

Control of Vector-Borne Diseases in Dogs and Cats

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TABLE OF CONTENTS

INTRODUCTION.....4

1. CONSIDERATION OF PET HEALTH AND LIFESTYLE FACTORS.....8

2. PREVENTION AND CONTROL OF VECTOR-BORNE DISEASES.....8

2.1. Insect-borne diseases8

2.1.1. Leishmaniosis8

2.1.2. Dirofilariosis and other filarial infections.....15

2.1.3. Bartonellosis.....22

2.1.4. Viral infections.....24

2.2. Tick-borne diseases25

2.2.1. Babesiosis (Piroplasmosis).....25

2.2.2. Ehrlichiosis29

2.2.3. Anaplasmosis.....31

2.2.4. Borreliosis - Lyme disease33

2.3. Vector-borne viral diseases.....35

Appendix 1 - Background.....38

FIGURES

Fig. 1: Approximate distribution of canine leishmaniosis in Europe10

Fig. 2: Approximate distribution of *Dirofilaria immitis* and *Dirofilaria repens* in Europe16

TABLES

Table 1: Insect-borne infectious agents of dogs and cats in Europe5

Table 2: Tick-borne infectious agents of dogs and cats in Europe.....6

Table 3: *Leishmania* species infecting dogs and cats in Europe9

Table 4: Chemotherapy of canine leishmaniosis12

Table 5: Filarial species infecting dogs and cats in Europe	15
Table 6: Morphological features of blood microfilariae from filarial worms of dogs and cats.....	18
Table 7: Prevention of dirofilariosis in dogs and cats in Europe	21
Table 8: <i>Babesia</i> species of dogs and cats and their vectors in Europe.....	25
Table 9: Distribution of canine <i>Babesia</i> spp. in Europe	26
Table 10: Clinical manifestations of canine babesiosis	26
Table 11: Chemotherapy of babesiosis in dogs	28
Table 12: Chemoprophylaxis of babesiosis in dogs caused by <i>Babesia canis</i>	28
Table 13: <i>Anaplasma</i> spp. affecting dogs and cats in Europe	31
Table 14: Distribution of pathogenic <i>Anaplasma</i> spp. in Europe.....	32
Table 15: Clinical and laboratory findings of pathogenic <i>Anaplasma</i> infections in dogs	32
Table 16: Vector-borne viruses which can affect dogs or cats in Europe	35
Table 17: Distribution of vector-borne virus infections in dogs and cats in Europe.....	36
Table 18: Clinical manifestations of vector-borne virus infections in dogs.....	36

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, Diptera (mosquitoes, ¹phlebotomine sand flies, muscid flies), lice and fleas.

Vector-borne pathogens or diseases are important because:

- They may be highly pathogenic in dogs and cats
- Their transmission is often unpredictable
- Their diagnosis and control are difficult
- Variable clinical signs can develop after long incubation periods and these are rarely pathognomonic
- Animals may have persistent infections and thus act as reservoirs
- Several are important zoonoses, such as leishmaniosis, borreliosis, rickettsiosis, bartonellosis and dirofilariosis

Climatic and ecological changes, national regulations on the management of stray dogs and cats together with the 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 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 wildlife, which act as reservoirs.

Effective control of vector-borne diseases requires a thorough knowledge of the infectious agents, their vectors and major hosts. Besides giving an overview of the majority of vector-borne diseases of dogs and cats, this guideline focuses on the following important infections/diseases: leishmaniosis, dirofilariosis, bartonellosis, babesiosis, ehrlichiosis, anaplasmosis and vector-borne viral diseases.

The following vector-borne diseases are not presented in detail in this guideline, but are mentioned here and in the tables:

- Rickettsiosis (e.g. *Rickettsia conorii*, *R. slovaca*, *R. felis* – these are small intracellular Gram-negative bacteria that typically cause fever in the acute phase in susceptible hosts; they are transmitted by many arthropods).
- Hepatozoonosis (e.g. *Hepatozoon canis* – protozoal pathogens of dogs transmitted by ingestion of an infected tick).
- Thelaziosis (*Thelazia callipaeda* – a nematode located in the conjunctival sac and transmitted by drosophilid flies).
- Haemoplasmosis (formerly haemobartonellosis, caused by small Gram-negative bacteria, Mycoplasmas or haemoplasmas, that attach to the surface of red blood cells, e.g., *Mycoplasma haemocanis* and *M. haemofelis*, in dogs and cats, respectively). Other less pathogenic species have been described in cats mainly: ‘*Candidatus Mycoplasma haemominutum*’ and ‘*Candidatus Mycoplasma turicensis*’ and in dogs, ‘*Candidatus Mycoplasma haematoparvum*’ and although their mode of natural transmission is still not known, ticks and fleas could be implicated.

¹ phlebotomine sand flies – within Europe Psychodid sand flies of the genus *Phlebotomus* are responsible for the transmission of leishmaniosis. These will be referred to throughout the text as phlebotomes.

Table 1: Insect-borne infectious agents of dogs and cats in Europe

Disease or infection	Causative agents	Vector ¹	Host	Geographic distribution	Severity of clinical signs
DISEASES CAUSED BY PROTOZOA					
Leishmaniosis	<i>Leishmania infantum</i>	Phlebotomes	Dogs, cats, foxes	southern Europe	Subclinical-severe
DISEASES CAUSED BY HELMINTHS					
Dipylidiosis	<i>Dipylidium caninum</i>	Fleas, lice	Dogs, cats, foxes	Europe	Subclinical
Filarioses	<i>Dirofilaria immitis</i>	Culicidae	Dogs, cats, foxes, humans	Europe	Subclinical-severe
	<i>D. repens</i>	Culicidae	Dogs, cats, foxes, humans	southern and eastern Europe	Minor-moderate
	<i>Acantocheilonema dracunculoides</i> & <i>A. reconditum</i>	Culicidae and (<i>Rhipicephalus sanguineus</i>)	Dogs, foxes	Spain, France, Italy, Portugal	Minor
Thelaziosis	<i>Thelazia callipaeda</i>	Drosophilid flies	Dogs, cats, foxes, wolves, humans, other mammals	Italy, France Switzerland, Spain	Minor-severe
BACTERIAL INFECTIONS OR DISEASES					
Rickettsiosis	<i>Rickettsia felis</i> other	Fleas	Dogs, cats, hedgehogs, humans	Europe	Subclinical-moderate
Bartonellosis (cat scratch disease)	<i>Bartonella henselae</i>	Fleas, (ticks)	Cats (reservoir host), humans	Europe	Subclinical-minor
Bartonellosis (dog endocarditis)	<i>Bartonella vinsonii</i> and others	Arthropod vectors	Dogs	Europe	Moderate-severe
Haemoplasmas	<i>Mycoplasma haemofelis</i> (cats); <i>M. haemocanis</i> (dogs)	Fleas (ticks) suspected	Cats, dogs	Europe	Cats: minor-severe Dogs: subclinical
Tularaemia	<i>Francisella tularensis</i>	Mosquitoes Tabanidae	Cats (dogs), humans	southern Europe	Subclinical-severe
VIRAL INFECTION					
West Nile virus	West Nile virus (WNV), Flavivirus	<i>Culex</i> spp. and other mosquitoes	Horses, humans, (dogs, cats); reservoir: birds	Romania, Czech Republic, Italy, France, Portugal	Subclinical-severe

¹ non-insect vectors given in parentheses

Table 2: Tick-borne infectious agents of dogs and cats in Europe

Disease	Causative agents	Hosts	Vectors	Geographic distribution in Europe	Severity of clinical signs
DISEASES CAUSED BY PROTOZOA					
Babesiosis (piroplasmosis)	<i>Babesia canis</i>	Dogs, wolves	<i>Dermacentor reticulatus</i>	western, southern and central Europe up to the Baltic	Moderate - severe
	<i>B. vogeli</i>	Dogs	<i>Rhipicephalus sanguineus</i>	southern Europe following distribution of vector	Mild - moderate
	<i>B. gibsoni</i> and <i>B. gibsoni</i> - like	Dogs, wolves	<i>Haemaphysalis</i> spp., <i>Dermacentor</i> spp.	sporadic and rare in Europe	Moderate - severe
	<i>Babesia (Theileria) annae</i>	Dogs, foxes	<i>Ixodes hexagonus</i> ²	north-western Spain, Portugal, Croatia	Moderate - severe
Hepatozoonosis	<i>Hepatozoon canis</i> ¹	Dogs	<i>Rhipicephalus sanguineus</i>	southern Europe	Mostly mild infection; subclinical
	<i>Hepatozoon</i> spp.	Cats	Unknown	Spain	Subclinical
DISEASES CAUSED BY NEMATODES					
Filariosis	<i>Acanthocheilonema (Dipetalonema) dracunculoides</i> , <i>Acanthocheilonema (D.) reconditum</i> , <i>Cercopitiphilaria</i> spp.	Dogs, cats	<i>Rhipicephalus sanguineus</i> ³	southern Europe	Minor

Table 2 contd: Tick-borne infectious agents of dogs and cats in Europe

Disease	Causative agents	Hosts	Vectors	Geographic distribution in Europe	Severity of clinical signs
DISEASES CAUSED BY BACTERIA					
Bartonellosis	<i>Bartonella henselae</i> , <i>Bartonella vinsoni</i> , <i>Bartonella</i> spp.	Many animals, dogs, cats, humans	Ticks suspected ³	throughout Europe	Commonly subclinical infection
Borreliosis (Lyme disease)	<i>Borrelia burgdorferi</i> complex (especially <i>B. garinii</i> and <i>B.</i> <i>afzelii</i> in Europe)	Many animals especially rodents, dogs, cats, humans	<i>Ixodes ricinus</i> , <i>I. hexagonus</i> , <i>I. persulcatus</i>	throughout Europe	Mostly subclinical
Ehrlichiosis (monocytic)	<i>Ehrlichia canis</i>	Dogs (cats)	<i>Rhipicephalus</i> <i>sanguineus</i>	southern Europe following distribution of vector	Moderate – severe
Neoehrlichiosis	<i>Candidatus</i> <i>Neoehrlichia</i> <i>mikurensis</i>	Rodents, humans, dogs	<i>Ixodes ricinus</i>	Europe	unknown
Anaplasmosis (granulocytic ehrlichiosis)	<i>Anaplasma</i> <i>phagocytophilum</i>	Many animals, dogs,cats, humans	<i>Ixodes ricinus</i> , (<i>I. trianguliceps?</i>)	throughout Europe	Mild and subclinical infections common
Anaplasmosis (infectious cyclic thrombocytopenia)	<i>Anaplasma platys</i>	Dogs	<i>Rhipicephalus</i> <i>sanguineus</i>	southern Europe following distribution of vector	Commonly asymptomatic
Rickettsial infections (Mediterranean spotted fever/MSF)	<i>Rickettsia conorii</i>	Dogs	<i>Rhipicephalus</i> <i>sanguineus</i>	southern Europe following distribution of vector	Commonly asymptomatic
Coxiellosis (Q Fever)	<i>Coxiella burnetii</i>	Ruminants, dogs, cats, humans	<i>Ixodes</i> spp., ³ <i>Dermacentor</i> spp.	throughout Europe	Subclinical infection
Tularaemia	<i>Francisella tularensis</i>	Lagomorphs, cats	<i>Ixodes</i> spp., ³ <i>Dermacentor</i> spp., <i>Haemaphysalis</i> spp., <i>Rhipicephalus</i> <i>sanguineus</i> ¹	southern Europe	Subclinical infection occasionally moderate to severe in young cats
DISEASES CAUSED BY VIRUSES					
European tick- borne encephalitis	TBE virus, (Flavivirus)	Many animals, rodents, dogs	<i>Ixodes ricinus</i> , <i>I. persulcatus</i>	central, eastern and northern Europe	can be moderate but not commonly reported
Louping ill	Louping-ill virus, (Flavivirus)	Many animals, mainly sheep, dogs	<i>Ixodes ricinus</i>	UK, Ireland	can be moderate- severe but not commonly reported

¹ Transmission of *Hepatozoon* spp. is by ingestion of an infected tick and not a tick bite.

² Not yet experimentally demonstrated.

³ Ticks are not the sole arthropod vectors for these diseases.

1. CONSIDERATION OF PET HEALTH AND LIFESTYLE FACTORS

Animals require care tailored to their individual needs. Certain factors may dictate more intensive monitoring and/or treatment, while others may suggest a less aggressive approach.

Animal

Age and health status of the animal are important including its history and origin. Some breeds or individuals have a genetically determined susceptibility to some diseases such as leishmaniosis, while other concomitant infections may predispose to or aggravate vector-borne diseases.

Environment

Dogs and cats in kennels or catteries or animals living outdoors may be at greater risk of acquiring vector-borne diseases than individual animals living indoors. The risk of transmission may also depend on various local conditions such as (micro-) climate and local topography.

Nutrition

Poor nutrition may contribute to susceptibility to many diseases including vector-borne diseases.

Location and travel

Dogs and cats living in or travelling to specific geographical areas endemic for certain vector-borne diseases are at a higher risk of infection; for example, animals travelling with their owners on holiday or when rehoming, going to boarding facilities, to dog and cat shows, to the field during countryside walks or during hunting activities.

Transmission by blood transfusion

Veterinary surgeons should be aware that some of these infections may be present in the blood of individuals that appear healthy. It is especially important to avoid causing iatrogenic infection from these individuals. In particular, animals that are going to act as blood donors should be screened and demonstrated to be seronegative for relevant infections prior to donating blood.

2. PREVENTION AND CONTROL OF VECTOR-BORNE DISEASES

2.1. Insect-borne diseases

2.1.1. Leishmaniosis

2.1.1.a. Agents and vectors

In Europe, canine leishmaniosis is predominantly caused by *Leishmania infantum* which comprises various enzymatic types (zymodemes); other species (*L.tropica*, *L. major*) have rarely been diagnosed (Table 3). The vectors are several species of blood-sucking flies of the genus *Phlebotomus* (Phlebotominae subfamily; phlebotomes = sand flies).

The dog is considered the main reservoir of *L. infantum* infection, but cats can also be hosts of *L. infantum*. Many other mammalian species can be infected including humans, and this parasite has been isolated from various rodents such as rats and squirrels. Horses, cattle, goats, sheep, cats and wild canids including foxes, wolves and jackals can be infected but the epidemiological role of these hosts has not yet been clearly established.

The development of phlebotomes takes place in terrestrial habitats; eggs are laid in soil rich in organic matter and the larvae develop through four instars before they pupate and the adults emerge. The seasonal dynamics of phlebotomes have not been fully explored; however, it is known that some

palaeartic species overwinter as 4th stage larvae. Phlebotomes have a nocturnal circadian activity, most species seeking their hosts immediately after sunset. Activities vary from species to species and within their habitat. During the day, adult phlebotomes rest in dark and humid places especially cracks and holes in stone walls, piles of wood, basements or dark cellars of houses and animal stables.

Phlebotomes are widespread in the Mediterranean region, Africa and the Middle East. They are well adapted, depending on the species, to tropical or subtropical climates and even to arid habitats. Furthermore, it has been known for decades that the *Phlebotomus perniciosus* endemic area extends up to northern France, and this species was found in localised areas in southern Germany and southern Switzerland.

Table 3: *Leishmania* species infecting dogs and cats in Europe

Causative agent	Vector	Hosts
<i>Leishmania infantum</i> (variety of zymodemes)	<i>Phlebotomus</i> spp. (sand flies) e.g.: <i>P. perniciosus</i> , <i>P. ariasi</i> , <i>P. perfiliewi</i> , <i>P. neglectus</i> , <i>P. tobbi</i> , <i>P. langeroni</i>	Dogs, foxes, jackals, rodents, cats, various other mammals and humans
<i>L. tropica</i>	<i>P. sergenti</i> , <i>P. arabicus</i>	Dogs and humans
<i>L. major</i>	<i>P. papatasi</i>	Rodents, dogs and humans.

2.1.1.b. Biology and transmission

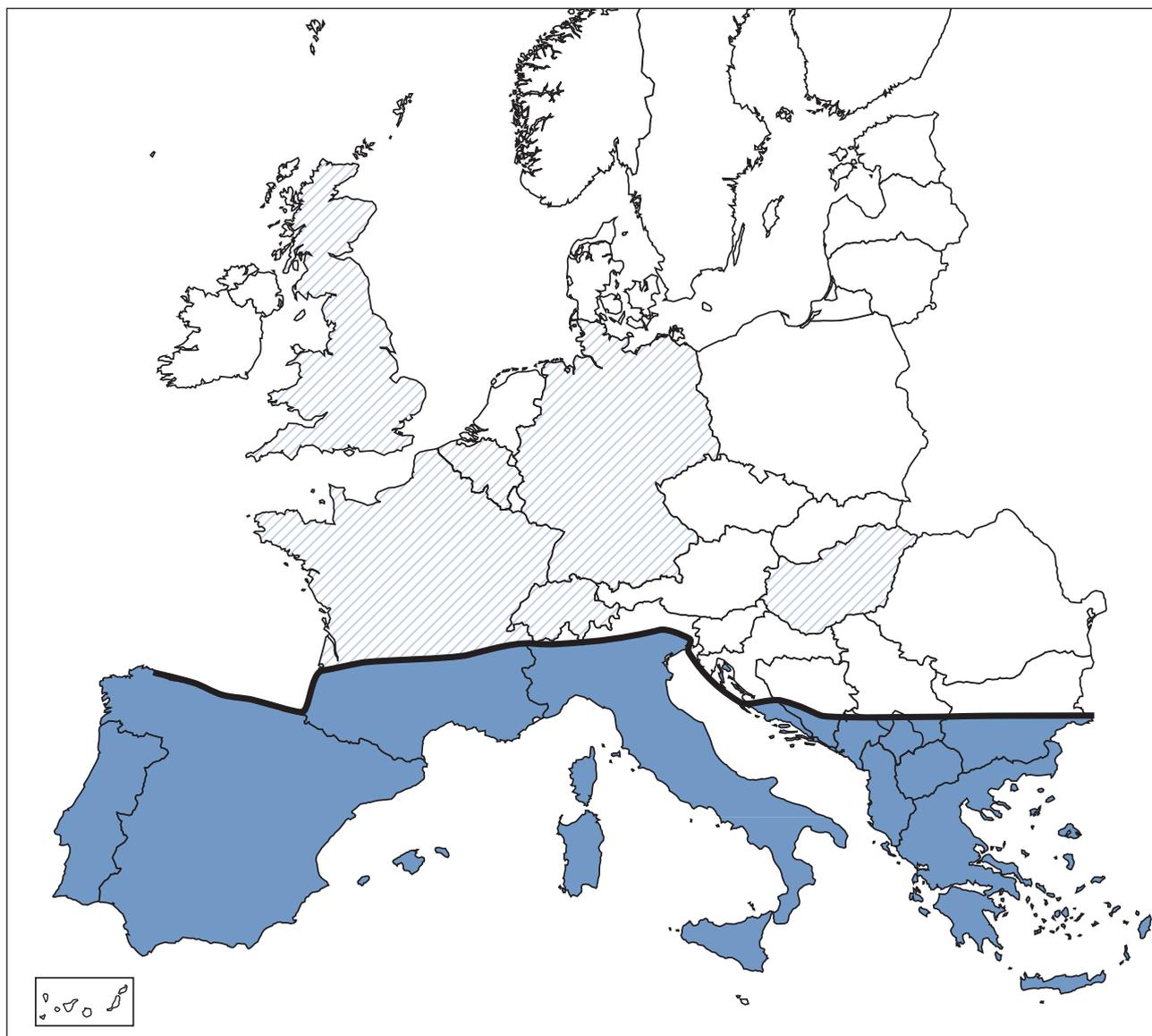
- *Leishmania* spp. occur and multiply in two well differentiated forms: intracellular amastigote stages infecting cells of the vertebrate host and extracellular flagellated promastigote stages in the gut of phlebotomes.
- *Leishmania* spp. are highly vector-specific and are transmitted by the blood-sucking females of several *Phlebotomus* species while feeding on their hosts. Vector activity is highest at dawn and at a minimum temperature of 18-22°C.
- The parasite's development in the vector is temperature dependent and lasts around 7-14 days at temperatures above 18°C.
- Other ways of transmission of *Leishmania* not dependent on phlebotomes such as from mother to offspring (intra-uterine), via infected blood donors or venereal transmission, have been observed but their epidemiological significance is low. Further, direct transmission by biting or through wounds or transmission with other hematophagous arthropods (e.g. ticks, fleas) have been postulated but remain unproven.
- There is some evidence of resistance in certain dog breeds (e.g. the Ibiza hound) as well as susceptibility of other breeds (e.g. German Shepherds, Rottweilers, Cocker Spaniels and Boxers) to disease development, but no sex- or age-dependent risks have been described. Infected dogs without clinical signs, including those which have undergone successful chemotherapy, may represent potential parasite carriers.
- The incubation period can vary between 3 months to years and is dependent on the individual immune response of the infected dogs.
- After local multiplication of parasites in dendritic cells and macrophages in the skin, dissemination primarily occurs via the lymphatic system and blood. Parasites can be found mainly in the skin, lymph nodes, spleen, liver, bone marrow and many other organs or body fluids (e.g. intestine, saliva, semen, urine).
- Clinical signs are only observed in a low proportion of infected dogs. Infected but clinically unaffected dogs represent an important reservoir of infection for phlebotomes.
- The main risks in endemic areas are related to vector exposure and abundance of reservoir hosts which include dogs living outdoors, stray dogs, dogs adopted from animal shelters in endemic areas and hunting dogs.

- Recent studies suggest that cats might act as an alternative reservoir host of *L. infantum* based on the PCR-detection of infections in peripheral blood in up to 20% of cats in Portugal and 60% in Sicily. Further investigations are required to confirm the possible role of cats in *L. infantum* transmission.

2.1.1.c. Distribution in Europe

Canine leishmaniosis is endemic in southern Europe with prevalence rates of infection of up to 75% in exposed populations. Fig. 1 shows the approximate northern limit of the endemic area. Outside this area, many imported cases of canine leishmaniosis and a few cases in cats have been diagnosed and treated. However, there are a few reports of isolated cases in dogs which did not travel through or stay for some time in endemic areas. Most probably, focal transmission can occur for a limited period of time if there is sufficient infection pressure from imported infected dogs.

Fig. 1 Approximate distribution of canine leishmaniosis in Europe:



2.1.1.d. Clinical signs

In endemic areas, a large number of infected dogs may be clinically unaffected.

Clinical signs are highly variable depending on immune responses, disease history and possibly many other as yet unknown factors. Local cutaneous lesions at the site of the initial phlebotome bites

are often the first signs observed before disseminated infection occurs. The typical sites of *Phlebotomus* bites are mainly the ear pinnae, the nose and the abdomen. The localised lesions sometimes go unnoticed or are misdiagnosed as tick or simply insect bites. They consist of single or several specific papular to ulcerative lesions, called chancres or “chancre d’inoculation”. They last several weeks but are self-limiting. During this period, infected dogs may remain seronegative but later, around 25% seroconvert and the disease becomes generalised. In affected dogs, enlargement of single or multiple lymph nodes may be evident accompanied by weight loss, anorexia and asthenia. More severe clinical signs may develop, and the disease can be fatal if therapy is not instituted. Severe clinical signs include skin lesions like alopecia, nodules, ulcers, hyperkeratosis, intense exfoliative dermatitis, mucocutaneous lesions, and onychogryphosis. Generalized cutaneous forms of the disease are normally non-pruritic and symmetrical and are most often keratoseborrhoeic, but may also be ulcerative, papular or pustular or, less frequently, nodular. General signs include: loss of body weight, asthenia, muscular atrophy, splenomegaly, epistaxis and haematuria. Other clinical signs include gastrointestinal disorders (vomiting, diarrhoea and chronic colitis), polyarthritis, glomerulonephritis (polyuria and polydypsia), ocular lesions (blepharitis, conjunctivitis, keratoconjunctivitis, anterior uveitis), and neurological disorders.

Although the clinicopathological abnormalities may be variable, there are many common findings such as a normocytic normochromic non-regenerative anaemia and, less frequently, a thrombocytopaenia, leukopaenia, plasma protein changes with hyperglobulinaemia and hypoalbuminaemia, proteinuria and a variable azotaemia with an increase in the protein/creatinine ratio due essentially to glomerulonephritis in some sick dogs.

2.1.1.e. Diagnosis

To reduce the potential for transmission of *Leishmania* from dogs to vectors, the diagnosis should be confirmed and treatment instituted as early as possible. Clinical signs together with relevant epidemiological information and various laboratory test abnormalities (CBC, biochemical profile and urinalysis including a urine protein/creatinine ratio should always be performed) strongly indicate a tentative diagnosis.

Direct diagnosis is possible by detecting the amastigote stages in Giemsa or Diff-Quick stained smears obtained from superficial lymph nodes or bone marrow aspirates or by observing the promastigotes after *in vitro* cultivation of samples. The sensitivity of parasite detection is lower with skin biopsies and is generally reduced in clinically healthy, infected dogs, but can be increased by molecular or immunohistochemical techniques.

Polymerase chain reactions (PCRs), mostly targeting repetitive sequences, have proven to be highly sensitive compared with the laborious *in vitro* cultivation, and they are not impaired by microbial contamination. However, the diagnostic sensitivity is dependent on the quality of the clinical samples. Lymph node aspirates, especially from animals with lymphadenopathy, are most convenient while bone marrow sampling is more invasive but may be indicated for special cases such as suspect but clinically unremarkable animals. Blood samples can be used in clinical cases but the diagnostic sensitivity is low while skin biopsies have been shown to be a useful alternative for sensitive molecular diagnosis. Quantitative PCR allows the parasitic load to be estimated in comparable tissues, which could be useful for follow-up during treatment although this approach needs to be thoroughly evaluated.

Serology is the most commonly used first step allowing the detection of a specific antibody response in dogs around 8-12 weeks after an initial infection. In subclinical infections this period may extend to years. Different laboratory based methods have been used to detect anti-*Leishmania* antibodies such as the indirect fluorescent antibody test (IFAT), enzyme linked immunosorbent assays (ELISAs), Western blot (WB) analysis or direct agglutination tests (DATs). Both the sensitivity and specificity of these tests vary according to the defined cut-off values in different laboratories. Point of care tests (“rapid tests”) based on immunochromatographic methods have been developed and many

commercial kits are now available for practitioners either for a diagnosis in the clinic or for use in epidemiological field studies. These tests have a reasonable sensitivity for the initial detection of untreated clinical cases. For the confirmation of clinical cases and for the clinical management after chemotherapy, especially in animals with low specific antibody reactions, methods allowing semi-quantitative estimations (e.g. IFAT, ELISA) are required. Serological results have to be carefully interpreted in vaccinated dogs!

2.1.1.f. Control

Treatment

Before initiating chemotherapy, animal owners should be informed about the prognosis, costs and the fact that the dog remains infected even when a clinical cure is achieved. Furthermore, there are certain country-specific veterinary public health regulations that have to be respected. Although euthanasia of infected dogs is not obligatory in any European country, there is an obligation for practitioners to communicate all new cases to the appropriate authorities in some countries such as Portugal, Italy and Greece.

Indications for treatment

Clinical signs and clinicopathological abnormalities associated with a positive serology and/or the evidence of the parasite in target organs. The drugs which are mostly used for treatment of clinical cases of canine leishmaniosis are listed in Table 4 (see www.esccap.org for details of approved products for specific countries). Generally, in non-endemic areas, single drug treatments with allopurinol or meglumine antimoniate or, more recently, with miltefosine, have been used successfully. In European endemic areas with a high seasonal infection pressure, combined therapy is recommended.

Besides specific therapy, symptomatic treatment together with an appropriate diet is recommended. A commercially available diet for clinically affected dogs without signs of renal disease is available containing moderate protein levels supplemented with omega acids, zinc sulphate and antioxidants.

An improvement may be observed within a few weeks after beginning chemotherapy but clinical cure is only achieved after several months. As the *Leishmania* infection is not eliminated by treatment with currently available compounds, relapses are common. First indicators of relapse are clinical signs and/or clinicopathological abnormalities associated with the disease, together with a significant rise in specific antibody reactions by ELISA or by IFAT (a 2-4 fold increase in titer) when examined by the same laboratory.

If there is no clinical improvement after a course of treatment, an alternative drug or a different dosage should be considered. Alternatively, the diagnosis should be queried or the animal should be examined for the presence of concomitant diseases such as ehrlichiosis, babesiosis, hepatozoonosis, neoplasias, or immunomediated diseases, all of which may affect the response to treatment.

Table 4: Chemotherapy of canine Leishmaniosis

Drugs	Dosage	Route of administration
Meglumine antimoniate	75-100 mg/kg daily for 4-8 weeks	Subcutaneous injection
Allopurinol*	10-20 mg/kg (BID or TID) for 6-18 months (minimal daily dose 20 mg/kg)	Oral
Miltefosine	2 mg/kg once daily for 4 weeks (with food)	Oral
Meglumine antimoniate + allopurinol*	see above for both compounds	Subcutaneous injection + oral
Miltefosine + allopurinol*	see above for both compounds	Both oral

*Not registered for veterinary use in Europe

Numerous pharmacokinetic studies have shown that administration of meglumine antimoniate by intramuscular or subcutaneous injection is more effective in maintaining sustained drug plasma concentrations than intravenous injections. After intravenous administration, plasma concentrations fall within 2 hours whereas they fall within 4 hours after intramuscular application. When injected subcutaneously, plasma concentrations rise after 5 hours and remain at therapeutic levels for at least 12 hours. It must be stressed that repeated intramuscular injections frequently lead to the development of painful oedematous reactions and myositis and are therefore not recommended; subcutaneous injections, being safer and painless, are preferred. Different dosages of meglumine antimoniate have been recommended but the most widely accepted regimen is indicated in Table 4.

Allopurinol is commonly administered twice or three times daily in a whole dose of 10-20 mg/kg bodyweight orally for 6-18 months with generally satisfactory results, a clinical cure being observed in most dogs within a few months of treatment. After a clinical cure has been achieved, it is advisable to stop treatment and monitor the dogs for possible relapses after 3 months and subsequently at 6-monthly intervals. As with all other drugs, relapses are relatively common but animals can generally be re-treated with the same compound. Some side effects have been reported including the development of xanthine nephrolithiasis (few reports) and dogs on long-term therapy with allopurinol should be checked using urinalysis and/or abdominal ultrasonography. Usually, xanthinuria has a good prognosis and this side effect disappears shortly after reducing the dosage or the cessation of treatment (if this is deemed necessary).

In the last few years, different clinical trials have been conducted in Spain, France and Italy with a new alkylphospholipid molecule (miltefosine). This drug has shown therapeutic effectiveness comparable with that of antimonial compounds. Side effects including vomiting, diarrhoea and anorexia of varying severity have been reported but these are quick to resolve if the drug is administered with food.

Recent clinical trials combining two compounds (see Table 4) have shown promising results with a reduced relapse rate.

Curative effects of many other drugs have been reported in the treatment of canine leishmaniosis, for example Amphotericin B, but this drug is not well accepted due to its nephrotoxicity and the invasive intravenous route of administration. Moreover, as it is an important compound used in human medicine, WHO and several public health committees argue for restricted use of Amphotericin B (liposomal formulations) to avoid selection for resistance.

Resistance to drugs used for the chemotherapy of *L. infantum* in dogs

Resistance has been observed against meglumine antimoniate *in vitro*, but no resistance has been recognised for the other recommended drugs.

Control strategies

Some control strategies used in the past such as the culling of sero-positive dogs in endemic areas, have been shown to be ineffective in reducing *Leishmania transmission*.

Prevention of phlebotome bites by the application of repellents/insecticides to dogs in the form of impregnated collars, spot-on and spray formulations is currently the most promising strategy; spray preparations appear to be the least effective in this respect. The basic objective is to interrupt parasite transmission and thus control the disease. The phlebotome season in endemic areas may vary from year to year and from region to region. As a general rule, the season starts in April and continues until November.

Numerous studies have assessed the efficacy of pyrethroids against phlebotome attack. For example, it has been observed that dog-collars impregnated with 4% deltamethrin possess a repellent effect against phlebotomes lasting from one week after application to over six months, thereby resulting in a significant decrease in the incidence of disease in endemic areas such as Italy or Spain over a

period of 2-3 years. Applications of permethrin alone or in combination with imidacloprid as a spot-on, have been shown to protect dogs against phlebotome bites within hours (i.e. after 24 hours) and for up to 3-4 weeks thus decreasing the incidence of canine leishmaniosis in endemic areas. These studies show that the interruption of *Leishmania* transmission through the external application of pyrethroids to dogs could be a major tool if incorporated into future disease control programmes in regions where pet dogs are the main reservoir of *L. infantum*.

Finally, other control measures to reduce disease transmission include keeping dogs indoors during dusk and dawn over the whole risk season, the use of insecticidal room sprays, protective nets in windows and doors (mesh size <0.4 mm²), and mosquito bednets treated with pyrethroids. Wherever they have been implemented, these measures have brought about a dramatic reduction in phlebotome populations. Moreover, reducing the breeding sites of phlebotomes by removing garbage and deposits of organic matter is also recommended in the vicinity of the houses and places where dogs are present.

Vaccination against canine leishmaniosis would undoubtedly represent the best strategy for controlling this disease. Recently, a vaccine based on a native antigen purified from medium supernatant of *L. infantum* cultures has been registered in some EU countries for use in non-infected dogs only. The vaccine can be used in dogs over six months of age, and is based on an initial vaccination with 3 doses once every three weeks and annual revaccination. The results of the preliminary field trials documented a reduction of clinical cases in vaccinated dogs as compared with control dogs, however these results need to be confirmed with more extensive field use.

Resistance to repellents and insecticides: There are no reports of resistance of phlebotomes to pyrethroids.

2.1.1.g. Public health considerations

Human visceral leishmaniosis caused by *L. infantum* is an important vector-borne zoonotic disease in southern Europe. Clinical cases of human leishmaniosis generally prove fatal without therapy, especially in children and immunocompromised patients. However, many infected immunocompetent people do not develop disease and are subsequently protected.

The responsibility of veterinary practitioners must be to adequately manage the disease in dogs and to reduce parasite transmission since dogs are the main reservoir of infection.

The following principles must be stressed:

- A thorough diagnostic procedure should be established to identify infected and sick dogs.
- The best treatment for sick dogs should be chosen, bearing in mind the potential risks for the development of resistance to “first line” drugs used in humans.
- The use of insecticides should be recommended for all dogs at risk and especially for infected clinically healthy or sick dogs even after successful chemotherapy; these should be applied throughout the risk season which depends on climatic conditions. In the southern European endemic areas, the risk season is between April and November.
- In endemic areas, kennels housing stray dogs, hunting dogs or breeding dogs should maintain a strict vector-borne disease monitoring programme; this should be combined with measures designed to prevent disease transmission by phlebotomes and thus avoid the risk of focal, highly endemic transmission.
- To avoid an extension of endemic areas, *Leishmania*-infected dogs should not be translocated to non-endemic areas where phlebotomes may be present.

2.1.2 Dirofilariosis and other filarial infections

2.1.2.a. Agents and vectors

Filarial worms are nematodes infecting connective tissues and the vascular system of dogs and cats. Mosquitoes, but also fleas and ticks, act as vectors for the different species (Table 5). *Dirofilaria immitis*, the canine and feline heartworm, is the most pathogenic species, while *D. repens*, which causes subcutaneous dirofilariosis, is the most important species responsible for zoonotic infections in Europe.

Table 5: Filarial species infecting dogs and cats in Europe (see Table 6 for morphology of microfilariae)

Filarial parasite	Vectors	Prepatent period	Length of Adult Worms	Location of adult worms
<i>Dirofilaria immitis</i>	Mosquitoes (Culicidae)	120-180 days	M: 12-18 cm F: 25-30 cm	Pulmonary arteries/right heart
<i>Dirofilaria repens</i>	Mosquitoes (Culicidae)	189-259 days	M: 5-7 cm F: 10-17 cm	Subcutaneous tissue/muscular fasciae
<i>Acanthocheilonema</i> (former: <i>Dipetalonema</i>) <i>reconditum</i>	Fleas and ticks	427-476 days	M: 9-17 mm F: 21-25 mm	Subcutaneous tissue/muscular fasciae, peritoneal cavity, kidney
<i>Acanthocheilonema</i> (former: <i>Dipetalonema</i>) <i>dracunculoides</i>	Fleas and ticks (<i>R. sanguineus</i>)	120 days	M: 15-31 mm F: 33-55 mm	Peritoneal cavity
<i>Cercopithifilaria</i> spp.	Ticks (<i>R. sanguineus</i>)	Unknown	M: unknown F: 23-24 mm	Subcutaneous tissue/muscular fasciae

M: male; F: female

2.1.2.b. Biology and transmission

Filarial nematodes are parasites of domestic and wild carnivores, mainly canids, but due to the low host specificity of their arthropod vectors, many mammalian hosts can be infected, including humans. In such hosts, the parasites generally do not develop to the adult stage.

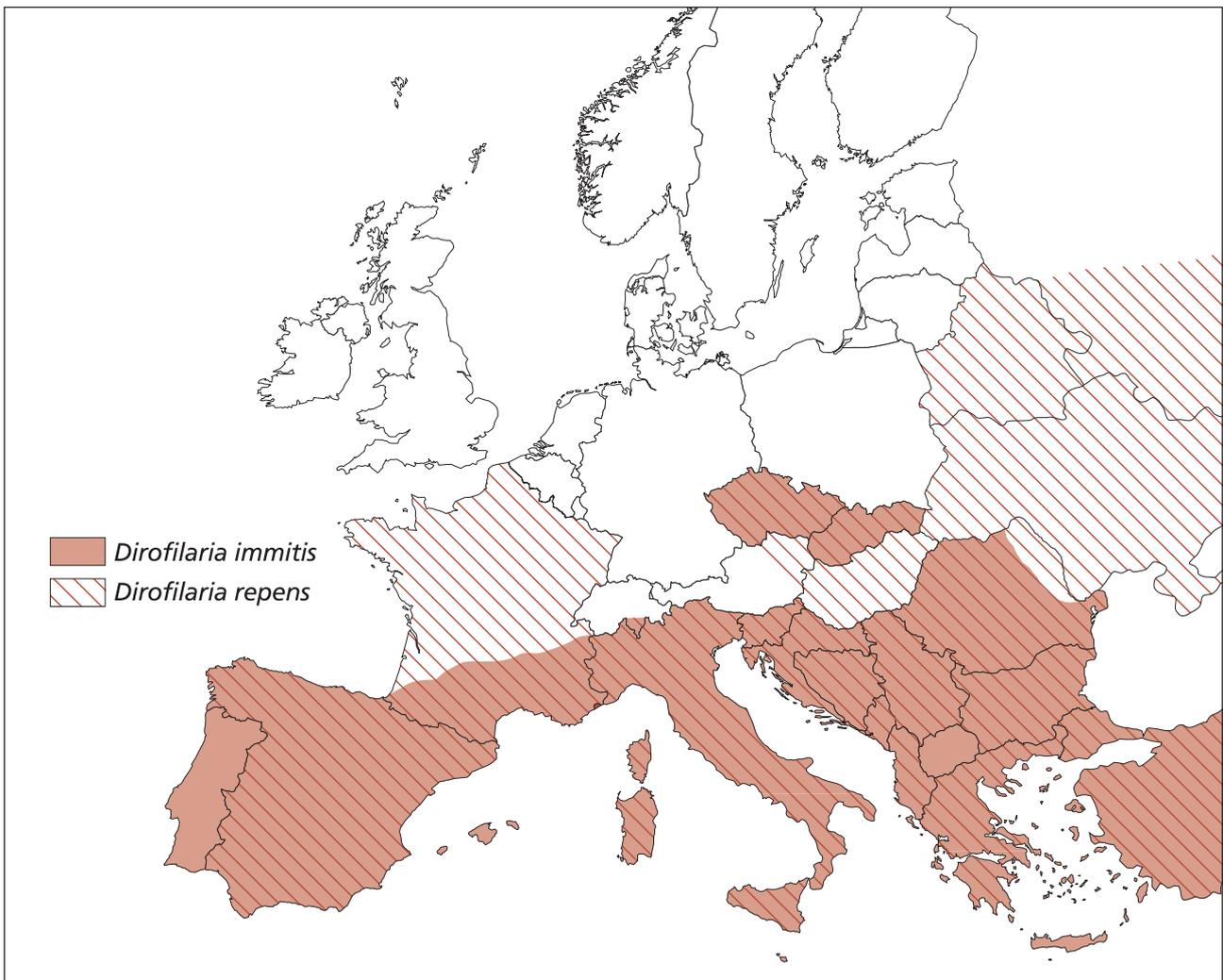
- *D. immitis* and *D. repens* microfilariae are released by female worms into the blood stream where they become available to blood-sucking mosquitoes. Microfilariae develop to the infective stage (L3) in the body of these vectors and are transmitted via their saliva during feeding. *D. immitis* larvae undertake an extensive migration through subcutaneous, subserosal and muscular tissues to reach the pulmonary arteries and the right heart where they develop to the adult stages and mate. In dogs, adult worms have a lifespan of up to 7 years (although survival in cats is shorter), and microfilariae survive 2-18 months in the blood stream. The infective larvae of *D. repens* migrate into the subcutaneous connective tissues where they reach maturity. Adult worms are found between subcutaneous and deep connective tissue layers in most parts of the body, sometimes forming non-inflammatory nodules. Adults can live for several years.
- *Acanthocheilonema* (syn. *Dipetalonema*) *reconditum* is found in subcutaneous tissues and fasciae, the peritoneal cavity and the kidneys of canids, *Cercopithifilaria grassii* is a parasite of the subcutaneous tissues and fasciae of canids and *A. dracunculoides* is a parasite of the peritoneal cavity. For diagnostic purposes, circulating microfilariae of these species must be differentiated from those of *D. immitis* and *D. repens*.

- Many mosquito species are competent intermediate hosts allowing the microfilariae to develop to infective stages which are transmitted to susceptible hosts immediately after initiating the bite. The most important vectors in Europe are species of the genera *Culex*, *Aedes*, and *Anopheles*. Recently, the Asian tiger mosquito *Ae. albopictus*, which is spreading in Europe, has been shown to be a competent vector.

2.1.2.c. Distribution in Europe

The frequency of transmission and the spread of *Dirofilaria* spp. infections depend on environmental factors such as temperature, the density of vector populations and the presence of microfilaraemic dogs, which are the main reservoirs of infections. Due to tourism and animal adoption, infected dogs are increasingly being moved from endemic areas such as Italy and Spain to non-endemic areas.

Fig. 2 Approximate distribution of *Dirofilaria immitis* and *Dirofilaria repens* in Europe



Dirofilaria immitis is endemic/hyperendemic in many countries of south-eastern Europe, including Greece, Turkey, the Czech Republic, Slovenia, Romania and Bulgaria (Fig. 2). The endemic areas of *D. immitis* and *D. repens* overlap in many regions. Recently, *D. repens* infections in dogs that had never left Germany, Austria or Poland have been documented.

Feline *Dirofilaria* infections occur in areas where canine infections are highly prevalent; the prevalence in cats, however, is generally only a tenth of that in dogs. Thus, in northern Italy, an area of high risk for canine heartworm infection, a prevalence of about 7% in pet cats was determined based on antigen detection and echocardiography.

Acanthocheilonema dracunculoides infections have prevalence rates of up to 14% in hunting dogs and dogs living outdoors in some countries and regions of Europe such as Spain and southern Italy (Sicily). *Acanthocheilonema reconditum* is quite frequently found in Sardinia (Italy).

2.1.2.d. Clinical signs

Infections with *D. immitis* may cause a severe and potentially fatal disease in dogs and cats. The adult heartworms live primarily in the pulmonary arteries but are occasionally found in the right heart and in adjacent large vessels such as the cranial and caudal venae cavae. Ectopic localizations in brain, eyes or aorta occur rarely, particularly in cats.

The cat is considered a susceptible, but not ideal, host. The infection in cats is characterized by a relatively low burden of adult worms (2 to 4 worms) which have a short lifespan (about 2 years), and by a low level and short duration of microfilaraemia.

Despite its name, heartworm disease is essentially a pulmonary disease because the worms are predominantly located in the pulmonary arteries and the right heart is involved only in the later stages.

Dirofilaria repens is the most frequent species associated with subcutaneous filariasis of dogs and cats. In some cases, subcutaneous, non-inflammatory nodules containing adult parasites or microfilariae can be observed. These “cold” nodules do not cause any pain and appear free within the skin. The parasite may also be observed during surgery in the perimuscular fasciae, in the perirenal fat or in the abdominal cavity. Rarely, in cases of heavy infection and sensitized patients, pruritus, pustular eruptions, ulcerative lesions and scabies-like dermatitis may be seen associated with microfilariae in the skin.

Infections with *A. reconditum*, *A. dracunculoides* and *C. grassii* are mostly asymptomatic. Differentiation of all species that produce microfilariae which can be found in the blood stream is necessary for specific diagnosis.

DOG

The clinical evolution of heartworm disease in dogs is usually chronic. Most infected dogs do not show any clinical signs for years. Clinical signs of the disease develop gradually and may begin with a chronic cough which may be followed by moderate to severe dyspnoea, weakness, and sometimes syncope after exercise or excitement. At this stage, auscultation may reveal abnormal pulmonary sounds (crackles) over the caudal lung lobes, and a split second heart sound can often be heard. Later, when right congestive heart failure is developing, oedema of the abdomen and less often in the limbs may be observed together with anorexia, weight loss and dehydration. Arterial damage is usually more severe in dogs that perform intensive physical exercise; sudden death is rare and usually occurs following respiratory distress or progressive emaciation.

During the chronic stages of the disease, there may be a sudden onset of acute signs. For example, after severe spontaneous thromboembolism following the natural death of many heartworms, dogs may show acute life-threatening dyspnoea and hemoptysis.

In small dogs, the displacement of adult worms from the pulmonary arteries to the right heart, due to pulmonary hypertension and a sudden fall in right cardiac output, is a common event. In this case, affected dogs present the so-called “caval syndrome”. Dyspnoea, a tricuspid cardiac murmur and haemoglobinuria, due to mechanical haemolysis in the right cardiac chambers, are the most typical signs and the outcome is usually fatal.

CAT

Most cats show no clinical signs for a long time after infection. These cats may undergo spontaneous self-cure without showing any signs or they may suddenly show a dramatic acute syndrome usually

with respiratory signs such as coughing, dyspnoea and haemoptysis; vomiting also frequently occurs. Sudden death in apparently healthy cats is not an infrequent consequence of infection. In most cases, the onset of clinical signs seems to be related to the natural death of parasites or to the arrival of pre-adult heartworms (L5) in the pulmonary arteries. Feline heartworm disease is now recognized as a significant pulmonary syndrome defined as Heartworm Associated Respiratory Disease (HARD). Clinical signs associated with HARD are anorexia, lethargy, weight loss, coughing, rapid heart rate, vomiting, diarrhoea, blindness, convulsions, collapse and sudden death.

2.1.2.e. *Wolbachia*/filarial worm symbiosis:

Gram-negative bacteria of the genus *Wolbachia* are obligate endosymbionts of both *D. immitis* and *D. repens*. These bacteria play an important role in the pathogenesis and immunology of heartworm infection and have been shown to provoke chemokinesis and pro-inflammatory cytokine production in canine neutrophils. They are released by live worms or following worm death through natural attrition, through microfilarial turnover or following pharmacological intervention. *Wolbachia* can be eliminated from filarial worms through antibiotic therapy of the infected host. Such depletion of *Wolbachia* is often followed by clear anti-inflammatory effects, thus, antibiotic treatment may be used concomitantly with the use of adulticidal therapeutic agents.

2.1.2.f. Diagnosis

DOG

Heartworm infection in dogs can be detected with blood tests which demonstrate the presence of circulating microfilariae or adult antigens in serum or plasma samples. Further diagnostic procedures are required to determine the severity of disease and possible treatment options. Morphological differentiation of the microfilariae by their length is often difficult because of overlapping sizes of most species (Table 6). However, microfilariae can be differentiated by the acid phosphatase stain (APh-S) or by molecular means (PCR).

Blood examination for microfilariae: Blood samples should be examined after concentration by the Knott or the filtration test (wet blood smears do not allow species identification and have a very low sensitivity). The identification of microfilariae provides conclusive proof of a specific infection, but up to 30% of dogs have no detectable circulating microfilariae even though they harbour adult worms. Thus, negative test results for microfilariae cannot rule out infection. It should be noted that the intensity of microfilaraemia is not correlated with the adult worm burden and in general, highly microfilaraemic dogs harbour few worms.

Table 6: Morphological features of blood microfilariae¹ from filarial worms of dogs and cats

Species	Length (μ)	Width (μ)	Features
<i>Dirofilaria immitis</i>	290-330	5-7	No sheath, cephalic end pointed, tail straight with the end pointed. APh-S: two activity spots located around the anal and the excretory pores
<i>D. repens</i>	300-370	6-8	No sheath, cephalic end obtuse, tail sharp and filiform often ending like an umbrella handle. APh-S: one spot around the anal pore
<i>Acanthocheilonema reconditum</i>	260-283	4	No sheath, cephalic end obtuse with a prominent cephalic hook, tail button hooked and curved. APh-S: activity throughout the body
<i>A. dracunculoides</i>	190-247	4-6.5	Sheath, cephalic end obtuse, caudal end sharp and extended. APh-S: three spots which include an additional spot in the medium body

¹Microfilariae measured after concentration by the Knott test; when using the DifiI® test, lengths are shorter. APh-S: acid phosphatase stain

Blood/serological tests for adult female antigens: Tests based on ELISA or on immunochromatographic methods designed to detect antigens of adult female heartworms are considered highly specific and some of them can be used in the clinic as “rapid tests”. These tests may provide information about worm burden. Antigen reactions are detected in the late prepatent period, 6-8 months post infection. The sensitivity of these tests is very high but false-negative results may occur in the case of prepatent or very light infections or when only male worms are present. Tests which detect antibodies directed against filarial antigens are non-specific and therefore have no diagnostic value in dogs.

X-rays: In the advanced stages of infection, thoracic radiographs may show enlargement of the pulmonary arteries, abnormal pulmonary patterns and in some severe cases, right-sided cardiomegaly. If congestive right heart failure is present, peritoneal and pleural effusions may be evident. X-rays can be useful to assess the severity of the disease.

Electrocardiography: As an electrocardiogram displays the electrical activity of the heart, abnormalities are usually only found in the later stages of the disease when severe damage to the right heart is present.

Echocardiography: Echocardiography allows direct visualisation of the cardiac chambers and major vessels and thus allows the detection of any parasites in the heart, main pulmonary arteries or caudal vena cava. The heartworms are visible as double, linear parallel floating objects.

CAT

Detection of microfilariae in the blood of infected cats is unlikely to be successful, and the sensitivity is very low.

Blood/serological tests for adult female antigens: Tests detecting adult female heartworm antigens have a very high specificity and can thus provide definitive proof of infection. In many cases, however, these tests yield false-negative results because of low worm burdens or the presence of only male or immature worms. A negative test therefore does not rule out infection.

Blood/serological tests for antibodies: Tests detecting heartworm-specific antibodies can be useful in the diagnostic process. These tests have a high sensitivity but are less specific. In addition, antibody tests may yield positive results in the case of abortive infections or after the spontaneous death of adult parasites. Tests become positive approximately two months after infection and remain positive until long after the elimination of both larval and adult stages. Consequently, antibody tests should be interpreted carefully and must take relevant clinical information into account.

X-rays: Although thoracic abnormalities may be absent or transient in cats, in some cases findings such as enlarged peripheral branches of the pulmonary arteries accompanied by varying degrees of pulmonary parenchymal disease are a strong indication of heartworm infection.

Electrocardiography: Since heartworm infection in cats does not cause pulmonary hypertension, deviation of electrical axis examined by electrocardiography is not affected and thus cannot provide useful clinical information.

Echocardiography: Cardiac ultrasound allows direct visualization of the parasites in the right atrium and ventricle, in the main pulmonary artery and in the origin of both its main branches.

The specificity is virtually 100%, and the sensitivity in cats is very high as only a short portion of the caudal pulmonary arteries cannot be examined. Cardiac ultrasonography should always be performed when feline heartworm infection is suspected.

2.1.2.g. Control

Treatment

Adulticidal therapy (*D. immitis*) in dogs: The organic arsenical compound melarsomine dihydrochloride is the only effective drug available for treating adult heartworm infections. The currently accepted regimen is a two-step treatment advised to reduce the risk of pulmonary thromboembolism: after one initial treatment of 2.5 mg/kg, given by deep intramuscular injection in the lumbar area, the recommended follow up treatment is administered 50-60 days later (2.5 mg/kg twice at an interval of 24 hours). Drug overdosage can cause pulmonary oedema but liver or kidney damage has not been described.

Pulmonary thromboembolism is an inevitable consequence of successful adulticide therapy. When several worms die, widespread pulmonary thrombosis may develop. Mild thromboembolism may be clinically inapparent but life-threatening respiratory distress can occur in severely affected cases. These complications can be reduced by the restriction of exercise during the 30-40 days following treatment and by the administration of heparin and high doses of glucocorticosteroids (prednisolone 2 mg/kg daily for 4-5 days). The empirical use of aspirin is not advised as there are no evidence-based reports of any beneficial antithrombotic effects.

Even though **not recommended**, ivermectin administered orally at the prophylactic dose of 6 µg/kg monthly throughout the year for a period of at least 2-2.5 years has been shown to kill adult parasites. For these reasons, this regimen should be restricted to selected cases, excluding active dogs, working dogs and heavily infected dogs. X-ray examination should be performed every 4-5 months throughout the treatment period to monitor the pulmonary patterns. It should be noted that throughout this period the infection could persist and pathology could continue to worsen. Furthermore the long-term use of a macrocyclic lactone in heartworm positive dogs could potentially lead to selection of resistant sub-populations of heartworms.

Recently, it has been shown that a combination of ivermectin (IVM), commencing at the beginning of the IVM regime, at 6 µg/kg given every 15 days for 180 days and doxycycline at 10 mg/kg once daily for 30 days is well tolerated, has good adulticide efficacy and reduces the risk of thromboembolism. Exercise should be rigidly restricted for the duration of the treatment process. An antigen test should be performed every 6 months and the combination treatment continued until two consecutive negative heartworm antigen tests have been obtained. Anecdotal reports on other macrocyclic lactones with adulticidal properties suggest similar results but no confirmatory studies have been published.

Surgical intervention is advised when several worms have been displaced into the right cardiac chambers producing sudden onset of caval syndrome. It can be accomplished under general anesthesia with flexible alligator forceps introduced via the jugular vein aided by fluoroscopic guidance which gives access not only to the right cardiac chambers but also to the major pulmonary arteries.

Adulticidal therapy (*D. immitis*) in cats: This is not advised in cats because of the high risk of severe thromboembolism and sudden death in the post-treatment period. Diminishing doses of prednisolone are advised in cats in order to relieve respiratory distress with an initial daily dose of 2 mg/kg. If a cat presents with severe signs, high doses of prednisolone orally (1-2 mg/kg, 3 times a day) are recommended.

Adulticidal therapy in canine and feline *D. repens* infection: No effective adulticide therapy is known for *D. repens*. Because of the zoonotic potential of *D. repens*, microfilaraemic dogs should be treated monthly for 12 months with preventative drugs able to kill microfilariae (see below).

Control strategies for dogs

The monthly administration of topical or oral macrocyclic lactones throughout the transmission season

is effective against *D. immitis* third stage larvae (L3) and L4 which have developed within the previous 30 days and thus prevents disease caused by the adult worms. Several compounds alone or in combination with other parasiticides are available for oral administration or topical application (Table 7); for approved compounds in individual countries see www.esccap.org. Toxic side effects described for macrocyclic lactones in Collies, Collie crosses and certain other dog breeds, do not occur with the low dosages used for heartworm prophylaxis. An injectable macrocyclic lactone sustained release formulation has been approved for use only in dogs older than six months and is registered to give six months protection.

Prevention through monthly administration of macrocyclic lactones should start well before the mosquito season in spring and should be continued until late autumn. In southern Europe, protection against heartworm should be carried out from May until the end of November.

Table 7: Prevention of Dirofilariosis in dogs and cats in Europe: minimal and maximal dosages of macrocyclic lactones

Compound	Presentation	Dog (min.-max dosage)	Cat (min.-max dosage)
Ivermectin	Tablets/chewables	6-12 µg/kg	24-71 µg/kg
Milbemycin oxime	Flavour tablets	0.5-1 mg/kg	2-4 mg/kg
Moxidectin	Tablets Injectable Topical	3-6 µg/kg 0.17 mg/kg 2.5-6.25 mg/kg	1-2 mg/kg
Selamectin	Topical	6-12 mg/kg	6-12 mg/kg

Before starting any prophylactic treatment, adult *D. immitis* or *D. repens* infections must be ruled out by testing for circulating antigen or microfilariae. Heartworm-infected animals should first be treated for adult worms; prophylactic treatment can begin around 4 weeks later. Currently, preventative drugs are fully effective against *D. immitis* but reports from the USA suggest that drug resistance is emerging. For this reason, testing for circulating antigens and microfilariae using the Knott test should be repeated every year before starting prophylactic treatment.

In the last few years, there has been an increase in lack of efficacy reports for different heartworm preventatives in North America. Furthermore, a number of reports have been published on the inability of macrocyclic lactones to clear microfilariae from heartworm-antigen-negative dogs from the Mississippi Delta Region. In vitro studies have shown an increased homozygosity of this microfilaria genotype, indicating possible macrocyclic lactone resistance in some areas of the USA. Even though such phenomena have not been reported from Europe, in view of the fact that the maintenance of macrocyclic lactone efficacy is critical for *Dirofilaria* control, there are some recommendations which may assist in decreasing the risk of resistance selection.

1. Dogs should be checked for both circulating antigens and blood microfilariae (Knott test) at the beginning of each preventative annual treatment.
2. Although *Dirofilaria* does not appear entirely dependent on its bacterial symbiont *Wolbachia*, which can be killed by a prolonged antibiotic treatment, the clearing of bacteria from circulating microfilariae seems to prevent infective larvae which develop in mosquitoes from continuing their development in dogs.
3. The combination of heartworm preventatives with products designed to prevent mosquito blood-feeding activity (repellents) during the heartworm transmission season could be useful in protecting dogs from infection and from ectoparasite infestations that often occur in the same season.

Control strategies for cats

Prophylactic larval treatments in the cat follow the same regimen as in the dog with monthly dosing regimes (see www.esccap.org for tables of approved compounds in individual countries).

Control strategies for canine and feline *D. repens* infections

Like heartworm infections, subcutaneous filariasis can safely and effectively be prevented in both dogs and cats by chemoprophylactic treatments. Although *D. repens* infections are frequently sub-clinical, microfilaraemic dogs act as reservoirs of infection. Monthly treatments with macrocyclic lactones (oral or spot-on formulations) or an annual treatment with an injectable sustained release formulation at the same dose rates used against *D. immitis*, i.e. once at the beginning of the risk season, have been found to be effective in preventing subcutaneous infection in dogs naturally exposed to *D. repens* transmitting mosquitoes.

Control strategies for travelling dogs and cats

Before travelling from endemic areas to non-endemic areas, dogs should be examined for dirofilarial infections, treated against adult heartworms and cleared of both *D. immitis* and *D. repens* microfilariae. Dogs and cats travelling from non-endemic to endemic areas should be protected against adult filarial infections. They should be treated within 30 days after arrival in the risk areas with macrocyclic lactone preventative drugs. For pets spending no more than one month in endemic areas, a single treatment, usually administered soon after returning home, is sufficient to assure complete protection. In the case of longer visits, a monthly regimen should be administered with the first treatment being administered 30 days after the pet enters the risk area and the last within one month after leaving.

Pets with an unknown history either coming from or having travelled for a long time in risk areas and which show no evidence of circulating antigen or microfilariae, should be treated twice, one month apart and tested for circulating antigens and microfilariae 6 and 12 months later.

2.1.2.h. Public health considerations

In Europe, *D. repens* is the most important cause of human filarial infection. Most cases are asymptomatic and infections are often diagnosed after the surgical removal of a nodule containing the worms. Pre-adult worms are also often observed localised in ocular subconjunctiva and even intravitreally. Furthermore, visceral localisations such as in the lung, the mesentery and intradurally, may mimic tumours, and are frequently found. The infection in humans is probably underdiagnosed because its presence is not generally considered by physicians.

2.1.3 Bartonellosis

2.1.3.a. Agents and vectors

The most important species involved in bartonellosis is the bacterium *Bartonella henselae* which is of relevance mainly as the causative agent of cat scratch disease (CSD) in humans. Cats are considered the main reservoirs of, amongst others, *B. henselae* and *B. clarridgeiae*. The vectors of many *Bartonella* species, especially *B. henselae*, are fleas, mainly the cat flea *Ctenocephalides felis felis*. *Bartonella* spp. have also been found in other blood-sucking arthropods such as ticks and flies but the role of these vectors in transmission of infection is still ill-defined. In the vast majority of humans with cat scratch disease, bacillary peliosis or bacillary angiomatosis, *B. henselae* or *B. quintana* have been isolated as the agents. On the basis of serological tests, *B. clarridgeiae* has been suspected as the cause of diseases similar to cat scratch disease.

2.1.3.b. Biology and transmission

Bartonella are haemotrophic bacteria which are facultative intracellular parasites of red blood cells and endothelial cells. They can be detected in samples of cat blood as well as in specimens from claws and in saliva. How *B. henselae* is transmitted has not been clearly defined. Crucial for an infection is both the contact with fleas and their faeces. The agent can survive and remain infectious for up to nine days in the faeces of infected fleas. For the infection of humans, scratches and bites from cats play a crucial role. It is assumed that the oral cavity and the claws of infected cats are contaminated with bacteria-containing flea faeces during grooming and that the agent is transmitted to humans through skin wounds. Another possibility is the iatrogenic transmission in blood transfusions.

2.1.3.c. Distribution in Europe

The agent *B. henselae* as well as the primary vector *Ctenocephalides felis felis* are distributed worldwide.

The highest probability of becoming infected with *Bartonella* has been associated with cats less than two years of age, cats with access to outdoor areas, stray cats and animals in multi-cat households. The observed prevalence of *Bartonella* infection varies between cat populations and is often dependent on the detection method used.

2.1.3.d. Clinical signs

Most infections with *Bartonella* spp. in cats remain asymptomatic. Generally, a bacteraemia develops within one to three weeks after the initial infection with chronic recrudescences for up to 21 months. Clinical signs are observed only in immunosuppressed cats which might show fever, lymphadenopathy, gingivitis, uveitis, and endocarditis; transient anaemia and persistent eosinophilia have also been described. Infection has also been associated with diseases of the urinary tract as well as with reduced reproductive performance.

In dogs, more than eight species of *Bartonella* have been associated with endocarditis, myocarditis, hepatitis and rhinitis, but *Bartonella*-associated disease is probably underdiagnosed.

2.1.3.e. Diagnosis

The following diagnostic procedure is recommended:

1. The presence of clinical signs that may be associated with bartonellosis.
2. Exclusion of other causes that might explain the clinical picture.
3. Laboratory tests:
 - a. The gold standard for the diagnosis of bartonellosis is blood culture. It is also possible to detect *Bartonella* DNA in samples of blood, tissue, cerebrospinal fluid or aqueous humor.
 - b. Antibodies can be detected serologically from approx. 10 to 14 days after infection. A positive serological finding only shows that the cat or dog has already had contact with *Bartonella* spp. For the diagnosis of clinical bartonellosis repeated testing of serum samples should show a rising antibody titre.
4. Response to treatment with an antibiotic effective against *Bartonella* spp. However, this may be complicated by the fact that the drugs effective against *Bartonella* spp. are broad spectrum antibiotics which are also effective against other possible infections which may have been included in the differential diagnosis. Despite following this procedure, a definitive diagnosis of bartonellosis is not always possible.

2.1.3.f. Control

Treatment

The therapy of bartonellosis with currently available drugs only reduces the bacteraemia but does not eliminate the pathogen. Treatment is therefore only recommended for animals that show clinical signs and/or have contact with immunocompromised persons.

Possible therapies include:

- Amoxicillin-clavulanic acid at a dose of 22mg/kg orally every 12 hours for 7 days
- Doxycycline at a dose of 10mg/kg every 12 or 24 hours for 2-4 weeks
- Enrofloxacin at a dose of 5mg/kg once daily for 2-4 weeks

If the cat or dog responds to the therapy, this should be continued for at least 28 days or for 2 weeks after remission of the clinical signs.

Should the animal still show clinical signs after 7 days:

- Azithromycin 10mg/kg orally once daily for approximately 10 days.

As above, the treatment should be continued up to two weeks after the signs have subsided.

Prevention

The primary measure for the prevention of *Bartonella* spp. infection is effective protection against flea infestations, including a prompt anti-flea treatment of infected animals and good hygiene to minimize the presence of flea faeces on the animal and in its environment (see ESCCAP Guideline 3: Control of Ectoparasites in Dogs and Cats). In households with immunocompromised persons, special precautions must be taken.

- New cats should only be introduced into the household if they are older than one year, confirmed to be free of fleas and preferably have also tested negative for *Bartonella* spp.
- The cats should only be kept indoors.
- Wounds caused by cat scratches or bites should be immediately washed and disinfected.

2.1.3.g. Public health considerations

Transmission to humans occurs by contact with subclinically infected cats usually by scratches or bites. Also, transmission through flea faeces contaminating skin lesions is possible. It is unclear whether transmission to humans directly through bites by the cat flea can occur.

Also in humans, infection with *B. henselae* does not always cause disease. Disease characteristics are significantly different in immunocompetent and immunosuppressed patients.

Immunocompetent patients usually suffer from the classical form of cat scratch disease (CSD) with pustule formation at the infection site, regional lymphadenopathy, abscess formation and possibly fever. Most cases of uncomplicated CSD are self-limiting but may last for months until complete resolution. This disease syndrome responds only minimally or not at all to antimicrobial therapy.

The course of disease is much more complicated in immunocompromised patients. Bacillary peliosis, bacillary angiomatosis, endocarditis, retinitis and encephalopathies can develop. In these cases, antimicrobial therapy is indicated and generally effective.

2.1.4. Viral infections

The reader is referred to section 2.3

2.2. Tick-borne diseases

2.2.1. Babesiosis (Piroplasmosis)

2.2.1.a. Agents and vectors

Babesia spp. (Table 8) are haemoprotozoa which exclusively infect erythrocytes and are transmitted by hard ticks.

Table 8: *Babesia* species of dogs and cats and their vectors in Europe

Causative agent	Size	Hosts	Tick vector
<i>Babesia canis</i>	Large ¹	Dogs	<i>Dermacentor reticulatus</i>
<i>B. vogeli</i>	Large	Dogs	<i>Rhipicephalus sanguineus</i>
<i>B. (Theileria) annae</i> ²	Small	Dogs ⁵	<i>Ixodes hexagonus</i> , <i>Ixodes ricinus</i> ³
<i>B. gibsoni</i> and <i>gibsoni</i> -like	Small ⁴	Dogs ⁵	<i>Rhipicephalus sanguineus</i> ³ <i>Haemaphysalis</i> spp. <i>Dermacentor</i> spp.
<i>Babesia</i> spp.	Small/large	Cats ⁵	<i>Rhipicephalus</i> spp. ³

¹ larger than half the diameter of an erythrocyte.

² synonym: *Theileria annae*.

³ role as vectors is suspected but has not been demonstrated.

⁴ smaller than half the diameter of an erythrocyte.

⁵ other species may also be important, like fox (*Vulpes vulpes*) and European wolf (*Canis lupus*).

2.2.1.b. Biology and transmission

Babesia spp. are generally highly host-specific with regard to both the transmitting tick species and the mammalian host.

After being ingested with a blood meal, *Babesia* stages penetrate the gut epithelium of the tick, multiply and migrate to different organs including the tick ovary and salivary glands. Transovarial transmission from infected adult female ticks to their progeny occurs with large *Babesia* spp. and thus their larvae (“seed ticks”) can be an important source of infection.

Female *Dermacentor* spp. generally require a period of initial feeding 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, they perform co-feeding with females and possibly feed from several different hosts.

Sporozoites infect erythrocytes where they differentiate into merozoites and divide by binary fission eventually causing cell lysis.

2.2.1.c. Distribution in Europe

Endemic areas of canine babesiosis (Table 9) are related to the distribution of the tick vector (for details see ESCCAP Guideline 3: Control of Ectoparasites in Dogs and Cats). In central Europe, canine babesiosis appears to be one of the most frequently imported diseases and the endemic area of *B. canis* seems to have expanded in central Europe up to the Baltic region in recent years. Besides *B. canis*, small *Babesia* spp. can sporadically occur in Europe. Babesiosis in cats has only occasionally been observed.

Table 9: Distribution of canine *Babesia* spp. in Europe

<i>Babesia</i> spp. in dogs	Distribution
<i>B. canis</i>	Endemic in northern Spain, Portugal, France, central and eastern Europe up to the Baltic region associated with the distribution of <i>Dermacentor</i> spp.
<i>B. vogeli</i>	Southern Europe, associated with the distribution of <i>Rhipicephalus sanguineus</i>
<i>B. gibsoni</i> or <i>B. gibsoni</i> - like spp.	Sporadic and rare in Europe, imported from Asia.
<i>B. (Theileria) annae</i>	Northwest Spain and Portugal (in foxes found in Croatia and Germany)

2.2.1.d. Clinical signs

Babesiosis may be subclinical or may follow a peracute, acute or chronic course. Furthermore, different species and subspecies or strains differ with regard to their pathogenicity. Several species or strains may be found in the same host, namely in overlapping areas of *Babesia* distribution, which makes diagnosis based on clinical signs rather difficult.

Table 10: Clinical manifestations of canine babesiosis

Causative agent	Clinical presentation
<i>B. canis</i>	<p>Acute disease: Incubation period 1-3 weeks: moderate to severe clinical signs Medium-high fever, lethargy, anorexia, jaundice, vomiting and in some cases, red coloured urine ("rusty urine"). Common clinicopathological findings are haemolytic anaemia, thrombocytopaenia, neutropenia and sporadic hemoglobinuria. If untreated, a long recovery period may be followed by relapses which may lead to shock, icterus and severe or even fatal renal failure</p> <p>Atypical forms may be associated with haemorrhage and disseminated intravascular coagulation with severe locomotor, cerebral, ocular, gastrointestinal and vascular disturbances</p> <p>Chronic disease: Clinical signs may include moderate depression, intermittent fever, anaemia, myositis and arthritis</p>
<i>B. vogeli</i>	Mild to moderate clinical signs; often subclinical but severe forms have been observed in puppies
<i>B. gibsoni</i>	Moderate to severe clinical signs
<i>B. (Theileria) annae</i>	Moderate to severe clinical signs, which may lead to renal failure, including apathy, anorexia, fever, severe anaemia, hemoglobinuria and thrombocytopaenia; a low parasitemia may be present which is not related to the severity of the clinical signs

Babesiosis in cats

Several *Babesia* spp. or subspecies have been reported in domestic cats from various parts of the world, particularly South Africa. Relatively few reports originate from Europe, and clarification of the species infecting cats in Europe is currently under investigation. Clinical cases of feline babesiosis reported are characterized by lethargy, anorexia, weakness and diarrhoea. Fever with icterus is not common, but signs may not be apparent until later stages of the disease. Most of the infected and clinically affected cats had babesiosis with other concurrent infections (mainly with retroviruses and/or mycoplasmas).

2.2.1.e. Diagnosis

Blood sampling: A diagnosis of acute babesiosis can be confirmed with high sensitivity by the examination of thin blood smears (Giemsa-stain or Diff-Quick) to detect large or small *Babesia* spp. Freshly prepared smears made from unclotted blood samples can be used. For *B. canis*, peripheral capillary blood taken from the ear pinna or the tip of the tail may yield higher numbers of parasitized

cells, and a rapid diagnosis of the acute disease is therefore possible when a sick animal is first presented. *Babesia canis* are large, pear-shaped organisms found singly or in pairs in erythrocytes. *Babesia gibsoni* and *B. annae* are generally single, rounded intracellular organisms, but can occasionally be seen as four linked organisms within single red cells (forming a “Maltese cross” shape); parasitaemia is normally low. The diagnosis of chronic infections or carrier dogs is a challenge under clinical settings, due to very low and often intermittent parasitaemia.

Serology: Specific antibodies can only be detected from two weeks after the first infection and acute infections will therefore be missed if relying on serology for diagnosis. For canine babesiosis, the indirect fluorescent antibody test (IFAT) using infected red blood cells either from infected dogs or from cell cultures, is the most common laboratory-based test, and antigen-coated slides are commercially available. In endemic areas, seropositivity may not be synonymous with disease and may be detected in a high number of dogs which have been in contact with the parasite but did not become ill.

Molecular diagnosis: Species- or subspecies-specific PCRs (including real-time PCRs) have been described and are being increasingly used in routine laboratory diagnosis. The sensitivity of the PCR has been proven to be higher than blood smear examination especially for the diagnosis of chronically infected dogs, but false-negative results cannot be completely excluded. Identification of species and subspecies can be important in terms of treatment options and prognosis.

2.2.1.f. Control

Treatment

Chemotherapy should be initiated immediately after confirmation of a diagnosis of babesiosis. Imidocarb dipropionate, and in some countries phenamidine, are the drugs commonly used for the therapy of *B. canis* infection and in many cases treatment with these drugs will eliminate the parasite. However, in endemic areas, treated dogs do not develop a specific immune response capable of protecting against re-infection. In all cases, adequate supportive therapy is strongly recommended including rehydration and, if appropriate, blood transfusion.

There is little information on the therapy of babesiosis caused by small *Babesia* spp. in dogs and by *Babesia* spp. in cats. However, currently available chemotherapeutic agents used at the recommended dosage can reduce both the clinical severity and mortality rate (Table 11).

Table 11: Chemotherapy of babesiosis in dogs

Drug	Dose	Efficiency and side effects
Imidocarb dipropionate ¹	5-6 mg/kg, i.m. or sc., administered 2 weeks apart are recommended	<i>B. canis</i> : clinical improvement within 48 hours in the absence of hepatic, renal and vascular complications Side effects: related to an anticholinesterase effect include hypersalivation, tachycardia, dyspnoea, vomiting and diarrhoea <i>B. gibsoni</i> : less effective, <i>B. annae</i> : not effective
Phenamidine ²	15-20 mg/kg, sc., a second administration after 48 hours is sometimes recommended	<i>B. canis</i> : clinical improvement within 48 hours in the absence of hepatic, renal and vascular involvement Side effects: injection site pain, hypotension, tachycardia and vomiting
Doxycycline ³	10 mg/kg orally daily for 4 weeks	Indicated only for small <i>Babesia</i> infections
Pentamidine ²	16.5 mg/kg im. once or twice 24 hours apart	Side effects: vomiting, hypotension and local irritation and pain at injection site
Atovaquone or Buparvaquone ²	13 mg/kg orally every 8 hrs for 10 days	High efficacy against <i>B. annae</i> infections
Azythromycin ²	10 mg/kg orally, once daily for 10 days	High efficacy against <i>B. gibsoni</i> infections

¹ To prevent or mitigate adverse reactions, atropine (0.05 mg/kg) can be administered before or within 30 minutes after administration of imidocarb.

² Not registered for veterinary use in Europe.

³ Not indicated for treatment of *Babesia* infections, but, besides efficacy on small *Babesia*, may have therapeutic effect on concomitant infections with other vector-borne diseases such as rickettsiosis, ehrlichiosis and anaplasmosis.

Resistance against compounds used for chemotherapy or prophylaxis of canine babesiosis has not yet been recorded.

Prevention

So far, no strategic control programmes have been developed for canine/feline babesiosis. The risk of infection with *Babesia* spp. for individual dogs in endemic areas, or for dogs travelling to or through such areas, can be significantly reduced by effective tick control (see ESCCAP Guideline 3: Control of Ectoparasites in Dogs and Cats).

Immunity resulting from repeated infection is incomplete and can be adversely affected by drug treatment. Chemoprophylaxis (Table 12) prevents disease but not infection and can be considered for all dogs entering an endemic area for short stays; this is especially important for splenectomised or immunocompromised animals or for dogs with a history of *Babesia* infection. It is also an alternative in cases where vaccination or tick control is contraindicated or for countries where vaccines are not available. Chemoprophylaxis can be administered hours before entering an endemic area.

Table 12: Chemoprophylaxis of babesiosis in dogs caused by *Babesia canis* prevents severe disease but not the establishment of infection

Drug	Dose	Duration
Imidocarb dipropionate ¹	5-6 mg/kg im. or sc. Single injection	Protection against severe disease lasts for approx. 4 weeks
Doxycycline ¹	10 mg/kg orally once daily	Protection against severe disease lasts for approx. 4 weeks

¹ Not registered for this indication

Two vaccines which can prevent severe disease but not the establishment of infection are available in some European countries. The level of immunoprotection may vary depending on the species,

subspecies and the antigenic structure of the strains, and this has to be considered in different endemic areas. Re-vaccination every year or every 6 months in highly endemic areas is advised. Vaccination of pregnant or lactating bitches is not recommended.

Post-vaccination side effects are diffuse swelling and/or hard painful nodules at the site of injection but these generally disappear within 4 days. Rarely, reactions following the second dose of vaccination may persist for up to 14 days. Vaccinated dogs may develop a stiff gait and reduced appetite for 2-3 days after vaccination.

2.2.1.g. Public health considerations

Infections with *Babesia* spp. of dogs and cats have not been reported in humans.

2.2.2. Ehrlichiosis

2.2.2.a. Agents and vectors

Ehrlichia are vector-borne, Gram-negative, obligate intracellular bacteria. In Europe, *Ehrlichia canis* is the aetiological agent of canine monocytic ehrlichiosis (CME). This pathogen infects mainly lymphocytes and monocytes wherein the typical, microscopically visible microcolonies (morulae) develop. The main host of *E. canis* is the dog (other canids can serve as reservoirs of infection); the vector is the tick *Rhipicephalus sanguineus*. *Ehrlichia canis* or a closely related species has been described in cats but has no veterinary relevance.

2.2.2.b. Biology and transmission

All stages (larvae, nymphs, adults) of *R. sanguineus* preferentially feed on canids and may acquire *E. canis* from bacteraemic animals. The pathogen may overwinter in infected ticks. Trans-stadial (from larva to nymph to adult), but probably no transovarial, transmission occurs. During the incubation period of 8-20 days, the pathogens multiply by binary fission in leukocytes of the dog, forming morulae within circulating mononuclear cells. Subsequently, they spread via the mononuclear phagocytic system to liver, spleen and lymph nodes. This may lead to impairment of platelet function, sequestration and destruction.

2.2.2.c. Distribution in Europe

The geographical distribution of *E. canis* generally corresponds to the distribution of its vector *R. sanguineus*. Countries with reported infections are France, Italy and Spain (in dogs and cats), Portugal (in dogs), Greece (in dogs) and Bulgaria (in ticks).

2.2.2.d. Clinical signs

DOG

During the acute phase of canine monocytic ehrlichiosis, which lasts around 1-3 weeks, dogs show apathy, depression, anorexia, dyspnoea, fever, lymphadenopathy, splenomegaly, petechiae and ecchymotic haemorrhages in the skin and the mucous membranes, epistaxis, and vomiting. Also typical are thrombocytopaenia, leukopaenia and mild to moderate normocytic, normochromic, and non-regenerative anaemia. In the subclinical phase, which may last for weeks or months, the dogs appear clinically normal. Thrombocytopaenia and hypergammaglobulinaemia are typical. Chronic canine monocytic ehrlichiosis is characterized by a very complex clinical picture. Noticeable are weakness, apathy, sustained weight loss, fever, lymphadenopathy, splenomegaly, peripheral oedema in the hind limbs and scrotum, pale mucous membranes, a predisposition for bleeding with haemorrhages in the skin and mucous membranes, mucopurulent ocular and nasal discharges, epistaxis, and hematuria. In addition, interstitial pneumonia with dyspnoea, renal dysfunction,

glomerulonephritis, arthritis, polymyositis and lameness may occur.

Typical changes in the eyes of the patients are anterior uveitis, corneal opacities and blood in the anterior chamber, subretinal haemorrhage, retinal detachment and blindness. With the involvement of the CNS, nystagmus, signs of meningoencephalomyelitis, pareses, ataxia and convulsions appear.

Typical laboratory abnormalities are an increase in liver enzyme activities (alanine aminotransferase (ALT) and alkaline phosphatase), as well as hyperproteinaemia, hypergammaglobulinaemia, moderate hypoalbuminaemia, proteinuria, thrombocytopaenia, leukopaenia and anaemia, less commonly also pancytopaenia. Cases of chronic severe CME in dogs have a poor prognosis.

CAT

Reports of *E. canis* infections in cats are rare. Clinical manifestations are not adequately described.

2.2.2.e. Diagnosis

The diagnosis of *Ehrlichia* infections in dogs is generally based on the combination of a thorough anamnesis to assess the possibility of an exposure to ticks, the assessment of clinical signs, haematological and clinical chemistry findings, and serology and/or PCR.

- Morphological diagnosis: A diagnosis is confirmed if the microscopic examination of blood smears reveals morulae in lymphocytes and/or monocytes.

During the course of canine monocytic ehrlichiosis, morulae are rarely found, in contrast to infections with *A. phagocytophilum* (see section 2.2.3.). Lymphocytes and monocytes (4% in the acute phase) are infected but no granulocytes.

In order to increase diagnostic sensitivity, buffy coat smears or thin blood smears of blood or lymph node aspirates should be performed. The diagnostic sensitivity of buffy coat and lymph node cytology was 66% and 61%, respectively.

- Serology: Antibodies may be detected by an indirect immunofluorescence test (IFAT) in the laboratory, using *E. canis* antigens. Seroconversion may occur one to four weeks after exposure to infection, thus, dogs and cats with acute infections can be serologically negative.

In endemic areas, positive IFAT results may be due to a previous infection and may not necessarily indicate a current acute infection. A repeat IFAT test after several weeks is recommended for patients from endemic areas and a rising titre is indicative of a current infection. Point of care tests ("rapid tests") based on immunochromatographic or ELISA methods have also been developed and many commercial kits are now available for practitioners for a diagnosis in the clinic.

- PCR: A positive PCR result generally confirms the presence of an infection. However, a negative PCR result does not exclude the presence of an infection.

2.2.2.f. Control

Treatment

The treatment of cases of canine ehrlichiosis consists of the administration of anti-rickettsial agents together with symptomatic therapy. Tetracyclines are the most commonly used compounds, with doxycycline at a dosage of 10 mg/kg/day over 4 weeks being the most commonly used treatment scheme.

Prevention

The primary measure for the prevention of *Ehrlichia* infections is an effective protection against tick infestation (see ESCCAP Guideline 3: Control of Ectoparasites in Dogs and Cats).

2.2.2.g. Public health considerations

E. canis is not considered a zoonotic agent.

2.2.3. Anaplasmosis

2.2.3.a. Agents and vectors

Anaplasma spp. are vector-transmitted, Gram-negative, obligate intracellular bacteria. In Europe, *A. phagocytophilum* (formerly *Ehrlichia phagocytophila*) and *A. platys* (formerly *E. platys*) have been reported from domestic dogs. They infect predominantly neutrophil and rarely eosinophil granulocytes (*A. phagocytophilum*) or platelets (*A. platys*), respectively, and develop into typical microcolonies (morulae) that are observable by light microscopy. An overview of the biological features of the two important species is given in Table 13.

Table 13: *Anaplasma* spp. affecting dogs and cats in Europe

Causative agent	Disease	Hosts	Reservoir	Tick vector
<i>Anaplasma phagocytophilum</i>	Canine granulocytic anaplasmosis (CGA)	Dogs, cats, humans, horses, sheep, goats, cattle, llamas	Roe deer, red deer, small rodents, lynx ¹	<i>Ixodes ricinus</i> , (<i>I. trianguliceps</i> ²)
<i>Anaplasma platys</i>	Canine cyclic thrombocytopaenia (CCT)	Dogs	unknown	<i>Rhipicephalus sanguineus</i> ³

¹ Partial list; additional species tested positive in serology and PCR.

² *A. phagocytophilum* has been demonstrated in *I. trianguliceps* in the UK.

³ Role as a tick vector suspected but not proven.

2.2.3.b. Biology and transmission

Anaplasma phagocytophilum

Trans-stadial but not transovarial transmission of *A. phagocytophilum* occurs in the *Ixodes* vector. Usually, tick feeding for 24-48 hours is required for the transmission of this agent to susceptible dogs.

The incubation period in the mammalian host is 1 to 2 weeks. After endocytosis, *A. phagocytophilum* develops by binary fission into morulae in the phagosomes predominantly of neutrophils and rarely eosinophils. Cells infected with *A. phagocytophilum* are found in the circulating blood as well as in the tissues of the mononuclear phagocytic system, such as the spleen, liver and bone marrow.

Anaplasma platys

The natural mode of transmission has not been definitely established, but ticks and other arthropod vectors are likely to be involved. In experimental infections, the incubation period ranges from 8 to 15 days. Infections lead to cyclic thrombocytopaenia, and the highest bacterial load is found during the initial peak. In subsequent cycles, only around 1% of the platelets are affected while thrombocytopaenic episodes remain approximately the same. Over time, the severity of the thrombocytopaenic response diminishes.

2.2.3.c. Distribution in Europe

The geographical distribution of infections with *A. phagocytophilum* and *A. platys* generally correspond to the distribution of their respective (or supposed) tick vectors (Table 14). With increasing travel of dogs with their owners, infections must also be expected to occur in previously non-endemic areas.

Table 14: Distribution of pathogenic *Anaplasma* spp. in Europe

Causative agent	Location	Countries with reported cases
<i>Anaplasma phagocytophilum</i>	Europe-wide	Norway ³ , Sweden ^{1,2} , Denmark ² , UK ^{1,2} , Ireland ² , Netherlands ³ , Germany ^{1,2} , Switzerland ^{1,2} , Austria ¹ , France ³ , Italy ^{1,2} , Spain ^{1,2} , Portugal ^{1,3,4} , Poland ¹ , Bulgaria ³ , Slovenia ¹ , Czech Republic ³
<i>Anaplasma platys</i>	Countries with Mediterranean climate ⁵	Italy ¹ , Spain ¹ , Portugal ¹ , France ¹ , Greece ¹

¹ Reported in dogs.

² Reported in cats.

³ Infection demonstrated in ticks.

⁴ Infection demonstrated in wild rodents.

⁵ In many European countries with cold or temperate climates, cases are seen only in animals imported from areas with a Mediterranean climate.

2.2.3.d. Clinical signs

Table 15: Clinical manifestations and laboratory findings of pathogenic *Anaplasma* infections in dogs

Causative agent (disease)	Clinical signs	Laboratory findings
<i>Anaplasma phagocytophilum</i> (CGA)	Non-specific clinical signs ¹ such as sudden onset of lethargy, anorexia and fever; lameness (polyarthritis), pale mucous membranes, tense abdomen, diarrhoea, vomiting, petechial haemorrhages, tachypnoea, splenomegaly, enlarged lymph nodes; rarely cough, uveitis, limb oedema, polydipsia, neurological signs	Most common laboratory abnormalities are thrombocytopaenia, anaemia, lymphopaenia, monocytosis, leukopaenia, and leukocytosis, hyperglobulinaemia, hypoalbuminaemia, increased liver enzymes and hyperbilirubinaemia
<i>Anaplasma platys</i> (CCT)	Fever, lethargy, pale mucous membranes, petechial haemorrhages, often asymptomatic or seen in conjunction with immunosuppression or concurrent infections	Cyclic thrombocytopaenia ² , anaemia

¹ Have been observed but are not always present

² Cyclic bacteraemia and cyclic thrombocytopaenia (< 20'000/μl) at 1 to 2-week intervals.

The clinical manifestations following *A. platys* infection may vary depending on the geographical region: in the USA it is considered to lead mainly to subclinical infections while distinct clinical syndromes have been reported in some countries of the Mediterranean basin. Concurrent infections, with *E. canis* or *Babesia* spp., have been reported which makes it difficult or almost impossible to attribute specific clinical signs to a single pathogen.

Reports of *Anaplasma* spp. infections in cats are rare. Cats with *A. phagocytophilum* infections suffer from lethargy, anorexia, fever, lymphadenopathy, anaemia and thrombocytopaenia.

2.2.3.e. Diagnosis

The diagnosis of *Anaplasma* spp. infections in dogs is generally based on the combination of a thorough anamnesis, to assess the possibility of a previous tick infestation, the clinical signs, haematological and clinical chemistry findings and serology and/or PCR.

- Serology: Antibodies may be detected by indirect immunofluorescent assay (IFA) using *A. phagocytophilum* or *A. platys* antigens. Seroconversion may occur 1-4 weeks after exposure and, thus, dogs and cats with acute infections can be serologically negative.

Additionally, in endemic areas, positive IFA results may result from a previous infection and may not necessarily be indicative of an acute infection.

Generally, two serological tests at an interval of 2-3 weeks need to be carried out to monitor if seroconversion has occurred. A positive result of a single serological test combined with clinical signs is not sufficient evidence for a diagnosis of anaplasmosis.

- PCR: Specific assays for the detection of *A. phagocytophilum* and *A. platys* are performed by specialized laboratories. A PCR-positive result generally confirms an infection. A PCR-negative result does not exclude the possibility of an infection.
- Morphological diagnosis: A definitive diagnosis is made when morulae can be observed in neutrophil (and more rarely eosinophil) granulocytes (*A. phagocytophilum*) or platelets (*A. platys*) on microscopical examination of blood smears.

To increase the diagnostic sensitivity, buffy coat smears should be examined. Positive results should be confirmed by PCR analysis.

2.2.3.f. Control

Treatment

The treatment of anaplasmosis consists of the administration of antirickettsial agents and symptomatic treatment. Tetracyclines are the most commonly used compounds, with doxycycline at a dosage of 10 mg/kg/day over 3-4 weeks being the most commonly used treatment scheme. With correct treatment, the prognosis of *A. phagocytophilum* infections is fairly good.

Prevention

The primary measure for the prevention of *Anaplasma* infections is an effective protection against tick infestations (see ESCCAP Guideline 3: Control of Ectoparasites in Dogs and Cats).

2.2.3.g. Public health considerations

Infections with *A. phagocytophilum* have been reported in humans. The transmission of the agent in all cases was by ticks; direct transmission from infected dogs to humans has not been reported.

2.2.4. Borreliosis - Lyme disease

2.2.4.a. Agents and vectors

There are currently 11 known species/genotypes of the *Borrelia burgdorferi* complex (=sensu lato) which are spirochaetes that infect many mammals and birds and are transmitted by ticks (*Ixodes ricinus*, *I. hexagonus*, and *I. persulcatus*). Human infections are of major public health importance and although infections have been demonstrated in dogs, they are not of major clinical importance. Humans as well as dogs acquire *Borrelia* infection when exposed to infected ticks but there is no interdependency between dogs and humans in terms of transmission. Positive serology in cats has also been reported, but disease in cats, if it occurs at all, is poorly understood and there is therefore little data concerning the prevalence of infection, clinical appearance and treatment options for cats.

2.2.4.b. Biology and transmission

- Currently, ticks of the family Ixodidae and mostly of the genus *Ixodes* are recognized vectors of *B. burgdorferi*.
- Larval, nymph and adult female tick vectors can acquire *Borrelia* when feeding on an infected "reservoir host", which is an animal harbouring the pathogen as a long-term infection. It is also the case that ticks can become infected with the spirochaetes when feeding next to infected ticks (co-feeding transmission).
- Several animal species have been identified as reservoirs of *Borrelia* in Europe, including many mammals and birds.

- *Borrelia* in ticks disseminate to the salivary glands and are transmitted trans-stadially but there is no transovarial transmission.
- The tick must be attached for at least 16-24 hours before pathogen transmission to a new host occurs.
- *Borrelia* remains in the skin of a host before travelling to other tissues. In some cases, it can take up to 4 weeks before a systemic infection develops.

2.2.4.c. Distribution in Europe

As one would expect, endemic areas of borreliosis are related to the distribution of the tick vectors. Over the past twenty years, a number of studies have been published on prevalences and genetic variability within the *B. burgdorferi* complex in Europe. Lyme borreliosis is present all over Europe, except in extremely hot southern or cold northern areas.

2.2.4.d. Clinical signs

Borreliosis is a well-recognized disease in humans but, as yet, is not clearly defined in dogs and most infected dogs are asymptomatic. "Lyme arthropathy" which is lameness in one or more joints has been described; puppies may be at higher risk of polyarthrits. "Lyme nephropathy": there are many reports of dogs seropositive for *Borrelia* which have immune-mediated glomerulonephritis but further studies are needed to clarify if there is any association. In some clinical cases, dogs could present fever associated with lameness.

Clinical manifestations in naturally-infected cats are uncommon.

2.2.4.e. Diagnosis

- **Direct diagnosis:** Detection of *Borrelia* by culture, cytology or PCR may be difficult, time-consuming and expensive. The organism is rarely found in blood, urine, joint fluid or CSF, but can be detected in skin and synoviae.
- **Serology:** Antibodies against *Borrelia* usually appear 3-5 weeks after infection and can be detected using several commercially available qualitative and quantitative immunochromatographic tests. However, positive results merely indicate exposure to the bacteria rather than an existing disease. If dogs suspected of having Lyme disease are seropositive, it is recommended to carry out a Western Blot immunoassay to check for specific banding patterns. Finally, specific antibody reactions to the C6 peptide are highly specific for exposure to *B. burgdorferi* in dogs.

2.2.4.f. Control

Treatment

Treatment studies for Lyme disease in dogs have produced variable results but a response to antibiotic therapy should be evident within 1-2 days in the case of polyarthrits. Studies in experimentally-infected dogs have shown that antibiotic treatment does not clear the infection from all dogs. The drug of choice is doxycycline, 10 mg/kg/day orally once daily for a minimum of 1 month.

Prevention

- Serological positivity in healthy dogs can lead to a misdiagnosis or the unnecessary treatment of many animals which will never develop Lyme disease.
- Serological screening can, however, provide seroprevalence and sentinel data which may increase owner awareness about tick infestations and control.

- The application of *Borrelia* vaccines is still a controversial issue due to the presence of several *Borrelia* spp. in the field and the fact that some vaccines protect only against *Borrelia burgdorferi sensu stricto*.
- Tick control is currently the method of choice for disease prevention.

2.2.4.g. Public health considerations

Dogs and cats are not reservoirs of *Borrelia burgdorferi* and thus do not present a public health concern with regard to the transmission of this disease to humans. However, ticks collected from dogs or cats may carry the pathogen and have to be carefully disposed of after removal to prevent their transmission of *Borrelia* to new hosts including humans.

2.3. Vector-borne viral diseases

2.3.1.a. Agents and vectors

Table 16: Vector-borne viruses which can affect dogs or cats in Europe

Disease	Causative agent	Hosts	Vector
European tick-borne encephalitis (TBE) ¹	TBE virus (Flavivirus)	Dogs, humans, horses; Reservoirs: rodents, birds, red foxes, ruminants; (not in cats).	<i>Ixodes ricinus</i>
Louping-ill	Louping-ill virus (LIV) ² , (Flavivirus)	Natural disease mainly in sheep and red grouse; occasionally also in dogs ³ , humans, horses, pigs, cattle, goats, farm-raised deer; (not in cats).	<i>Ixodes ricinus</i> (possibly other modes of transmission)
West Nile virus infection	West Nile virus (WNV) ⁴ , (Flavivirus)	Horses, humans, dogs and cats ⁵ ; reservoir: birds	<i>Culex</i> spp. and other mosquitoes (WNV also isolated from ticks)

¹ Also known as early summer meningo-encephalitis.

² Closely related to the TBE virus.

³ Most frequently in working sheepdogs or gun dogs.

⁴ Belongs to the Japanese encephalitis virus complex.

⁵ WNV has been associated with sporadic disease in small numbers of other species including dogs and cats during intense periods of local viral activity.

2.3.1.b. Biology and transmission

Infections are usually initiated by the bite of an infected tick or mosquito.

TBE virus: *Ixodes ricinus* larvae, nymphs and adult ticks can be infected and trans-stadial and occasionally transovarial transmission occurs. Due to low host specificity of *I. ricinus*, the virus can be transmitted to a wide range of vertebrates but most infections remain subclinical. Infections in humans via unpasteurized milk have been reported.

LIV: transmission through bites of *I. ricinus* but also by exposure to tissues of infected animals and by aerosols, for example in slaughterhouses or laboratories. Food-borne transmission is possible via unpasteurized milk, pig meat or carcasses. Ticks become infected by feeding on animals, usually sheep or grouse, with high blood virus loads. Trans-stadial but usually no transovarial transmission occurs.

For WNV, wild and domestic birds act as the main hosts but there is a great diversity of potential

hosts and vectors. Humans and other mammalian species (mainly horses) are dead-end hosts. Infections, which are often asymptomatic, are seasonal in temperate climates and peak in early autumn in the northern hemisphere.

2.3.1.c. Distribution in Europe

European TBE may occur in areas wherever its tick vector *I. ricinus* is present. Endemic regions have been documented within many European countries. WNV appears to be ubiquitous, existing in a variety of climatic zones. Presently, in Europe, the virus seems to be restricted to Mediterranean and eastern European countries.

Table 17: Distribution of vector-borne virus infections in dogs and cats in Europe

Disease	Countries with reported cases
European tick-borne encephalitis (TBE)	Sweden, Norway, Switzerland, Austria, Germany, Czech Republic, northern Italy, eastern France, Greece
Louping-ill	UK, Ireland ¹
West Nile Virus infection	No clinical cases reported in dogs and cats in Europe so far. Waves of outbreaks in other species have been reported over the past two decades in various European countries ²

¹ A virus, presumably originating from a British Louping-ill virus isolate also caused disease in livestock and humans in Norway. Closely related but distinctly different viruses have also been found in diseased sheep or goats in other European countries, such as Spain, Turkey, Greece and Bulgaria.

² Romania (humans, 1996-97), Czech Republic (humans, 1997), Italy (horses, 1998), France (horses, 1962, 2000, 2006), Portugal (horses, 2010).

2.3.1.d. Clinical signs

Table 18: Clinical manifestations of vector-borne virus infections in dogs

Infection	Clinical presentation
European tick-borne encephalitis (TBE)	Peracute lethal (3 to 7 days), acute (1 to 3 weeks), chronic asymptomatic ¹ (months). Rottweiler dogs seem to be over represented in reported TBE cases. Fever, apathy, depression, anorexia, ² ± severe encephalitis: multifocal neurological signs including myoclonic convulsions, paresis, stupor, hyperaesthesia, cranial nerve deficits and reduced spinal reflexes
Louping-ill (LIV)	Acute viral encephalomyelitis but may also be asymptomatic. ¹ Muscle tremors, spasms, ataxia, fever, depression, paresis. Louping-ill viruses are primarily associated with disease in sheep, cattle or people but disease has also been reported in horses in LIV areas. Infections of domestic animals have been mainly reported in the British Isles but could also be expected in other countries with areas endemic for <i>I. ricinus</i>
West Nile Virus infection	Clinical disease in dogs appears to be rare with only few reported cases in the USA and Africa. Fever, apathy, anorexia, progressive neurological signs including stiff gate, ataxia, paresis, tremors, altered behaviour, and conscious proprioceptive deficits

¹ Infection with flaviviruses and seroconversion in the absence of apparent disease is common.

² In dogs, there is no biphasic course as described in humans.

2.3.1.e. Diagnosis

- TBE is a seasonal disease which depends on the climate-related activity of *I. ricinus*. A possible diagnosis is based on clinical evidence and on the known risk of exposure to tick bites in virus endemic regions. A rise in specific antibody titres in samples taken 2 to 3 weeks apart, or specific antibodies in the CSF, may confirm a diagnosis. Cross-reactivity among different flaviviruses has been reported. In contrast to other flaviviruses, viraemia in TBE is usually very short-lived and is not present at the time of clinical presentation. In cases with rapid disease

progression, diagnosis is confirmed at necropsy examination by histopathology.

- In viral CNS infections e.g. TBE and WNV, mononuclear pleocytosis is found in the CSF of infected dogs.
- In LIV infection, there is an increase in serum antibody titre measured by the haemagglutination inhibition test.
- Immunohistochemistry, virus isolation and RT-PCR and serology are used to detect infection with WNV.
- Flaviviruses are usually eliminated by the immune system.

2.3.1.f. Control

Treatment

Clinically apparent TBE infections are treated using non-steroidal anti-inflammatory drugs (NSAIDs) and broad-spectrum antibiotics; adequate supportive therapy including rehydration is recommended. Treatment with glucocorticoids is controversial.

Prevention

- Safe and effective vaccines against TBE are available for use in humans at risk of exposure but no vaccines or vaccination schedules are available for dogs or cats. Some dogs in endemic regions have been vaccinated, but vaccine efficacy was not assessed. The main control measure is based on prevention of exposure to ticks.
- Animals that survive a LIV infection and eliminate the virus by an effective humoral immune response remain seropositive, and the protection is probably lifelong.
- Avoiding mosquito bites by vector control strategies, such as repellents is the most important way to prevent WNV infection. Vaccines are available for horses at risk, and an experimental vaccine for dogs and cats is being evaluated.

2.3.1.g. Public health considerations

Recently, there has been an increasing awareness of TBE-related risk to humans and dogs. Human cases of Louping-ill are very uncommon but are occasionally seen, mainly in slaughterhouse or laboratory workers. There is a rising concern of the potential of WNV to further spread in Europe and there is an increased risk linked to possible virus transmission by blood transfusion and organ transplantations.

Appendix 1 - Background

ESCCAP (European Scientific Counsel Companion Animal Parasites) is an independent, not-for-profit organisation that develops guidelines and promotes good practice for the control and treatment of parasites in companion animals. With the proper advice the risk of diseases and parasitic transmission between animals and humans can be minimised. ESCCAP aspires to see a Europe where companion animal parasites no longer threaten the health and wellbeing of animals and humans.

There is a great diversity in the range of parasites and their relative importance across Europe and the ESCCAP guidelines summarise and highlight important differences which exist in different parts of Europe and, where necessary, specific control measures are recommended.

ESCCAP believes that:

- **Veterinarians and pet owners must take measures to protect their pets from parasitic infestations**
- **Veterinarians and pet owners must take measures to protect the pet population from risks associated with travel and its consequent potential to change local parasite epidemiological situations through the export or import of non-endemic parasite species**
- **Veterinarians, pet owners and physicians should work together to reduce the risks associated with zoonotic transmission of parasitic diseases**
- **Veterinarians should be able to give guidance to pet owners regarding risks of parasite infestation and diseases and measures which can be taken to minimise these risks**
- **Veterinarians should attempt to educate pet owners about parasites to enable them to act responsibly not only for their own pet's health but for the health of other pet animals and people in their communities**
- **Veterinarians should wherever appropriate undertake diagnostic tests to establish parasite infestation status in order to provide the best possible advice**

To achieve these objectives, ESCCAP produces:

- ***Detailed guidelines for veterinary surgeons and veterinary parasitologists***
- ***Translations, extracts, adaptations and summarised versions of guidelines which address the varied requirements of European countries and regions***

Versions of each guideline can be found at www.esccap.org.

Disclaimer:

Every effort has been taken to ensure that the information in the guideline, which is based on the authors' experience, is accurate. However the authors and publishers take no responsibility for any consequence arising from the misinterpretation of the information herein nor is any condition or warranty implied. ESCCAP emphasises that national, regional and local regulations must be borne in mind at all times before following ESCCAP advice. All dose-rates and indications are provided for guidance. However, vets should consult individual data sheets for details of locally approved treatment regimens.

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