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ABSTRACT BOOKLET

AN UPDATE ON EUROPEAN TICK-BORNE DISEASE IN DOGS AND CATS

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Introduction

Vector-borne diseases are caused by a wide range of infectious agents including viruses, bacteria and parasites (protozoa and helminths), which are transmitted by a variety of arthropod vectors such as ticks, lice, fleas and Diptera (mosquitoes, phlebotomine sand flies, muscid flies).

Vector-borne pathogens or diseases may be pathogenic for dogs and cats and their transmission is often unpredictable. The diagnosis and control of vector-borne diseases are difficult, while the variable clinical signs can develop after long incubation periods and are rarely pathognomonic. Animals may have persistent infections and thus act as reservoirs. Several are important zoonoses, such as leishmaniosis, borreliosis, rickettsiosis, bartonellosis, anaplasmosis and dirofilariosis. Climatic and ecological changes, national regulations on the management of stray dogs and cats together with an increase in pet travel and translocation of pet animals can influence the epidemiological situation of vector-borne diseases in Europe. Rare diseases may increase in frequency in certain areas, either due to the increased importation of infected animals or because the causative agents and their vectors spread to and establish in previously non-endemic areas. Such an expansion of endemic areas has been recorded for various parasitic diseases such as dirofilariosis, babesiosis and leishmaniosis. Babesiosis, for example, has been observed across central Europe in the past few years, emerging from previous endemic regions in Europe. Another important feature of these diseases is their increasing occurrence in wild animals, which could act as reservoirs. The effective control of vector-borne diseases requires a thorough knowledge of the infectious agents, their vectors and major hosts. This lecture will give an overview of the most relevant tick-borne diseases of dogs and cats in Europe.

Babesiosis

Babesia spp. are haemoprotozoa that exclusively infect erythrocytes and are transmitted by hard ticks. They are generally highly host-specific with regard to both the transmitting tick species and the mammalian host. Females of different species of Ixodidae, generally require a period of initial feeding (24–48 hours) before *Babesia* sporozoites are available for transmission within their saliva to the dog. In male ticks, transmission may be more rapid as they repeatedly feed taking only small amounts of blood, and they perform co-feeding with females and possibly feed from several different hosts. Endemic areas of canine babesiosis are related to the distribution of the tick vector. In central Europe, canine babesiosis appears to be one of the most frequently imported diseases and the endemic area of *B. canis* (large species), seems to have expanded in central Europe up to the Baltic region in recent years. Besides, small *Babesia* spp. can sporadically occur in Europe. Babesiosis in cats has only occasionally been observed in Europe and the species and vectors are still unknown. Babesiosis may be subclinical or may follow a peracute, acute or chronic course. *B. canis* can cause acute disease after an incubation period of 1–3 weeks. Sometimes the symptoms occur when the owner returns home to a non-endemic area, which makes recognition and diagnosis more difficult. A diagnosis of acute babesiosis can be confirmed by blood examination (blood smears, serology, PCR). Treatment should be initiated immediately after confirmation of a babesiosis diagnosis with

imidocarb dipropionate, and in some countries phenamidine. Adequate supportive therapy is strongly recommended including blood transfusion and, if appropriate, rehydration. The risk of infection with *Babesia* spp. can be significantly reduced by effective tick control. Vaccination can prevent severe disease, but not infection. A vaccine is available in some European countries. Re-vaccination every six months in highly endemic areas is advised.

Hepatozoonosis

The protozoan parasite *Hepatozoon canis* is transmitted to the dog by the brown dog tick *Rhipicephalus sanguineus*. The infection occurs in central Europe and Mediterranean countries. Unlike most tick-borne diseases, hepatozoonosis is not transmitted through the bite of an infected tick but rather by the ingestion of an infected tick. Hepatozoonosis is uncommon because most infected dogs have a low level of parasitaemia (less than 5% of circulating leukocytes infected with gamonts). Symptoms are seen when the parasitaemia approaches 100% of infected circulating leukocytes. These dogs may have hepatitis, glomerulonephritis or pneumonitis in addition to severe anaemia, fever, and cachexia. *H. canis* infections are treated with imidocarb dipropionate (5 to 6 mg/kg SC or IM every 14 days) until gamonts are no longer seen on blood smear examination. Despite its name, canine hepatozoonosis is not a zoonotic disease and rarely affects the liver. The only prevention consists of optimal tick control.

Borreliosis (Lyme disease)

Borrelia burgdorferi are spirochaetes that infect many mammals and birds and are transmitted by Ixodes spp. Lyme borreliosis is present all over Europe, except in extremely hot southern or cold northern areas. Ticks must be attached for at least 16–24 hours before pathogen transmission to a new host occurs. The bacteria remain in the skin of a host before disseminating to other tissues. In some cases, it can take up to four weeks before a systemic infection develops. Human infections are of major public health importance and although infections have been demonstrated in dogs, they are not of major clinical importance. There is no interdependency between dogs and humans in terms of transmission. Most infected dogs have subclinical status, and it is difficult to correlate naturally acquired *B. burgdorferi* infection with clinical signs in dogs such as fever, lameness, myalgia and lethargy. “Lyme arthropathy” which is lameness in one or more joints has been described; puppies may be at higher risk of polyarthritis. The term “Lyme nephropathy” has been used for a syndrome of protein losing immune complex nephropathy that occurs in 2% of seropositive dogs. Positive serology in cats has also been reported but disease is poorly understood. The drug of choice for treatment is doxycycline orally for a minimum of one month. Tick control is the only prevention.

Ehrlichiosis

Ehrlichia canis bacteria are transmitted by the tick *Rhipicephalus sanguineus* and infects mainly lymphocytes and monocytes. *Ehrlichia* has been described in cats but has no veterinary relevance. Disease in dogs can occur fairly quickly after infection, but also years later. Transmission of *E. canis* by blood transfusion has been described, therefore screening of canine blood products is strongly recommended in endemic areas. The geographical distribution of *E. canis* generally corresponds to the distribution of its vector. During the acute phase, which lasts around 1–3 weeks, dogs show apathy, weakness, lethargy, anorexia, dyspnoea, fever, lymphadenopathy, splenomegaly, weight loss and vomiting. Chronic canine monocytic ehrlichiosis is characterised by a very complex clinical picture. Reports of *E. canis* infections in cats are rare and clinical manifestations are still not very well described. *Ehrlichia* is diagnosed with blood tests. The treatment consists of tetracyclines for 4 weeks. The primary measure for the prevention is an effective protection against tick infestation. *E. canis* is not considered a zoonotic agent.

Anaplasmosis

In dogs, the bacteria *Anaplasma phagocytophilum* and *A. platys* infect predominantly neutrophil, and rarely eosinophil granulocytes (*A. phagocytophilum*) or platelets (*A. platys*). The natural mode of transmission has not been definitely established, but Ixodes ticks and other arthropod vectors are likely to be involved. Usually, tick feeding for 24–48 hours is required for the transmission of this agent to susceptible dogs. The geographical distribution corresponds to the distribution of the tick vectors. The incubation period in the mammalian host is 1–2 weeks. Anaplasmosis is diagnosed with blood tests. The treatment consists of the administration of tetracyclines for 3–4 weeks. The primary measure for the prevention of anaplasmosis is an effective protection against tick infestations. Infections with *A. phagocytophilum* have been reported in humans. The transmission was via ticks and direct transmission from infected dogs to humans has not been reported. Dogs may also carry infected ticks to human beings. Blood from infected dogs should be handled with caution.

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A SYSTEMATIC APPROACH TO EXOTIC PARASITE ASSESSMENT IN THE IMPORTED PET AND THE FUTURE OF THE PET PASSPORT SCHEME

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Introduction

Over the past 10 years increased numbers of legally and illegally imported pets have been relocated to other countries, both within and from outside Europe. Part of this trend has been driven by pets being rescued in foreign countries and then rehomed abroad. This in combination with expanding global parasite distributions, increases the risk of exotic parasites being introduced. The rapid identification of parasitic infection and disease in imported rescue dogs is essential in limiting these risks. Due to the ongoing risk that imported parasites represent to individual pets, owners, the wider public and UK biosecurity, it is important to limit this risk through four fundamental steps (The "four pillars").

Checking for ticks and identifying any found

Identification of ticks allows the introduction and distribution of exotic tick species to be monitored but also indicates which tick-borne pathogens the imported pet may have been exposed to. *Rhipicephalus* ticks on imported dogs may indicate that households have been infested so if these are identified, investigation and treatment of the house should also be considered. Treat dogs with praziquantel within 30 days after entering the country and treat for ticks if treatment is not already in place. *Echinococcus multilocularis*, the cause of cystic echinococcosis, is a severe zoonosis and considered a neglected disease by the World Health Organisation. Zoonotic infection occurs through ingestion of eggs passed in the faeces of dogs and foxes which are immediately infective. This can occur though association with infected dogs and therefore, *E.multilocularis* infected dogs represent a significant zoonotic threat to individuals living with them. Treating dogs with praziquantel within 30 days after entry to Poland ensures that *E.multilocularis* is eliminated from imported pets. Tick treatment will increase the likelihood of attached ticks being killed if they are missed on examination. This is particularly important to try and prevent *Rhipicephalus sanguineus* infestation in households if these ticks are present. Recognise clinical signs relevant to diseases in the countries visited or country of origin. A thorough clinical exam of imported pets will identify clinical signs which can then be compared to common exotic parasitic diseases in the countries that the pet has visited.

Screening for *Leishmania* spp., heartworm and exotic tick-borne pathogens in imported dogs

Both *Leishmania infantum*, tick-borne diseases and heartworm can have long incubation periods and carrier states. Infection can be lifelong and in some cases, can carry a poor prognosis. Screening for these parasites in dogs imported from endemic countries will lead to early diagnosis. This in turn, will help prepare the owner for what could be a lifetime of potential treatment, any associated zoonotic risk and implementation of steps to limit wider spread. Light microscopy of blood smears is a useful test to perform in all imported dogs. Dogs infected with *Hepatazoon canis* typically have high numbers of gametocytes present in peripheral blood smears. Piroplasms of *Babesia* spp. in red blood cells as well as morulae of *Ehrlicihia canis* and *Anaplasma platys* may also be seen. These will be present in lower numbers however, so a negative blood smear does not rule out the possibility of these infections being present.

Screening for tick-borne pathogens

Blood smear examination carries a high sensitivity and specificity for *Hepatazoon canis* detection and *Babesia canis* in clinical cases. Screening for *Ehrlichia canis* and *Anaplasma platys*, however, requires PCR or serology testing. Serology is highly sensitive and specific for detecting exposure to the parasite. Quantitative serology is useful in the case of *E. canis* infection where a fourfold increase in test titres taken 2 weeks apart is indicative of active infection. Blood PCR is also a highly specific and sensitive test for both parasites.

Screening for *Leishmania*

Fine needle aspirate techniques can unambiguously demonstrate the presence of the amastigote stage of *Leishmania* in Giemsa or DiffQuick stained smears obtained from superficial lymph nodes or bone marrow aspirates of clinically affected animals (Maia and Campino, 2008). However, this method can be insensitive and time-consuming. Histology performed on tissue samples carries a higher sensitivity. The main histopathological finding in the affected tissues is lymphoplasmacytic inflammatory reaction associated with macrophages infected with a large number of *Leishmania* amastigotes. Histopathological lesions are most commonly found in the spleen, lymph nodes, bone marrow, liver, gastrointestinal tract, and skin. Serological detection of specific anti-*Leishmania* antibodies can be used for routine screening. It permits the detection of a specific antibody response in dogs at around 6–8 weeks of infection. (IFA) and enzyme linked immunosorbent assay (ELISA) tests are commercially available. They do not confirm or rule out active infection, but quantitative serology allows the size of response to be measured. If this climbs over time, then it indicates active infection and is suggestive that clinical leishmaniosis is present or about to develop. These assays may give false-positive reactions with sera of dogs imported from endemic areas that have been vaccinated against *Leishmania* and in dogs infected by *Trypanosoma cruzi*, another protozoan that infects dogs in the Americas. PCR allows detection of *Leishmania* DNA in animal's tissue. Aspirates of lymph node, bone marrow, spleen, skin biopsies, and conjunctival swabs provide better diagnostic sensitivity compared to samples from blood, buffy coat or urine (Maia et al., 2009; Solano-Gallego et al., 2011). PCR assays are available at some veterinary diagnostic laboratories and can be used to confirm infection. Many pets that have spent prolonged periods of time in endemic countries, however, will be positive by PCR as low-level sub clinical carriers and a positive result in these pets does not mean that *Leishmania* is necessarily the cause of any presenting clinical signs.

Screening for heartworm

Testing for uterine antigen secretions of female adult *D. immitis* heartworms is a highly specific diagnostic test in infected dogs and highly sensitive in cases of large worm burdens. A second test in newly imported animals 6 months after arrival is useful to rule out infection. The modified Knott's test carried out by some external labs concentrates microfilariae in the blood and is a useful test in positive dogs to quantify the numbers of circulating microfilariae. Dogs with high circulating numbers are more likely to suffer anaphylaxis if treated with macrocyclic lactones.

Future changes to the pet travel scheme

Currently the only legal requirements for dogs, cats and ferrets travelling within EU and listed non-EU countries are as follows. The pet must be microchipped. Vaccination against rabies at least 21 days before travel. There is a minimum age of 12 weeks for rabies vaccination. A valid EU Pet Passport Travel with an approved transport company on an authorised route. Dogs entering any of the UK, Ireland, Finland, Norway or Malta must be treated for tapeworms by a vet with a product containing praziquantel (or equivalent) not less than 24 hours and not more than 120 hours (between 1 and 5 days) before its arrival. These rules are concerned with limiting the spread of rabies and *E. multilocularis*. With the spread of many other vector-borne pathogens across Europe, the addition of rules such as compulsory tick treatment and screening for tick-borne pathogens, heartworm and *Leishmania infantum* have been considered. For any new rules to be added

however, there must be consensus and supporting evidence that the spread of zoonotic pathogens would be significantly limited by introduction of new rules. Additional requirements would also make pet travel more difficult. All of these things must be taken into account when lobbying for changes to the scheme in the future.

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ANGIOSTRONGYLUS VASORUM PLUS FELINE LUNGWORMS: UNDER-DIAGNOSED OR TRULY EMERGING?

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Introduction

The apparent increase in the prevalence of canine and feline lungworms, their emergence in previously free areas, and the rise in clinical cases in dogs and cats during the past decade have attracted attention of veterinary practitioners. Canine angiostrongylosis and feline aelurostrongylosis are globally widespread important respiratory diseases affecting dogs and cats. The cardiopulmonary nematode *Angiostrongylus vasorum* infects red foxes (Vulpes vulpes) and domestic dogs (*Canis familiaris*), though several other wild canids involving wolves, coyotes and jackals can serve as reservoir hosts. *Aelurostrongylus abstrusus*, the "cat lungworm" resides in the bronchioles and alveolar ducts of domestic cats and feral felids. *A vasorum* and *A. abstrusus* have an indirect life cycles with a wide range of gastropods (slug and snails) as obligatory intermediate hosts. Dogs and cats acquire infection through the ingestion of intermediate hosts or frogs or possibly birds acting as paratenic hosts. Clinical manifestations of canine angiostrongylosis and feline aelurostrongylosis may range widely from subclinical to a variety of respiratory signs including life-threatening respiratory disease. In cats, other lungworm species, *Troglostrongylus brevior* has been increasingly reported in the Mediterranean. The nematode previously affiliated to wild felids have gained increased attention since recent reports of its infections and co-infections with *Aelurostrongylus abstrusus* in domestic cats.

Angiostrongylus vasorum

Adult worms reside in the pulmonary artery and its branches and the right side of the heart in dogs and other carnivores (excluding cats). Larvae and rarely adult specimens have been recovered occasionally in ectopic locations like the brain, bladder, kidney, femoral artery or anterior chamber of the eye. The distribution of *A. vasorum* is heterogeneous and includes endemic foci and areas in several European countries. Recent reports describe larger endemic areas with the expansion of the parasite in dogs and wildlife. Foxes in particular are considered an important reservoir in Europe, for instance in Denmark *A. vasorum* regional prevalence of 1.7–37.5% was recorded (Lemming et al. 2020). Several other wild carnivores can serve as *A. vasorum* hosts, i.e. raccoon dogs (*Nyctereutes procyonoides*), American mink (*Neovison vison*), beech marten (*Martes foina*) and polecats (*Mustela putorius*) (Lemming et al. 2020). Dogs acquire infection through the ingestion of intermediate hosts (terrestrial slugs and snails) or paratenic hosts (frogs and birds). Infective larvae develop and migrate to the right side of the heart and pulmonary artery to reach the adult stage. Prepatent period lasts 38–60 days. Eggs moved with bloodstream to the lungs hatch rapidly and larvae penetrate the alveoli. They are then coughed up and excreted in faeces as first stage larvae. More than 20 species of slugs and snails have been found to be potential as intermediate hosts. The prevalence of A. vasorum in terrestrial slugs can be significant; for example in south Wales, Great Britain 29.4%–41% were found infected (Aziz et al. 2016) and in Denmark 4%–26% (Ferdushy et al. 2009). *A. vasorum* is considered highly pathogenic, however in some cases the signs remain unnoticed for months. A frequent clinical manifestations include respiratory signs such as coughing and dyspnoea (caused by verminous pneumonia and inflammatory response to migrating larvae), coagulation defects and haemorrhagic diathases, neurological and gastrointestinal signs. Infected dogs may develop severe

pulmonary lesions which can potentially lead to pulmonary hypertension (Corda et al. 2019). In chronic infections, coughing, vomiting, anorexia, anaemia, weight loss, decrease exercise tolerance, pulmonary hypertension and signs of coagulopathy, i.e. subcutaneous haematomas and prolonged bleeding from minor injuries can be seen. In rare cases sudden death may occur. Diagnosis is by (1) detecting larvae in fresh faeces using the Baermann method, (2) microscopic detection of first stage larvae in bronchial lavage material or (3) serology with commercial test to detect circulating antigen. The treatment of canine angiostrongylosis is based on the use of macrocyclic lactones with different treatment protocols or repeated daily administration of benzimidazoles (fenbendazole) for 3 weeks. In severe clinical cases supportive treatment (antibiotics, glucocorticoids) with substitute fluids may be needed. In high-endemic areas and/or for hunting dogs or the dog eat grass/plant material harbouring the infective gastropods, the monthly administration of macrocyclic lactones is recommended to prevent infection. *Aelurostrongylus abstrusus*, the cat lungworm is distributed worldwide with a frequency that varies depending on the geographic area and the lifestyle of an animal (indoors/outdoors). The final hosts of *A. abstrusus* are domestic cats and other felids. In Europe, the prevalence in cats varies from 1.2% in domestic cats to 35%–50% in feral and stray cats (Elsheikha et al. 2016). Cats acquire infection by the ingestion of paratenic hosts (amphibians, reptiles, birds or small mammals) or less frequently intermediate hosts (gastropods). After ingestion, the larvae penetrate the stomach or intestine wall and with the bloodstream migrate to the lungs. Adult worms occupy the alveolar ducts and terminal bronchioles. The prepatent period is on average six weeks. Lung infection with *A. abstrusus* in cats may be asymptomatic or it can elicit serious respiratory signs such as dyspnoea, coughing, lethargy, interstitial bronchopneumonia and respiratory failure. In severe cases death may occur. Basic diagnosis is the isolation larvae from fresh faeces using the Baermann technique. As diagnostics for larvae detection is not routinely performed in cats and due to large daily variation in larval excretion, the detection rate of *A. abstrusus* in cats is underestimated. Treatment with spot-on preparations licensed for cats (eprinomectin 0.5 mg/kg b.w.) is recommended. Other, may also be used: fenbendazole (50mg/kg b.w. for 5–15 days), spot-on moxidectin, emodepsid or selamectin. Troglostrongylus brevior has been increasingly reported in domestic cats in the Mediterranean (Spain, Sicily, mainland Italy, Greece, Bulgaria, Albania). Adult worms reside in the trachea and the bronchial tree. *T. brevior* display a similar biology to *A. abstrusus* and clinical picture resembles that of the cat lungworm infection. For *T. brevior* and *A. abstrusus* mixed infections, the diagnosis is difficult due to similarity of the larvae excreted in the faeces. Treatment options include spot-on eprinomectin, moxidectin or emodepsid. The combination product of eprinomectin, praziquantel, fipronil and (S)-methoprene was demonstrated to be 100% efficacious against developing larval and adult *T. brevior* in experimentally infected cats (Knaus et al. 2020).

Summary

Canine angiostrongylosis and feline aelurostrongylosis should always be included in the differential diagnosis of respiratory diseases in pet animals living in areas endemic for lungworms. A coproscopy for larvae should be considered as the first diagnostic step in dogs and cats at risk of lungworm infections. Red foxes serving as an important sylvatic reservoir of *A. vasorum* may contribute to emerging cases in dogs.

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