the field of antigen detection for use in recombinant protein enzyme-linked immunosorbent assays (ELISA).[32–38]

Therapy lacking parasite elimination

In general, neither treatment nor the host immune response completely eliminate the infection. Animals thus become chronic carriers of the organism and potential reservoirs of infection.

Imidocarb dipropionate and diminazine aceturate are widely used anti-piroplasm drugs. Besides these drugs, other compounds have been used with varying degrees of success for the management of clinical signs of piroplasmosis (quinuronium sulphate, trypan blue, pentamidine, phenamidine and parvaquone). At best, all these drugs result in amelioration of clinical signs; they rarely achieve a true sterilization of the infection.[6] Treatment of small *Babesia* species infections, especially *B. gibsoni*, has been tested with diverse combinations such as clindamycin, metronidazole and doxycycline[39] or azithromycin and atovaquone[11] with reasonable clinical efficacy. Generally, supportive therapy such as intravenous fluids and blood transfusions should be employed whenever necessary in both, large and small *Babesia* spp. infection. Besides these approaches to treat an established *Babesia* infection, prevention is also of major importance. This is especially the case for dogs traveling into endemic areas but also for local animals, considering the transmission period.

Vaccination and further prevention

Vaccination against *Babesia* infection has been a subject of discussion for a long time and is registered in some countries. However, cross-immunity experiments have shown in provisional results that the antigenic differences between the large *Babesia* species have important implications for the development of a vaccine, as there is none or no complete cross-protection between the individual pathogens. A combination of soluble parasite antigens (SPA) from both European *B. canis* and South African *B. rossi* in form of a vaccine offered greater protection against heterologous challenge.[40] Currently, suitable immunodominant and protective antigens are also under investigation in Japan for use in vaccines against *B. gibsoni*.[41] Similarly, several drugs have been investigated for their prophylactic potential against babesiosis, but none has been consistently reliable in this regard.[6]

As with other vector-transmitted diseases, the best prevention is achieved by avoiding exposure to the vector.[6] This should be considered when deciding to take a dog from non-endemic into (highly) endemic areas. The dog should be protected with broad-spectrum ectoparasiticides with repellent properties, such as the synthetic pyrethroid permethrin.[42] Inside an endemic area, regular treatment during the transmission period, e.g. in the form of monthly spot-on applications, supports the prevention of tick bites and thus potential *Babesia* spp. transmission. In Eurasia, the combination of imidacloprid and permethrin has proven activity against the vector of *B. canis*, *Dermacentor reticulatus*. The combination also prevents the biting of other vectors such as ticks, sand flies, mosquitoes and stable flies and therefore minimizes the interaction between host and vector. This in turn results in a decreased risk of CVBD transmission.

Fig. 5a Babesiosis distribution based on recent scientific publications in Asia-Pacific.

Fig. 5: Canine babesiosis belongs to the so-called canine vector-borne diseases (CVBD). This group of infectious diseases with worldwide importance comprises the major infections transmitted by ticks, mosquitoes or sand flies. Bayer HealthCare has collected data from recent scientific publications to provide a comprehensive view of the endemic situation of several CVBD including also babesiosis in Asia-Pacific (Fig. 5a) and Europe (Fig. 5b). In most countries, the corresponding map is available as a poster for the veterinary practice; the interactive versions of the Asia-Pacific and the Europe map can be found at www.cvbd.org.

BABESIOSIS OCCURRENCE MAP EUROPE

Fig. 5b Babesiosis distribution based on recent scientific publications in Europe.

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