If one investigates the market trends, it becomes very obvious that a high standard of efficacy against ectoparasites is a given, and the demand from the veterinary profession is directed more into the prevention of host-parasite interaction, vector-borne pathogens and the health and well-being of the animals. Especially in the field of vector-borne pathogens and the diseases they cause, one can clearly state an accelerating amount of knowledge generated in the last couple of years. New diagnostic tools, implemented in small animal clinics, provide previously unknown knowledge about pathogens. Knowledge on the severity of the diseases caused by many of these pathogens increased the demand to implement ectoparasite control strategies, preventing ectoparasite-host interaction in the first place. Thus ectoparasicides, especially those with fast onset and repellent activity, offer reliable tools in the prevention of vector-borne diseases.

We at Bayer HealthCare, Animal Health, are pleased to work together with experts in the field to increase scientific information about canine vector-borne diseases (CVBD). The first CVBD symposium was held in April 2006, in Billesley, UK. On behalf of the entire Management Committee of Bayer HealthCare’s Animal Health Division, I am pleased to see the demand for the contingency working in this fascinating field of veterinary and human medicine. We at Bayer are committed to delivering high-quality products, together with service and scientific knowledge. In saying that, we hope you all enjoy the CVBD World Forum Symposium, to be held this year at Mazara del Vallo in Sicily, Italy.
Introduction to the CVBD World Forum

It gives me great pleasure to announce the CVBD World Forum Symposium, to be held in April 2007, in Mazara del Vallo on Sicily, Italy. And Sicily in particular, is a truly endemic area for a variety of vector-borne pathogens, including canine leishmaniosis and several tick-borne diseases.

Bayer HealthCare, Animal Health called for the 1st Canine Vector-Borne Disease Symposium last year. The meeting was held in April 2006, in Billesley, UK. As one can see from last year’s proceedings, the meeting covered a wide range of vector-borne pathogens. It was our aim to exchange the current scientific knowledge as well as future trends and needs concerning the clinical presentation, diagnosis, pathogenesis and prevention of pathogens transmitted by blood-feeding arthropods. Thus the 1st Canine Vector-Borne Diseases Symposium mirrored the diversity of pathogens transmitted by arthropods: viruses, bacteria and protozoa.

Furthermore, the participants of the 1st CVBD Symposium voted to continue working in this field and formed a group, which was named the CVBD World Forum. Thus it was more than logical that the 1st symposium was not a stand-alone meeting. Your demand for another meeting fitted well with our intention to show continuity and to call for the follow-up meeting.

Topics like “international travel” and “climate change” are currently debated worldwide and are of great public interest. There is no doubt that today, climate and travel have an impact on the distribution of the vector and thus the pathogens they harbour. Effects caused by global climate changes are well documented, and whilst climate change is global in nature, it may pose unknown future risks to both the health of animals and man. On a more local scale, changes often occur more rapidly. Therefore, climate data are essential at a local level to evaluate the dynamics of vector-borne diseases using, for example, health-climate modelling.

Environments that have previously been unsuitable for certain arthropods may now become suitable, with the result that arthropods may be endemic in the future. In addition to the climate, changes in the environment may impact on vector distribution, too. Current publications on, for example, the distribution of Derma
center reticulatus ticks in Germany or sandflies in northern Italy may already be the documented signs on the changing landscape of arthropod-vector distribution. International tourism and travel – as humans often travel together with their four-legged companions – pose the risk of the relocation of arthropods, especially ticks, into environments where these ectoparasites have never been endemic.

In the UK, due to their island situation, dog numbers entering and re-entering are well documented by the so-called “Pet Travel Scheme” (PETS). Under the scheme, previously unknown vector-borne pathogens like L. infantum and Babesia canis are well documented. The spread of pathogens by the vector itself, or via the infected host animal to an unprotected, naïve population, is currently a European phenomenon for canine leishmaniosis. Asymptomatic dogs from Leishmania infantum-endemic areas are relocating by the thousands to Central and Northern European countries. The impact this has on both dog and human health, is currently not fully understood. Another example of relocation of animals and the impact on spreading diseases happened after hurricane Katrina. Homeless dogs have been shipped all over the United States in order to secure shelter for these animals. Knowing that the Gulf area is a highly endemic area for Dirofilaria immitis, one can easily imagine the number of unprotected, thus infected, dogs that are being relocated, too.

Today, with the veterinary medicinal ectoparasitcides and even some vaccines available, it is possible to reduce the risk of CVBD to a minimum. The principle of prevention has become firmly established in veterinary medicine and thus should be the basic rule, especially in small animal clinics. Prophylaxis is preferred over therapy. In the absence of effective preventative strategies against most of the vector-borne pathogens in most parts of the world, the logical consequence can only be ectoparasite control. Prevention, by means of repellent plus acaricidal/insecticidal efficacy, reduces the arthropod-host interaction, starting from first interaction of the arthropod with the host, to skin attachment and blood feeding, and can thus help to reduce the risk of infection. Prophylaxis of tick attachment and against sandfly bites must be in place in any dog either living in tick- and/or sandfly-endemic areas, traveling with their owners to such regions. The veterinary and medical view on vector-borne diseases fits well with the demand from a growing number of pet owners asking for the best standard of care for their animals. The veterinary and medical profession is the source of information not only for pet owners but the local society in general, in terms of infectious diseases (incl. CVBD), parasites and their zoonotic risks. The use of ectoparasiticides in companion animals should thus reflect the needs of the individual pet, its owner’s habits, the number of pets in the household and many other factors. Ectoparasite control in companion animals may thus lean more towards parasite management.

At the venue site of the 2nd CVBD Symposium in Sicily, the prevalence of canine leishmaniosis and a variety of tick-borne diseases is high. Within the scientific meeting, we will discuss the current scientific knowledge of these diseases, how we diagnose, how we prevent and how we educate pet owners and clinicians. This all happens under a real-world situation, with the dogs around the venue site to remind us instantly: “please doc, care for our well-being”.

Enjoy the conference!

Norbert Mencke
Bayer HealthCare AG, Animal Health
Head of Global Veterinary Services

References


Canine Vector-Borne Diseases website: http://www.cvbdb.org

Canine and Feline Anaplasmosis: Emerging Infectious Diseases

Introduction

Although tick-borne pathogens are of tremendous historical importance to both veterinary and human medicine, recent events emphasise an expanding role for newly discovered, as well as previously recognised tick-transmitted organisms, as a cause of animal and human suffering. One of the most important new developments related to anaplasmosis and ehrlichiosis is the realisation that a given mammalian species can be infected simultaneously or sequentially by several Ehrlichia species. As an example, both dogs and people can be infected with Ehrlichia chaffeensis, Ehrlichia canis, Ehrlichia ewingii and Anaplasma phagocytophilum (see reclassification below).

During the past decade, observations related to anaplasmosis and ehrlichiosis in animals have contributed substantially to the rapid expansion of new knowledge related to human anaplasmosis and ehrlichiosis. Increasingly, veterinarians in practice are being called upon to provide comparative medical information about anaplasmosis and ehrlichiosis in animals and to discuss the zoonotic risks that are attributable to members of the Anaplasma and Ehrlichia genera. Without question, the increased spectrum of human and companion animal recreational activities continues to bring each of us, as well as our pets, into contact with competent tick vectors. Therefore, so as to decrease disease transmission, drug manufacturers should continue to search for effective acaricides and products with strong repellent characteristics, so as to prevent tick attachment. These preventive efforts must occur in conjunction with the development of enhanced diagnostic techniques and improved therapeutic management of tick-transmitted diseases.

Reclassification of Anaplasma and Ehrlichia species

Genetic analyses of 16S rRNA genes, heat shock and surface protein genes have resulted in a reclassification of the genera Anaplasma, Ehrlichia, Cowdria, Neorickettsia and Wolbachia (Fig. 1). As a result, the genus Ehrlichia is now comprised of Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia muris and Ehrlichia ruminantium. The genus Anaplasma is now comprised of Anaplasma phagocytophilum, (previously Ehrlichia equi, Ehrlichia phagocytophilum or the human granulocytic ehrlichiosis, i.e. the HGE agent), Anaplasma bovis and Anaplasma platys. Ehrlichia risticii has been transferred to the genus Neorickettsia, which includes: Neorickettsia sennetsu, Neorickettsia helminthoeca, Neorickettsia risticii and the Salmon Fever agent.

Because of this reclassification, clinicians will have to reorganise the nomenclature that involves pathophysiology, diagnosis, treatment and preventive strategies related to these organisms. Although a currently cumbersome task, the recent reclassification will result in enhanced clarity, when considering similarities and differences amongst organisms in the same or different genera. The terms ehrlichiosis, anaplasmosis and neorickettsial infections continue to take on a new clinical meaning.

Comparative medical importance of Anaplasma and Ehrlichia species infections

Of comparative medical interest, cats, dogs, horses and humans, as well as other domestic and wild animal species, can all be infected with the same Anaplasma or Ehrlichia sp. For example, E. chaffeensis has been shown to infect dogs, goats, deer and human beings. Similarly, A. phagocytophilum can be detected in blood samples from a wide range of wild and domestic animals and can cause an acute febrile illness in cats, dogs, horses and humans. With the recent application of new molecular diagnostic techniques, the study of vector-borne disease problems has been enhanced. This technology continues to result in substantial clarification of the role of established agents in the pathogenesis of previously undiagnosed disease sequelae. In many respects, the immunopathogenic consequences of tick-borne infections, such as anaplasmosis and ehrlichiosis, are nearly identical amongst infected animals, as well as human patients. The experimental characterisation of the immunopathological response of a specific Anaplasma or Ehrlichia sp. in animals has provided important insights as to the potential pathogenic consequences induced when the same organism infects human patients. Conversely, observations in human patients have contributed to the recognition of an increased spectrum of disease manifestations in animals, such as acute renal failure or acute respiratory distress syndrome (ARDS) in dogs with ehrlichiosis, for example.
Feline Anaplasmosis and Ehrlichiosis

In general, our knowledge of tick-borne diseases in cats is substantially less than our knowledge of the comparable disease in dogs or human patients. Recent molecular evidence indicates that cats can be infected with *A. phagocytophilum* and an *E. canis*-like organism. The infrequent diagnosis of anaplasmosis and ehrlichiosis in cats may be related to a number of factors including a general under-recognition of tick-borne diseases in cats, decreased pathogenicity of tick-borne pathogens in cats as compared to other animals, or the more rapid removal of ticks from cats resulting in decreased opportunity for disease transmission. Most tick-transmitted pathogens require a 24 to 48 hour period of attachment to the host before there can be successful transmission of infectious organisms. Compared to other tick-borne pathogens, *A. phagocytophilum* has a relatively rapid transmission time following tick attachment. Fastidious grooming may result in the early removal of most ticks from cats and thereby the prevention of disease transmission.

Although various *Ehrlichia*, *Anaplasma* and *Neorickettsia* species have been reported to cause disease in cows, sheep, dogs, horses and human beings, the role of any specific species as a pathogen in cats remains less clearly defined. The first evidence for naturally occurring feline ehrlichiosis was provided by Charpentier and Groudale in France. Feline ehrlichiosis was subsequently reported in 1989, by Buoro and colleagues, when they described intracytoplasmic inclusions in monocytes and lymphocytes derived from three cats in Kenya. By both light and electron microscopy, the inclusions were morphologically similar to *Ehrlichia* sp. morulae, as observed on blood smears obtained from other animals. Subsequently, morulae were described in stained blood smears obtained from cats in the United States, France, Brazil and Serbia. To date, no *Ehrlichia* species has been cultured from the blood of a cat, however, Bijorisdoff and colleagues amplified and sequenced 16S rDNA from an EDTA blood sample obtained from a 14-month-old shorthaired cat from Sweden that was 100% similar to canine and equine *A. phagocytophilum* strains from the same region.

In dogs and people with ehrlichiosis, bone marrow cytopenia can vary substantially, particularly in relation to the duration of infection prior to sampling. The mechanisms by which ehrlichial organisms induce changes in the bone marrow, particularly hypoplasia or myelofibrosis, is poorly understood. The extent to which immunosuppression should be used concurrently with doxycycline in *E. canis*-infected cats remains uncertain. However, ehrlichial infection should be considered as a differential diagnosis in cats with bone marrow hypoplasia, particularly when accompanied by dysplastic changes. When used concurrently, corticosteroids or other immunosuppressive drugs may interfere with the therapeutic effectiveness of doxycycline for the elimination of *E. canis* in cats. Therefore, whenever possible, treatment with only antibiotics (doxycycline) should be attempted.

To further define the spectrum of feline anaplasmosis and ehrlichiosis, PCR testing will be necessary, until such time as serologic testing is thoroughly validated in experimentally or naturally infected cats. In addition, *E. canis* has been isolated from cats and several isolates are available from disparate geographic regions for detailed comparative genetic study, the molecular evidence presented supporting *E. canis* infection in cats must be interpreted with caution. As tick exposure was not clearly established in these cats, it is possible that an *Ehrlichia* genotype, with complete or partial 16S rDNA homology with *E. canis* is capable of infecting cats and may have evolved with a different mode of transmission, as compared to tick transmission of *E. canis* to dogs.

Canine Anaplasmosis and Ehrlichiosis

Canine ehrlichiosis is an infectious rickettsial disease of dogs, caused by *E. canis*, *E. chaffeensis* and *E. ewingii* and potentially *E. ruminantium*. Investigators from South Africa have obtained molecular evidence (16S rDNA sequencing) that supports infection of dogs and people with an organism that is identical or closely related to *E. ruminantium* (previously *Cowdria ruminantium*). The implications of this recent finding could prove to be of great importance, if *E. ruminantium*, the organism that causes Heartwater in cattle in Africa, was introduced into the United States by way of dog transport.

Although the clinicopathologic course of disease will vary depending upon the infecting *Ehrlichia* species, illness is typically characterised by an acute reduction in cellular blood elements, most often thrombocytopenia. Historical synonyms for canine ehrlichiosis have

![Fig. 2: Six-year-old male hound-mixed dog evaluated because of chronic lameness and neutrophilic polyarthritis that was co-infected with *E. chaffeensis* and *E. ewingii* based upon PCR testing.](image-url)
Canine ehrlichiosis, caused by *Ehrlichia canis*, is documented only within the United States. All three Ehrlichia spp. are encountered most frequently in dogs living in the southern states. Based upon recent research, canine ehrlichiosis may be attributable to concurrent infection with a Bartonella species. In addition to clinical signs, which may be suggestive of ehrlichiosis, mild to severe laboratory abnormalities can contribute to the index of suspicion for the disease. Haematologic abnormalities, including pancytopenia, aplastic anaemia or thrombocytopenia, would be consistent with *E. canis* infection. Thrombocytopenia is the most consistent haematologic abnormality in both the acute and chronic stages of ehrlichiosis. However, some dogs will have normal or increased platelet counts. Pancytopenia is documented in less than 25% of cases in retrospective clinical studies. Because *E. canis* causes defects to platelet function, bleeding can be detected in dogs with normal or mildly suppressed platelet counts. Lymphocytosis, which can be profound and mimic lymphocytic leukaemia, has been observed in dogs with ehrlichiosis. Finding *E. canis* morula in peripheral blood smears or buffy coat smears is diagnostic, however, morula are found only during the first two weeks following infection and generally in very low numbers. Anaemia, if present, will vary in degree of severity amongst affected dogs.

Positive Coombs' tests suggest that immune-mediated damage, due to circulating anti-erythrocyte antibodies, can contribute to an acute haemolytic crisis in some dogs with ehrlichiosis. In this situation, a regenerative anaemia may be encountered, however, a non-regenerative anaemia is most frequently documented in chronically infected dogs. Serum proteins are abnormal in approximately half of reported canine ehrlichiosis cases. Hyperglobulinemia is characterised by increased beta and gamma globulins. Serum protein electrophoresis may reveal a polyclonal or monoclonal gammapathy. A monoclonal gammapathy may be attributable to concurrent infection with a plasma cell myeloma. Hyperglobulinemia occurs in one in five dogs with chronic ehrlichiosis, in dogs with a normal or a reciprocal decrease in albumin associated with progressive hyperglobulinemia. Less frequently encountered laboratory abnormalities include increased alanine aminotransferase, serum alkaline phosphatase, total bilirubin, azotemia and proteinuria. *Ehrlichia* canis does appear to induce a protein-losing nephropathy, most likely related to immune complex glomerulonephritis.

Serologic diagnosis utilising the indirect fluorescent antibody technique (IFA) is currently recommended for confirming a diagnosis of ehrlichiosis. The IFA test for *E. canis* is sensitive and specific, however, based upon Western blot (WB) analysis, low IFA titers are not diagnostic and may represent exposure to other infectious organisms. Current modalities to detect *E. canis* antibodies in serum samples obtained from dogs for diagnostic purposes, such as the microimmunofluorescent assay (MIFA), do not facilitate differentiation of the infecting *Ehrlichia* species. There is substantial serologic cross-reactivity between *E. canis* and *E. chaffeensis*, whereas *E. ewingii*-infected dogs generally do not recognise *E. canis* antigens or do so at very low titers. Serologic cross-reactions to *E. canis* antigens have not been reported in association with *Rickettsia rickettsii*, *Babesia canis*, *A. platys* or *A phagocytophila*. Due to extensive serologic cross-reactivity, a positive *E. canis* IFA titer is indicative of infection with *E. canis*, *E. chaffeensis* or to a lesser extent *E. ewingii*.

Dogs generally become seronegative within 3 to 9 months after effective treatment, although some dogs maintain persistently stable titers for years. Although the clinical utility has not been clearly established, polyclonal bone marrow reaction (PCR) amplification can facilitate a molecular confirmation of the diagnosis of canine ehrlichiosis, determine the infecting *Ehrlichia* species or help to confirm the therapeutic elimination of infection. EDTA blood is

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**Clinical manifestations, associated with the chronic phase of the disease, would be characterised as mild to absent in some dogs, while severe and life-threatening in other dogs. For example, it is not unusual in endemic areas to detect haematologic abnormalities due to chronic *E. canis* infection in clinically healthy dogs being evaluated for heartworm adulticide therapy. Undetected thrombocytopenia in these patients might potentiate the severity of pulmonary haemorrhage associated with thromboembolism. A combination of bleeding tendencies, palpable due to anaemia, severe weight-loss debilitation, abdominal tenderness, anterior uveitis, retinal haemorrhages and neuropsychiatric signs consistent with meningoencephalitis typify dogs that are chronically affected. Immunosuppression has been historically attributed to infection with *E. canis* in dogs, however, a recent experimental infection study did not induce detectable immunosuppression.**

Secondary bacterial infections may be documented. Numerous patterns of haemorrhage may occur in dogs with ehrlichiosis. Epistaxis, once considered a hallmark of the disease, occurs infrequently in dogs in the United States and may be attributable to concurrent infection with a Bartonella species.

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required for PCR and should optimally be collected prior to or after cessation of antibiotics.

Tetracycline (22 mg/kg given every eight hours) or doxycycline (5 mg/kg every twelve hours), administered daily for four weeks, represent the treatment of choice for canine and feline ehrlichiosis.13,20 Oxytetracycline is also effective, but is nephrotoxic. Clinical improvement may be observed with penicillin, sulfonamides, enrofloxacin or imidocarb dipropionate, but the therapeutic response is incomplete and, therefore, these antibiotics cannot be recommended.13,21 Dramatic clinical improvement generally occurs within 24 to 48 hours after initiation of a tetracycline derivative in dogs with acute phase or mild chronic phase disease. Haemorrhage, immunosuppression and concurrent infections with Babesia or Bartonella species may contribute to the death of chronically affected dogs despite the initiation of tetracycline therapy. The duration of treatment of chronically affected dogs with severe pancytopenia or aplastic anaemia is controversial. Despite clinical improvement and clearance of infection, bone marrow regeneration may require up to 120 days following treatment. Supportive therapy, including fluids, blood transfusion, vitamins and anabolic steroids are required in some patients. Long-term tetracycline prophylaxis (6.6 mg/kg once daily), reposi tolytetracycline (200 mg IM twice weekly) or doxycycline have been utilised in military working dogs or dogs maintained in tick-infested kennels to prevent ehrlichiosis. Following therapeutic elimination of the organism, dogs do not develop protective immunity and can be reinfected when reintroduced to a vector-competent tick.

Experimentally, dogs have been reinfected by both homologous and heterologous challenge. Tick control is critically important, but does not assure prevention of the disease or elimination of reinfection. Although not well characterised, the long-term prognosis following treatment for ehrlichiosis does not appear to be predictable.15 The reasons for variability in post-treatment outcomes remains to be established through long-term follow-up studies.

**Simultaneous infection with multiple vector-transmitted pathogens**

Recently, simultaneous infection with more than one tick-borne pathogen has been recognised with increasing frequency in human and canine patients (fig. 4).

![fig. 4: Three-year-old female spayed Labrador retriever that was referred for evaluation of chronic polyarthritis, seizures, epistaxis and endocarditis. The dog was co-infected with *Ehrlichia canis* and *Bartonella* spp. (berkhoff)](link)

Obviously, simultaneous infection with more than one tick-transmitted pathogen has important diagnostic, therapeutic and prognostic implications for the individual patient. The pathophysiologic consequences of co-infection in dogs with various combinations of bacteria, rickettsia and protozoa have not been characterised clinically or experimentally. Although retrospective seropidemiologic studies suggest that dogs may experience simultaneous infection with multiple tick-borne pathogens, microscopic (culture) or molecular (PCR) evidence of such infection in dogs is currently limited. In nature, the risk of exposure to ticks, fleas, mosquitoes and biting flies is far greater for dogs than for human beings. In addition, dogs can be infected with hundreds of ticks, and at times infestation may involve different tick species. Therefore, the unknown influences of concurrent infection with multiple tick-borne pathogens, including *Anaplasma, Ehrlichia, Bartonella, Babesia* and *Rickettsia* species, on factors such as pathophysiology, diagnosis, prognosis or therapeutic outcome, could be more readily characterised in dogs. Of 27 dogs that were investigated in a kennel due to increased mortality, 25 were seroreactive to an *Ehrlichia* sp., 20 to a *Bartonella* sp., 17 to a *Babesia* sp. and 22 seroconverted to *R. rickettsii* antigen.11 Based upon PCR analysis, several dogs were co-infected with multiple *Ehrlichia* species, as well as a *Bartonella*, *Babesia* or *Rickettsia* species. Prospective evaluation of sick dogs, managed in our teaching hospital, has yielded molecular evidence of co-infection with multiple tick-transmitted pathogens. Our recent experience indicates that dogs with heavy tick exposure can be infected at a high rate with multiple, potentially zoonotic, tick-borne pathogens.

**Causality and infection with vector-borne pathogens**

From an evolutionary perspective, it is obvious that vectors, vector-borne organisms and animal and human hosts have developed a highly adapted form of interaction. In general, vectors need blood for nutrition. Bacterial, rickettsial and protozoal organisms need an intracellular environment to survive. Immunologically, most hosts appear to be able to support chronic infection with many vector-borne organisms for months to years without obvious deleterious effects. For these reasons, establishing causality associated with highly fastidious vector-transmitted pathogens will remain a challenge for the foreseeable future. As recent serologic and molecular evidence indicates that co-infection in dogs with *Anaplasma, Ehrlichia, Bartonella*, *Rickettsia* and *Babesia* spp., may be more frequent than previously realised, the extent to which infection with *Bartonella* affects the pathophysiology of ehrlichiosis, a disease of much longer historical venue, deserves critical reappraisal. For example, infection with *Bartonella* in dogs concurrently infected with *Ehrlichia canis* may contribute to the tendency to develop epistaxis.10 Historically, epistaxis has been attributed to ehrlichiosis, rather than bartonellosis.

**Zoonotic implications of Anaplasmosis and Ehrlichiosis**

Based upon isolation from patients, *E. canis*, *E. chaffeensis* and *E. ewingii* can all cause human ehrlichiosis. *Anaplasma phagocytophilum*, transmitted by *Ixodes scapularis*, *I. pacificus* and *R. ricinus* can also infect people and induce disease manifestations that are very similar to those caused by *Ehrlichia* spp. However, the zoonotic role of dogs as a reservoir for human infection has not been clearly established for any *Ehrlichia* species. In South America, *E. canis* causes human monocytic ehrlichiosis and dogs are the probable reservoir host. It is probable that deer, rodents and other small mammals serve as the major reservoir for other *Ehrlichia* sp., with dogs playing only a minor role in the maintenance of the organism in a given geographic location. Recently, the detection of *E. chaffeensis* DNA by PCR amplification provided the first documentation for natural infection of dogs residing in animal shelters or in a kennel in south-eastern Virginia. Subsequently, we documented *E. chaffeensis* infection in dogs that was clinically and serologically indistinguishable from *E. canis* or *E. ewingii* infection.9 Treatment with doxycycline resulted in therapeutic elimination of *E. canis*; however, based upon species-specific PCR amplification, *E. chaffeensis* DNA could be detected in all three dogs for up to one year following treatment, potentially due to frequent re-exposure and reinfection by *E. chaffeensis*-infected *Amblyomma* (fig. 5). The clinical or zoonotic implications of this observation await additional clarification.

![fig. 5: *Amblyomma americanum* ticks, which are prevalent throughout the south-eastern United States, transmit *Ehrlichia chaffeensis* and *Ehrlichia ewingii* but are not known to harbor *Anaplasma phagocytophilum*.](link)
References

Spread of Companion Animal Vector-Borne Parasitic Disease in the US and Europe: Concerns relative to Travel, National Disasters, Shelter-source Animals and Wildlife

Abstract

This paper discusses three vector-borne animal parasitic diseases that occur in the United States: canine heartworm, canine visceral leishmaniosis and feline cytazuxoniosis. The paper examines the history of the spread of the infections, the association of the spread with various wildlife species and the potential of spread of these and similar infections in Europe. Heartworm is now endemic throughout the United States, having taken only about 50 years to reach all the 50 states of this country; the United States has a wildlife reservoir host, the coyote, that has probably facilitated the spread of this parasite on the North American continent. Visceral leishmaniosis seems to be making inroads into the United States, but it remains unclear why infections are disproportionate in some areas. It appears that as people are moving into more and more areas with “natural” surroundings, and as the population of the bobcat increases in many areas, that more and more cats are becoming infected with this parasite over a wider geographic range.

Heartworm disease

In the case of heartworm, the spread of the infections has been dramatic. For what was probably some 450 years, the disease was fairly well restricted to the southeastern states of the United States. Then, in a matter of 50 years, it spread to the point where it is believed that autochthonous transmission may have occurred in all 49 states of the US in North America and most of the Canadian provinces. The story of the spread of American heartworm (Fig. 1) is an example of how a vector-borne disease can rapidly engulf a continent in a short period – even in the face of highly successful pharmaceutical agents for its prevention.

Although there is no reason to believe that heartworm had not come to the United States soon after the discovery of the Americas, heartworm was first identified in a dog in the United States in 1847.11 In 1936, Maurice C. Hall first called attention to the beginning of its spread when he noted that the development of “speedy modern transportation” had been the cause of its appearance in New York, New Jersey, Pennsylvania and Illinois.11 In 1950, it was reported that more and more cases were being seen in veterinary practices in the north and west, due to increased awareness by the veterinary community and because of the increased transport of southern dogs for hunting and breeding, and the movement of dogs...
belonging to army personnel.19 Survey results published in 1955, reported the enzootic area of infection being around large bodies of water (lakes and rivers) and mainly along the eastern coast and the Mississippi River.14 Roncalli felt that the development of the Eisenhower Interstate highway system, after WWII, greatly contributed to the diffusion of heartworm disease, and changed its geograph- ic distribution in the United States.15,16

Early in its spread, one of the surprise appearances of heartworm was in the northern state of Minnesota,20,21 a state with cold winters and hot summers (the record temperatures span 79 degrees C), and depending on location, the average annual precipitation is from 48 to 187 cm. In this state, the veterinary dogma in 1956 had been that “the less temperate climates of the northern latitudes in the United States were thought to prevent transmission of the parasite by its culicine vectors.” In 1937, several dogs were found infected with heartworms in the area of Minneapolis and Saint Paul, twin cities near the northern source of the Mississippi River. Suddenly, in 1956, a canine heartworm epizootic was noted in this area, and a survey in 1960, revealed that it was restricted to the greater Metropolitan area. From 1960 to 1977, the disease spread throughout much of the state known as the “Land of 10,000 Lakes”.

Heartworm was first reported from California, from near San Francisco, in indigenous dogs that had never left the region in 1949.22 The next report was not until 1970 (McGreevy et al., 1970). 1 of 800 dogs housed outdoors in a Beagle colony in Davis California, and 13 native dogs with no history of travel from ten northern counties.23 It has since been accepted that heartworm is present in three foothills regions of central California: the foothills of the Sierra Nevada, the North Coast Range and the South San Francisco Bay ranges. It has now been reported in autochthonous dogs from Los Angeles County,24 and from San Diego County.6 Thus, in about 50 years, heartworm has spread throughout the state of California.

One reason heartworm may have spread through California is the burgeoning coyote population. The coyote, Canis latrans, is a canid that is indigenous to the Americas and, unlike the fox, will support the growth and development of Dirofilaria immitis. It has been shown that heartworms have increased in the coyote population in the northern California counties around San Francisco between an initial sam- pling in 1975 and 1980 and a more recent survey in 2000 to 2002.25 In the 2000 to 2002 survey, some 24 % to 57 % of the coyotes were infected. A survey of coyotes from throughout the state of California has revealed that some 5 % to 60 % of the coyote population is infect- ed and spread throughout the state; thus, there are now unprotected reservoir hosts through- out the state.24

In Canada in 1976, heartworm was endemic in a single area around Windsor, Ontario, across from Detroit, MI, with two small foci also around Brantford and Sarnia.25 Then, new foci appeared increased in size, affecting dogs found in an increasingly wider area of southern Ontario. The first focus to appear in Canada in 1976 was on the southern border of Winnipe where it initially remained small and began to decrease, but then the focus increased and dogs were found infected across southern Manitoba. In Quebec, the original focus in Montreal expanded towards the west. So, in a quarter of a century, heartworm became indigenous throughout most of the Canadian provinces.

The concern about heartworm spread was focused on the US humane societies and shelter systems after the devastation that occurred after hurricane Katrina struck New Orleans, Louisiana. It was discovered by those working in the triage units in Louisiana that up to 80 % of the dogs in the shelters were heartworm-posi- tive. It is estimated that some 5,000 to 10,000 dogs or more were shipped to other parts of the nation to different animal shelter systems. Some shelters had trouble accepting the dogs, because owners and communities were upset that infected dogs were placing other dogs at risk in areas with low levels of heartworm where many local dogs were not on prevention (Kate Hurley, UC Davis, personal communica- tion). This was a case of massive awareness, because the dogs all moved at once from a highly endemic area, but many dogs move every year, perhaps even a similar number of dogs, with families, military personnel and visiting students and tourists. It should also be remembered that some 50 % of these dogs also had hookworms,19 some had whipworms and an unknown number may have had various other “tropical” diseases such as Ehrlichia, Rickettsia or Babesia.

In Europe, heartworm still appears to be con- fined, for the most part, to Southern European countries. Portugal, Spain, France, Italy, and Slovenia, Bulgaria, Romania, Greece and Turkey.26 Gench et al. (2005) summarise the cur- rent situation in Europe and point out that infections in Serbia and Croatia have increased from sporadic to occurring, and cases are now appearing to occur autochthonously in south- ern Switzerland. Gench’s group then presents maps showing where and when transmission could theoretically start and stop seasonally each year in parts of Europe. There is not a great deal of Europe not included on the maps, and as people travel more and more with their pets, there may be an increased risk of spread. Maybe, Europe will, not need to worry because of the lack of a good reservoir host such as the coyote in North America. However, some coun- tries are approaching the condition with cau- tion and Sweden has put in place guidelines such that dogs being brought into Sweden from other EU countries will besides being required to have ID-marking, rabies vaccination, blood sampling and de-worming for tapeworms, also require protection against distemper, canine hepatitis, canine parovirus infection and lep- tosiosis and treatment to eliminate Rhipice- phalus sanguineus and heartworms.10 The exist- ing highly effective preventives and the lack of a good reservoir for heartworm in Europe would suggest that the proactive approach would not be the study of the spread of the infection and worrying about what new coun- tries are developing autochthonous spread, but rather an eradication plan to remove the “cruel” filiarid from Europe.

Visceral leishmaniosis

Autochthonous visceral leishmaniosis in dogs (fig. 2) in the United States has a fairly long but confusing history that stretches back some 27 years. There seems to be two major sets of dogs, foxhounds and sporadic cases in dogs other than foxhounds. Transmission is unexplained in almost all the cases. In all the cases where the dogs had been typed, they have been found to be Leishmania infantum.

The first report in foxhounds was from Oklahoma in 1980.27 Four of 16 dogs in a kennel in Oklahoma were infected based on serology and culture. It turned out that some of these dogs had been born in a kennel in Kansas and some were from a third kennel in Okla- homa, and some were from either the kennel in Kansas or other locations. All in all, there were 55 foxhounds examined with 17 being positive on serology, and of these, 13 were positive on culture. In 1988, an English Foxhound from a closed research colony was diagnosed as infect- ed with visceral leishmaniosis.28 In 1989, a Foxhound from a hunting club in Michigan was diagnosed with visceral leishmaniosis, and serology on the other 64 dogs in the club kennel revealed four additional dogs that were positive, and of these, two had organisms iso- lated by culture. Serological testing at the club 8 to 24 months later revealed that 16 dogs in the club seroconverted after the initial serolog- ical assessment.29 Two other hunt clubs in the area were sampled, and all dogs were negative. In 1991, 5 of 48 foxhounds from a hunt club in Alabama were shown to be positive. Then in 2000, visceral leishmaniosis was diagnosed in 46 of 112 foxhounds in a club in Dutchess County New York, and further examination revealed several thousand dogs infected in foxhound hunt clubs in some 21 US states and two Canadian provinces.30 New cases kept turning up during the investigation, suggesting that active transmission was occurring throughout the period of examination. People and other breeds of dogs at the clubs were not infected. There have been other cases of visceral leish- maniosis somehow acquired autochthonously in the United States. A Basenji in Texas in 1991, was found infected; this dog was only five months old.31 In 2000, a Toy Poodle was found infected in Maryland (Eddlestone, 2000). In 2000, a Newfoundland was admitted to Cornell University that was found to be infected with visceral leishmaniosis, and the dog had never travelled further than Canada.32 This New- foundland had been housed for a period of time with another intact Newfoundland male.
that had been born in Italy via caesarean section from a known infected dam; this male later died. In 2001, a female Spinone Italiano was diagnosed in NY as being infected with visceral leishmaniosis; this dog was born in Italy via caesarean section and was later found to have leishmaniosis in North Carolina. Serology on 300 additional Spinone Italianos breeds of dogs in the US and Canada did not reveal any additional infections. A Cocker Spaniel was diagnosed as infected at Auburn University, and it was later found that it had lived with its owners in Italy, along with another Cocker Spaniel that was euthanised due to chronic renal failure. A Beagle that had lived with the infected Cocker Spaniel, along with another dog brought into the home soon after the Cocker Spaniel died, both became seropositive and had leishmanial DNA isolated from their bone marrow. A Dobermann pinscher from Massachusetts was found to be infected with visceral leishmaniosis; the dog’s parents were both from Italy and the dam was later shown to be seropositive.

As of yet, a vector has not been identified in the area of New York where the large number of foxhounds in Dutchess County were found to be infected. However, many of these foxhounds travelled extensively to hunt, and shared facilities with many other dogs from around North America. The lack of vectors in some areas, the possibility of congenital transmission, the potential of direct transmission by contact and, perhaps, the reuse of needles for multiple vaccinations have all been considered as potential sources of transmission in dogs, but as of yet, the epizootiology of canine visceral leishmaniosis in the United States remains very unclear.

In Europe, it appears that there is a northward spread of leishmaniosis from its current Mediterranean distribution. There is also a wildlife reservoir for this infection in the red fox, Vulpes vulpes, that has the capability of serving as a very efficient source of infection with very high prevalence rates. The increase in the red fox population could serve also as a means of enhancing the spread of this zoonotic disease within Europe, much as it has done for Echinococcus multilocularis. Thus, it would seem that now would be a time to be vigilant about this disease in areas where it has not been apparent in the past, both in the dog and the fox populations.

Cytauxzoonosis

Cytauxzoon felis (fig. 3) is transmitted to cats from bobcats, Lynx rufus, by the bite of the American Dog Tick, Dermacentor variabilis. This parasite first appeared in domestic cats in Missouri in 1973, and the concern at that time was that it was an introduced species of importance to agriculture, perhaps related to Theileria parva. It was also, at this time, described as a new species: Cytauxzoon felis (Kier, 1979). Two life cycle stages, schizonts and merozoites, occur in the feline host. Schizonts are found in histiocytes and macrophages of the bone marrow, and veins and venules of various organs, including the lungs, liver, spleen, lymph nodes, brain and kidneys. Merozoites occur in circulating red blood cells later in the infection, and therefore, often will not be found in cats dying of acute disease.

The bobcat is the natural definitive host of C. felis. In domestic cats that are infected through the bite of the tick vector, schizonts develop within macrophages, which become markedly enlarged. Cats with acute disease typically develop anemia, depression, fever, dehydration and icterus. The majority of cats die within 9 to 15 days of infection. The cause of death is occlusion of veins and venules with schizont-laden macrophages. Haematologic changes may be severe, and result from displacement of haematopoietic tissue within the bone marrow. If the cat survives for more than six days, erythrocytes become infected and the merozoite stage develops, typically with no more than 1% to 4% of red blood cells infected. In the case of the bobcat, the schizogonous stage is shortened, and they become prolonged carriers of the erythrocytic stage (Blouin et al., 1987). In a recent survey of cases from the mid-Atlantic states of the US, of the 34 cats infected with C. felis, 32 succumbed to the infection. The most common signs are pancytopenia and icterus. During the acute stage, organisms can be identified in smears of bone marrow or in biopsy specimens. Chronic disease is diagnosed by finding the merozoites in red blood cells. No treatments are consistently efficacious during the acute stage of the disease. Antiprotozoals with supportive care and antibiotics are often administered, but the prognosis remains poor. This is a disease that is currently best prevented by keeping cats indoors in areas where the ticks are active or using products that will kill or repel ticks.

Natural cases of cytauxzoonosis in cats have typically been described from the south-eastern and south-central United States, with cases being reported from Kansas, Oklahoma, Missouri, Arkansas, Texas, Louisiana, Mississippi, Georgia and Florida.

As more and more people move into housing developments on the fringes of tick areas, it can be assumed that more and more cases of CYB and other tick-transmitted diseases will be seen. This is due in part to the continued expanding range of the American bobcat, particularly in the Midwestern and several mid-Atlantic states (U.S. Fish and Wildlife Service website). The range is increasing and the numbers are increasing. It is estimated that there are now at least some 1.5 million bobcats in the United States, but this may be an underestimate due to poor animal sighting abilities by census takers. The annual harvest for fur was about 35,000 per year in the mid 1990s (US Fish and Wildlife Service website). In recent years, there has been an increase in the harvest due to the demand of the fashion industry, but this does not seem to be decreasing populations in any areas. The expanding range of the host of the parasite and the vector would be expected to expand the range of both agents. Thus, it should be expected that cats will be under greater and greater risk of disease due to this pathogen throughout the United States.

Conclusions

The consideration of these and other parasitic diseases make it easy to view the inter-connection of all sorts of different inputs in the outcome of emerging disease. The activities damming against the wearing of fur and the protection of wildlife may have increased the number of cats dying from cytauxzoonosis, and now the fashion industry’s ability to make fur “trendy” again may work to kill bobcats, but reduce risk to cats. In the United States, foxes have been multiplying to very large populations, not because of wildlife rabies control, but because there has not been an epizootic in foxes since the 1950s. The increased fox populations may increase the potential of the establishment of a sylvatic cycle of leishmaniosis (and as in Europe, echinococcosis). Heartworm has

Fig. 3: Cytauxzoon Felis. Histological selection (H&E) of the lung of the cat that died of cytauxzoonosis showing the enlarged macrophages containing the schizonts in the larger blood vessels.
spread throughout the United States, helped by travel, people moving pets, coyotes and the military. This may have been unfortunate, most of the spread seems to have occurred after the awareness of the severity of the disease and the ability to prevent the disease was available with diethylcarbamazine and then macrocylic lactones. Hopefully, Europe will be more proactive and work towards containment and eradication rather than being complacent and waiting to see what happens.

References

Dermacentor reticulatus in Germany and the Spread of Canine Babesiosis

Abstract

In Germany canine babesiosis was usually regarded as a “travel-related disease” in dogs brought from countries bordering the Mediterranean sea or parts of Eastern Europe, such as Hungary. Within the past 15 years, there have been a few cases of autochthonous babesiosis reported in dogs which had never left Germany. In this study, 2,322 Dermacentor ticks were collected by the public between September 2004 and December 2006, from all over Germany. All ticks used for this study were unfed adult ones. Those ticks were screened by PCR for the presence of Babesia sp., twelve of these ticks (0.52 %) were Babesia sp.-positive. In addition to the figures, a distribution map is presented.

Introduction

About 40 years ago Dermacentor reticulatus (Figs. 1, 2) was a rare species in Germany.3,21 It was assumed that specific biotopes are missing. In the 1970s, the first local foci of Dermacentor reticulatus were discovered.15,17 At the end of the 1970s, it was stated that babesiosis is of minor significance in Germany.4 Ten years later the first autochthonous babesiosis focus was reported from south-western Germany (Odenburg/Lahr/Freiburg). D. reticulatus was suspected to be the vector.7 For a few years more babesiosis cases were reported also in other parts of Germany. Now babesiosis is known to be endemic in Germany.5,33,24

There is more or less only one recent publication available covering the distribution of Dermacentor ticks in eastern Germany.1 A little more is known about the local occurrence of D. reticulatus in some foci, such as oak woodland or heath in eastern Germany.15,17 or some areas along the Rhine valley in south-western Germany.5,15,24

One aim of this study was to get an overview of the current distribution of Dermacentor ticks in Germany.

Canine babesiosis was imported from Hungary and the Mediterranean countries into Germany more than 20 years ago.5,12 Dermacentor reticulatus is thought to be the vector and reservoir of Babesia canis and the Mediterranean tick Rhipicephalus sanguineus is thought to be the reservoir and vector of Babesia vogeli.11 Two further Babesia species are known to be endemic in Germany, Babesia microti and B. divergens.14 The latter are thought to be transmitted by Ixodes ricinus.11

Another aim of this study was the isolation and determination of Babesia sp. from adult Dermacentor ticks. Only ticks, which had not yet taken a blood-meal, were investigated, to be sure that these ticks were not contaminated by infected dogs.

Material and methods

In Summer 2004, the project to collect Dermacentor ticks was launched. To get representative results for the whole of Germany, the public (with the main focus on dog owners) was asked to collect Dermacentor ticks in nature and from their pets. The appeal was started with the distribution of “wanted” circulars via the Internet. The circulars were mainly found on the homepages of the different associations of animal lovers, but were also quickly spread by discussion groups. The next step was the distribution of “wanted” posters amongst approximately 3,000 veterinary practices, to be placed in the waiting rooms. A number of different articles on the subject were also published in magazines.15,20,21 Finally a film was produced and was transmitted, completely or in part, at least eleven times, and reached around four million individuals.

The ticks were delivered by post and the collectors were requested to describe the biotopes of the ticks as accurately as possible. All ticks were catalogued and the species of each was determined,13 and then kept in 70 % alcohol. They were then stored at 4°C for further investigation, where they were analysed by PCR for the presence of Babesia sp. All Babesia-positive samples were then sequenced.

<table>
<thead>
<tr>
<th>Number</th>
<th>Location</th>
<th>Female</th>
<th>Male</th>
<th>D. reticulatus</th>
<th>Babesia spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 a</td>
<td>Prenden (forest)</td>
<td>x</td>
<td>x</td>
<td>Babesia canis</td>
<td></td>
</tr>
<tr>
<td>107 a</td>
<td>Magdeburg</td>
<td>x</td>
<td>x</td>
<td>Babesia microti</td>
<td></td>
</tr>
<tr>
<td>111 a</td>
<td>Schönbuch (Tübingen)</td>
<td>x</td>
<td>x</td>
<td>Babesia canis</td>
<td></td>
</tr>
<tr>
<td>164 a</td>
<td>Charlottenburg (Berlin)</td>
<td>x</td>
<td>x</td>
<td>Babesia vogeli</td>
<td></td>
</tr>
<tr>
<td>202 a</td>
<td>Leipzig</td>
<td>x</td>
<td>x</td>
<td>Babesia microti</td>
<td></td>
</tr>
<tr>
<td>212 e</td>
<td>Elbtal</td>
<td>x</td>
<td>x</td>
<td>Babesia vogeli</td>
<td></td>
</tr>
<tr>
<td>219 b</td>
<td>Viernheim (Hessen)</td>
<td>x</td>
<td>x</td>
<td>Babesia canis</td>
<td></td>
</tr>
<tr>
<td>225 a</td>
<td>Bensdorf (Brandenburg)</td>
<td>x</td>
<td>x</td>
<td>Babesia canis</td>
<td></td>
</tr>
<tr>
<td>261 a</td>
<td>Ranndorf (Teltow)</td>
<td>x</td>
<td>x</td>
<td>Babesia microti</td>
<td></td>
</tr>
<tr>
<td>283 a</td>
<td>Roedental/Einberg</td>
<td>x</td>
<td>x</td>
<td>Babesia canis</td>
<td></td>
</tr>
<tr>
<td>332 d</td>
<td>Bensdorf (Brandenburg)</td>
<td>x</td>
<td>x</td>
<td>Babesia canis</td>
<td></td>
</tr>
<tr>
<td>352 j</td>
<td>Aken</td>
<td>x</td>
<td>x</td>
<td>Babesia canis</td>
<td></td>
</tr>
</tbody>
</table>

Tab. 1: Babesia sp.-positive ticks.
Results

Most of the Dermacentor ticks were collected in the areas of Berlin, Magdeburg, Leipzig, Frankfurt/Main and Tuebingen. *D. marginatus* was found exclusively in Baden-Württemberg. Fig. 3 demonstrates the *Dermacentor* sp. distribution in Germany.

12 out of 2,322 ticks investigated were positive for Babesia sp. From seven ticks *Babesia canis* could be sequenced, three ticks were positive for *Babesia microti* and two ticks for *Babesia vogeli* (fig. 4). Nine of these were males. Tab. 1 shows the details of Babesia sp.-positive ticks.

All these Babesia-positive adult *Dermacentor reticulatus* ticks were unfed and should, therefore, have acquired their Babesia infection in their larval or nymphal stage or transovarial.
References

Changing Paradigms in Understanding Transmission of Canine Tick-Borne Diseases: the Role of Interrupted Feeding and Intrastadial Transmission

Overview
A large number of different pathogens are transferred from infected ticks to dogs following tick attachment and feeding. However, transmission of tick-borne disease agents does not appear to occur immediately when the tick begins feeding for the first time in a given life cycle stage. Rather, a reactivation period is required for the pathogens to begin replicating, migrate to, or be activated in, the salivary glands, and be transmitted to the host when the tick regurgitates excess fluid back into the bite wound. In most tick-borne disease systems, an initial attachment and feeding period of at least 24–48 hours is thought to be required to allow transmission of most tick-borne pathogens and subsequent transmission. However, some data suggest that, in nature, there may be exceptions to this apparent rule for some tick-borne disease agents that relate to both innate characteristics of the pathogens themselves and behaviour and biology of the respective tick vectors. Understanding the timing involved in the dynamic process of acquisition and transmission of tick-borne disease agents is critical to the formation of accurate, effective recommendations that can help protect both pets and people from infection with tick-borne pathogens.

Biology of ticks transmitting canine disease agents
Pathogens are transmitted to dogs by a variety of different species of ixodid (hard) or argasid (soft) ticks, each of which has a distinct life history pattern. For example, within the hard ticks, the prostriate Ixodes spp., responsible for transmitting Borrelia burgdorferi and Anaplasma phagocytophilum to dogs, may mate prior to infestation and feeding.1,2 Because they can mate off the host, Ixodes spp. males are often present in fewer numbers than females on a given infested animal;3,4 indeed, in some nest-dwelling Ixodes spp., the males do not feed at all.5 In contrast, males of metastriate tick genera, such as Rhipicephalus, Amblyomma and Dermacentor, which are commonly associated with transmission of a variety of rickettsial and protozoan pathogens of dogs, require a blood meal prior to sexual maturation and mating.6 Because metastriate males actively feed prior to, and during, mating, and because females leave the host to deposit eggs in the environment after engorging for 9–14 days, dogs infested with metastriate ticks often harbour more male than female ticks of a given species.7 These actively feeding metastriate males, which remain on the dog after the females have detached, may serve as an important source of infection when they move directly to new dogs or survive in the environment long enough to eventually find another host.

The ixodid (hard) ticks, which commonly infest dogs, transmit a number of diverse types of pathogens. The specific pattern of transmission is dependent both on the species of tick and the nature of the pathogen involved in a given cycle. For example, motile spirochetes of B. burgdorferi are present only in the midgut of recently molted Ixodes spp. ticks. Upon attachment and initiation of feeding, the spirochetes multiply in the gut, penetrate into the haemocoel, and then migrate to salivary glands.8 For rickettsia, such as the Rickettsiellaaceae (Rickettsia spp.) or Anaplasmataceae (Anaplasma spp. and Ehrlichia spp.), infection is also harboured in the midgut of newly molted ticks, but organisms may also be found in salivary glands. For example, A. phagocytophilum has been reported from the salivary glands of unfed Ixodes spp., suggesting it may be transmitted to hosts more quickly upon attachment, although experimental transmission times reported to date vary widely.9,10 For Babesia spp., zygotes form in the midgut, penetrate into the haemocoel, and migrate to the salivary glands where sporogony occurs; sporozoites are then transmitted to the host via saliva upon subsequent initiation of tick feeding.11

Time required to allow transmission
Spirochetes of B. burgdorferi are not found in the tick salivary glands until at least 36 hours after initial attachment to a host, although both numbers of organisms and efficiency of transmission increase dramatically after 54 hours of attachment.12 Similarly, sporozoites of Babesia spp. are not thought to be transmitted from the salivary glands until at least 48 hours post feeding.13 In contrast, Rickettsia rickettsii, the agent of Rocky Mountain spotted fever, reportedly can be released from the salivary glands as soon as 2 hours after tick attachment. However, transmission may take longer in some cases, and these historic studies, whilst important, should be repeated using currently circulating strains of R. rickettsii and modern confirmatory detection methods.14,15 Surprisingly, the minimum time required for transmission of most Ehrlichia spp. and Anaplasma spp., including E. canis, has not been evaluated.16 Colonies of Anaplasma marginale were not detected in salivary glands of Dermacentor andersoni until 48 hours after initial attachment, although infection did persist in the salivary glands for the remainder (> 30 days) of tick feeding.17 Published transmission times for A. phagocytophilum by I. scapularis were 24 hours to more than 40 hours, with recent unpublished reports suggesting organisms can be transferred as soon as 4 hours post attachment.14,18 However, due to the inherently complex nature of the required studies, for most ixodid tick-borne pathogens of dogs, including E. canis, E. ewingii, A. platys, A. phagocytophilum, B. canis, B. gibsoni and R. rickettsii, the minimum transmission times to dogs by ticks have not been determined.19

Interrupted feeding and the effect on transmission time
For the well-characterised B. burgdorferi transmission system, spirochetes cannot be transmitted to the host until at least 36 hours following initial tick attachment; a minimum time is required for the spirochetes to be activated, increase in number, and migrate from the midgut to the salivary glands. However, once transmission is actively occurring, if feeding ticks are dislodged from one host and attach to another host, transmission times have been shown to be considerably shorter.20 Partially fed ticks will readily reattach to new hosts and resume feeding.14,17 This interrupted feeding route, in which ticks move to a second host while still in the same instar stage, not only accelerates transmission times, but also allows intrastadial transmission to occur.14,15

Intrastadial transmission of tick-borne disease agents
Transstadial and transovarial transmission are terms commonly used to describe routes by which disease agents are maintained within tick populations as immature ticks molt to more advanced stages, or as adult female ticks pass infections to larval offspring, respectively. However, intrastadial transmission, in which the same
feeding tick instar acquires the infection, moves to another host, and then transmits the infection without molting or developing to the next stage, may also be important in the natural infection cycle of some tick-borne disease agents. For example, uninfected nymphal l. scapularis have been shown to acquire B. burgdorferi from spirochetemic mice, and, then, upon resuming feeding on naive mice, are able to transmit that infection in less than 24 hours.16 Uninfected adult male D. andersoni were able to acquire and then transmit A. marginale to as many as five subsequently infested calves over a 35-day period. Although minimum transmission time was not evaluated in this study, A. marginale was detected in the salivary glands of feeding ticks immediately following attachment to each of the calves.16 More recently, adult male R. sanguineus have been shown capable of acquiring and then transmitting E. canis intrastadially between dogs.17

Natural models for interrupted feeding

Although interrupted feeding has been known for some time both to allow intrastadial transmission and to accelerate transmission times, the relevancy of this route in tick-borne pathogen transmission cycles in nature has not been fully evaluated. For intrastadial transmission to be ecologically relevant, interrupted feeding of infected ticks must occur naturally. In natural infestations of hosts with metastriate ticks, males outnumber females by ratios of 2:1 to as high as 8:1,19 and thus may be encouraged to leave an infested animal in search of additional females to mate. To evaluate the extent to which this phenomenon may occur in the canine R. sanguineus system, we recently conducted two experimental trials in which we infested dogs with colour-coded R. sanguineus ticks, co-housed them for five or seven days, and monitored tick movements between dogs. In the first study, tick sex ratios were skewed to males, with an average of 10.8 % of ticks emigrating from each of the four dogs, and a 9.4 % average immigration rate recorded to three of the four dogs (Little et al., unpublished data). We suspect that recorded rates of tick movement in the second trial were lower than in the first due to the shorter observation period and the less severely skewed tick sex ratios. Nonetheless, male ticks readily changed dogs in both trials, most likely in order to continue to mate females. This movement of male ticks between dogs represents a naturally occurring form of interrupted feeding that may influence disease transmission.

Conclusion

Interrupted feeding of male ticks on dogs, as we have shown, occurs with R. sanguineus, it may also occur with other metastriate tick species and could profoundly influence transmission dynamics of tick-borne pathogens in natural cycles. If adult ticks acquire pathogens from dogs and then move to another host, either dog or human, to resume feeding, transmission of disease agents is likely to be accelerated. A recent paper from Brazil documenting four cases of R. sanguineus feeding on people found only adult male ticks,20 suggesting that this stage may be more likely to move from dogs or the environment to people. Similarly, if a tick infected as a larva or nymph begins transmitting to one host as a nymph or adult, respectively, and then moves to a second host, faster transmission times are likely to occur. Understanding the influence of intrastadially migrating ticks on maintenance cycles in general, and on transmission times in particular, is critical to developing a comprehensive picture of how these tick-borne pathogens create a disease threat to both dogs and people. Detailed knowledge regarding transmission dynamics is needed to make informed recommendations about best practices for preventing tick-borne diseases.

Acknowledgements

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Exposure to Arthropod-Borne Pathogens in Healthy or Leishmania infantum-Infected Dogs from Spain

Introduction
Canine leishmaniosis (CL) is a severe systemic infectious disease of dogs that is caused by protozoan parasites of the genus Leishmania. The dog is considered the main peridomestic reservoir of the parasite, which results in zoonotic transmission to man. When left untreated, leishmaniosis is usually fatal in people and in dogs. 

Co-infections and leishmaniosis
Clinically, Giger and Greene (1998) considered that the clinical signs of immunodeficiency are recurrent and/or chronic course of infection; presence of infections with common non-pathogenic organisms; severe and often atypical infectious disease manifestations; delayed, incomplete or lack of response to antimicrobial therapy; adverse reactions to modified live virus vaccines. The first four points are commonly observed in dogs affected by leishmaniosis. Furthermore, there are several other findings that suggest that dogs affected by leishmaniosis have a depressed cell-mediated immunity (reduced skin hypersensitivity to leishmanin, alteration in the production of several cytokines and alteration in ratio CD4+/CD8+).

In recent years, an increasing number of publications have described simultaneous infection with another vector-borne pathogen in dogs that had a classical presentation for leishmaniosis. A few published examples include co-infections of Leishmania and Neospora, Ehrlichia, Hepatozoon, Bartonella, Babesia or Dirofilaria (Nocardia) for some organisms, the association between the two infectious entities occurs with a greater frequency than would be expected, based upon the incidence of each respective infection. For instance, an epidemiological study performed in the Campania region (Italy) found N. caninum seroreactivity as a major risk factor for L. infantum seroreactivity. Co-infections should be expected in dogs living in areas that are highly endemic for several vector-borne organisms, in dogs that are maintained predominantly outdoors (enhanced vector transmission) and in dogs that are not routinely treated with acaricides or other ectoparasiticides. For example, serological evidence of exposure to multiple vector-borne organisms was recently reported in hunting dogs living outdoors on the island of Mallorca.

Co-infections and immune response
Although co-infections could occur merely as a function of lifestyle, it is also possible that one or more vector-borne organisms suppress the host’s immune response to other organisms that would typically be eliminated immunologically. As examples, infection with L. infantum can induce suppression of the immune system or promote an abnormal response, and result in an imbalance between Th-1 and Th-2 responses. Infection with Bartonella vinsonii (berkhoffii) can result in CDB+ lymphocytopenia, impaired monocyte phagocytosis and impaired antigen presentation to helper T cells. Survival and multiplication of ehrlichial organisms in infected cells rely on the organism’s ability to inhibit phagosome-lysosome fusion. Apparently, T-cell-induced immunity and interferon (IFN) secretion play a predominant role in recovery from ehrlichial infections, and decreased CD4+CD8+ ratios have been encountered in ehrlichia-infected dogs. Moreover, immunopathologic consequences may have a detrimental effect on progression of the disease, as some pathologic and clinical manifestations are immune-complex mediated.

Clinically, when we are approaching a patient with leishmaniosis (fig. 1), it is important to consider the possibility of co-infections. Probably they are relatively common. It is always necessary to adequately investigate any clinical sign of the patient and suspect a co-infection: leishmaniosis in old dogs which have been living for years in endemic areas; atypical clinical signs; a lack of response to conventional treatment (persistence of hypergammaglobulinemia or high titer of antibodies) or an affected dog from a prevalent area of any pathogen.

Co-infections in Spain
Currently, there is limited information regarding the prevalence of many vector-borne infections in dogs residing in specific geographical areas of Europe, especially in Spain where leishmaniosis is an endemic infectious disease.

The different studies that have been done in Spain about co-infections in dogs show a wide presence of different infectious agents, such as Rickettsia conorii, Ehrlichia canis (fig. 2), Anaplasma phagocytophilum, Bartonella henselae, Bartonella vinsonii ssp. berkhoffii, Leishmania infantum (fig. 3), Borrelia burgdorferi, Dirofilaria immitis.
Through serologic testing, substantial evidence of vector-borne diseases was found in these dogs. Of note, 98% of the dogs in some of these studies were seroreactive for at least one organism for which ELISA or IFA testing was performed. \(^{2}4\)

Total seroreprevales for *Rickettsia conorii* (56.4%), *Leishmania infantum* (30%), *Ehrlichia canis* (16.7%), *Anaplasma phagocytophilum* (11.5%), *Bartonella henselae* (16.8%), *Borrelia burgdorferi* (0.6%) and *Dirofilaria immitis* (0.6%) antigens were similar among the different studies. \(^{10,24,27,30,31}\)

These studies indicate that exposure to *Rickettsia conorii*, *Leishmania infantum*, *Ehrlichia canis* (or related *Ehrlichia* spp.), *Bartonella henselae* and *Anaplasma phagocytophilum* (or related *A. phagocytophilum*), *Bartonella henselae* spp., *Borrelia burgdorferi* and *Borrelia burgdorferi* is uncommon amongst dogs from the Mediterranean basin. \(^{10,24,27,30,31}\)

In another study circumscribed to the Barcelona (Spain) area, \(^{10,24,27,30,31}\) the seroreactivity was most frequently detected to *R. rickettsii* antigens and *L. infantum* antigens, followed by *B. burgdorferi* (berkholferii) antigens and *E. canis* antigens. No dog’s serum reacted to *Borrelia burgdorferi* C6 peptide and all *D. immitis* antigen test results were negative. Serum from healthy dogs lacking clinical and serological evidence of *L. infantum* infection were reactive to *R. rickettsii* and *B. burgdorferi* antigens, and to *E. canis* antigens. Serum from dogs that had clinical manifestations of leishmaniosis was reactive to *R. rickettsii*, *B. burgdorferi* (berkholferii) and *E. canis*. Seroreactivity to both *E. canis* and *R. rickettsii* antigens was found in two samples (one each from group two and three) and reactivity to *R. rickettsii* and *B. burgdorferi* (berkholferii) was found in ten samples (two from group one, five from group two and three from group three). When reciprocal antibody titers of the various organisms were stratified according to gender, age, breed and date of blood collection, no serological associations were noted.

These serological surveys illustrated the potential for exposure to *R. rickettsii*, *L. infantum*, *B. burgdorferi* and *E. canis* from dogs in the same area. However, as serological reactivity may exist (for instance, between *Ehrlichia* and *Anaplasma* species), these results may in fact represent exposure to other tick-borne pathogens.

Therefore, the primary purpose of new studies is to further characterise the degree of co-infection by molecular identification of multiple tick-borne pathogens. Perhaps we can finally answer these questions: Is that a real immunodepression? Or is that just a co-infection?

References

**Ehrlichia and Anaplasma Infections in Dogs and Cats in Spain**

**Ehrlichia canis infection in dogs**

Canine ehrlichiosis has been considered a well-known disease in Spain since the nineties. The first clinical cases were diagnosed in the north-east of the country (Catalonia) in 1988, using the immunofluorescent antibody test (IFAT). Since then, many cases have been reported in almost all regions of the country.

In our laboratory, antibody titers against *E. canis* have been detected in canine serum samples from every region of Spain, including the Canary and Balearic Islands. This wide distribution of the agent can be explained by the presence of *Rhipicephalus sanguineus* in most parts of Spain. This tick is especially prevalent in dogs living in areas with Mediterranean and Continental climates (central, eastern and southern Spain) and can also be detected in the north of the country, where the temperature is lower.

The suggested chronic evolution and the long periods of subclinical phases of the disease lead to the detection of clinical cases throughout the year. However, acute cases are especially common in summer, spring and autumn, the seasons in which *R. sanguineus* is mainly active in our latitude.

Seroprevalence studies have been performed in different regions, showing rates ranging from 1.9 % to 19.2 %. Significant differences have been found when comparing seropositivity to *E. canis* in two different canine populations living in the same area (Madrid), with similar environmental conditions (temperature, humidity, rainfall rate, etc.), but with different prophylactic measures.

*E. canis* has traditionally been accepted as the major etiological agent of canine ehrlichiosis in Spain. In fact, most clinical and serological studies about *Ehrlichia/Anaplasma* infection in Spain have been performed using *E. canis* as antigen. Nevertheless, the first isolation and molecular characterisation of *E. canis* in dogs in Spain was achieved in 2004 (fig. 1).1

Dog sera from Majorca recognised *E. canis* antigens by IFAT (17.6 %) that were not detected by a commercial diagnostic test that uses *E. canis* synthetic peptides as antigens (0 %). These divergent results could suggest the presence of a unique or new *Ehrlichia* spp. on this island.13

Concurrences of ehrlichiosis with many other illnesses, especially infectious and parasitic diseases, are relatively common in dogs in Spain (around 30 % of cases). The co-infection most frequently detected is amongst canine ehrlichiosis and leishmaniosis (69 % of the animals with any co-infection). The existence of co-infections in dogs in Spain is not statistically associated with a non-favourable response to therapy. Bone marrow aplasia and renal failure are usually associated with the worst prognosis, the severity of this infection being similar to that reported in other latitudes.

Doxycycline and imidocarb dipropionate have been routinely used by Spanish clinicians to treat dogs with ehrlichiosis, with good clinical results. However, platelet count and serum protein electrophoresis results returned to normal values much slower in dogs that received imidocarb compared to those treated with doxycycline. These differences can be explained by the recent study in which the lack of efficacy of imidocarb when treating dogs with experimental ehrlichiosis is reported.

**Anaplasma spp. infection in dogs**

**Anaplasma platys in dogs**

The detection of inclusion bodies compatible with *A. platys* (fig. 2) in canine blood platelets, and the serological evidence of the presence of this organism, were first reported in Spain in 1999. The pathogenic role of *A. platys* in dogs is not clearly defined. Different degrees of severity have been described depending on the geographic origin of the *A. platys* strain. Experimental *A. platys* infection using a Greek strain seems to be more virulent than the inoculation with American strains. In a clinical study performed in Spain on thrombocytopenic dogs seropositive to *A. platys*, no other clinical signs compatible with ehrlichiosis were found. However, genetic characterisation of this agent has been recently achieved in a dog from central Spain negative to *E. canis* with a clinical history compatible with ehrlichiosis. These findings could suggest that the variability detected in the clinical signs caused by this divergent results could suggest the presence of a unique or new *Ehrlichia* spp. on this island.13 concurrence is reported.

**Anaplasma phagocytophilum**

This agent is transmitted by *Ixodes* ticks, species typically found in northern Spain where the climate is colder and more humid than in the rest of the country. *Ixodes* ticks are rarely found in the Mediterranean basin and in the south of Spain due to the environmental conditions of these areas.

Because of that, most reports of *A. phagocytophilum* comes from northern Spain. *A. phagocytophilum* has been found in *Ixodes* ticks from these areas (mainly in *Ixodes ricinus*). There are also some reports about the presence of this agent in *Neotrombicula autumnalis*, cattle, sheep, goat, horse, deer, wild small mammals and birds. This agent has been associated with abortions in sheep, and with non-specific illness in cattle.

The first case of human anaplasmosis has been detected recently, confirmed by PCR in north-
was no statistical association between seropositivity to *A. phagocytophilum* and clinical status. However, *E. canis* seropositivity was associated with clinically healthy status in one of the studies.

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**Fig. 3:** Immunofluorescent antibody test positive to *Ehrlichia canis*.

The pathogenic importance of these organisms in cats has also been evaluated in different works. In the studies performed in Spain, there
Tick-Borne Diseases in Canada: Assessing Canine Risk through Human Surveillance Activities

In Canada, there are only about 40 species of ticks, including members of the Argasidae and Ixodidae. Although populations of important vector species such as Dermacentor variabilis, D. andersoni and Ixodes scapularis are established (or introduced annually in the case of I. scapularis) across large geographic regions of Canada, the range of most of the species of ticks in Canada is discontinuous and focal. Partly as a result of this, tick-borne diseases in humans and companion animals are relatively uncommon. For example, tick paralysis and tick-borne infections such as relapsing fever, tularemia, Q fever, Rocky Mountain spotted fever, and Lyme disease are less common in parts of central and eastern Canada. In endemic areas, pathogens associated with it: Borrelia burgdorferi, the agent of Lyme borreliosis, and Anaplasma phagocytophilum, the agent of granulocytic anaplasmosis. In the last decade, the epidemiology of infectious diseases has grown rapidly, including the development of a national tick surveillance network, which has allowed public health officials to gain a better understanding of the distribution of tick vectors, the pathogens they may transmit and the factors that limit their distribution. This passive surveillance programme has focused mainly on the blacklegged tick, I. scapularis, and the program is established in all provinces west of Albertan. Ticks removed from humans and domestic or wild animals are submitted to the National Microbiology Laboratory by a variety of stakeholders across central and eastern Canada including: public health institutions, physicians, wildlife biologists, university staff, veterinarians and the general public. Submitted ticks are identified to species and screened for a variety of pathogens. The application of molecular diagnostics for screening ticks collected from 1997 to the present has documented the existence of infectious blacklegged ticks throughout western, central and eastern Canada and the prevalence of B. burgdorferi in these ticks is ~15%. Despite the apparent limited distribution of established populations of blacklegged ticks in Canada, these results show that there is risk of exposure to ticks infected with this important human and animal pathogen throughout non-endemic areas of Canada. Screening of ticks from a blacklegged tick-endemic locality in Ontario resulted in the identification and genetic characterization of A. phagocytophilum in these arthropods. The detection of A. phagocytophilum in these ticks was the first study to show that I. scapularis populations in Canada are infected with this organism, and subsequent testing of ticks from the passive surveillance programmes have documented this pathogen in ticks collected in Manitoba, Quebec, Prince Edward Island and Nova Scotia. The results of the passive surveillance programme are provided to local public health officials and/or other stakeholders (e.g., veterinarians and even the general public) through various provincial and federal collaborators. Passive surveillance data is used as a central component of regional public health, physician/veterinarian awareness campaigns or vaccination strategies (veterinarians). In addition, information about the infectious status of attached ticks is often used in decision-making concerning the need for patients or clients to receive antimicrobial therapy. In addition, data from the passive surveillance programme have been used to define potential localities where establishment of blacklegged ticks seems probable, and where intensive field studies (i.e., active surveillance) are warranted. Newly established populations of blacklegged ticks have been detected at several localities along the north shore of Lake Erie and Lake Ontario, and most recently in Nova Scotia, Manitoba and eastern Ontario, and this detection was aided, in part, by passive surveillance. Developing an inventory of indicators that should trigger active surveillance for established tick populations is one of the ongoing objectives of the overall tick surveillance programme. In response to the expanding range of blacklegged ticks, tick control projects with stakeholders from Manitoba and Nova Scotia have been initiated. These projects will employ an integrated approach of landscape management, targeted acaricide treatments and the deployment of self-application devices for small mammals and white-tailed deer, to reduce ticks in these localities. Ultimately, the goal of the tick surveillance programmes is to help define the factors (i.e., microclimate, habitat suitability, host availability, etc.) that limit distribution of vector tick species, such that models can be built to help predict the potential distribution of ticks, and the subsequent risk of exposure of people and other animals throughout Canada. To date, several models have been developed that incorporate tick developmental temperature thresholds and tick dispersion patterns via migratory birds to predict the most likely localities in Canada where blacklegged tick populations will become established, in the short term, as well as under anticipated climate change scenarios. Based on these models, the risk of tick-borne disease for humans and companion animals is predicted to expand throughout much of eastern and central Canada, in the near and distant future.

References

Immunoglobulin G subclass – Distribution in Canine Leishmaniosis: What does it Mean?

Infection with *Leishmania* may have different outcomes in genetically distinct individuals, and the course of infection is determined by the nature of the host's innate and adaptive immune response. Thus in experimentally infected mice (of CS7Blk, CBA or C3H strains), and in naturally infected dogs or humans, the protective (self-healing or asymptomatic) phenotype is associated with the induction of Th1-regulated (self-healing or asymptomatic) phenotype. By contrast, a Th2-regulated humoral immune response is associated with severe symptomatic disease. In the murine model system there is a strong correlation between clinicopathological phenotype and the nature of the antigen-specific humoral immune response. 34 Symptomatic infection and Th2 regulation is associated with elevation in antigen-specific IgG1 and IgGf, whereas asymptomatic infection with Th1 regulation is linked with IgG2a production. These IgG2a antibodies may play an adjunct role in the clearance of pathogen by neutralising amastigotes that are released from ruptured infected macrophages.

IgG subclass restriction is less clear in the human disease, with only some clinical forms being correlated to a specific serological profile. 9, 31 Other studies in *Leishmania*-infected humans have failed to show a distinct skewing of the IgG subclasses – in terms of features such as preferential reactivity with protein or carbohydrate antigen, complement fixation, opsonization, role in antibody-dependent cell-mediated cytotoxicity or action in a reaginic fashion by binding to mast cell or basophil Fc receptors.

Moreover, there are no data that suggest either biochemical or functional equivalence between murine and canine IgG subclasses – a point which is often misunderstood in studies of canine IgG subclass distribution in disease. There is greater knowledge of comparative function between murine and human subclasses – for example, the Th2-regulated allergic phenotype is characterised by IgG1 production in the mouse, and by IgG1 and IgG4 production in humans.

Although fundamental immunological investigations of canine IgG subclass function are lacking, there are clinical studies which suggest that subclass bias is associated with specific types of immune response in this species. For example, there are numerous reported investigations of canine atopic dermatitis and a range of autoimmune diseases (pemphigus, sub-epidermal blistering disease, immune-mediated haemolytic anaemia, lymphocytic thyroiditis, autoimmune diabetes) in which there is clear bias towards IgG1 and IgG4 production. 2, 10–13, 15, 17, 19

The second problem which has clouded the field of canine IgG subclass distribution in clinical disease relates to the nature of the detecting reagents used to identify these molecules. This is nowhere more an issue than in studies of canine leishmaniosis. There are two sets of reagents that are currently employed for the detection of canine IgG subclasses. The most widely utilised is the set of two polyclonal antisera marketed by Bethyl Laboratories. These are claimed to detect canine ‘IgG1’ and ‘IgG2’, but have never been independently validated for their specificity. The second panel of antibodies is the group of monoclonal reagents prepared by the University of Bristol in the early 1990s. 7, 16 These reagents specifically detect canine IgG1, IgG2, IgG3 and IgG4 in serological, flow-cytometric or immunohistochemical assay. It is these latter reagents that have been employed in all of the studies of allergic or autoimmune disease described above.

It is most important to recognise that the ‘IgG1’ and ‘IgG2’ detected by the Bethyl reagents are not equivalent to the IgG1 and IgG2 defined by the Bristol monoclonal antibodies. Moreover, the former polyclonal antisera appear not to be IgG subclass-specific. In an early study, we demonstrated that both of these reagents, in fact, detect all four canine IgG subclasses when
tested in ELISA with subclass proteins purified by protein G and A affinity chromatography, using the FPLL system. These factors may go some way towards explaining why, in the current literature, there is no consensus as to whether dogs naturally or experimentally infected with, recovering from, or vaccinated against, Leishmania infection display a particular IgG subclass profile. Only a single investigation to date has employed the panel of Bristol monoclonal antibodies to examine the Leishmania-specific IgG response in a cohort of naturally infected dogs. This study showed elevation, with increasing duration of infection, in specific antigen serum antibody of all four IgG subclasses – a pattern which suggests that canine humoral immune responses to this pathogen may be more akin to those of man than mice. A current investigation is examining the vaccine IgG subclass response but these data have not yet been finalised.

References

Canine Leishmaniosi s and Concurrent Infection with other Vector-Pathogens in Southern Italy

Introduction

Domestic dogs represent the main reservoir hosts for zoonotic human visceral leishmaniasis in both the Old and New Worlds. In Italy, Canine Leishmaniosis (canL) is highly endemic along the Mediterranean coasts and in the islands; however, several autochthonous cases are being reported also in northern regions of the country, probably due to climatic changes. The appearance and severity of clinical signs depend upon the dog’s immunological response and disease stage. In foci of canL, symptomatic disease occurs in less than 50% of infected dogs, and is characterised by chronic evolution of viscerotrophic and increased levels of renal parameters alterations. Chronic CME signs are characterised by aplastic pancytopenia renal dysfunction, ocular involvement, polymyositis and central nervous system signs. In this state, haemorrhagic tendencies are very frequent, including epistaxis, dermal petechiae and ecchymoses. These alterations are due to thrombocytopenia and thrombocytopenthia. Platelets-bound auto-antibodies have been demonstrated in these pathological conditions.

Usually a mild non-regenerative anaemia is also present, together with lymphocytosis. Other frequent laboratory abnormalities are hyperproteinemia with polyclonal or monoclonal gammopathies, proteinuria and renal parameters alterations. Polyarthritis is also described but this sign has been associated with canine granulocytic ehrlichiosis (CGE) more often than with the other species of *Ehrlichia*. In Italy, there are very few cases of CME and all are attributed to *Anaplasma phagocytophilum*. The early manifestations of CGE are usually mild and consist in acute onset of fever and depression with or without thrombocytopenia. *Anaplasma platys* has also been recognised as a pathogen of dogs in southern Italy; infection results in moderate thrombocytopenia that usually causes minimal clinical illness. Uveitis is associated, with this infection in very few cases. The role of this agent has to be better investigated. There are very few studies about clinicopathological findings of CB in Italy. Here, this infection is caused by *Babesia canis* (fig. 2) (large form of parasite) subspecies *B. canis* and *B. canis voglei*.

These two subspecies are morphologically indistinguishable. Clinicopathological signs reflect the intravascular and extravascular haemolysis due to the life cycle of the parasite. The most common haematological abnormalities found in canine babesiosis are anaemia and thrombocytopenia. In contrast with that usually observed in ehrlichias infections, the anaemia is macrocytic and hypochronic and regenerative as the disease progresses. However, it is to be considered that in the very early stage of the infection, anaemia is mild, normocytic and normochromic. Reticuloctysis increase reveals the regenerative stage of the anaemia. Erythrocyte autoantibodies are involved in the haemolytic form of the disease. There can be erythrocite autoagglutination and many dogs may show a positive Coombs’ test. Biochemical profile abnormalities are an increase of hepatic enzymes, hyperbilirubinaemia, hypoalbuminaemia, electrolyte and acid-base abnormalities. There are a large variety of clinical signs depending on the parasite species/subspecies involved and in view of the host immune response. The most frequent clinical signs are fever, lethargy, jaundice and rarely, central nervous system involvement, acute renal insufficiency and multiple organ dysfunction syndrome (MODS).

These severe conditions are more frequently described in *B. canis* infections recognized in South Africa. It is important to point out that co-infection between *Leishmania infantum*, *Ehrlichia canis* and *Babesia canis* is relatively frequent, especially in dog kennels where there is poor control of the vector agents. In this case, it is very difficult to attribute the clinical signs and haematological and/or biochemical abnormalities to a single specific agent.

A possible mode to suspect the presence of two or more agents is to point out the clinicopathological findings typical of each of these pathologies. In our experience, lymph adenomegaly, cutaneous signs, ocular and renal involvement are mainly due to *Leishmania infantum* infection. Fever and splenomegaly are more frequent in CME and in CB than in canL. When thrombocytopenia is present, CME has to be always suspected because it is the first haematological alteration observed in CME. Trilinear bone marrow cytopaenia is usually due to *Ehrlichia canis*, too. It is important to remember that the
anaemia usually described in CB is a regenerative anaemia, together with the presence of jaundice.

Evolution of canL and CME co-infection

An additional point of discussion is to understand the influence that every infection could have on the evolution of other pathologies. For this reason, we performed a 28-month longitudinal study in a cohort of 41 beagles in order to evaluate any relationship between Ehrlichia canis and Leishmania infantum infection acquired naturally. By the end of the study, 100% and 95.1% of dogs were found infected by Ehrlichia and Leishmania, respectively, at some point (fig. 3). The main clinical sign identified was thrombocytopenia, which appeared very early in the study in 58% of dogs, despite low seropositivity to Ehrlichia (25%) and the absence of detectable Leishmania infections.

The appearance of progressive forms of leishmaniosis late in the study (up to 64% of dogs at month 28) did not correlate with the peak of associated thrombocytopenia + Ehrlichia seropositive condition (98% and 95%, respectively), found 12 months after the study start.

Due to the high prevalence of E. canis infection, several studies are necessary to investigate the role of this agent to influence the infection and the progression of other diseases. Our preliminary data seem to demonstrate that acute onset of E. canis infection does not influence the infection and the evolution of canine leishmaniosis.

Further Reading

Visceral leishmaniosis (VL) is a zoonotic vector-borne disease, which involves interaction between three major elements: the sandfly vector, the canine reservoir host and humans who become infected by the disease agent Leishmania infantum. VL is a severe disease that is generally fatal in people if not diagnosed and treated in time. The dog is the primary peridomestic reservoir host and the disease is transmitted to humans by phlebotomine sandflies. The availability of computer-based geographic methods and sensor technologies has created a growing use of satellite environmental assessment tools to study the epidemiology of vector-borne diseases and to aid in disease control programs. Landscape features that are mapped over large areas using remote sensing data can integrate geographic data and provide an insight that is not available from the ground. Geographic information system (GIS) analysis provides a tool to link epidemiologic data on disease systems with features in the environment and develop models that can then be used to predict the risk of disease over broad areas, where data may not be available.

The application of GIS has been used to study a number of diseases and disease vectors. These include: malaria, trypanomiasis, schistosomiasis, Lyme disease, cutaneous leishmaniosis and rabies.1,3,5,6,8,11,12,14,15,17,18 Beck and colleagues (1997) used remote sensing-based models, generated for one area, for prediction of malaria transmission in another comparable area.4 They created two remote sensing-based models for predicting populations of the malaria vector Anopheles albimanus in villages in southern Chiapas, Mexico. These populations of adult mosquitoes are an indicator for malaria transmission risk. Using the models created in one set of villages, they then tested their applicability for the prediction of risk in another set of 40 villages that were geographically distinct and randomly selected. One model accurately predicted 79% of the villages with a high abundance of the malaria vector and 50% of the villages with a low abundance of the mosquito. The other model correctly identified seven of ten villages with the highest Anopheles albimanus abundance.

Another study employing GIS technology examined the environmental risk for schistosomiasis in the state of Bahia, Brazil.5 A GIS database that was created included: information on host snails distribution, elevation, population density, duration of annual dry period, maximum rainfall, total precipitation during three consecutive months, vegetation types, soil types, annual maximum and minimum temperatures and diurnal temperature differences. Sample municipalities were ranked by prevalence of schistosomiasis into low-, medium- and high-prevalence categories. Analysis of the results from the study indicated that the length of the dry season was the most important factor influencing the host snail distribution and the suitability of a site for life cycle development and transmission. Most of the municipalities classified as having high- or medium-prevalence rates of schistosomiasis were clustered around the coast, where the dry period was shorter than in areas of low prevalence. Other significant findings were that a higher prevalence of the disease was found in municipalities with higher population densities, and that the high prevalence of disease was also associated with certain soil types that tend to be richer in clay and have a lower drainage capacity, allowing accumulation of surface drainage waters where snails may occur in abundance.

Recent studies on VL based on remote sensed data have been carried out in Brazil.14,15 In addition, a system for epidemiological health surveillance has been used for analysis of human and canine leishmaniosis cases in Messina, Italy.7 Poor socio-economic conditions and increased vegetation were associated with a high incidence of human VL in Teresina, Brazil. Increasing prevalence of canine infection predicted a high incidence of human VL and poor socio-economic conditions were found to have an amplifying effect on the association between canine infection and the incidence of human VL.16 High altitudes, a relatively large number of phlebotomine sandflies were trapped, indicating that control measures against VL should be focused on canine leishmaniosis foci at the above altitudes within this region.17 VL has emerged, during the last fifteen years, in central Israel and the Palestinian Authority (PA), where it had been reported mostly in children under the age of three years.1,2,18 This outbreak warranted a study on the interaction between environmental conditions and the spread of VL to predict the potential for further disease spread, and to facilitate prevention measures.

The objectives of this study were to investigate the epidemiology of human and canine VL in Israel and the PA, and to define ecological risk factors for the disease. Databases of the human and canine cases of VL in Israel and the PA during 1994–2004 were constructed and analysed for the year of diagnosis, geographic location and type of community (rural vs. urban). Trapped sandflies, and previous collections from multiple locations throughout Israel and the PA, were analysed for species and sex. Locations of sandfly trapping, infected humans and dogs were mapped using the Arcview® GIS software. In all, 176 people from the PA and 14 from Israel were reported with VL. Three cases of HIV/leishmaniac co-infection from Israel were related to immigration from Ethiopia and may not be autochthonous. The majority of human VL cases were from children in the Jenin and Hebron districts in the PA. One hundred and eighty nine dogs were detected with VL (128 by passive surveillance), 25% were from northern Israel and...
75% from the central region and the PA (fig. 1). Ninety per cent of the dogs were from rural settings and 10% from urban locations. The number of infected dogs is probably underestimated due to lack of diagnosis and asymptomatic infection. A clear overlap was found between the regions in which canine and human VL were reported, however, no clinical human cases have been found in some of the active canine foci. Sandfly collections from 63 locations in Israel and the PA indicated that Phlebotomus tobbi, Ph. perfiliewi and Ph. syriacus, considered vectors of L. infantum in the eastern Mediterranean, were present in the disease foci. The distribution of these sandfly spp. roughly paralleled the geographic spread of VL, whereas other sandfly spp. were present in areas where VL is not found. A significant statistical difference in the annual precipitation was found between locations where suspected sandfly vectors were trapped vs. locations where non-vector spp. were trapped (P=0.0226) (figs. 2a,b).

Extensive land use, a relatively high annual rainfall, low average temperatures in October, high elevation and high wind speeds at ground levels were significantly associated (p<0.05) with the incidence of human VL in the West Bank (PA). Although previous studies have shown a considerable rate of asymptomatic exposure to VL in Israel, the reasons for the difference in the number of human cases in Israel and the PA should be investigated further, and preventative measures should be implemented in disease foci.1, 2, 9

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Asymptomatic and Symptomatic Dogs in Endemic Areas, their Role in the Epidemiology of canL

Introduction

Canine leishmaniosis (canL) caused by the api-complexa protozoan *Leishmania infantum* (syn. *L. chagasi*) is a chronic and serious vector-borne disease endemic in the Mediterranean basin, Asia and Latin America. Vectors are phlebotomine sandflies of the genus Phlebotomus in the Old World and of the genus Lutzomyia in the New World.

canL can be considered an emergent infection in the Mediterranean area.\(^7\) A review of the literature of the distribution of canL in Southern Europe leads to the conclusion that the infection is still in evolution. Many regions considered free from canL in the 90s, have, in the last two decades, experienced new and sometimes stable foci.\(^11\) This epidemiological pattern evolves likely from the interaction of several factors, mostly related to humans and the environment.

CanL is characterised by a spotted distribution, usually overlapping the presence and density of the phlebotomine vectors, which need strict ecological requirements in order to survive. Prevalence in very close areas can vary widely from less than 2 % to more than 50 %, depending on the intensity of parasite transmission (force of infection) in unstable and stable foci, but depending also on methods used (serology, different parasite detections) and even on the period of the survey.

Dogs as a reservoir

Dogs are the most important reservoir of the disease and are mainly responsible for the persistence of canL, and also human visceral leishmaniosis (VL) in the Palearctic and Neotropical regions.\(^1\) The dog is incontestably the main reservoir of *L. infantum* zymodeme MON-1. Other eight zymodemes have been isolated in dogs, but their reservoir system remains unknown.\(^36\) Fisa et al.\(^11\) tried but failed to find the reservoir of the zymodeme MON-29 which was isolated from humans and sandflies, while Gallego et al.\(^11\) were able to determine the life cycle of MON-27 (isolated in dogs, humans and the vector).

Other hosts have been found naturally infected (foxes, cats, rodents, horses),\(^39\) or serologically positive, but it is unlikely that they can act as effective reservoirs. Isolation of the parasite from a host and its ability to infect the vector are necessary but not conclusive steps in order to define a new reservoir. This data must be supported by a consistent prevalence in that particular host and by a basic reproductive number (R0) > 1.\(^37, 40\)

A good example is the study of Courtney et al.\(^10\) on the crab-eating fox *Cerdocyon thous* in the transmission of *L. infantum* in the Amazon region of Brazil: a total of 37 wild foxes were immunologically and clinically monitored, and 26 foxes were exposed to laboratory colonies of the sandfly vector (*Lutzomyia longipalpis*), over a 15-month period. In total, 78 % of foxes were seropositive and 38 % had infections confirmed by PCR and/or by culture. None of the foxes were infectious to the 1,469 sandflies dissected from 44 feeds. A conservative estimate of the possible contribution of foxes to transmission was 9 %, compared to 91 % by sympatric domestic dogs. These results show that crab-eating fox populations do not maintain a transmission cycle independently of domestic dogs. Recently Maroli et al.\(^28\) succeeded to transmit *L. infantum* from a symptomatic cat to the sandfly Phlebotomus perniciosus, but still only a few dozen infected cats have been reported in literature, compared to the hundreds of thousands of infected dogs in endemic areas. Neither cat or fox are likely to introduce the parasite into Leishmania-free dog populations.

The failure of control programmes for human VL in some states of Brazil, that depend on the elimination of infected dogs, have suggested that other reservoir hosts may participate in the transmission cycle. To determine whether persons infected with *L. chagasi* can infect the vector sandfly, Costa et al.\(^7\) fed laboratory-reared *Lutzomyia longipalpis* on Brazilian subjects with active, cured and asymptomatic VL. Of 3,747 insects fed, 26 (0.7 %) acquired infection from 11 of the 44 persons with active VL, but none acquired infection from the 137 asymptomatic persons. The conclusion is that humans, in this epidemiological situation, can contribute to the life cycle of leishmaniosis only if patent and left untreated for long time.

Symptomatic and asymptomatic dogs

The dog is such an excellent reservoir of *L. infantum* for three main reasons:

1. Very long pre-patent period of infection
2. High concentration of protozoan amastigotes in the skin
3. High percentage of relapses together with uncertain parasitological sterilisation after treatment

CanL is usually characterised by a long pre-patent period. This condition has been recently confirmed by Olivá et al.\(^16\) in a three-year longitudinal study on 43 beagles naturally infected in a well-known, highly endemic area of the Campania region (Italy).

Results showed that the time course of infection was highly variable in each dog, and three patterns were identified: (i) rapid establishment of a patent condition (0 to 2 months from detection of infection); (ii) a prolonged sub-patent condition (4 to 22 months) before progression; (iii) a transient sub-patent condition followed by 10 to 21 months of apparent Leishmania-negative status before progression.

Both asymptomatic and symptomatic dogs are able to infect sandflies. A study on *L. major* and its vector *P. duboscqi*,\(^1\) has suggested that one amastigote is sufficient to cause an infection to a sandfly and, as a result of multiplication in the gut and the existence of mechanisms that increase the number of infective bites delivered by a female sandfly, they are able to sustain the transmission of leishmaniosis in an area.

Whether asymptomatic dogs have lower or equal capacity than symptomatic ones to infect the vector is still controversial. Some studies state that symptomatic dogs present the highest infection rate and intensity of infection,\(^1, 15, 45\) whereas others found that the infectivity to sandflies is independent of the extent of symptoms in the dogs,\(^1, 20, 48\) and that there is often no correlation between clinical condition, parasitological condition and infectivity to sandflies.\(^1\)

One of the reasons for this discrepancy is the difficulty to define an asymptomatic dog: they can be defined as dogs without external clinical signs or they can be defined as dogs without clinical signs, including haematological alteration (such as anaemia, thrombocytopenia, etc.), alteration of protein profile or renal functions. From an epidemiological point of view, the most important asymptomatic dogs are those that are considered healthy by the owners (without evident clinical signs), and so far, they do not have a chance to be diagnosed as infected or potenti ally infected by veterinarians. These dogs are often detected within random surveillance pro-
Sensitivity and specificity of IFAT test in relation to the time course of the infection (redrawn from Dye et al., 1993)

Fig. 1: Sensitivity and specificity of IFAT test in relation to the time course of the infection.

The development of ELISA-based assays for the detection of antibodies against recombinant leishmanial antigens (namely, the rK26 and rK39 from Leishmania infantum and the R2 protein from L. donovani) compared to ELISAs employing crude parasite lysates (Tab. 1). The results further indicate that all three recombinant proteins must be used in parallel to detect essentially all infected dogs. Many PCR assays have been proven to be reliable and very sensitive techniques, but a number of variables affect even PCR sensitivity, such as target copy number, DNA extraction method, PCR protocol or detection method. It is unlikely that PCR can be used in large epidemiological studies due to budgetary constraints. Probably neither technique identifies all infected animals.

Another factor that can affect PCR performance is the choice of the biological samples to be tested. Solano-Gallego et al. showed that sensitivity of PCR in the bone marrow, the conjunctiva and the skin were 17.8%, 32%, and 51%, respectively, in 100 dogs living in an area endemic for canL. In this study, prevalence of the infection, 67%, was calculated combining serology and the PCR performed on all samples and indicated that the majority of dogs living in an area where canL is endemic are infected by Leishmania and that the prevalence of infection is much greater than the prevalence of overt Leishmania-related disease. Similar results are reported by De Andrade et al. (Tab. 1).

Conclusions

Apart from untreated diseased dogs and free-ranging ones (which can be a problem not easily managed in many endemic areas of canL), asymptomatic dogs are the most important reservoir of canL, due to their ability to infect sandflies, to remain asymptomatic for years, and due to the sensitivity of diagnostic tests, which may fail to detect canL carriers, especially in large-scale surveys. The protection of dogs with repellents during the vector season, as well as an efficacious vaccine, protective against infection and not only clinical disease, may positively contribute to reduction of the proportion of asymptomatic dogs in endemic areas, resulting in the control of canL and human VL. Finally, it has to be reminded that asymptomatic dogs are most likely responsible for the introduction of the disease in Leishmania-free areas.
References


Epidemiology of the European Foci of Human Leishmaniosis

Introduction

The leishmaniases are endemic in Southern Europe, where they are almost exclusively due to the species *Leishmania infantum*. This parasite is transmitted by Phlebotomine sandflies of the genus Phlebotomus, sub-genus Larroussius, and is responsible for a zoonosis of domestic and wild canids.

*L. infantum* is also responsible for visceral leishmaniosis in humans (VL), a form classically infecting domestic and wild canids. *L. infantum* infrequently infects domestic animals, and is responsible for cutaneous manifestations.

Geographical distribution

The endemic areas of leishmaniosis in Europe are restricted to the Mediterranean bioclimatic zones, of which thirty countries are concerned, including Portugal, Spain, France, Italy, Malta, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Albania, Greece, Turkey and Cyprus.

Epidemiology

Cases of leishmaniosis are registered differently according to each country and their clinical form. Human VL is a notifiable disease in Spain, Greece, Italy and Portugal, while CL is only notifiable in Greece and Italy. However, for all countries, it is presumed that the available figures for incidence are grossly underestimated, making the mean annual numbers of reported cases globally weak in all European countries. Between 300 and 400 new cases of human leishmaniosis are reported annually, in the whole of Southern Europe (fig. 1).

Parasite

*Leishmania infantum* is almost the only species present in Europe, and, with 33 different zymodesmes, it shows a high level of enzymatic polymorphism. *L. infantum* MON-1 is the most common zymodeme in dogs, the main reservoir, and in humans.

Vector

Four sandfly species are vectors for *L. infantum* in Europe. Out of them, Phlebotomus perniciosus has the largest geographical distribution, extending from west Portugal to Crete and Turkey. It is the main vector of the occidental part of Southern Europe. *P. ariasi* is more restricted in its distribution, where it is only found in Portugal, Spain, France and north-western Italy. In the oriental part of Southern Europe (Italy, the former Yugoslav Republic, Albania and Greece), there coexists *P. perfiliewi*, a north African sandfly species, and the oriental *P. neglectus*.

Reservoir

*Leishmania infantum* commonly infects domestic and wild canids, the dog, *Canis familiaris*, being its main reservoir. Canine leishmaniosis is zoonotic in Southern Europe, with its incidence higher than that of human leishmaniosis (annual incidence of cases reaching several tens of thousands). A few apparently autochthonous cases of canine leishmaniosis have been reported outside the common enzootic areas: Belgium, Germany, the Netherlands, Switzerland and the United Kingdom. In these cases the elucidation of transmission routes remain problematic.

Wild canids have been found harbouring *L. infantum*, particularly the fox, *Vulpes vulpes*, in southern France, Italy and Portugal. The position of the fox within the natural life cycle of the parasite remains unclear, as the number of cases reported is reduced. Leishmaniosis in the cat, *Felis catus*, first described in 1912 in Algeria, has, for a long time, been an exceptional phenomenon, but now appears more commonly in a few counties of Southern Europe (France and Spain), with its prevalence higher in Italy.

The epidemiological status of the cat within the natural life cycle remains to be elucidated: occasional host or true reservoir?

Clinical outcome

*L. infantum* is a viscerotropic parasite, mainly responsible for VL, but occasionally involving skin and, in rare cases, affecting the mucosa. The clinical outcome of VL during AIDS, in 1985, *L. infantum* appears

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as an opportunistic parasite occurring during HIV infection, particularly in Spain, Italy, France and Portugal, where 1,911 cases were detected between 1981 and 2001.1

The main risk factors for Leishmania/HIV co-infection in Europe are age (77.3% aged between 31 and 50 years), and intravenous drug use. In Spain, the sharing of syringes is suspected to be responsible for human-to-human transmission, which has lead to the occurrence of a possible anthropoponic cycle of L. infantum free of a vector transmission stage.1

While the prevalence of co-infection is decreasing, following the use of highly active anti-retroviral therapy (HAART) in the treatment of AIDS, the multitude of therapeutic immunosuppressions favours occurrence of VL cases in endemic areas. A recent review reported 57 VL cases in organ-transplanted patients, of which 49 were from countries of Southern Europe.2

L. infantum can also be responsible for localised cutaneous leishmaniosis, particularly in some foci, such as that of the Pyrénées-Orientales in southern France.7 Moreover, it can also be responsible for isolated mucosal lesions, without any cutaneous extension or visceral involvement (Fig. 2, 3).

Asymptomatic infection appears frequently in populations in endemic areas of Southern Europe, and was demonstrated in 1974 by Pampiglione et al.3 In the Alpes-Maritimes focus (southern France), 30% of the human population of an enzootic canine leishmaniosis area showed a positive skin test to the leishmanial antigen.5 In the same focus, some blood donors were also harbouring parasites.

Conclusion

L. infantum has a large geographic extension in Mediterranean Europe and is responsible for a large number of asymptomatic infections, which are susceptible to develop into symptomatic cases, in the event of immunosuppression. Symptomatic forms mainly include VL, less frequently, CL cases and rarely, mucosal lesions, which is illustrative of the complexity of the leishmaniasis pathology, related not only to the species tropism (visceral in principle), but also to host immunity.

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