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A challenge for the practitioner – co-infection with vector-borne pathogens in dogs

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Introduction

When Bayer Animal Health called for the 1st International CVBD® Symposium in 2006, this 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. Especially with increasing international travel and emerging climate change, CVBD have become a global issue and 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 – being affected to a high extend by and serving as a host for some of the zoonotic pathogens.

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 *CVBD® Digest,* relevant findings from the International CVBD® Symposia are presented periodically to veterinary practitioners. While the first edition was on "asymptomatic leishmaniosis in dogs", the second edition is about co-infection with CVBD-causing pathogens. During the three symposia so far, it became clear that beside the transmission of multiple pathogens by one vector, high attention has to be paid to co-infection with pathogens arising from different vectors, like ticks and sand flies. Furthermore, the difficulties in clinical diagnosis and the complex interaction of different infectious agents, e.g. via the canine immune system, make co-infection with CVBD-causing pathogens a substantial concern for veterinarians throughout the world.

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A challenge for the practitioner – co-infection with vector-borne pathogens in dogs

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Blood-feeding arthropods can transmit a plethora of pathogens to dogs. These canine vector-borne diseases (CVBD) vary in their clinical appearance. Often more than one pathogen is transmitted to the host by the same or different vectors, resulting in double or even multiple infections. These vector-borne co-infections have important implications for diagnosis, therapy and prognosis of the patient. In endemic areas, they should always be considered and ruled out in dogs showing unspecific clinics. Control and prevention can be achieved by continuous use of ectoparasiticides that inhibit blood-feeding.

Ectoparasites referred to as vectors – such as ticks, fleas, sand flies and mosquitoes – can transmit bacteria, protozoa, viruses or helminths to dogs. These transmitted pathogens may lead to a variety of serious infections, e.g. leishmaniosis, babesiosis, ehrlichiosis or heartworm disease. Some of these vectors, esp. ticks, are capable of transmitting more than one pathogen, and a single vector can harbor more than just one type of pathogen. Moreover, similar clinical signs of different CVBD complicate the problem of simultaneous infection causing diagnostic, therapeutic and prognostic implications for the veterinary practitioner, and subsequently the individual patient.

Predisposing factors

Concerning living conditions and handling, some external factors are discussed that predispose dogs to infections with two or multiple vector-transmitted pathogens:

1. Living in areas that are highly endemic for several vector-borne pathogens.
2. Maintenance of animals predominantly outdoors, thus facilitating enhanced vector transmission.
3. Irregular or missing use of ectoparasiticides.

Besides these, a suppressed immune response, due to old age, underlying infection or immunosuppressive therapy can promote an infection that might have been controlled in immunocompetent dogs, and thus is suspected to be a major predisposing internal factor.

The impairment of the immune response can be a consequence of an immunosuppressive effect of other co-infections. This has been shown for *Leishmania infantum* infections, where immunsuppression or promotion of an abnormal immune response can result from an imbalance between cell-based Th-1 and Th-2 responses.[1,2] Furthermore, it may be caused by a pathogen challenging the host's immune system in general, like it is discussed

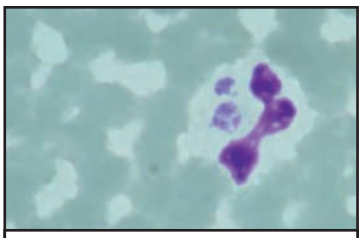

Fig. 1: *Anaplasma phagocytophilum* morula in a neutrophilic granulocyte (® photo by Institute for Comparative Tropical Medicine and Parasitology, LMU Munich, Germany)

for the infection with *Anaplasma phagocytophilum* (Fig. 1) and *Borrelia burgdorferi*[3] (Fig. 2). In these cases, the initiation of two different lines of defense (humoral and cell-based adaptive immune response) may lead to apparent clinical signs. Finally, impairment of the immune response can be the result of an immunosuppressive therapy due to other underlying diseases. Remarkably, most hosts appear to be able to support chronic infection with vector-borne pathogens for months or even years without displaying obvious deleterious effects.[4]

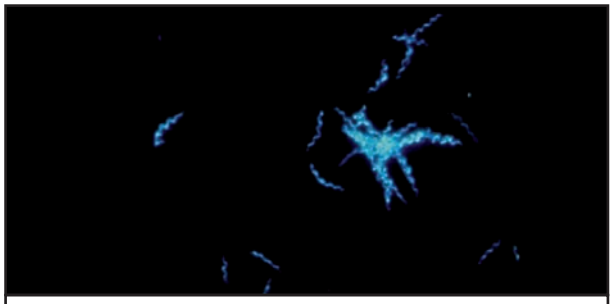

Fig. 2: *Borrelia burgdorferi* (dark field microscopy); typical helical shaped structure of spirochaetes visible (® photo by Bayer Animal Health)

Co-infection with tick-borne pathogens

Among the vectors, which are transmitting disease-causing pathogens, ticks are the most important ones, as they harbor the largest number of different entities (Tab. 1). Investigations have even shown that there is a co-evolution between ticks and some pathogens, which lost the capability of a direct host-to-host transmission over the time. Ticks are especially suitable for pathogen transmission, by attaching securely to their hosts and facilitating effective transmission of infectious pathogens over a couple of days.

As shown in table 1, different pathogens can share the same tick vector for transmission. Double or even triple infections not only with different species of the same genus, but also with completely different pathogens have been reported.[6] Serologic and molecular evidence indicates that co-infection in dogs with *Anaplasma, Ehrlichia, Babesia, Rickettsia* and *Bartonella* spp. may be more frequent than previously realised.[4] One of the best studied combinations, *Anaplasma phagocytophilum* and *Borrelia* spp., share the same tick vector (e.g. in Europe *Ixodes ricinus*, Fig. 3). Equally, this also applies for some Rickettsiae. Another example of a shared vector is *Rhipicephalus sanguineus*, which can harbor *Babesia* spp., *Ehrlichia canis*, *A. platys* and *Rickettsia conorii*.

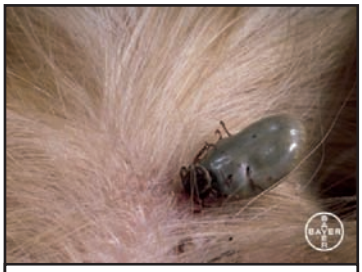

Fig. 3: Adult *Ixodes ricinus* (Castor Bean tick), a known vector for *Borrelia* spp., *Anaplasma phagocytophilum* and *Rickettsia* spp. (® photo by Bayer HealthCare AG)

Many of the diseases caused by these tick-borne pathogens possess a wide variety of clinical features and share non-specific signs such as wasting, weight loss, fever and poor appetite or anorexia, all in all challenging the veterinary practitioner in stating a definite diagnosis.[5]

However, in co-infections with pathogens that have different clinical signs, the extent to which different infections might influence each other's pathophysiology still is not clear. Experimental studies in mice[7] and humans[8] have already demonstrated more severe and complex clinical signs in co-infections. A recent study of Beall and colleagues[9] found dogs, which were positive for antibodies of *A. phagocytophilum* and *B. burgdorferi*, to be nearly twice as likely to have clinical signs similar to anaplasmosis and/or borreliosis, when compared to dogs that were seroreactive to only one of these pathogens. In a subgroup of dogs exhibiting illness compatible with anaplasmosis or borreliosis, antibodies to only *A. phagocytophilum* were detected in 29 %, to

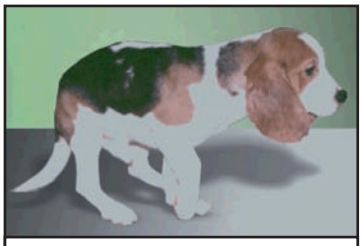

Fig. 4: Beagle with acute forelimb lameness (® photo by Straubinger R.K., Leipzig, Germany)

only *B. burgdorferi* in 9% and to both pathogens in 43% of the dogs. A cardinal sign of borreliosis, lameness (Fig. 4), was found to be more often associated with co-infection (in 32 from 38 seropositive dogs) than with single *B. burgdorferi*-infection (in 5 out of 8 seropositive dogs).

Co-infection with pathogens of different arthropods

Canine leishmaniosis is one of the major vector-borne diseases in dogs. The clinical features of this sand fly-transmitted protozoal disease can vary

TICK SPECIES	PATHOGEN	TICK DISTRIBUTION
***Ixodes* spp.**	***Anaplasma* sp.** ***Borrelia* spp.** **some *Rickettsia* spp.** ***Hepatozoon canis***	
Ixodes ricinus (Castor Bean tick)	*Anaplasma phagocytopilum*[1] *Borrelia* spp. Rickettsia spp.	Central Europe, Northern Africa
Ixodes pacificus (Western black-legged tick)	*Anaplasma phagocytopilum*[1] *Borrelia burgdorferi*	Western North America
Ixodes scapularis (Black-legged Deer tick)	*Anaplasma phagocytopilum*[1] *Borrelia burgdorferi*	Eastern North America
***Dermacentor* spp.**	***Babesia* spp.** ***Rickettsia rickettsii*[2]** ***Ehrlichia chaffeensis***	
Dermacentor marginatus	*Babesia canis*	Central Europe, China, Iran, Afghanistan
Dermacentor reticulatus (Marsh tick or Ornate Cow tick)	*Babesia canis*	Central and Southern Europe
Dermacentor variabilis (American Dog tick)	*Rickettsia rickettsii*[2] *E. chaffeensis* suspected additional vector for *Ehrlichia canis*[3]	North and Central America
***Rhipicephalus* spp.**	***Babesia* spp.** ***Ehrlichia canis*[3]** ***Anaplasma platys*[4]** ***Rickettsia* spp.** ***Hepatozoon canis***	
Rhipicephalus sanguineus (Brown Dog or Kennel tick)	***Babesia* spp.** ***Ehrlichia canis*[3]** ***Anaplasma platys*[4]** ***Rickettsia conorii*** ***Hepatozoon canis***	Worldwide, more commonly in warmer climates; can be established inside buildings
***Amblyomma* spp.**	***Ehrlichia chaffeensis*** ***Ehrlichia ewingii*[5]** ***Rickettsia rickettsii*[2]** ***Hepatozoon americanum***	
Amblyomma americanum (Lone Star tick)	*Ehrlichia chaffeensis* *Ehrlichia ewingii*[5] *Rickettsia rickettsii*[2]	America
Amblyomma maculatum (Gulf Coast tick)	*Hepatozoon americanum*	North America
***Haemaphysalis* spp.**	*Babesia* spp. *Ehrlichia canis*[3]	
Haemaphysalis leachi	*Babesia canis rossi* *Ehrlichia canis*[3]	Southern Africa
Haemaphysalis longicornis	*Babesia gibsoni*[6]	East Asia (Japan, Korea)

[1] canine granulocytic anaplasmosis; [2] Rocky Mountain spotted fever; [3] canine monocytic ehrlichiosis (CME); [4] canine cyclic thrombocytopenia; [5] mild form of canine granulocytic ehrlichiosis (CGE); [6] also confirmed from dogs in Europe, USA, and Australia (via unknown vector transmission or direct infection)

Tab. 1: Canine tick-borne pathogens. Listed are genus and species of transmitting ticks, important transmitted pathogens and their endemic regions. Many of these pathogens are also causing diseases in humans.

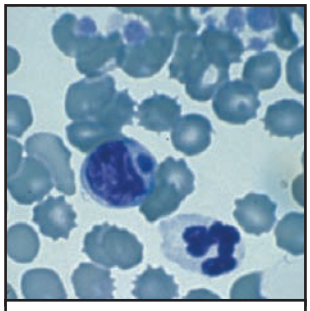

Fig. 5: Inclusion bodies of *Ehrlichia canis* from a dog also infected with *Leishmania infantum* (® photo by Roura X., Barcelona, Spain)

widely and are often non-specific, such as chronic wasting, weight loss, poor appetite, fever, anemia, non-pruritic alopecia and skin erosions or ulcerations. Variation is expected to be a consequence of pathogen- or host-specific factors, but can also be related to co-infection with other vector-borne pathogens in some individuals.[10] An increasing number of publications report on simultaneous infections with additional vector-borne pathogens in *Leishmania*-infected dogs[11–13], like *Ehrlichia* (Fig. 5), *Anaplasma, Babesia, Bartonella, Rickettsia* and *Hepatozoon* species as well as mosquito-transmitted *Dirofilaria repens*.

Even though some authors do not expect concurrent infections e.g. with *L. infantum* to substantially influence the clinical course and final outcome of chronic canine ehrlichiosis[11], others presume the immunosuppression caused by cutano-visceral leishmaniosis to promote the occurrence of co-infection with other pathogens[14] and discuss a synergism between leishmaniosis and ehrlichiosis in altering platelet function by different pathways.[15] Likewise, an epidemiological study from Italy found *Neospora caninum* seroreactivity to represent a major risk factor for *L. infantum* seroreactivity.[16]

Diagnosis of vector-borne co-infections

The clinical signs of dogs infected with more than one pathogen are often non-specific and very variable. Thus, when approaching a dog, e.g. with leishmaniosis, any clinical sign of the patient should be investigated and co-infection should be clarified in dogs that

- lack response to conventional treatment (e.g., persistence of hypergammaglobulinemia, persistence of high antibody titer);
- show atypical clinical signs of the suspected disease;
- live in endemic areas for years without any signs of disease and suddenly fall ill with a suspected mono-infection of a CVBD.

Anamnesis and searching for typical clinical-pathological findings, accompanied by laboratory results, are key clinical diagnostic approaches. However, these findings might be mimicked and altered by co-infection as it is suspected for epistaxis. It has long been thought and taught a cardinal sign in ehrlichiosis, but is possibly caused by an underlying *Bartonella* infection in *E. canis*-positive dogs[4] (Fig. 6). Thus, it may become very difficult to attribute the clinical signs and hematological and/or biochemical abnormalities to a single specific pathogen. Nevertheless, the veterinary practitioner should follow a standard examination procedure: detailed anamnetic report, profound clinical and laboratory examination, including search for typical signs, and additional serological and molecular identification of multiple pathogens, which offer a more successful diagnostic approach apart from only clinical and laboratory parameters. It should be considered, however, that molecular or serological evidence of a pathogen alone, without any clinical signs, does not represent a proof for a disease.

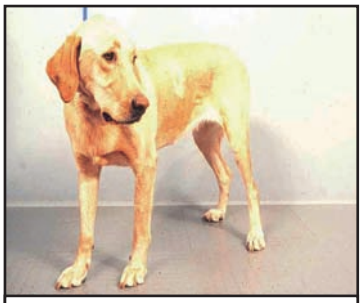

Fig. 6: Labrador retriever referred for evaluation of chronic polyarthritis, seizures, epistaxis and endocarditis. The dog was co-infected with *Ehrlichia canis* and *Bartonella vinsonii* subspecies *berkhoffii*. (® photo by Breitschwerdt, E.B., Raleigh, USA)

Control of vector-borne co-infections

The different infection scenarios with vector-borne pathogens in dogs call for a comprehensive control program. Sequential transmission, concurrently or over a time, by ticks and other vectors such as mosquitoes (*Dirofilaria* spp. transmission) and

especially sand flies (*L. infantum* / *L. chagasi* transmission) has to be taken into account. Furthermore, epidemiological studies revealed new distribution patterns of vectors, so that previously non-endemic regions may be endemic today. As a consequence, veterinary practitioners are advised to bear in mind differential diagnoses of diseases formerly not occurring in the respective region.

Prevention of arthropod bites is mainly achieved by preventing the attachment and thus further blood-feeding if possible. Broad-spectrum ectoparasiticides with repellent properties, such as the synthetic pyrethroid permethrin, are ideal compounds to reach this goal[17], as they prevent the biting of different vectors like ticks, fleas, sand flies and mosquitoes and therefore minimize the host-parasite interaction, thus resulting in a decreased risk of disease transmission. A regular treatment with these compounds during the transmission period, e.g. in form of monthly spot-on applications, is crucial for the prevention of single as well as multiple CVBD.

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