ESCCAP

EUROPEAN SCIENTIFIC COUNSEL COMPANION ANIMAL PARASITES

Control of Vector-Borne Diseases in Dogs and Cats

ESCCAP Guideline 05 – September 2009

ESCCAP

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Background

ESCCAP 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 infection status in order to provide the best possible advice.*

To achieve these objectives, ESCCAP produces guidelines in two formats:

- *A detailed guideline for veterinary surgeons and veterinary parasitologists*
- *A summarised guideline which can be used by both veterinarians and pet owners*

Both versions of each guideline can be found at www.esccap.org. Various guidelines for treatment and control of parasitic infection in companion animals have been provided in other countries for example by organisations such as the CAPC (Companion Animal Parasite Council) in the USA. However, to date no comprehensive guidelines have been developed for Europe.

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.

Control of Vector-Borne Diseases in Dogs and Cats

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, [1]phlebotomine sand flies, Muscidae), lice and fleas. It has also been shown that pathogens (e.g. *Leishmania, Anaplasma, Ehrlichia*) may be transmitted via blood and so potential blood donors should be tested regularly for these infections prior to any transfusion.

Vector-borne 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 management of stray dogs and cats together with the increase in pet travel and translocation of pet animals can influence the present 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.

Vector-borne diseases can only be effectively controlled through knowledge of the infectious agents and their vectors. Besides giving an overview of the majority of vector-borne diseases of dogs and cats, this guideline focuses on the following important infections/diseases:

1 Babesiosis (piroplasmosis)
2 Dirofilariosis and other filarial infections
3 Leishmaniosis
4 Ehrlichiosis/Anaplasmosis
5 Borreliosis
6 Viral diseases

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

- Bartonellosis – (e.g. *Bartonella henselae* – arthropod transmitted Gram-negative bacteria typically with mammalian reservoir hosts)
- 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)
- Hepatozoonosis (e.g. *Hepatozoon canis* - protozoal infection of dogs transmitted when dogs ingest an infected tick)
- Thelazioses (*Thelazia callipaeda* – a nematode infecting the surface of the cornea)

[1]phlebotomine sand flies – in 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.

I. 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 climate, microclimate 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 relocating, going to boarding facilities and to dog and cat shows.

II. Prevention and control of vector-borne diseases

II.1. Babesiosis (Piroplasmosis)

II.1.a Agents and vectors:

Babesia spp. are haemoprotozoa which exclusively infect erythrocytes and are transmitted by hard ticks.

Table 1: *Babesia* species of dogs and cats and their vectors in Europe

Causative agent	Size	Hosts	Tick vector
Babesia canis canis	Large[1]	Dog	*Dermacentor reticulatus*
B. canis vogeli	Large	Dog	*Rhipicephalus sanguineus*
B. annae[2]	Small	Dog[3]	*Ixodes hexagonus, Ixodes ricinus*[4]
B. gibsoni and *gibsoni* like	Small[5]	Dog[3]	*Rhipicephalus sanguineus*[4] *Haemaphysalis* spp. *Dermacentor* spp.
Babesia spp.	Small/large *Rhipicephalus* spp.[3]	Cat[3]	*Rhipicephalus* spp.[3]

[1] larger than half the diameter of an erythrocyte.
[2] synonym: *Theileria annae* = *Nicollia annae*.
[3] other species may also be important.
[4] role as vectors are suspected but have not been demonstrated.
[5] smaller than half the diameter of an erythrocyte.

II.1.b Biology and transmission:

- *Babesia* are generally highly host-specific with regard to both the transmitting tick species and the mammalian host.
- After ingestion 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 gland.

- Transovarial transmission from infected adult females to their progeny occurs with large *Babesia*, and thus their larvae ("seed ticks") can be an important source of infection.
- Female *Dermacentor* generally require a period of initial feeding before *Babesia* sporozoites within their saliva are available for transmission to the dog. It has been demonstrated that male ticks may transmit *Babesia* spp., however the epidemiological importance of male ticks in transmission has yet to be established.
- Sporozoites only infect erythrocytes, where they differentiate into merozoites and divide by binary fission eventually causing cell lysis.

II.1.c Distribution in Europe:

Endemic areas of canine babesiosis are related to the distribution of the tick vector (for details see ESCCAP Guideline 3: Ectoparasites Part 1: Control of Parasitic Insects and Ticks in Dogs and Cats). In central Europe, canine babesiosis appears to be one of the most frequently imported diseases and in recent years, the endemic area of *B. canis canis* seems to have expanded in central Europe up to the Baltic region. Besides *B. canis* small *Babesia* spp. can sporadically occur in Europe. Babesiosis in cats has only been observed occasionally.

Table 2: Distribution of canine *Babesia* spp. in Europe

Babesia species in dogs	Distribution
B. canis canis	Endemic in northern Spain, Portugal, France, central and eastern Europe up to the Baltic region associated with the distribution of *Dermacentor* spp.
B. canis vogeli	Southern Europe, associated with distribution of *R. sanguineus.*
B. gibsoni or *B. gibsoni* - like species	Sporadic and rare in Europe, imported from Asia.
B. (Theileria) annae[1]	Northwest Spain.

[1] synonym: Theileria annae = Nicollia annae.

II.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.

Table 3: Clinical manifestations of canine babesiosis

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

Babesiosis in cats:

Several *Babesia* species 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 cats had babesiosis with concurrent other infections (mainly retroviruses and mycoplasmosis).

II.1.e Diagnosis:

- Blood sampling: A diagnosis of acute babesiosis can be confirmed with a high level of sensitivity by the examination of thin blood smears (Giemsa-stain or Diff-Quick) to detect large or small *Babesia*. 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, thus a rapid diagnosis of the acute disease is therefore possible on first presentation of the sick animal. *B. canis* are large, piriform-shaped organisms found singly or in pairs in erythrocytes. *B. gibsoni* and *B. annae* are generally single, rounded intracellular organisms, but can occasionally be seen as four linked organisms within single red cells (Maltese cross forms; 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 first infection and acute infections will therefore be missed if relying on serology for diagnosis. In 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 test and antigen-coated slides are commercially available.
- Molecular diagnosis: Genus-, 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 examination especially for diagnosis of chronically infected dogs, however it does not completely eliminate false-negatives. Identification of the species and subspecies can be important for therapy design and prognosis.

II.1.f Control:

So far, no strategic control programmes have been developed for canine babesiosis. The risk of infection with *Babesia* 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: Ectoparasites Part 1: Control of Parasitic Insects and Ticks in Dogs and Cats).

Immunity resulting from repeated infection is incomplete and can be adversely affected by drug treatment. Chemoprophylaxis can be used for dogs entering an endemic area for short stays; this is especially important for splenectomised or immunocompromised animals or in dogs with a history of *Babesia* infection. One treatment that has been used for prophylaxis is imidocarb diproprionate at 5-6 mg/kg im or sc as a single injection which provides protection against severe disease but not infection caused by *B. canis* for approximately four weeks. In an experimental study Doxycycline 5 mg/kg po daily as a single dose had a similar effect as long as the antibiotic was administered, however this compound should be used as a last resort. Specific recommendations for individual countries can be found on www.ESCCAP.org. It is also an alternative in cases where vaccination or tick control is contraindicated or for countries where vaccines are not available. Chemoprophylaxis is best administered a few hours before entering an endemic area.

Two vaccines (Table 4) which can prevent severe disease with *B. canis* 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 strains, and this has to be considered in different endemic areas. Re-vaccination every year, and every 6 months in high endemic areas, is advised. Use in pregnant or lactating bitches is not recommended.

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

Table 4: Vaccines against babesiosis caused by *B canis* in dogs (not available in all European countries)

Vaccine	Application	Indications and efficiency*
Pirodog®	Two subcutaneous injections at an interval of 3-6 weeks.	Used to protect healthy non-*Babesia* infected dogs over 5 months of age (not within 15 days of application of other vaccines apart from rabies and leptospirosis). In case of an earlier *Babesia* infection, vaccination should be delayed for at least 8 weeks after the end of treatment. Onset of protection occurs several days after the second injection. The vaccine should not be used in pregnant bitches.
Nobivac® Piro	Two subcutaneous injections at an interval of 3-6 weeks.	Used to protect healthy, non-*Babesia* infected dogs over 6 months of age (excluding pregnant and lactating bitches). Onset of protection against severe disease occurs 3 weeks after the second injection. Booster vaccinations are recommended at 6 monthly intervals.

* According to company data and literature.

II.1.g Chemotherapy:

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 therapy of *B. canis* infection and in many cases treatment with these drugs will eliminate the infection. However, in endemic areas, treated dogs do not develop a specific immune response able to protect against re-infection. In all cases adequate supportive therapy is strongly recommended including rehydration and if appropriate, blood transfusion.

There is little information on therapy of babesiosis caused by small *Babesia* species in dogs and by *Babesia* spp. in cats. However, currently available chemotherapeutic agents used at the recommended dosage can decrease both the clinical severity and mortality rate (Table 5).

Table 5: Chemotherapy of babesiosis in dogs

Drug	Dose	Efficiency and side effects
Imidocarb diproprionate*	Recommended dose varies according to country. Generally dose ranges of 4-6.6 mg/kg im or sc repeated after 2 weeks.	*B. canis*: clinical improvement within 48 hours in the absence of hepatic, renal and vascular complications. Side effects: related to an anticholinesterase effect includes hypersalivation, tachycardia, dyspnoea, vomiting and diarrhoea. *B. gibsonii*: less effective, *B. annae*: not effective.
Phenamidine**	15-20 mg/kg, sc, a second administration after 48 hours is sometimes recommended.	*B. canis*: clinical improvement within 48 hours in the absence of hepatic, renal and vascular involvement. Side effects: injection site pain, hypotension, tachycardia and vomiting.
Doxycycline***	10 mg/kg po daily for 4 weeks.	May be useful to treat small *Babesia* infections.
Pentamidine**	16.5 mg/kg im once or twice 24 hours apart.	Side effects: vomiting, hypotension and local irritation and pain at injection site.
Atovaquone**	13 mg/kg po every 8 hrs for 10 days.	High efficacy against *B. annae* infections.
Azythromycin**	10 mg/kg po once daily for 10 days.	High efficacy against *Babesia gibsoni* infections.

* To prevent or treat adverse reactions, atropine (0.02 - 0.04 mg/ kg sc) can be administered before or within 30 minutes after administration of imidocarb.
** Not commercialized as a vet product in EU.
*** Commercialised as a vet product in EU but not for this indication.

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

II.1.h Public health considerations:

Infection with *Babesia* spp. of dogs and cats has not been reported in humans.

II.2. Dirofilariosis and other filarial infections

II.2.a Agents and vectors:

Filarial worms are nematodes infecting connective tissues and the vascular system of dogs and cats. Most species are transmitted by mosquitoes, and a few by fleas and ticks (Table 6). *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 6: Filarial species infecting dogs and cats in Europe (see Table 7 for morphology of microfilariae)

Filarial parasite	Vectors	Prepatent period	Length of Adult Worms M-F	Location of adult worms
Dirofilaria immitis	Mosquitoes (Culicidae)	120-180 days	12-18 cm 25-30 cm	Pulmonary arteries/right heart
Dirofilaria repens	Mosquitoes (Culicidae)	189-259 days	5-7 cm 10-17 cm	Subcutaneous tissue/muscular fasciae
Acanthocheilonema (former: *Dipetalonema*) *reconditum*	Fleas and ticks	427-476 days	9-17 mm 21-25 mm	Subcutaneous tissue/muscular fasciae[4]
Acanthocheilonema (former: *Dipetalonema*) *dracunculoides*	Flies and ticks (*R. sanguineus*)	120 days	15-31 mm 33-55 mm	Peritoneal cavity
Cercopithifilaria (former: *Acanthocheilonema*) *grassii*	Ticks (*R. sanguineus*)	unknown	unknown 23-24 mm	Subcutaneous tissue/muscular fasciae

M: male; F: female.

II.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 vectors, many mammalian hosts can be infected, including humans. In such hosts, the parasite does not generally develop to the adult stage.

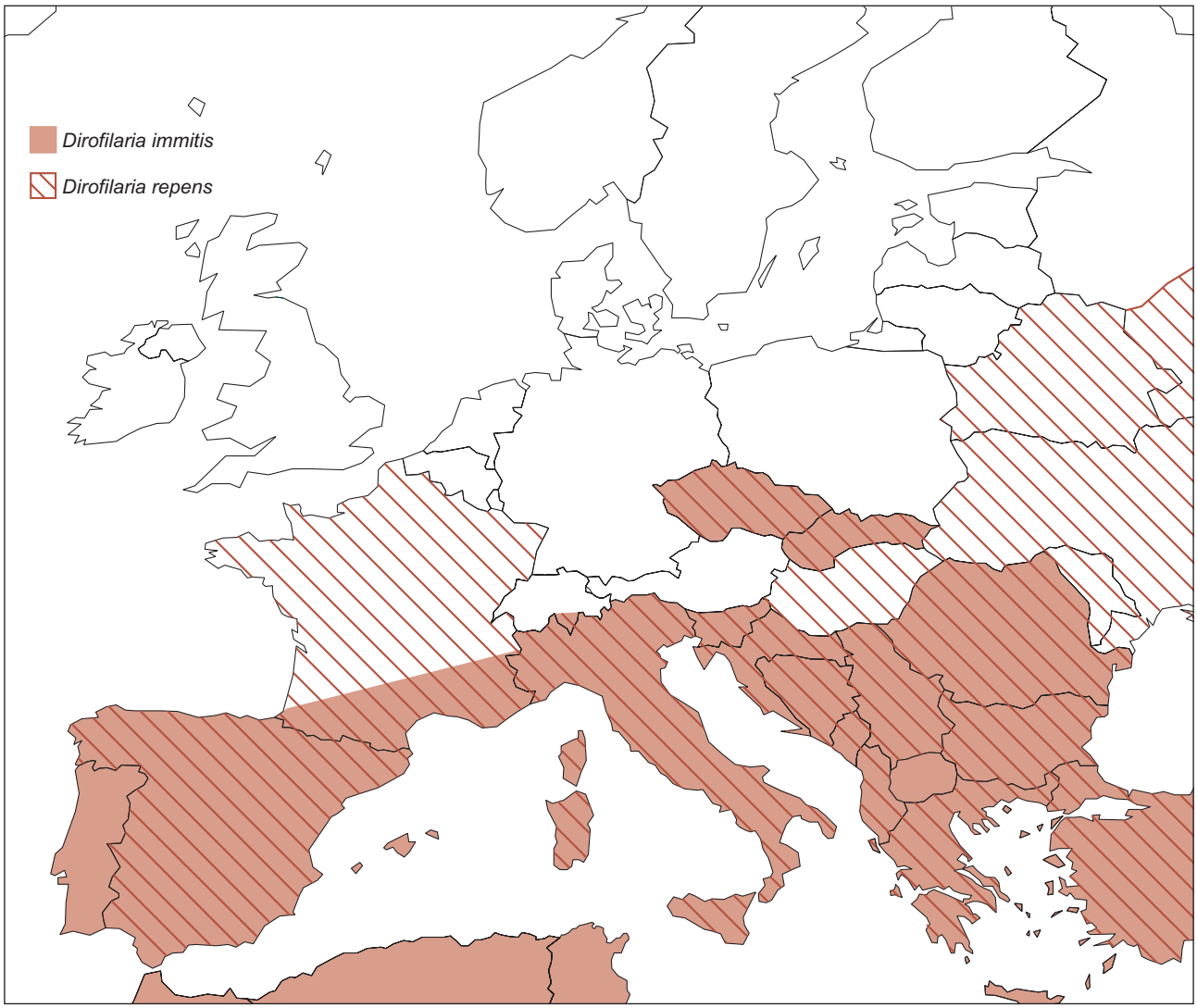
- *D. immitis* and *D. repens* microfilariae develop in the uterus of female worms and are excreted into the blood stream where they become available to blood-sucking mosquitoes. Microfilariae develop to the infective stage (L3) in the body of these vectors and are transmitted via their saliva during 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 stage and mate. In dogs, adult worms are able to survive up to 7 years, whereas microfilariae can only survive 2-18 months in the blood stream. *D. repens* infective larvae do not migrate far in the subcutaneous connective tissues and reach maturity there. 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* and *Cercopithifilaria grassii* are parasites of the subcutaneous tissues and fasciae of canids, including dogs and jackals and *A. dracunculoides* is a parasite of the peritoneal cavity of canids. For diagnostic purposes, circulating microfilariae of *A. reconditum* and *A. dracunculoides* must be differentiated from those of *D. immitis* and *D. repens*.
- Many mosquito species are competent intermediate hosts and allow the development of microfilariae to infective stages in the Malpighian tubules. Vectors

of *Dirofilaria* spp. transmit infective third-stage larvae to susceptible hosts immediately when they bite. The most important vectors in Europe are species of the genera *Culex*, *Aedes*, and *Anopheles*. Recently, *Aedes albopictus*, the Asian Tiger Mosquito which is currently spreading in Europe, has been shown to be a competent vector for *Dirofilaria* spp. under field conditions.

II.2.c Distribution in Europe:

The frequency of transmission and spread of *Dirofilaria* spp. infections depend on environmental factors such as temperature and the density of the vector population. Socio-economic factors are also important such as the density and the movement of microfilaraemic dogs, which are reservoirs of infection, due to tourism or animal adoption and their translocation from endemic areas such as Italy and Spain.

Fig. 1 Approximate endemic areas of *Dirofilaria immitis* and *Dirofilaria repens* in Europe



Recently, *D. repens* infections in dogs that had never left Germany, the Netherlands or Austria have been documented.

Dirofilaria immitis is endemic/hyperendemic in Spain, the Canary Islands, Portugal, the south of France, parts of southern Switzerland, Italy, the Adriatic coast from Italy to Greece, Turkey, the Czech Republic, Slovenia, Romania and Bulgaria (Fig. 1). The endemic areas of *D. immitis* and *D. repens* overlap in many regions. However, *D. repens* is the only species occurring in areas such as northern France and Hungary.

It should be noted that mosquito density and the rate of *Dirofilaria* maturation to infective third-stage larvae in the mosquito vector depend mainly on environmental temperature and humidity. A rise in average temperatures tends to extend the risk areas and the risk season for infection and this contributes to an increase

in prevalence rate. Thus prevention should be started well before the mosquito season in spring and to be continued until late autumn.

Feline *Dirofilaria* infection occurs in areas where canine infections are highly prevalent; the prevalence in cats, however, is generally 10 times lower than in dogs in such areas. *D. immitis* infections in cats have been frequently diagnosed in northern Italy where seroprevalence studies for specific antibodies indicate prior exposure to infection in approximately 18% of pet cats; the actual infection prevalence based on a positive antigen test and echocardiography is about 7%.

A. dracunculoides infection has a prevalence rate of up to 14% in hunting dogs and dogs living outdoors in some European countries such as Spain. This parasite has also been reported from the south of Italy (Sicily) but with lower prevalence rates. *A. reconditum* is quite frequently found in Sardinia with prevalence rates ranging from 3 to 19%.

II.2.d Clinical signs:

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

The life span of adult heartworms is 5-7 years in the dog, which is the primary definitive host. The cat is considered a susceptible, but not ideal, host. Infection in cats is characterized by a relatively low burden of adult worms, (2 to 4 worms), a longer prepatent period (8 months), a low level and short duration of microfilaremia, and a shorter life span of adult worms (maximum 2 years).

Despite the name of the disease which suggests a primary cardiac condition, heartworm disease is essentially a pulmonary disease, because the main worm location and initial damage is in the pulmonary arteries and only in the later stages is the right heart involved.

Infections with *A. reconditum, A. dracunculoides* and *Cercopithifilaria grassii* are mostly asymptomatic. A few cases of dermatitis, pruritus and microgranulomas have been described in dogs with patent (microfilaraemic) *A. dracunculoides* infection. *D. repens* is the most frequent species associated with subcutaneous filariosis of dogs and cats. In some cases, subcutaneous nodules containing adult parasites or microfilariae can be observed on the body surface of infected hosts, mainly on the trunk. These "cold" nodules do not cause pain and appear free within the skin. The parasite may be observed in subcutaneous tissues, in the perimuscular fasciae, in peri-renal fat or in the abdominal cavity during surgery. Rarely, in cases of heavy infection and sensitized patients, pustular eruptions, ulcerative lesions and scabies-like dermatitis may be seen associated with the presence of microfilariae in the skin.

Differentiation of all species that produce microfilariae which can be found in the blood stream is necessary for diagnosis.

DOG:

The clinical evolution of heartworm disease in dogs is usually chronic. Most infected dogs do not show any clinical signs for years depending on the worm burden, individual sensitivity and level of exercise; arterial damage is usually more severe in dogs that perform intensive physical exercise. 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 the limbs may be observed together with anorexia, weight loss and dehydration. Cardiac murmurs over the right side of the thorax due to tricuspid valve insufficiency and abnormal cardiac rhythm due to atrial fibrillation are common findings. 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 haemoptysis.

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:

Clinical signs in cats are quite different from those in dogs. Most cats show no clinical signs for a long time after infection. These cats may undergo spontaneous self-cure due to the natural death of parasites without showing any signs or they may suddenly show a dramatic acute syndrome where respiratory signs such as coughing, dyspnoea and haemoptysis are usually seen; vomiting also occurs frequently. Sudden death in apparently healthy cats is not an infrequent consequence of infection.

Chronic illness including coughing, vomiting, diarrhoea and weight loss is less frequently observed. In contrast to the situation in dogs, clinical signs related to right ventricular heart failure are not considered consistent with heartworm infection in cats.

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.

II.2.e Wolbachia/filarial worm symbiosis:

Endosymbiotic Gram-negative bacteria of the genus *Wolbachia* play an important role in the pathogenesis and immunology of heartworm infection. These bacteria are released by live worms or following worm death through natural attrition, through microfilarial turnover or following pharmacological intervention. *Wolbachia* from *D. immitis* have been shown to provoke chemokinesis and pro-inflammatory cytokine production in canine neutrophils. Cats with heartworm disease also produce antibodies to *Wolbachia*.

Wolbachia can be eliminated from filarial worms through antibiotic therapy of the infected host. Treatment with tetracyclines or their synthetic derivatives can drastically reduce albeit not completely remove the endosymbionts from their worm hosts. Such depletion of *Wolbachia* is often followed by clear anti-inflammatory effects, further inhibition of larval development, female worm sterility and some adulticidal effects. Thus antibiotic treatment may be used concomitantly with the use of adulticidal therapeutic agents and effective protocols are currently being developed.

II.2.f Diagnosis:

DOG:

- Heartworm infection in dogs can be detected with blood tests which demonstrate the presence of circulating microfilariae (in wet blood smears or Knott test) or adult antigens (in whole blood, serum or plasma samples), but further diagnostic procedures are usually required to determine the severity of disease and possible treatment options. Morphological differentiation of the microfilariae is often difficult because of overlap in lengths of most species (Table 7). However, microfilariae can be differentiated in specialized laboratories by the acid phosphatase stain (APh-S) or by molecular investigations (PCR).
- **Blood test for microfilariae:** Blood samples are examined after concentration for the presence of microfilariae (Knott or filtration test). If microfilariae are seen and identified as *D. immitis* based on morphology, this is considered conclusive proof of specific infection. However, up to 30% of dogs do not have circulating microfilariae even though they harbour adult worms. This may be due to the age of the adult

worms as fecundity decreases in ageing females or to the host immune response against the parasites. The administration of microfilaricidal drugs or, infrequently in dogs, the presence of single sex infections, may also be responsible for the absence of circulating microfilariae. The sensitivity of tests for microfilariae is therefore not considered sufficient to rule out an infection in the case of a negative result. 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 7: Morphological features of blood microfilariae[1] from filarial worms of dogs and cats

Species	Length µm	Width µm	Features
Dirofilaria immitis	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.
D. repens	300-370	6-8	No sheath, cephalic end obtuse, tail sharp and filiform often ending as an umbrella handle. APh-S: one spot around the anal pore.
Acanthocheilonema reconditum	260-283	4	No sheath, cephalic end obtuse with a prominent cephalic hook, tail button hooked and curved. APh-S: activity throughout the body.
A. dracunculoides	190-247	4-6.5	Sheath, cephalic end obtuse, caudal end sharp and extended. APh-S: show three spots which include an additional spot in the middle of the body.

[1]microfilariae measured by Knott test; when measured by Difil® Test lengths are shorter, APh-S: acid phosphatase stain.

- **Blood/serological tests for adult female antigens:** Tests based on ELISA or on colloidal gold staining techniques designed to detect antigens of adult heartworms, are considered highly specific as cross-reactivity with other related canine parasites such as *D. repens* and *Acanthocheilonema* spp., does not occur. These tests allow detection of adult heartworm antigens produced by female worms and may provide information about worm burden. Antigen reactions are detected in the late prepatent period which is 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 effusion 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 such as electrical axis right deviation and atrial fibrillation are usually only found in the later stages of the disease when severe damage is present in the right heart.

- **Echocardiography:** Echocardiography allows a direct visualization of the cardiac chambers and major vessels and thus allows the detection of any parasites in the heart, the main pulmonary arteries or the caudal vena cava. The heartworms are visible as double, linear parallel objects floating in the chambers of the right heart or in the lumen of the major vessels. It is performed mainly in cases where clinical and radiographic findings suggest severe disease. Cardiac ultrasound can increase

the accuracy in staging the disease and estimating the worm burden, both of which affect the selection of the treatment regime and the prognosis.

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 can provide definitive proof of infection in cats as in dogs because of their very high specificity. In many cases however, these tests yield false negative results because the worm burdens in cats are often either very low or consist of only male heartworms or because clinical signs may be due to the presence of 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 the specificity is reduced, possibly due to cross reactivity with other parasites. 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 elimination of both larval and adult stages. Consequently, antibody tests should be interpreted carefully and must take into account other relevant clinical information.
- **X-rays:** Thoracic radiographs may be useful in the diagnosis of feline heartworm disease. Although thoracic abnormalities may be absent or transient 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. Enlargement of the main pulmonary artery cannot be observed because the origin of this vessel is obscured by the cardiac silhouette. Right sided cardiomegaly is not considered a typical finding in cats with heartworm infection.
- **Non-selective angiocardiography:** Non-selective angiocardiography is useful for visualization of the gross morphology of the pulmonary arteries. Rarely, heartworm infection can be suspected if negative filling defects are observed within opaque arteries.
- **Electrocardiography:** Since heartworm infection in cats does not involve the right heart, electrocardiography can provide little useful clinical information.
- **Echocardiography:** Cardiac ultrasound allows the 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.

Specificity is virtually 100%, and sensitivity in cats is very high as only a short portion of the caudal pulmonary arteries cannot be examined because of acoustic impedance of the air inflated lungs. Based on these considerations, cardiac ultrasonography should always be performed when feline heartworm infection is suspected.

II.2.g Control:

D.immitis prevention

DOG:

Monthly administration of 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 prevent disease caused by the adult worms. Several compounds alone or in combination with other parasiticides are

available for oral or topical application or injection (see www.esccap.org for tables of approved compounds for individual countries). Toxic side effects described for macrocyclic lactones particularly ivermectin in certain 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 in dogs from six months of age onwards and is registered for a six months protection period. Currently in southern Europe protection against heartworm should begin in May and finish at the end of November.

In endemic areas, testing of dogs is recommended at the beginning of each transmission season in order to identify infections with adult worms which may have resulted from inadequate control measures during the preceding season. Before starting any prophylactic treatments, animals should be tested for circulating antigen and microfilariae of both adult *D. immitis* or *D. repens* infections. Infected animals should first be treated against adult worms; prophylactic larval treatment can begin around 4 weeks later. Testing for circulating antigens should always be carried out after the first season of prophylactic treatment because regular use of the macrocyclic lactones will usually clear any microfilariae from the blood but if an infection were to become patent during the therapy, it would not be detected by screening only for microfilariae. Some microfilaraemic dogs may be observed in cases where treatments were inadvertently omitted towards the end of the transmission season. Testing should therefore be performed at least 6 months after the last drug administration. Whilst there are parasiticidal products that can kill and/or repel mosquitoes, these cannot be relied upon to prevent transmission of *Dirofilaria* infection. See ESCCAP Guideline 3: Ectoparasites Part 1 for more information on mosquitoes and their control.

CAT:

Prophylactic larval treatments in the cat follow the same regimen as in the dog with monthly dosing beginning within one month from the start of the transmission season and the last dose being given within one month from the end of the risk period. (see www.esccap.org for tables of approved compounds for individual countries).

Monthly larvicidal treatment in cats infected with adult stages carry a low risk of adverse reactions, with the exception of rare cases of microfilaraemic cats; as for dogs, pre-treatment testing of cats for adult heartworm infection is advisable at the beginning of each new transmission season using both *D. immitis* antibody and antigen tests. Whilst there are parasiticidal products that can kill and/or repel mosquitoes, these cannot be relied upon to prevent transmission of *Dirofilaria* infection. See ESCCAP Guideline 3: Ectoparasites Part 1 for more information on mosquitoes and their control.

CAT AND DOG:

Canine and feline *D. repens* infections

Like heartworm infection, subcutaneous filariosis can be safely and effectively prevented in both dogs and cats by chemoprophylactic treatments. Disease due to *D. repens* infection is much less severe than that due to heartworm infection and is frequently sub-clinical. However, infected dogs can present with cutaneous disorders of varying severity, such as pruritus, dermal swelling and subcutaneous nodules which contain the parasites. Severe disease has been associated with allergic reactions probably against microfilariae. The main concern relating to *D. repens* in Europe is its ability to cause human infection. Monthly treatments with macrocyclic lactones (oral or spot-ons formulations) or an annual treatment with an injectable sustained release formulation at the same doses used against *D. immitis*, 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.

Adulticidal therapy (*D. immitis*):

DOG:

The organic arsenical compound melarsomine dihydrochloride is the only effective drug available for use against adult heartworm infections. The standard regimen is two doses of 2.5 mg/kg given at an interval of

24 hours by deep intramuscular injection in the lumbar area. In heavily infected dogs, a more gradual two step treatment is advised to reduce the risk of pulmonary thromboembolism: after one initial injection, the recommended two dose injection regime is administered 50-60 days later. It is known that one administration of melarsomine at the dose of 2.5 mg/kg kills about 90% of male worms and 10% of female worms, resulting in a 50% reduction of the worm burden. This drug has a better efficacy and safety profile than the previously used thiacetarsamide. 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. If 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 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) to reduce the clinical signs associated with thromboembolism. The empirical use of aspirin is not advised as there are no convincing reports of any beneficial antithrombotic effects.

Surgical intervention is advised when several worms have been displaced into the right cardiac chambers producing the sudden onset of severe clinical signs (caval syndrome). It can be accomplished under general anaesthesia 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. In heavily infected dogs, the surgical removal of as many worms as possible greatly reduces potential thromboembolism and the clinical signs associated with adulticide treatment.

Prophylactic ivermectin therapy at 6 µg/kg monthly throughout the year for a period of at least 2-2.5 years, has been shown to kill adult parasites. This regimen should be restricted to selected cases which excludes very active dogs, working dogs or other animals where adulticide therapy is contra-indicated.

CAT:

Diminishing doses of prednisolone are advised in cats in order to relieve respiratory distress with an initial dose of 2 mg/kg daily. If a cat presents with severe signs due to embolism of dead worms, high doses of prednisolone (1-2 mg/kg 3 times a day) are recommended.

Adulticide therapy is generally not advised in cats because of the high risk of severe thromboembolism and sudden death in the post-treatment period.

Canine and feline *D. repens* infection

No effective therapy is known for this *Dirofilaria* species. Because most infections are asymptomatic, no therapy is advised but chemoprophylactic drugs administered monthly for 12-24 months will reduce microfilaraemia. Parasitic nodules can be removed surgically. In the case of skin lesions resulting from sensitization, doxycycline should be administered to reduce inflammatory reactions associated with *Wolbachia* depletion.

Canine *Acanthocheilonema* infection

There is no known effective therapy against *Acanthocheilonema*. Because most infections are asymptomatic, no therapy is advised but, if deemed necessary, the chemoprophylactic regime used for *D. repens* may be administered.

TRAVELLING DOGS AND CATS

Dogs and cats travelling from non-endemic to endemic areas should be protected against adult filarial infections. Whilst there are parasiticidal products that can kill and/or repel mosquitoes, these cannot be relied upon to prevent transmission of *Dirofilaria* infection. (See ESCCAP Guideline 3: Ectoparasites Part 1 for more information on mosquitoes and their control). For convenience it may be easiest to start prophylactic treatment prior to entry into an endemic area otherwise they should be treated within 30 days after arrival in risk areas with the indicated heartworm prevention (larvicidal) doses of macrocyclic lactones. For pets spending no more than one month in endemic areas, a single treatment, usually soon after returning home, is sufficient to assure

complete protection. In the case of longer visits, a monthly regimen should be applied with the first treatment 30 days after the pet enters the risk area and the last at least 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 once with a macrocyclic lactone; in such cases, repeated testing for circulating antigens and microfilariae is recommended every 6 months. To avoid the risk of parasite transmission in non-endemic areas, a microfilaricidal treatment should be administered to all *D. immitis* microfilaraemic dogs after adulticide therapy. The administration of macrocyclic lactones over several months have markedly reduced numbers of *D. immitis* microfilariae; in the case of *D. repens* infections, monthly treatment for 12-24 months reduces microfilaraemia. The efficacy of the treatment regimen should be checked 30 days after the last drug administration.

No resistance against drugs used for therapy or prophylaxis of dirofilariosis has so far been documented.

II.2.h Public health considerations:

In Europe, *D. repens* is the most important agent of human filarial infection. Most cases are asymptomatic and do not require any therapy; in many cases infection has only been diagnosed after surgical removal of a nodule containing the worms.

More than 450 cases of *Dirofilaria* infection in humans have been recorded in the literature. The country with the most recorded cases of *D. repens* human infection is Italy (70%), followed by France (17%), Spain (15%) and Greece (9%). Cases have also been reported from Hungary, Turkey, Russia, Serbia and Croatia. Women are more often affected than men and the age distribution reveals a much higher incidence for both parasites in patients of both sexes over 40 years of age.

Apart from the classical presentation of solitary pulmonary nodules, it has been shown that transitory pulmonary nodules can be a frequent manifestation of infection. Furthermore, several cases of ocular localization have been reported and also in deep body tissues, sometimes mimicking a tumor. In a two year case-finding study carried out in western Spain, eight cases of pulmonary dirofilariosis were diagnosed in a population of approximately 50,000 where the seroprevalence rate was 21%. The calculated incidence of 4 cases/100,000 people/year is a clear indication that the infection is widely underdiagnosed probably because its presence is not generally considered by human physicians.

II.3. Leishmaniosis

II.3.a Agents and vectors:

In Europe, canine leishmaniosis is caused by *Leishmania infantum* which comprises various enzymatic types (zymodemes). The vectors are blood-sucking flies of the genus *Phlebotomus* (Phlebotominae sub family; phlebotomes = sand flies), see Table 8.

The dog is considered the main host of *L. infantum* infection, but cats can also be hosts of *L. infantum* transmitted by several phlebotome species. Many other mammal 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, but the epidemiological role of the infection in 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 pass through four instars before pupation and adult emergence. The seasonal dynamics of phlebotomes have not been fully explored, however it is known that some palaearctic 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 cool and humid places especially cracks and holes in stone walls, dark cellars of houses and animal stables.

Phlebotomes are widespread in the Mediterranean Region, Africa and the Middle East and well adapted,

depending on the species, to tropical and subtropical climates and even arid habitats. Furthermore, it has been known for decades that the *P. perniciosus* endemic area extends up to northern France and this species was found in localised areas in southern Germany and southern Switzerland.

Table 8: *Leishmania* species infecting dogs and cats in Europe

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

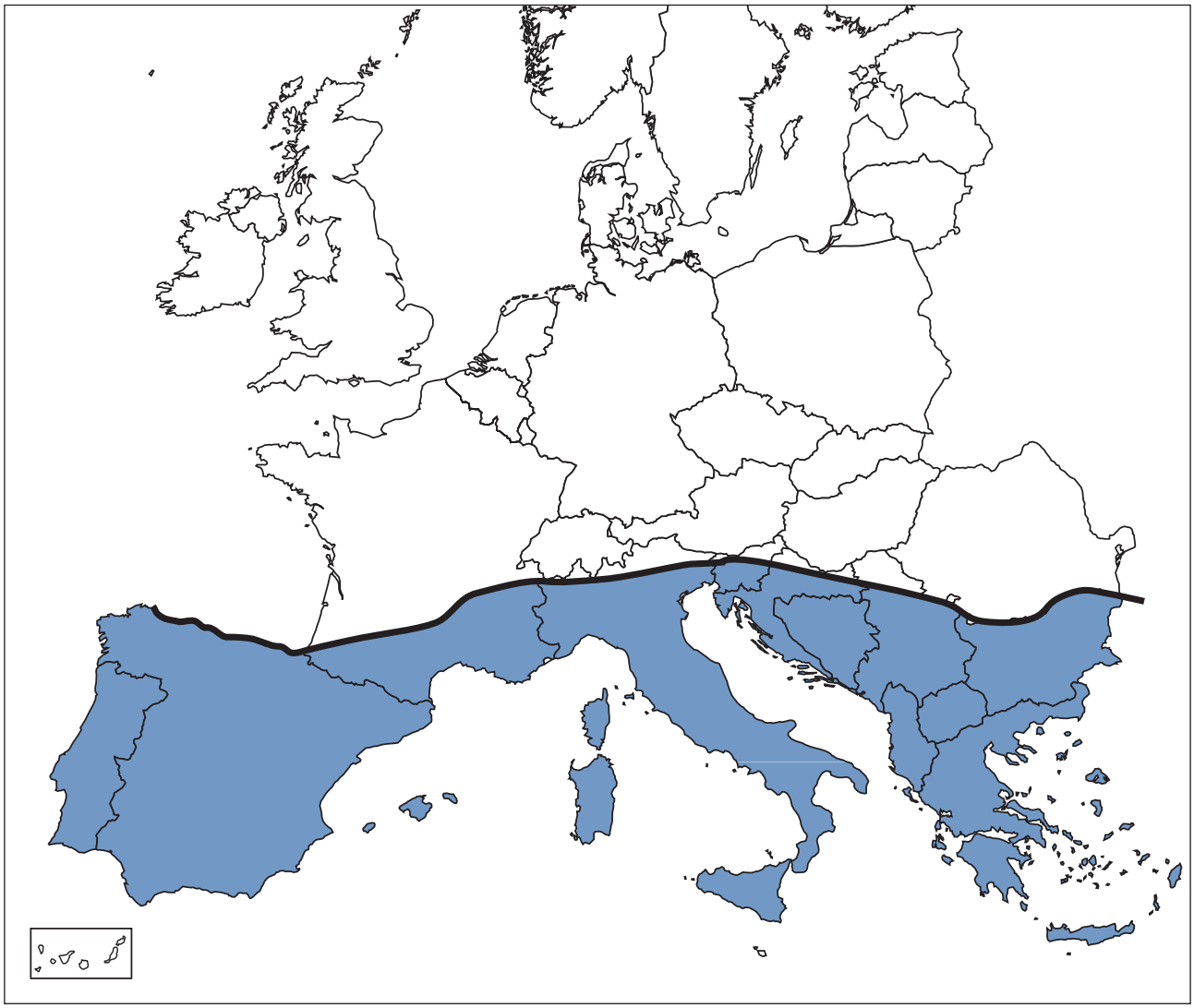
II.3.b Biology and transmission:

- *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 during the night and at a minimum temperature of about 18- 22°C.
- The parasite's development in the vector is temperature dependent and takes around 7-14 days at temperatures above 18°C.
- Transmission of *Leishmania* by arthropods other than phlebotomes (e.g. ticks) as well as horizontal transmission from mother to offspring or by biting has also been reported, (but these events are epidemiologically insignificant).
- There is some evidence of resistance in certain dog breeds (e.g. the Iberian hound) as well as susceptibility of certain dog breeds (eg. German shepherds, Rottweilers 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 reservoirs.
- The incubation period can vary between 2 months and 8 years and is dependent on a number of immunological mechanisms.
- After a 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 in the skin, lymph nodes, spleen, liver, bone marrow and many other organs.
- The main risks in endemic areas are related to vector exposure and abundance of reservoir hosts which include dogs living outdoors, a high stray dog population, dogs adopted from animal shelters in endemic areas and hunting dogs.

II.3.c Distribution in Europe:

Canine leishmaniosis is endemic in southern Europe with prevalence rates up to 75% in exposed populations. Fig. 2 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 time limited focal transmission can occur if there is sufficient infection pressure from imported infected dogs.

Fig. 2 Approximate distribution of canine leishmaniosis in Europe



The black line denotes the approximate northern boundary of the endemic area. North of this line there are many imported cases together with rare descriptions of local transmission.

II.3.d Clinical signs:

In endemic areas, a large part of the infected population may be asymptomatic.

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 mis-diagnosed as tick or simply insect bites. They consist of a single or several ulcerative lesions, called chancres or "chancre d inoculation". They last several months but are self-limiting. During this period, infected dogs may remain seronegative but later, around 25% seroconvert and the disease becomes patent and generalised. In affected dogs, enlargement of single or multiple lymph nodes may be evident accompanied by weight loss, anorexia and debility. More severe clinical signs may develop, and the disease can prove fatal if therapy is not applied. Severe clinical signs include skin lesions like alopecia, skin ulcers, hyperkeratosis, intense squamous dermatitis, and general signs such as muscular atrophy, splenomegaly, epistaxis, haematuria and haemorrhagic enteritis have also been reported. Other clinical signs include gastrointestinal disorders, polyarthritis, glomerulonephritis, meningitis and uveitis. 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.

Although the clinical pathology may be variable, there are many common findings such as a normocytic normochromic non-regenerative anaemia and, less frequently, a thrombocytopaenia, plasma protein changes with hyperglobulinaemia and hypoalbuminaemia, proteinuria and a variable azotaemia with an increase in the protein/creatinine ratio due to renal failure.

II.3.e Diagnosis:

- To reduce the potential for transmission of *Leishmania* from dogs to vectors, a diagnosis of the disease should be confirmed and a course of treatment instituted as early as possible.
- Clinical signs together with relevant epidemiological information can strongly indicate a putative diagnosis. However, clinical signs are only observed in a low proportion of infected dogs and clinically healthy infected dogs represent an important reservoir of infection for phlebotomes.
- Direct diagnosis is possible by detection of the amastigote stages in Giemsa or Diff-Quick stained smears obtained from superficial lymph nodes or bone marrow aspirates or after culture of samples to allow the development of promastigotes in vitro. If skin biopsies are examined for parasite detection the sensitivity is lower. The sensitivity of parasite detection can be increased by immunohistochemistry techniques such as immunoperoxidase staining.
- PCRs, mostly targeting repetitive sequences, have proven to be highly sensitive compared with laborious in vitro cultivation and have the advantage that there is little risk of bacterial contamination. The diagnostic sensitivity however, is dependent on the quality of the clinical samples. Lymph node aspirates, especially from animals with a lymphadenopathy, are the most convenient while bone marrow sampling is more invasive but may be indicated for special cases such as suspect asymptomatic 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.
- Serology is the most commonly used first step because it is relatively non-invasive and permits the detection of a specific antibody response in dogs at around 6-8 weeks after an initial infection. In subclinical infections this period may extend to years. Different 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 sensitivity and specificity of these tests vary according to the defined cut-offs. Immunochromatographic devices have been developed and many commercial kits are now available either for practitioners for a quick diagnosis or for use in epidemiological field studies. These tests have a reasonable sensitivity for clinical cases but are not suitable for the detection of the infection in healthy dogs.

II.3.f Control:

Some control strategies used in the past such as the culling of sero-positive dogs in endemic areas, have not been very successful in reducing *Leishmania* transmission.

Prevention of phlebotome bites by the application of repellents/insecticides to dogs in the form of impregnated collars, sprays and spot-on preparations is currently the most promising strategy. The basic objective here 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 phlebotomes. For example, it has been

observed that dog-collars impregnated with deltamethrin possess a repellent effect against phlebotomes lasting from one week after application to over six months, thereby resulting in a significant decrease of infection incidence 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 24 hours after drug application; this protection lasts for 3-4 weeks, and has been shown to be effective in 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 over night throughout the whole risk season (especially during dusk and dawn), the use of insecticidal room sprays, protective nets in windows and doors (mesh 0.3-0.4 mm^2), and mosquito bednets treated with pyrethroids. Wherever they have been implemented, these measures have brought about a dramatic reduction in phlebotome populations.

Vaccination against canine leishmaniosis could represent the best strategy for controlling the disease but no vaccine is currently available in Europe.

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

II.3.g Chemotherapy:

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 has been 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, Spain, Italy and Greece.

Indications for treatment are:

Clinical signs and clinical pathology associated with a positive diagnosis. The drugs which are mostly used for treatment of clinical cases of canine leishmaniosis are listed in Table 9 (see www.esccap.org for details of approved products for specific countries). Generally, in non endemic areas, single drug treatments with meglumine antimoniate or more recently with allopurinol have been successfully used, but in European endemic areas, with a high seasonal infection pressure, combined therapies are recommended.

Besides specific therapy, symptomatic treatment together with a healthy diet is recommended. There is a special diet commercially available containing moderate protein levels supplemented with omega acids, zinc sulphate and antioxidants for clinically affected dogs.

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 a relapse are hypoalbuminaemia/ hypergammaglobulinaemia associated with a rise in specific antibody titres in two serial samples taken several months apart and examined at the same laboratory.

If there is no clinical improvement after a course of treatment, a change of drug or 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 or hepatozoonosis or of immunosuppression, all of which may affect the response to treatment.

Table 9: Chemotherapy of canine *leishmaniosis*

Drugs	Dosage	Route of administration
Meglumine antimoniate	75-100 mg/kg daily for 4-8 weeks	Subcutaneous injection
Allopurinol*	20-50 mg/kg divided into two or three doses daily for 6-18 months	Oral
Miltefosine	2 mg/kg once daily for 4 weeks (with food)	Oral
Meglumine antimoniate + allopurinol*	For both compounds see above	Subcutaneous injection + Oral
Miltefosine + allopurinol*	For both compounds see above	Both oral

*Not commercialized as a vet product in the EU.

Numerous pharmacokinetic studies have shown that administration of meglumine antimoniate by intramuscular or subcutaneous injections is more effective in maintaining sustained drug plasma levels than intravenous injections. After intravenous administration, plasma levels fall within 2 hours whereas they fall within 4 hours after intramuscular application; when injected subcutaneously, plasma levels rise after 5 hours and remain above 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 are not therefore 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 9.

Allopurinol is commonly administered twice or three times daily in doses 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 retreated with the same compound. Some side effects of treatment have been reported including the development of xanthine urolithiasis. However, this side effect disappears spontaneously shortly after cessation of treatment.

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 been tested in dogs with natural *L. infantum* infection and 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, when it is administered with food.

Recent clinical trials combining two compounds (see Table 9) have shown promising results with a reduced relapse rate. However, no carefully controlled clinical studies comparing the different regimes have so far been published.

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 mode of administration.

Resistance against 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 in the other recommended drugs.

II.3.h 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 immunologically protected.

The responsibility of veterinary practitioners must be the clinical management of the disease in dogs and the reduction of parasite transmission since dogs are the main reservoir of infection.

The following principles must be stressed:

- A thorough diagnostic procedure should be established to evaluate infected and sick dogs.
- The best treatment for sick dogs should be chosen, bearing in mind the potential risks for the development of resistance in "first line" drugs used in humans.
- The use of insecticides should be recommended for all dogs at risk and especially for infected dogs even after successful chemotherapy; these should be applied throughout the risk season which depends on climatic conditions in each geographical area. In the Southern European endemic areas the risk season is between April and October.
- 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 or ticks and thus avoid the risk of focal high endemic transmission.
- To avoid an extension of endemic areas, *Leishmania*-infected dogs should not be translocated to non endemic areas particularly where phlebotomes may be present.

II.4. Ehrlichiosis/ Anaplasmosis

II.4.a Agents and vectors:

Ehrlichia and *Anaplasma* are vector-transmitted, Gram-negative, obligate intracellular bacteria of the order Rickettsiales, family Anaplasmataceae. In Europe, *Ehrlichia canis*, *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*), and *Anaplasma platys* (formerly *Ehrlichia platys*) have been reported from domestic dogs. These three organisms infect primarily leucocytes or platelets and form typical microcolonies (morulae) that may be seen by light microscopy in infected cells.

Table 10: Pathogenic Anaplasmataceae affecting dogs and cats in Europe

Causative agent	Disease	Hosts	Reservoir	Tick vector
Ehrlichia canis	Canine monocytic ehrlichiosis (CME)	Canids (Cat)[1]	Canids	*Rhipicephalus sanguineus*
Anaplasma phago-cytophilum	Canine granulocytic anaplasmosis (CGA)	Dog, cat, humans, horse, sheep, goat, cattle, llama	Roe deer, red deer, small rodents, lynx[2]	*Ixodes ricinus, (I. trianguliceps[3])*
Anaplasma platys	Canine cyclic thrombocytopenia (CCT)	Dogs	–	*Rhipicephalus sanguineus**

[1] *E. canis*, or a closely related agent, has also been reported in some cats.
[2] Partial list; seropositivity and PCR-positivity have also been demonstrated in other species.
[3] *A. phagocytophilum* was demonstrated in *I. trianguliceps* in the UK.
* Role as a tick vector suspected but not proven.

II.4.b Biology and transmission:

Ehrlichia canis

- All stages (larva, nymph, adult) of *R. sanguineus* preferentially feed on canids and may acquire *E. canis* from bacteraemic animals. Trans-stadial transmission occurs (from larva to nymph to adult). *E. canis* may overwinter in the infected tick, which

may survive indoors even in countries with cold or temperate climates. There is no reported transovarial transmission.

- During the incubation period of 8 to 20 days, the infectious agents multiply by binary fission forming morulae within circulating mononuclear cells. Subsequently, they spread via the mononuclear phagocytic system to liver, spleen and lymph nodes. Circulating infected cells adhere to the vascular endothelium especially in the lungs, kidneys and meninges, and induce vasculitis and subendothelial tissue infection. This may lead to platelet damage, sequestration and destruction.

Anaplasma phagocytophilum

- Trans-stadial but not transovarial transmission of *A. phagocytophilum* occurs in the *Ixodes* spp. vectors (or *Ixodes ricinus* vector). Normally several hours of tick feeding are required for transmission of this agent to susceptible dogs.
- The duration of the incubation period is 1 to 2 weeks. After endocytosis, *A. phagocytophilum* multiplies by binary fission into morulae in the phagosomes mainly of neutrophil but also eosinophil granulocytes. Cells infected with *A. phagocytophilum* are found in the circulating blood and 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 or other arthropod vectors are likely to be involved. In experimental infections, the incubation period ranges from 8 to 15 days. Infections lead to cyclic thrombocytopenia and the highest bacterial load is found during the initial peak; in subsequent cycles, only ~1% of the platelets are affected while thrombocytopenic episodes remain approximately the same. Over time, the severity of the thrombocytopenic response diminishes.

II.4.c Distribution in Europe:

The geographical occurrence of infections with *E. canis, A. phagocytophilum* and *A. platys* generally corresponds to the distribution of their respective vectors *R. sanguineus* and *I. ricinus*. Although only 2-4% of the ticks may carry these pathogens, with increasing travelling of people and dogs, infections must also be expected to occur in non-endemic countries.

Table 11: Distribution of pathogenic Anaplasmataceae in Europe

Causative agent	Location	Countries with reported cases
Ehrlichia canis	Europe-wide*	France[1,2], Italy[1,2], Greece[1], Spain[1,2], Portugal[1], Bulgaria[3].
Anaplasma phago-cytophilum	Europe-wide	Norway[3], Sweden[1,2], Denmark[2], UK[1,2], Ireland[2], Netherlands[3], Germany[1], Switzerland[1], France[3], Italy[1,2], Spain[1,2], Portugal[3], Poland[1], Bulgaria[3], Slovenia[1], Czech Republic[3].
Anaplasma platys	Countries with Mediterranean climate*	Italy[1], Spain[1], France[1], Greece[1].

[1] Reported in dogs.
[2] Reported in cats.
[3] Infection demonstrated in ticks.
* In many European countries with cold or temperate climates, cases are only seen in animals imported from areas with a Mediterranean climate.

II.4.d Clinical signs:

Table 12: Clinical manifestations of pathogenic Anaplasmataceae infections in dogs

Causative agent (disease)	Clinical signs	Laboratory findings
Ehrlichia canis (CME)	Acute phase: fever, anorexia, depression, lethargy, weight loss, epistaxis, haemorrhages (petechiae, echymoses)[2], lymphadenomegaly, splenomegaly, ocular signs[2], neurological signs[2]. Subclinical phase: no clinical signs. Chronic phase (severe form): lethargy, weight loss, haemorrhagic disorders, secondary infections.	Thrombocytopenia, anaemia, leukopenia, mononuclear pleocytosis[1], hypoalbuminaemia, hyperproteinaemia, hyperglobulinaemia, proteinuria, haematuria, elevated ALT, ALP.
Anaplasma phagocytophilum (CGA)	Fever, anorexia, lethargy, depression, reluctance to move[2], lameness, stiffness, splenomegaly[2], hepatomegaly[2], neurological signs[2], often asymptomatic or seen in conjunction with immunosuppression or concurrent infections.	Thrombocytopenia, mild hypoalbuminemia, elevated ALP, occasional neutropenia or regenerative left shift, neutrophilic polyarthritis, predominantly neutrophilic cerebrospinal fluid, pleocytosis.
Anaplasma platys (CCT)	Fever, lethargy, pale mucous membranes, petechial haemorrhages, often asymptomatic or in conjuction with immunosuppression or concurrent infections.	Cyclic thrombocytopenia[3], anaemia.

[1]Granular lymphocytosis may be confused with well-differentiated lymphocytic leukaemia; the hypergamma-globulinaemia reported in some of these dogs may sometimes lead to a misdiagnosis of lymphocytic leukaemia.
[2] Has been observed but is not always present.
[3] Cyclic bacteraemia and cyclic thrombocytopenia (<20'000/µl) at 1 to 2 week intervals.

The acute phase of CME is due largely to a vasculitis and usually lasts 2 to 4 weeks. Lack of appropriate treatment may lead to a subclinical phase and dogs may remain carriers for months or years. The chronic stage of the disease reflects bone marrow suppression. Concurrent infections affect the severity and clinical manifestations of the disease. Immunodeficiency may lead to more severe clinical signs and a higher number of circulating morulae when compared with immune competent animals. German shepherd dogs seem to be more susceptible, the disease syndrome being more severe and the prognosis poorer than in other breeds.

For *A. phagocytopilum*, susceptibility to infection may increase with age.

The clinical manifestations following *A. platys* infection may vary depending on the geographical region: for example, 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 *E. canis* and *A. phagocytophilum* infections in cats are rare. Clinical manifestations are not well known but are likely to be similar to those reported in dogs.

II.4.e Diagnosis:

- Diagnosis of *Ehrlichia/Anaplasma* infections in dogs is usually based on the combination of a good history to ascertain the potential exposure to tick infestation, clinical signs, haematological and clinical chemistry results, serology and/or PCR. Positive results from serology and/or PCR should be interpreted with caution with regard to any *Ehrlichia/Anaplasma* being the sole causative agent of the observed clinical syndrome. Indeed, ticks can harbour multiple infectious agents, thus it is possible for dogs to be infected with multiple pathogens. This complicates the diagnosis as the diagnostic features (both clinical and test results) associated

with single infections may be significantly different in multiple infections. In particular, since they have the same vector and some clinical signs are similar, Lyme borreliosis and granulocytic anaplasmosis may be misdiagnosed.

- Morphological diagnosis: a definitive diagnosis may be made, when blood smears are examined and morulae can be seen in lymphocytes, monocytes, granulocytes or platelets. In CME, in contrast to *A. phagocytophilum* infection, morulae are rarely seen and if so, they are more frequently found in lymphocytes than in monocytes. To increase diagnostic sensitivity, buffy coat smears or thin blood smears of capillary blood should be performed. Morulae may also be demonstrated in smears of aspirates from tissues, such as lymph nodes or spleen. The detection of morulae is time-consuming and must be performed by experienced personnel. Due to the cyclic bacteraemia of *A. platys*, its morulae can be particularly difficult to find.
- Serology: antibodies may be detected by indirect immunofluorescent assay (IFA) using *E. canis, A. phagocytophilum* or less frequently *A. platys* antigens. Seroconversion may be demonstrated one to four weeks after exposure and an acutely infected dog or cat might therefore still be serologically negative. Additionally, in endemic areas, positive IFA results may result from a previous exposure and may not necessarily represent an acute infection. Repeat testing by IFA after one to several weeks is recommended in these cases. Cross-reactivity may occur with this test depending on the geographical area and the prevailing strains of the organisms. *A. phagocytophilum* and *A. platys* strains are known to cross-react to some degree, while little or no cross-reactivity is usually found between *E. canis* and *A. phagocytophilum*. Antibodies to *A. phagocytophilum* and *E. canis* usually decline within six to nine months after successful treatment. If titres are followed over time, samples should be assayed by the same laboratory to ensure comparable results; this is advisable due to differences in the assays and strains used by different laboratories. Several ELISAs and point-of-care tests have been evaluated for the detection of *E. canis* infections. For the Snap 3Dx test, which is commercially available in some European countries and which simultaneously also screens dogs for *D. immitis* and *B. burdorgferi* infections, sensitivities and specificities of 71-93% and 89-100%, have been reported. Sensitivity was higher with high-titre (> 1:320) than with low-titre sera. The Snap 4Dx test which also includes *A. phagocytophilum* is now also available in some European countries.
- PCR: specific assays for detection of *E. canis, 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 infection; it may be due to factors such as inappropriate sample selection as the agent is located in different body compartments at different periods after infection or the actual time of sampling as there is a decrease in the bacterial load after therapy. For samples from cats with suspected ehrlichiosis or anaplasmosis, PCR assays with broad specificity, able to detect several members of the family *Anaplasmataceae*, should be used.

II.4.f Control:

Currently no preventative measures, e.g. vaccines, are available to protect against disease caused by *E. canis, A. phagocytophilum* or *A. platys* infections in dogs and cats. The primary method of prevention of infection is based on effective tick-control (see ESCCAP Guideline 3: Ectoparasites Part 1). For dogs outside endemic areas, it is recommended not to travel to or through endemic regions. When travel to *E. canis* or *A. phagocytophilum* endemic areas cannot be avoided, tick control measures should be applied prior to departure. Prophylactic chemoprophylaxis has also been shown to be effective but such measures should be reserved for selected cases (splenectomised and/or immunocompromised dogs).

Dogs that have recovered from ehrlichiosis or anaplasmosis may be susceptible to re-infection as a protective immunity does not always develop.

II.4.g Chemotherapy:

The treatment of canine ehrlichiosis, anaplasmosis and cyclic thrombocytopenia consists of antirickettsial agents and supportive care. Tetracyclines are the most commonly used compounds, with doxycycline once daily at 10 mg/kg for 4 weeks being the most commonly used therapeutic regimen. Chloramphenicol, imidocarb dipropionate, and amicarbalide have also demonstrated efficacy but are rarely used. Imidocarb leads to clinical improvement but not to clearance of the infectious agents while tetracyclines appear to be more efficacious in clearing infection. In *E. canis*-infected dogs, resolution of thrombocytopenia is a good indicator of a positive response to therapy. An increase in the platelet count may be seen within 48 hours after initiating therapy and counts are normalized within 14 days. Cases of chronic severe CME in dogs have a poor prognosis.

II.4.h Public health considerations:

Confirmed infections of humans with *E. canis* or a closely related organism are very limited, and *E. canis* is not considered a significant zoonotic risk. Human monocytic ehrlichiosis is usually due to *Ehrlichia chaffensis*, which has not yet been reported in Europe. However, infections with *A. phagocytophilum* have been reported in humans. This agent is naturally transmitted to humans, dogs and cats by *Ixodes* spp. The potential of *A. phagocytophilum*-infected dogs or cats to be a zoonotic risk for humans by direct transmission is not known.

II.5. Borreliosis - Lyme disease

II.5.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 in dogs have been demonstrated, they are not of major clinical importance. Humans as well as dogs acquire *Borrelia* while exposed to ticks, there is not interdependency between dogs and humans in terms of transmission. Positive serology in cats has also been reported, but any disease in cats, if it occurs, is poorly understood and there is therefore little data concerning the prevalence of infection, clinical appearance and treatment options for cats.

II.5.b Biology and transmission:

- Currently, ticks of the family Ixodidae and mostly of the genus *Ixodes* are recognized vectors of *B. burgdorferi* sensu lato.
- Larval, nymph and adult female tick vectors can acquire *Borrelia* when feeding on an infected competent "reservoir host", which is an animal harbouring the pathogen as a long-term infection. Ticks can become infected while having a blood meal on such an animal as well as by co-feeding transmission when spirochetes pass from infected to uninfected ticks which feed simultaneously on the same host.
- Several animal species have been identified as competent 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 transmission to a new host can occur.
- *Borrelia* remain in the skin of a host before disseminating. In some cases, it can take up to 4 weeks before the systemic infection develops.

II.5.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.

II.5.d Clinical signs:

Borreliosis is a well-recognized disease in humans but as yet, it is not clearly defined in dogs and around 95% of infected dogs are asymptomatic. "Lyme arthropathy": which is a lameness in one or more joints has been described; puppies may be at higher risk of such a polyarthritis and there is a possible breed predisposition in Retrievers. "Lyme nephropathy": there are many reports of dogs seropositive for *Borrelia* which have immune-mediated glomerulonephritis but further studies are needed to clarify any association. Some dogs present with fever associated with lameness.

Clinical manifestations in naturally infected cats are uncommon.

II.5.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 true disease. If dogs suspected of having Lyme disease are positive by serology, a Western Blot immunoassay is recommended to check for specific banding patterns. Finally, specific antibody reactions to the C6 peptide are highly specific for *B. burgdorferi* sensu lato exposure in dogs.

II.5.f Control:

Serological positivity for infection 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 infestation and their control. The use of *Borrelia* vaccines is still a controversial issue due to the presence of several *Borrelia* species in the field and the fact that vaccines protect only against *B. burgdorferi* sensu stricto. Tick control is currently the disease prevention method of choice.

Therapy:

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 polyarthritis, but responses will take longer in dogs with suspected Lyme nephropathy. Studies in experimentally-infected dogs have shown that antibacterial treatment does not clear the infection from all dogs. The drug of choice is doxycycline,10 mg/kg po once daily for a minimum of 1 month.

II.5.g Public health considerations:

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

II.6. Viral Diseases

II.6.a Agents and vectors:

Table 13: 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 fox, ruminants; not in cats	*Ixodes ricinus*
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	*Ixodes ricinus* (possibly other modes of transmission)
West Nile virus infection	West Nile virus (WNV)[4], Flavivirus	Horses, humans, dogs and cats[5]; reservoir: birds	*Culex* 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.

II.6.b Biology and Transmission:

- Infections are usually initiated by the bite of an infected tick or mosquito.
- TBE virus: *I. ricinus* larvae, nymphs and adult ticks can be infected and trans-stadial and occasionally transovarial transmission occurs. Due to the low host specificity of *I. ricinus*, the virus can be transmitted to a wide range of vertebrates but most infections remain clinically inapparent. Infections of 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 laboratory workers. Food-borne transmission is possible via unpasteurized milk or pig meat or carcasses. Ticks become infected by feeding on animals with high blood virus loads such as sheep or grouse. Trans-stadial but usually no transovarial transmission occurs in ticks.
- For WNV, wild and domestic birds act as the main hosts but there is a great diversity of potential hosts and vectors. Humans and several 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.

II.6.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, it seems to be restricted to Mediterranean and eastern European countries.

Table 14: Distribution of vector-borne virus infections in dogs and cats in Europe

Infection	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]. Europe : up to 1999: http://www.cdc.gov (vol. 5 no.5 Emerging Infectious Diseases publication Sep-Oct 1999)

[1] A virus, presumably originating from a British Louping-ill virus isolate also caused disease in livestock and humans in Norway. Closely related but distinct 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).

II.6.d Clinical signs:

Table 15: 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	Acute viral encephalomyelitis but may also be asymptomatic[1] Muscle tremors, spasms, ataxia, fever, depression, paresis. Louping-ill viruses (LIV) 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. ricinus*.
West Nile Virus infection	Clinical disease in dogs appears to be rare with only five reported cases in the USA and Africa. Fever, apathy, anorexia, progressive neurological signs including stiff gate, ataxia, paresis, tremors, altered behavior, and conscious proprioception deficiencies.

[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.

II.6.e Diagnosis:

- TBE is a seasonal disease which depends on the climate-related activity of *I. ricinus*. A tentative diagnosis is based on clinical evidence and on the known risk of exposure through 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, can confirm the 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 histophathology.
- 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 of titre in the haemagglutination inhibition test.
- Immunohistochemistry, virus isolation, RT-PCR and serology are used to detect infection with WNV.
- Flaviviruses are usually eliminated by the immune system.

II.6.f Control:

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. Some dogs in endemic regions have been vaccinated, but vaccine efficiencies were 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 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. No vaccines are currently available for use in dogs and cats.

II.6.g Chemotherapy:

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.

II.6.h Public health considerations:

Recently, there has been increasing awareness of TBE-related risk to humans and dogs.

Louping-ill: human cases are very uncommon but are occasionally seen mainly in slaughterhouse or laboratory workers.

There is a rising concern of the potential for WNV spreading in Europe and an increased risk linked to possible transmission by blood and organ transplants.

Table 16: Overview of insect-transmitted pathogens causing vector-borne diseases (VBDs) in Europe

Disease or Infection	Causative Agents	Vector[1]	Host	Geographic Distribution in Europe
DISEASES CAUSED BY PROTOZOA				
Leishmaniosis	*Leishmania infantum*	Phlebotomes	Dog, cat	southern Europe
DISEASES CAUSED BY HELMINTHS				
Dipylidiosis	*Dipylidium caninum*	Fleas, chewing lice	Dog, cat	throughout
Filarioses	*Dirofilaria immitis*	Culicidae	Dog, cat	southern and eastern Europe
	D. repens	Culicidae	Dog, cat	southern and eastern Europe
	Acantocheilonema dracunculoides & A. reconditum	Culicidae and *(Rhipicephalus sanguineus)*	Dog	Spain, France, Italy
Thelaziosis	*Thelazia callipaeda*	Muscid flies	Dog, cat	Italy, France Switzerland
BACTERIAL INFECTIONS OR DISEASES				
Rickettsiosis	*Rickettsia felis* other	Fleas	Dog, cat, hedgehog	Europe
Bartonellosis (cat scratch disease)	*Bartonella henselae*	Fleas, ticks	Cat (reservoir host)	throughout
Bartonellosis	*Bartonella vinsonii* and others	Arthropod vectors	Dog	throughout
Tularaemia	*Francisella tularensis*	Mosquitoes Tabanidae	Cat (dog)	southern Europe
VIRAL INFECTION				
West Nile virus	West Nile virus (WNV), *Flavivirus*	*Culex* spp. and other mosquitoes	Horse, humans, (dog, cat); reservoir: birds	Romania, Czech Republic, Italy, France

[1] non insect vectors in ()

Table 17: Overview of tick-transmitted pathogens causing tick-borne diseases (TBDs) in Europe

Disease	Causative Agents	Hosts	Vectors	Geographic Distribution in Europe	Severity of Clinical Signs
DISEASES CAUSED BY PROTOZOA					
Babesiosis (piroplasmosis)	*Babesia canis canis*	Dog	*Dermacentor reticulatus*	Southern and Central Europe up to Baltic	moderate - severe
	B. canis vogeli	Dog	*Rhipicephalus sanguineus*	Southern Europe following distribution of vector	mild - moderate
	B. gibsoni and gibsoni like	Dog	*Haemaphysalis* spp., *Dermacentor* spp.	Sporadic and rare in Europe	moderate - severe
	Babesia (Theileria) annae	Dog	*Ixodes hexagonus***	North - Western Spain	moderate - severe
Hepatozoonosis	*Hepatozoon canis**	Dog	*Rhipicephalus sanguineus*	Southern Europe	mostly mild infection; subclinical
	Hepatozoon spp.	Cat	Unknown	Spain	subclinical

Table 17: Overview of tick-transmitted pathogens causing tick-borne diseases (TBDs) in Europe *cont'd*

Disease	Causative Agents	Hosts	Vectors	Geographic Distribution in Europe	Severity of Clinical Signs in dog and cat
DISEASES CAUSED BY NEMATODES					
Filariosis	*Acanthocheilonema (Dipetalonema) dracunculoides* *Acanthocheilonema (Dip.) grassii* *Acanthocheilonema (Dip.) reconditum*	Dog	*Rhipicephalus sanguineus*†	Southern Europe	minor
DISEASES CAUSED BY BACTERIA					
Bartonellosis	*Bartonella* spp.	Many animals, dog, cat, human	Ticks suspected†	Throughout Europe	commonly subclinical infection, chronic endocarditis
Borreliosis (Lyme disease)	*Borrelia burgdorferi* complex (especially *B. garinii* and *B. afzelii* in Europe)	Many animals especially rodents, dog, cat, human	*Ixodes ricinus* *I. hexagonus* *I. persulcatus* *D. reticulatus*	Throughout Europe	mostly subclinical, sometimes clinical signs typically malaise and lameness in dogs
Ehrlichiosis (monocytic)	*Ehrlichia canis*	Dog (cat)	*Rhipicephalus sanguineus*	Southern Europe following distribution of vector	moderate – severe
Anaplasmosis (granulocytic ehrlichiosis)	*Anaplasma phagocytophilum*	Many animals, dog,cat, human	*Ixodes ricinus, (I. trianguliceps?)*	Throughout Europe	mild and subclinical infections common moderate with lethargy
Anaplasmosis (infectious cyclic thrombocytopenia)	*Anaplasma platys*	Dog	*Rhipicephalus sanguineus*	Southern Europe following distribution of vector	commonly asymptomatic
Rickettsial infections (Mediterranean spotted fever/MSF)	*Rickettsia conorii*	Dog	*Rhipicephalus sanguineus*	Southern Europe following distribution of vector	subclinical infection or moderate with lethargy
Coxiellosis (Q Fever)	*Coxiella burnetti*	Ruminants, dog, cat, human	*Ixodes* spp.† *Dermacentor* spp.	Throughout Europe	subclinical infection
Tularaemia	*Francisella tularensis*	Lagomorphs, cat	*Ixodes* spp.† *Dermacentor* spp.† *Haemaphysalis* spp.† *Rhipicephalus sanguineus*†	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, dog	*Ixodes ricinus* *I. persulcatus*	Central, Eastern and Northern Europe	clinical signs neurological and can be moderate but not commonly reported
Louping ill	Louping-ill virus, (Flavivirus)	Many animals, mainly sheep, dog	*Ixodes ricinus*	UK, Ireland	clinical signs neurological and can be moderate-severe but not commonly reported

*Transmission of *Hepatozoon* spp. is by ingestion of an infected tick and not a tick bite.
** Not yet experimentally demonstrated.
† Ticks are not the sole arthropod vectors for these diseases.

ESCCAP

EUROPEAN SCIENTIFIC COUNSEL COMPANION ANIMAL PARASITES

Control of Vector-Borne Diseases in Dogs and Cats

ESCCAP Guideline 05 – September 2009

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