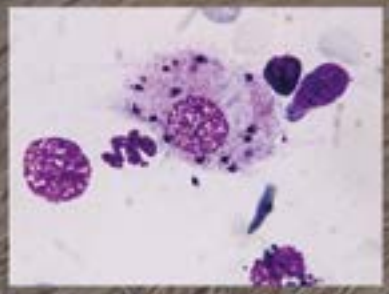
Textbook of Clinical Parasitology in dogs and cats

Frédéric Beugnet
Lénaïg Halos
Jacques Guillot

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Textbook of clinical parasitology in dogs and cats

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Textbook of
Clinical Parasitology
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The authors would like to thank the following teachers warmly for sharing and transmitting their passion for parasitology: Professors Jean Gevrey (Lyon), René Chermette (Alfort), Michel Franc (Toulouse), and Jacques Euzéby (Lyon). This textbook is dedicated to them.

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AUTHORS

Prof. Frédéric Beugnet

DVM, PhD, *Agrégé en Parasitologie et Maladies Parasitaires*, Dip. EVPC

Frédéric Beugnet graduated as a Doctor of Veterinary Medicine from the National Veterinary School of Lyon (France) in 1991. He holds a PhD in Parasitology, is a Diplomate of the European Veterinary Parasitology College (EVPC), and is also *Agrégé en Parasitologie et Maladies Parasitaires* and holds an HDR (*Habilitation à Diriger les Recherches*). He spent more than 10 years teaching and researching at university before joining Merial and still gives university lectures. He specialises in veterinary parasitology for dogs, cats and horses, as well as antiparasitic drugs, mathematical modelling of flea and tick dynamics, and epidemiology of vector-borne diseases in pets and horses. Frédéric is Head of the Global Technical Services for Parasitology and Parasiticides at Merial (now Boehringer Ingelheim Animal Health).

Dr. Lénaïg Halos

DVM, PhD, Dip. EVPC

Lénaïg Halos graduated from the Alfort Veterinary School (France) in 2001. During her MSc, PhD and postdoctoral studies, she further specialised in parasitology, with a specific interest in arthropods and arthropod-borne diseases. From 2007 to 2010, she was Assistant Professor in the Parasitology Department at the Alfort Veterinary School, before joining Merial, where her work is dedicated to Pet Parasitology as Global Technical Director at Merial (now Boehringer Ingelheim Animal Health).

Prof. Jacques Guillot

DVM, PhD, Dip. EVPC

Jacques Guillot graduated from the Alfort Veterinary School (France) in 1991. He is a member of the French Society of Parasitology (SFP), the French Society of Medical Mycology (SFMM), the European Confederation of Medical Mycology (ECMM), and the International Society for Human and Animal Mycology (ISHAM). From 2003 to 2007, he was the scientific director of the Alfort Veterinary School. Jacques Guillot is an active member of the European Scientific Counsel Companion Animal Parasites (ESCCAP), an independent, non-profit organisation whose objective is to develop guidelines for the diagnosis, treatment and control of major parasitic infections in companion animals in Europe. His primary research areas focus on the diagnosis and treatment of fungal and parasitic infections in domestic and wild animals. Jacques Guillot is currently Head of the Parasitology Department at the Alfort Veterinary School.

PREFACE

This book is the second edition of a group of two volumes edited in 2005, and addresses clinical aspects of canine and feline parasitoses.

The objectives of this book are:

- To give a complete view of the main parasites that can be observed and diagnosed in dogs and cats, with particular focus on the European region.
- To confirm the appropriate methods of diagnosis and to emphasise the importance of parasitological diagnosis (coproscopy, parasite observation), which is often forgotten by veterinarians.
- To discuss the treatment and prevention of parasitoses, and the zoonotic risks linked to these diseases.

This book concentrates on the most important general information and does not discuss taxonomy, morphology or biology in any great detail.

The pictures in this book have been sourced from the French Veterinary Schools, other European veterinary faculties, and the authors. Not all photos are of the same size or proportions due to the variation in microscopy equipment used, processing techniques, magnification, zoom, etc.

The life cycles are adapted and inspired from an original version published by Doug Carithers and Guadalupe Miró in Pet Owner Educational Atlas. Parasites, Ed. Servet, 2012.

The authors focused on Parasitology *sensu stricto* (helminthology, protozoology and entomo-acarology), so fungal infections are not included in this book.

This book has been developed specifically for veterinarians and veterinary students so that they may rapidly access information about infestation, clinical studies, diagnosis, therapy, prevention and zoonotic risks.

The authors

SPECIFIC NOTE FROM THE AUTHORS

In line with the World Association for the Advancement of Veterinary Parasitology (WAAVP), we decided to follow the international recommendations for the Standardised Nomenclature of Animal Parasitic Diseases. Consistency in the use of terminology is an important requirement for clear communication in any field of science. In contrast to the basically homogeneous terminology of bacterial and fungal diseases, different names are being used with variable frequency in the nomenclature of parasitic diseases to denote the same disease entity, such as leishmaniasis and leishmaniosis, dirofilariasis and dirofilariosis, toxocariasis and toxocarosis, etc. To address this issue, the Standardised Nomenclature of Parasitic Diseases (SNOPAD) guidelines were published in 1988. Their proposal was endorsed in 1990 by the World Federation of Parasitologists for all parasitic diseases, including human parasitoses.

- When disease names are formed from the taxonomic name of the parasite, the suffixes "-asis" and "-iasis" used to describe a disease or infestation should be discontinued, and only the suffix "-osis" ("-oses" in the plural) should be used.
- Another major source of confusion in the nomenclature originates from variations in the stems of words which are formed either from the nominative genus (e.g., trypanosomosis, hypodermosis) or from the family name (e.g., trypanosomatosis, hypodermatosis). SNOPAD offers a simple solution for uniform usage by proposing that the suffix "-osis" be added to the stem of the parasite taxon, which is usually formed from the nominative case of the taxon with the last one or two letters removed (e.g., *Toxocara*/toxocarosis; *Dirofilaria*/dirofilariosis; *Aelurostrongylus*/aelurostrongylosis; *Isospora*/isosporosis; *Leishmania*/leishmaniosis, etc.).
- When taxa end with -x in the nominative, the stem is formed from the genitive and the disease name is derived from this stem (e.g., *Pulex*/pulicosis).
- In some cases, the disease name is formed by adding the suffix "-osis" to the full name of the parasite taxon (e.g., *Hepatozoon*/hepatozoonosis).

As a rule, all parasitic diseases are denominated using the suffix "-osis" or "-iosis" in this book.

Finally, there is some debate between the words "infection" and "infestation": we decided to follow the zoological definition and to split based on the taxonomy; "infection" applies to viruses, bacteria, protozoa and fungi, while "infestation" applies to multicellular organisms, i.e., Metazoa.

This logo indicates a risk of transmission to humans.

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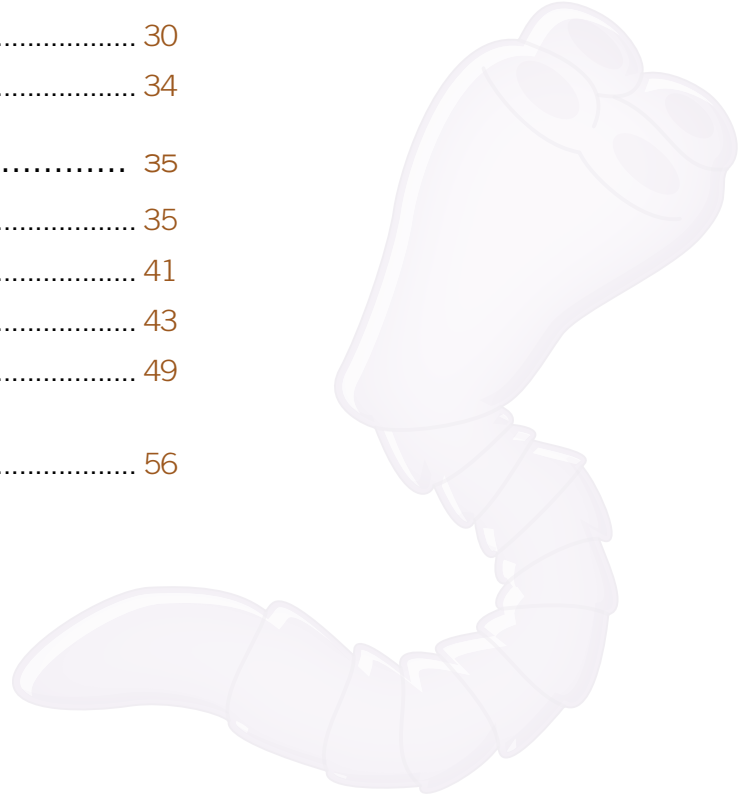

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GASTROINTESTINAL PARASITOSES

Oesophageal and gastric parasitoses

Ollulanosis

General comments

Ollulanus tricuspis is a nematode belonging to the order Strongylida (superfamily Trichostrongyloidea). It is very small in size (approximately 1 mm in length), very slender and coiled like a watch spring at the anterior end (Fig. 1).

This parasite is found in the stomachs of wild cats and foxes, and can occasionally be found in domestic cats.

Biology

Ollulanus is an unusual type of strongylid nematode because the female is viviparous and releases the infective third-stage larvae (L3) into the stomach lumen. The L3 can continue to develop into the immature L4, and then the adult stage, in the cat's stomach, which makes an endogenous life cycle possible.

The L3 larvae are often expelled into the environment in the parasite-induced vomit of infested cats and can infest other cats which ingest that vomitus. The vomit can also contain L4 larvae and adult parasites which may also infest other cats when ingested soon after being expelled from the previous host. L3 larvae can survive for up to 12 days in the environment and are sometimes found in paratenic hosts. The prepatent period is approximately 30–35 days after the animal ingests the L3 larvae or a paratenic host. The adult parasites live in the stomach lumen or in the crypts of the gastric glands and feed on gastric mucosal debris and secretions.

Epidemiology

Ollulanus is a rare helminth which is usually detected sporadically and incidentally. This gastric parasite seems to be more common in warm climates and is more commonly found in wild cats than domestic cats.

Figure 1. Female *Ollulanus* observed in the vomit from a cat. Courtesy of Michael Dryden.

Clinical signs and diagnosis

Ollulanus tricuspis is a small parasite with low pathogenicity which lives on the surface of the gastric mucosa and infestations are mostly asymptomatic although chronic gastritis may develop during massive infestations or in particularly susceptibility cases, presenting as irregular appetite, salivation, abdominal pain and frequent vomiting. Infestation causes thickening and ulceration of the gastric mucosa, with increased production of mucus. Antemortem diagnosis is difficult because parasites are not found in the faeces, however L3 and L4 larval stages or adults may be detected in vomitus. Definitive diagnosis is only possible by necroscopic examination of the gastric mucosa for parasites.

Control measures

There is no recent data on the treatments available. The use of a benzimidazole over several consecutive days or avermectin/milbemycin should eliminate the parasite.

Spirocercosis

General comments

Spiruroses are caused by the presence and development of gastrointestinal Spiruroidea nematodes in the anterior digestive tract in carnivores. The life cycle of spiruroid nematodes is indirect and requires the intervention of intermediate coprophagous hosts. Dogs can be infested with several species of spiruroids, but ***Spirocerca lupi*** is the most common and the most pathogenic.

Other parasites of the superfamily Spiruroidea belong to the genera *Physaloptera* and *Gnathostoma*. Cats can also be infested by ***Spirura rytipleurites*** (see *Other gastrointestinal spiruroses*, page 10).

Geographical distribution

Spirocerca lupi can be seen sporadically in cold or temperate countries such as France; however, the species tends to be more widespread in tropical and subtropical countries, including Africa, Madagascar and the island of Reunion, India, Asia, China, the Caribbean, Indonesia and Malaysia.

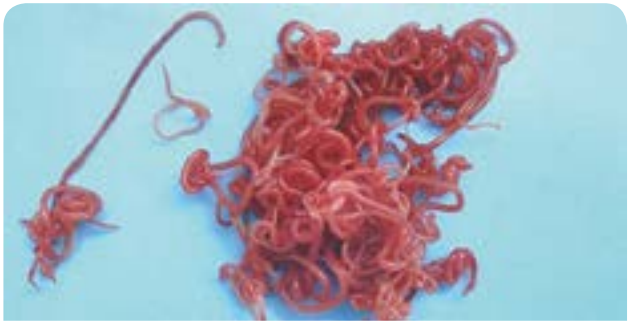


Figure 1. *Spirocerca lupi.* Adults extracted from nodules.

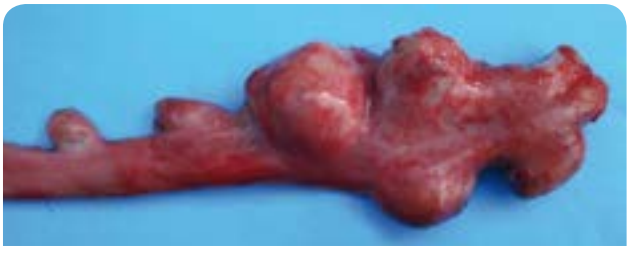


Figure 2. *Spirocerca* nodules in the aortic wall.

Hosts

Wild and domestic dogs can be infested with *S. lupi*.

Importance

Spiruroids can be the dominant pathogens in countries where they are frequently found, such as South Africa, Madagascar, the island of Reunion, India and Guyana. Canine spirocercosis can have serious clinical consequences and may lead to death of the animal.

Morphology

Adult *Spirocerca lupi* are 3–8 cm long, and approximately 1 mm in diameter. It is a round, fairly large, reddish-brown worm with a well-developed buccal vestibule with thick walls (Fig. 1), as are all spiruroids.

Spiruroids produce small eggs which contain larvae. *S. lupi* eggs measure 40 × 10–15 µm.

Biology

Spirocerca lupi lives in fibrous nodules formed in the walls of the oesophagus and stomach. They are sometimes also found in the lymph nodes, lungs, bladder or arteries (Figs. 2 and 3).

The females burrow into these nodules to lay their eggs. which are then excreted into the environment, where they must be ingested by an intermediate host to develop from the L1 to L3 larval stage. In the case of spiruroids, these intermediate hosts are coprophagous arthropods, often dung or scarab beetles (e.g., *Geotrupes, Scarabeus*).

If these intermediate hosts are ingested by small mammals, insectivores, or reptiles, these creatures become paratenic hosts.

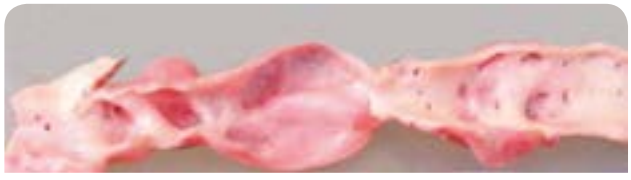
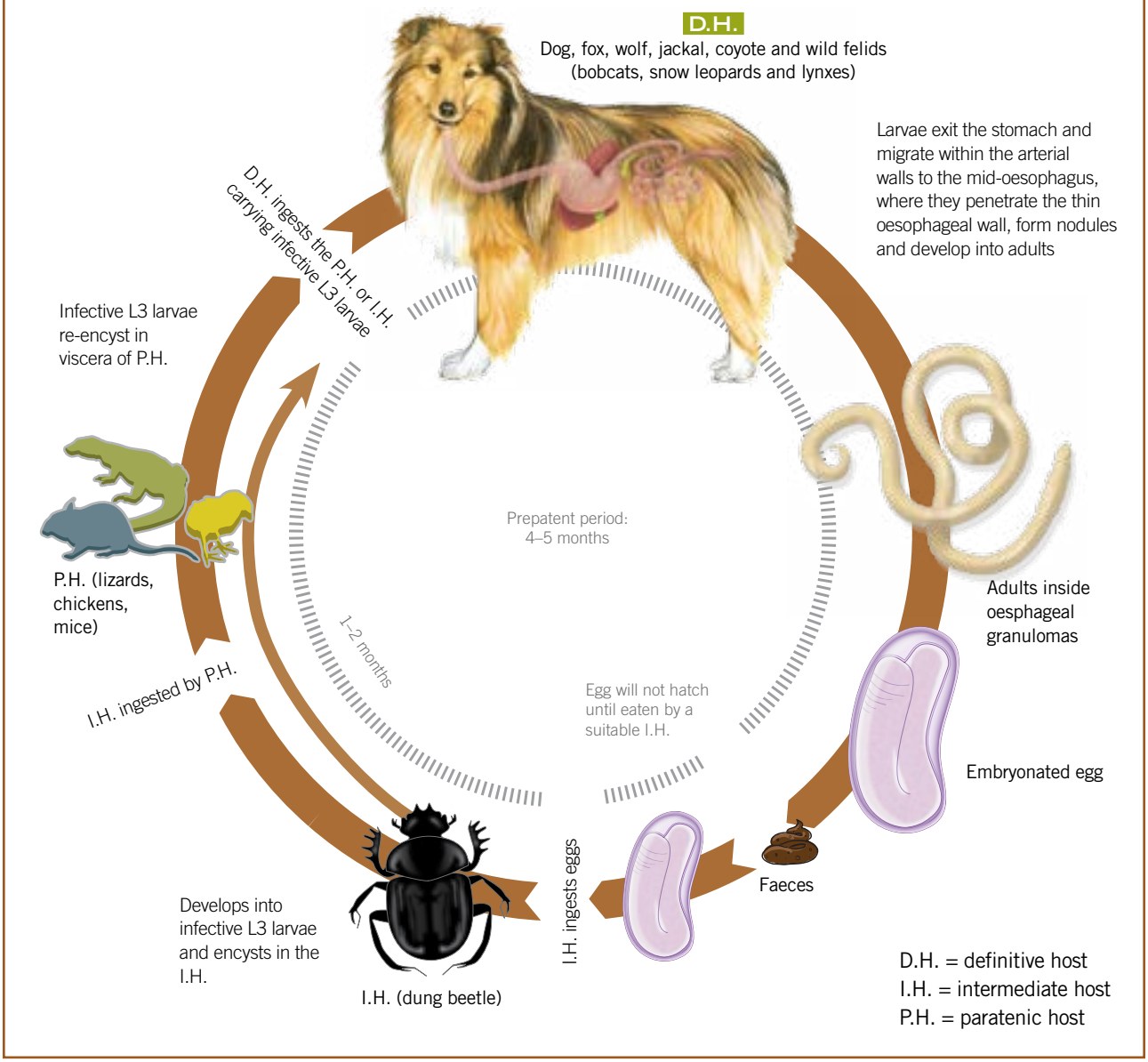


Figure 3. Aortic *Spirocerca* lesions.

Dogs become infested by consuming these intermediate or paratenic hosts. Intermediate hosts are digested in the dog's stomach and release L3 larvae which burrow into the stomach wall, towards the gastroepiploic artery and the aorta. Once they reach the aorta, they travel along the aortic wall and then migrate to the oesophagus where they form granulomatous nodules in the oesophageal wall (Figs. 4–8).

The life cycle takes approximately 4 months. The adults are located inside granulomas that measure 4–10 cm in diameter, and have an opening where the females lay their eggs. This location makes it difficult for anthelmintic agents to reach them because such agents do not penetrate granulomatous tissue well.

Life cycle of *Spirocerca lupi*



S. lupi can cause significant damage to the arteries. This parasite may cause thickening of arterial walls and fibrosis which increases the risk of verminous aneurysm, rupture and internal haemorrhaging.

Beetles or Dictyoptera insects can be intermediate hosts and paratenic hosts are also possible, especially rodents (e.g., mice, voles). Excreted eggs measure 55–60 × 36–38 µm.

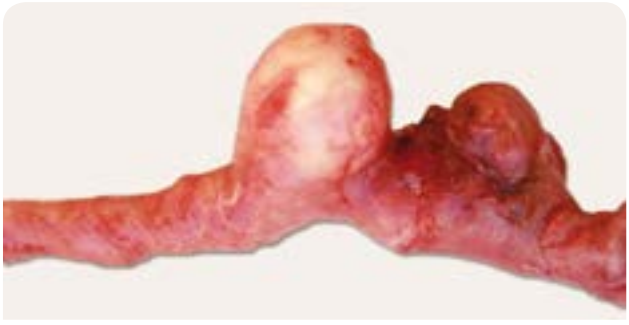


Figure 4. Oesophageal *Spirocerca* nodules.

Figure 5. *Spirocerca* nodule inside the oesophagus.

Figure 6. Oseophageal *Spirocerca* nodule.

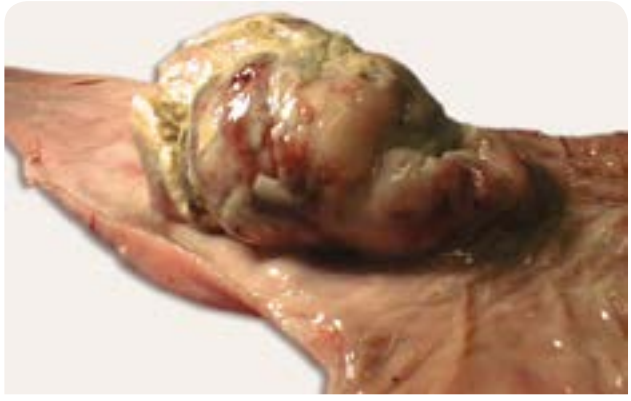


Figure 7. Necrotic oesophageal *Spirocerca* nodule.

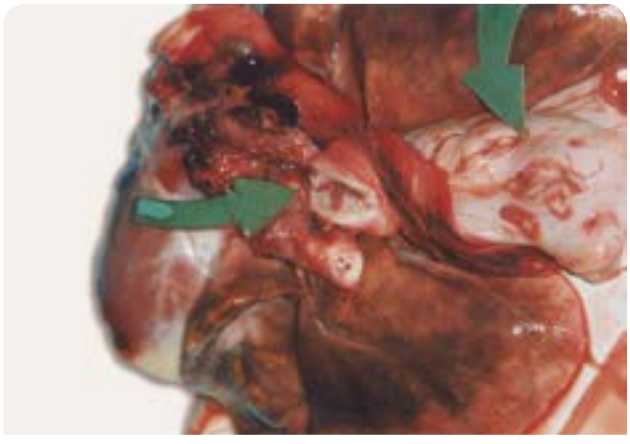


Figure 8. Free adult *Spirocerca lupi* in the oesophagus.

Epidemiology

Canine spirocercosis and feline spirurosis are sporadic in temperate climates and enzootic in warm climates. Spiruroids which affect carnivores are mainly seen in rural environments because they must be ingested by an intermediate host, usually a beetle, or a paratenic host (e.g., lizard, bird, small mammal, etc.).

Parasite sources

- **Intermediate hosts:** dung beetles (*Geotrupes, Scarabeus*), Dictyoptera (cockroaches).
- **Paratenic hosts:** amphibians, reptiles, birds or small mammals.

Mechanism of infestation

Ingestion of intermediate or paratenic hosts.

Host susceptibility

Carnivores living in rural environments are more likely to hunt paratenic hosts.

Clinical signs and lesions

Classic form

- Intestinal signs include salivation, frequent regurgitation and dysphagia. Vomiting and nausea often occurs at the sight of food and dogs are often polydipsic due to fluid loss from vomiting. Polyphagia is sometimes seen because the animal regurgitates any food that is ingested and can therefore be very hungry. The difficulty in swallowing and keeping food down will gradually lead to malnutrition, weight loss and eventually, cachexia.
- Respiratory signs are caused by the presence of larvae in the aorta or the tracheobronchial lymph nodes, which leads to vagal nerve neuritis, and signs including coughing, dyspnoea and syncope.
- Neural signs, including convulsions and paralysis, have also been described.
- Arterial disease, including deterioration of the walls of the aorta or gastroepiploic artery, can lead to aneurysm. Rupture may also occur, resulting in moderate haemorrhage, haemoptysis, melaena, anaemia or even massive haemorrhage and subsequent death.
- Chronic spirocercosis will often cause cachexia, where the animal becomes weak and anaemic and suffers considerable weight loss.

Complications

- Possible aortic rupture, followed by fatal internal bleeding.
- Moderate bleeding from the aorta, causing chronic anaemia.
- Rupture of the oesophagus (rare).
- Development of oesophageal neoplasia: although rare in dogs, oesophageal neoplasms seem to be closely linked to *S. lupi* infestation. These are most commonly fibrosarcomas, which often metastasise to the lungs, and the prognosis in such cases is poor.
- Development of hypertrophic pulmonary osteoarthropathy with characteristic signs of marked hypertrophy of the long bones, oedema in the legs and considerable locomotion difficulty (Fig. 9). This syndrome, called the Cadiot syndrome, is characteristic of canine spirocercosis in regions where the parasitosis is enzootic.

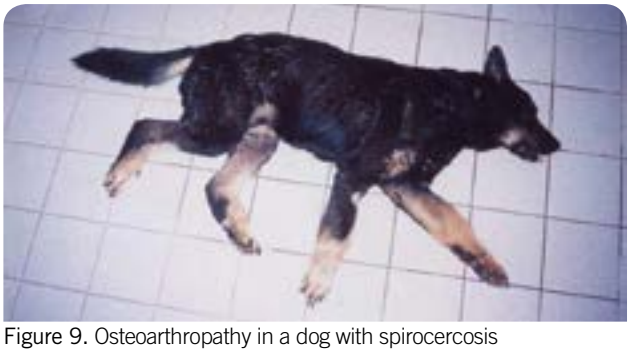


Figure 9. Osteoarthropathy in a dog with spirocercosis (Cadiot syndrome).

Lesions

- **Oesophagus:** thickening of the wall and presence of fibrous granulomatous nodules (4–10 cm diameter) that may block the lumen. These nodules have a circular opening, 1–4 mm in diameter, in which the female parasite lays her eggs. These granulomas consist of fibrous tissue and contain a bloody liquid and 5–6 parasites, sometimes more. Nodules are generally located cranially to the diaphragm and caudally to the aortic arch.
- **Stomach:** formation of nodules similar to those observed in the oesophageal mucosa.
- **Aorta:**
  - Punctuated endarteritis: separate depressions of 1 mm in depth forming a sinuous pattern. *S. lupi* larvae may be found in the wall.
  - Adventitial fibrosis and infiltration of the aortic wall.

- Development of nodules, 2–7 mm in diameter, containing larvae and growing to 1 to 2 cm in size.
- Widely dispersed thickening of the *tunica intima* and *tunica media*, which may weaken the arterial wall and result in aneurysm and potential rupture.

Diagnosis

In regions where the parasite is enzootic, clinical suspicion must arise when dogs present with chronic gastric disorders and and their general condition has changed. Endoscopic examination and visualization of the pathognomonic fibrous nodules will confirm diagnosis. Endoscopic examination can also be used to follow the progress of lesions following treatment: nodules should regress and become whitish in colour (they are usually pinkish-red to start with). This demonstrates a reduction in parasite viability. Faecal examination is possible, but at this stage, the eggs are often only found in small quantities and are laid irregularly (Fig. 10). Faecal flotation in a saturated sugar solution seems to be the most sensitive test.

Control measures

Treatment

Their location makes it difficult for anthelmintic products to reach the nodules containing *Spirocera* because these products do not circulate well in this kind of tissue.

Subcutaneous injection of 10 mg/kg nitroxynil is effective. It binds to plasma proteins and is active against the majority of haematophagous nematodes.

Topical administration of 2.5 mg/kg moxidectin is also effective. 0.5 mg/kg milbemycin oxime administered orally at weekly intervals for one month has also proven to be active.

Prevention

A monthly topical administration of 2.5 mg/kg moxidectin prevents infestation effectively.

Oral milbemycin oxime, administered monthly at 0.5 mg/kg, has been shown to prevent infestations in some studies.

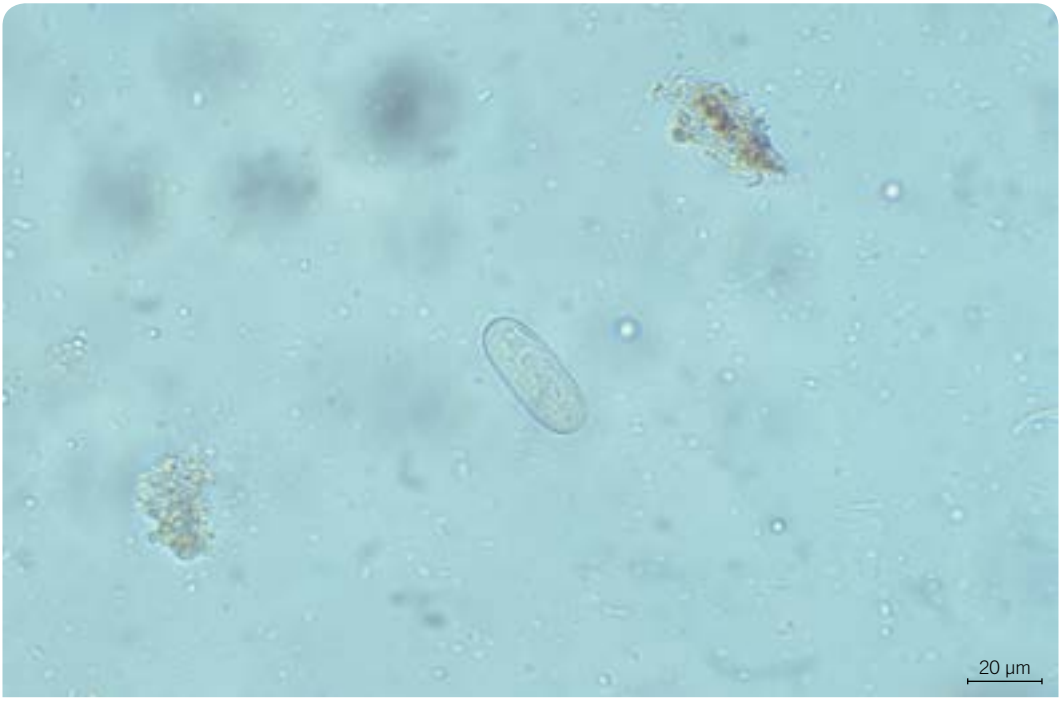


Figure 10. *Spirocerca lupi* egg.

Other gastrointestinal spiruroses *

General comments

Dogs and cats can become infested with several species of spiruroid, mostly exotic. Only *Spirocerca lupi* is found in dogs in many countries (see *Spirocercosis*, page 5). Cats can be infested by ***Spirura rytipleurites***.

Species found in hot climates belong to the genera ***Physaloptera*** and ***Gnathostoma***. ***Rictularia***, a fox spiruroid, can also infest other canids, including dogs.

Geographical distribution

Cases of spirurosis occur sporadically in temperate to cold climates around the world but they are widespread in tropical regions (America, the Caribbean, Asia, India, China, Indonesia, Malaysia, Pacific Islands, Africa, Madagascar and the island of Reunion). Feline spirurosis is frequent in North Africa. Gnathostomosis is mainly found in Asia, while physalopterosis is reported in Asia and Africa, and also in America.

Hosts

S. rytipleurites is found in the oesophagus and stomach of cats.

Physaloptera praeputialis, which is 15–45 mm long, is found in the stomachs of domestic and wild felids. ***Physaloptera canis*** infests dogs (Fig. 1).

Gnathostoma spinigerum is mainly found in cats, but sometimes in dogs and wild carnivores too.

Importance

Intestinal spirurosis is usually asymptomatic in carnivores, but some species may be transmitted to humans, such as *G. spinigerum* in the Far East, India, America and Australia. This spiruroid causes severe visceral or cutaneous (especially facial) *larva migrans* in humans.

Morphology

Spirura rytipleurites is 20–30 mm long and has a diameter of 0.6–0.8 mm.

Physaloptera praeputialis is 15–45 mm long. Physalopteridae have a characteristic ring around their anterior extremity.

Gnathostoma spinigerum is 10–30 mm long. Gnathostomidae have a characteristic spiny dome in the cephalic region.

Figure 1. *Physaloptera* egg. Coproscopy.

Biology

Adult forms of *S. rytipleurites* live in the wall of oesophagus and stomach. Adult forms of the parasites *Gnathostoma (G. spinigerum)* and *Physaloptera (P. praeputialis)*, are found in the stomach or small intestine, depending on the species. Their life cycles involve tissue migration, but this does not affect the arteries, which means that the medical consequences are less serious than those of canine spirocercosis.

Gnathostoma spinigerum lives in bloody pockets in the stomach wall, and comes into contact with the gastric contents via an orifice in the pocket.

Epidemiology

The life cycle of *Spirura* and *Physaloptera* is terrestrial and involves arthropods as first intermediate hosts, and mammals or reptiles as paratenic host. In contrast, the *Gnathostoma* life cycle involves freshwater intermediate hosts (e.g., crustaceans of the genus, *Cyclops*) and fish are paratenic hosts.

- **Parasite sources:** intermediate hosts include dung beetles (*Geotrupes*, *Scarabeus*), Dictyoptera (cockroaches), and copepods.
- **Paratenic hosts**: amphibians, reptiles, birds and small mammals (*Spirura*, *Spirocerca* and *Physaloptera*) and fish (*Gnathostoma*).
- **Mechanisms of infestation**: ingestion of the intermediate or paratenic host.
- **Susceptibility**: animals living in rural areas and carnivores that hunt paratenic host species are at higher risk of infestation.

Clinical signs

- **Feline spirurosis:** *Spirura rytipleurites* is less pathogenic than *Spirocerca lupi*, but gastritis can still lead to chronic vomiting, sometimes tinged with blood, and weight loss. Infested cats are often asymptomatic.
- **Physalopterosis**: often asymptomatic, but can cause chronic granulomatous gastritis with vomiting in infested cats or dogs.
- **Gnathostomosis**: causes nodular gastritis, generally well tolerated by the final host. Necrotic hepatic lesions connected with larval migration are possible.

Control measures

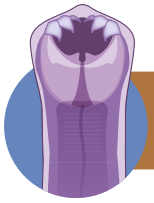
- **Anthelmintic treatment**: no publication with recent anthelmintic drugs. Historically, nitroxynil at 10 mg/kg was recommended. Macrocyclic lactones are considered active.
- **Prevention** is challenging: limit the reservoir of wild carnivores and control paratenic hosts (rats, mice, lizards, etc.).

Risk to humans

Humans become infested by eating undercooked fish containing *G. spinigerum* larvae (may occur occasionally by drinking water that has been contaminated with infective larvae from *Cyclops* crustaceans).

Intestinal parasitoses

NEMATODOSES



Ancylostomoses *

General comments

Ancylostomosis is a helminth infestation caused by the penetration or ingestion and migration to the small intestine of Ancylostomatidae nematodes (hookworms). The clinical signs are a general loss of condition (weight loss, anaemia), intestinal disorders (diarrhoea) and more rarely, skin or respiratory conditions. In temperate countries, dogs are infested by ***Ancylostoma caninum*** and ***Uncinaria stenocephala***, while cats are infested by ***Ancylostoma tubaeforme*** and rarely, ***U. stenocephala***. In Asia, the most important species infesting both dogs and cats is *A. ceylanicum*. These are nematodes of the order Strongylida, suborder Ancylostomatoidea.

Ancylostoma are small, round, slender and white in colour, measuring approximately 10 mm in length. At the anterior extremity they have a buccal capsule with either hooks (*Ancylostoma*) or cutting plates (*Uncinaria*) around its edge (Fig. 1).

Synonyms

- Hookworm infestation.
- Ancylostomosis (when caused by *Ancylostoma*).
- Uncinariosis (when caused by *Uncinaria*).

Hosts

- Dogs and other canids (*A. caninum*, *A. braziliense*, *A. ceylanicum, U. stenocephala*).
- Cats (*A. tubaeforme*, *A. braziliense*, *A. ceylanicum*).

Geographical distribution

Although ancylostomosis occurs worldwide, parasites of the genus *Ancylostoma* are mainly found in warmer regions. *Uncinaria* seems to be more adapted to temperate and cold regions and is thought to have originally been a parasite of the fox.

Importance

Ancylostoma are of great medical significance because of the their pathogenic nature.

They are of economic significance when they affect communities of dogs (breeding kennels, rescue shelters, hunting kennels).

Hookworms are of zoonotic significance because humans may become infested by *A. caninum*, *A. ceylanicum* and *A. braziliense*, which can cause *larva migrans*. *A. ceylanicum* (Fig. 2) is unique in that it not only causes *larva migrans*, but can also develop into an adult worm in the intestine of humans.

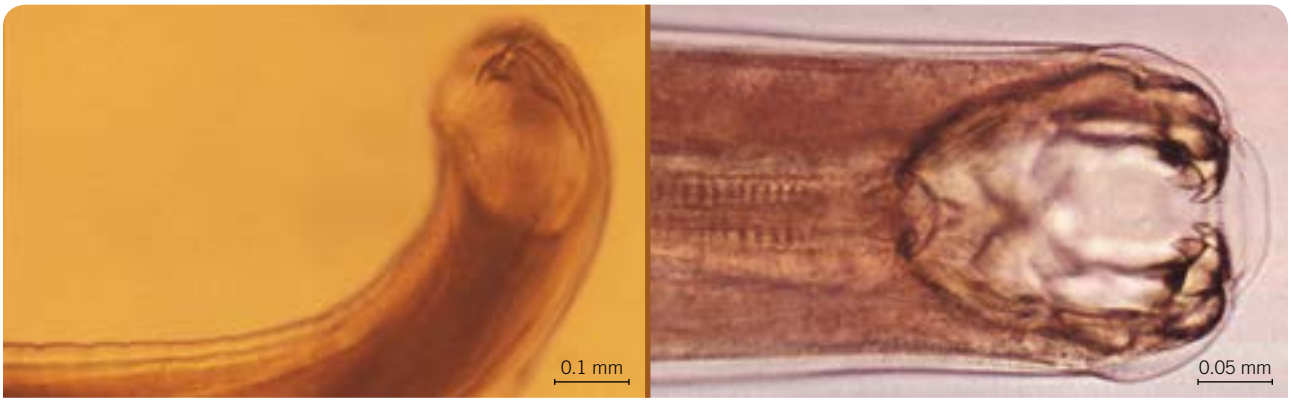


Figure 1. Anterior end of *Ancylostoma*.

*Mainly *Ancylostoma* spp.

Biology

Hookworms are parasites of the small intestine and are partially haematophagous (especially *Ancyclostoma*). The females lay strongyle-type eggs which are then shed in faecal matter and develop in the environment. Adults have a life span of approximately 6 months.

The strongyle-type eggs are oval with a thin, smooth shell and they enclose one morula containing only 8 to 16 cells when they are shed. They are approximately 30–40 × 55–75 µm in size (Figs. 3 and 4).

The eggs hatch in the environment and release a stage 1 (rhabditiform, L1) larva which, after two moults, will become the stage 3 (filariform, L3) infective larva. Larval development requires moist, warm soil at least 16 °C, and can occur in as little as 7 days at optimum temperatures (22 °C).

Larval formation generally occurs in the environment, on grassy soils, as it does in the parasitic strongylids of ruminants and horses. It cannot occur on concrete or hard mud/clay surfaces. Infective larvae can survive for a number of weeks in a favourable environment.

Dogs and cats will either ingest the larvae (which frequently occurs with *Uncinaria*), or the larvae will penetrate the skin and migrate subcutaneously (especially *Ancylostoma*). The stage 3 larvae will then rapidly find their way into the lymph vessels or the blood stream and migrate to the heart and pulmonary arterioles. They then penetrate the pulmonary alveoli, ascend the bronchial tree and are then swallowed into the gastro-intestinal tract, where they reside and develop into adults. The migratory cycle is similar to that of ascarids and the life cycle takes approximately 6 weeks to complete.

In female dogs and cats, a few larvae will continue their migration through the blood stream after leaving the lungs and become disseminated in various tissues and organs, as it occurs with *Toxocara*. They will then encyst and remain quiescent for several months or years but, if a bitch or queen is pregnant, the larvae may mobilise at birth and infest the young through the mother's milk. *In utero* infestations seem to be rare.

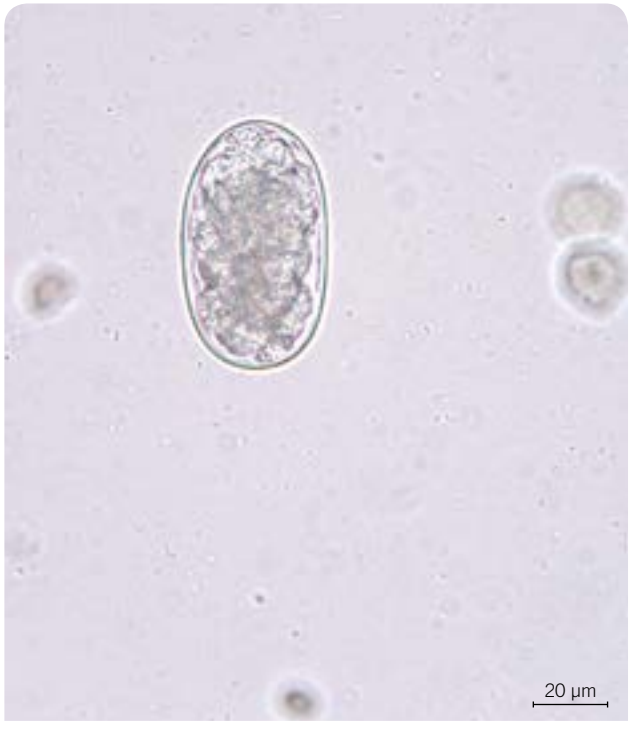


Figure 3. *Ancylostoma tubaeforme* egg.

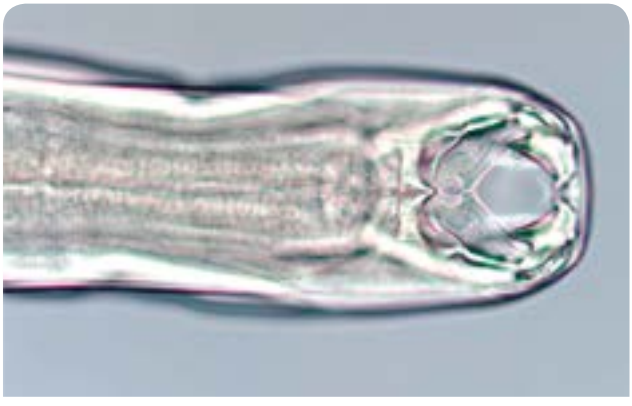


Figure 2. Anterior end of *Ancylostoma ceylanicum*.

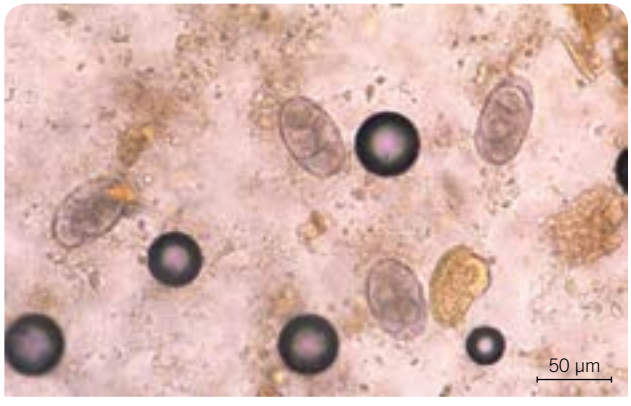
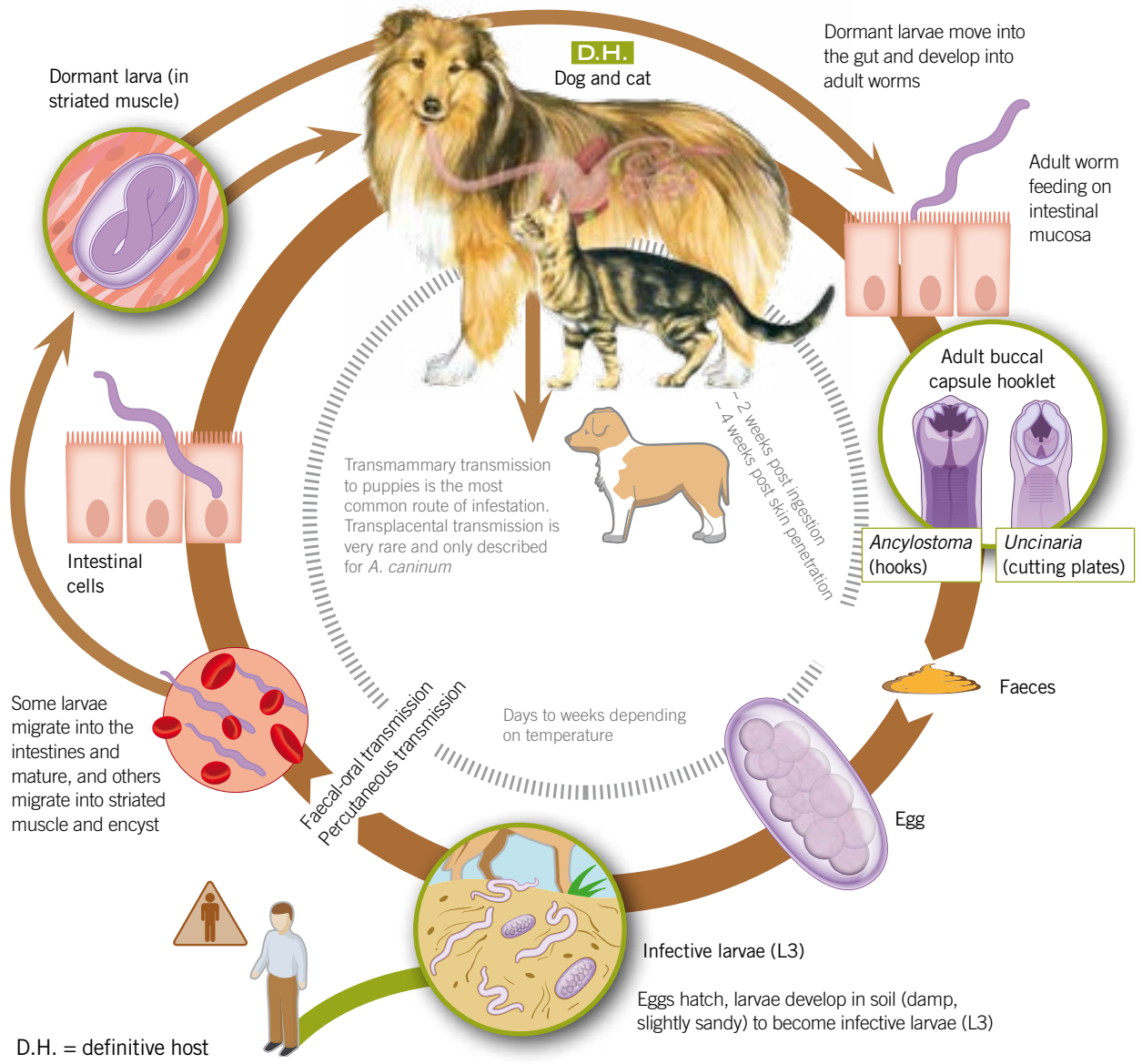


Figure 4. *Ancylostoma* sp. eggs.

Life cycle of *Ancylostoma/Uncinaria*



Epidemiology

Ancylostomosis can be contracted by all types of carnivores, but generally affects stray animals, or animals living in communities. It is often found in hunting dogs and in dogs kept in kennels, and it is commonly seen in rural areas.

The source of the parasites are dog and cat carriers, and soils contaminated by stage 3 larvae. When the filariform larvae are ingested by small mammals (e.g., rats, mice) they become encysted in their tissues and remain infective. These paratenic hosts will then infest any carnivores that eat them. L3 larvae need damp, grassy areas to survive as they do not resist dry conditions well. They are also sensitive to ordinary disinfectants. As with the majority of parasitic diseases, young carnivores are the most susceptible and other factors, such as malnutrition or fatigue (hounds), will increase susceptibility.

Clinical signs and lesions

- **Cutaneous signs**: penetration of the skin by the L3 larvae can result in papular lesions covered with scales on the legs and ventral areas. These inflamed areas become itchy and the lesions can become infected and develop into pyoderma. Superficial adenitis is often seen (popliteal lymph node, in particular).
- **Respiratory signs**: migration of the larvae causes coughing and signs of pneumonia, as it does in toxocarosis in puppies and kittens. Other signs of ancylostomosis in dogs include loss of sense of smell (often occurring in hunting dogs), change to the bark (to a higher pitch) and epistaxis.
- **Intestinal disorders**: adult parasites will cause congestive haemorrhagic enteritis and sometimes, diarrhoea, which is often abundant and haemorrhagic.
- **General disorders**: the continued parasitic burden in some dogs will lead to chronic weight loss, muscular atrophy and development of a wasting syndrome.

Lesions

Congestive haemorrhagic enteritis, with worms found in the mucosa (Fig. 5).

Diagnosis

Ancylostomosis should be considered in dogs with epistaxis, associated gastrointestinal disorders and weight loss. Differential diagnosis may include other parasitic or wasting diseases, such as leishmaniosis or other helminthoses (e.g., trichuriosis).

However, diagnosis based on clinical signs is not possible, and intestinal disorders and weight loss will only suggest possible parasite infestation. A definitive diagnosis can only be made by examination of faeces and identification of eggs.

Control measures

Treatment

Ancylostoma are relatively sensitive to nematodicides, such as pyrantel, benzimidazoles, emodepside, eprinomectin, milbemycin, moxidectin, and selamectin. In some communities, populations of *Uncinaria* may be chemoresistant to benzimidazoles. However, this phenomenon seems to be limited, and is not comparable to the levels of resistance found in horse or ruminant strongyles.

Prevention

Regular deworming of carnivores with anthelmintics that have a larvicidal effect is essential to prevent ancylostomosis. Gestating females should also be treated 15 days prior to giving birth.

Environmental control may include covering mud/dirt areas with gravel, regularly removing faeces and cleaning concrete areas, and rodent control. Boiling water or disinfectants may be used to clean at weekly intervals.

A vaccine, involving inoculation with irradiated stage 3 larvae was developed, providing protection for 1.5 years, but it was abandoned for economic reasons such as cost of production, supply challenges, and stability issues.

Risk to humans

Hookworms from dogs and cats may infest humans with a complete life cycle (*A. ceylanicum*) or cutaneous *larva migrans* after penetration through the skin (*A. braziliense, A. caninum,* and rarely *U. stenocephala*).

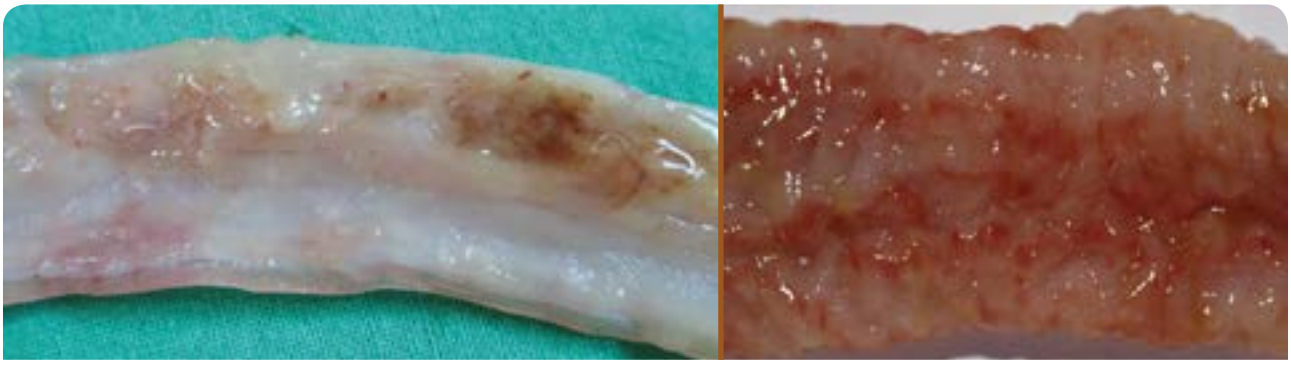


Figure 5. Ancylostomosis lesions in a cat (left) and a dog (right).

Toxocaroses *

General comments

Toxocarosis is a parasitic disease caused by the presence and development of large nematodes of the order Ascaridida, genus ***Toxocara***, in the small intestine. Dogs and cats may also be infested by ***Toxascaris leonina***.

Roundworms (or ascarids) are the most common helminths in dogs and cats: 10–20 % of dogs and cats in urban and rural areas are infested, and infestation levels are approximately 60 % in kennels. 20–40 % of dogs and cats with parasitic worm burdens have ***Toxocara canis*** or ***Toxocara cati***, 5–20 % of dogs have hookworms (*Ancylostoma* and *Uncinaria*) and 10–30 % of dogs are infested with whipworms (*Trichuris vulpis*). Due to the nature of their life cycle, roundworms are mainly found in young carnivores and are responsible for a variety of clinical signs, such as coughing, diarrhoea, vomiting, pot-belly and abdominal pain. Subclinical signs, such as growth retardation and fragile bones, may also occur. Nevertheless, roundworms can be diagnosed in adults and are not restricted to young puppies or kittens.

Roundworms are the primary parasites that can cause growth retardation in young carnivores. Roundworm control requires hygienic measures and regular treatment of breeding stock and young animals.

Toxocarosis is a significant public health issue because it can infest humans when embryonated eggs of *T. canis* and *T. cati* are ingested.

Synonyms

The infestation should be called ascaridiosis after the parasitic order Ascaridida.

The accepted terms could then be ascaridosis (for all infestations by roundworms) or toxocarosis (only for infestation due to *Toxocara*).

The term ascaridiosis is sometimes found in the legal documents for certain dewormers (e.g., summary of product characteristics) which is totally incorrect as ***Ascaridia*** are bird parasites.

Figure 1. Anterior end of *Toxocara*. Electron microscopy.

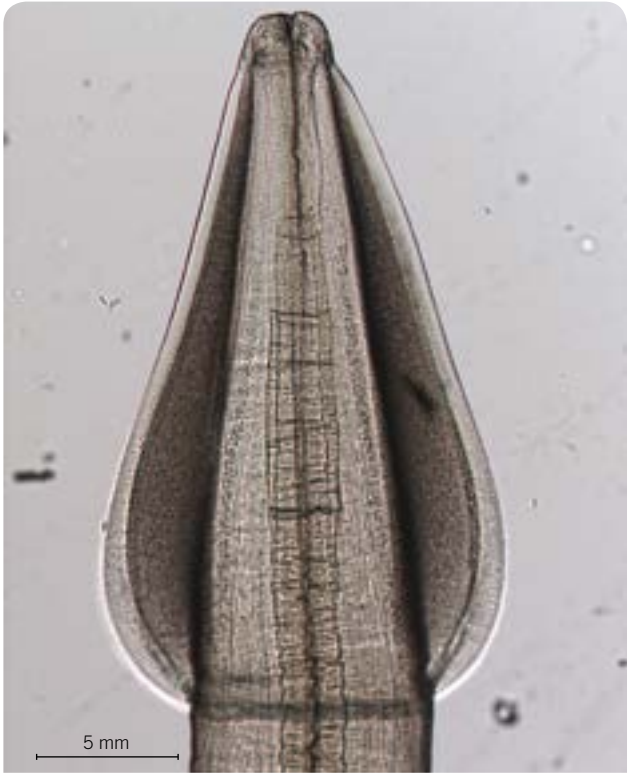


Figure 2. Anterior end of *Toxocara cati*. Light microscopy.

* *Toxocara canis* and *Toxocara cati*

Morphology

Three species of roundworms infest dogs and cats: *T. canis* (dog only), *T. cati* (cat only) and *Toxascaris leonina* (dog and cat).

Infestations caused by *T. canis* and *T. cati* (Figs. 1–3) are by far the most significant, because of their prevalence, zoonotic potential and serious consequences in puppies and kittens.

Toxascaris leonina can infest both dogs and cats, and is usually seen in rural environments, in carnivores that hunt mice. The importance of the paratenic host is such that some authors suggest that it acts more like an intermediate host with a dixenous life cycle (Fig. 4).

Adult roundworms are found during autopsies of young carnivores, or when they are shed in the faeces or vomitus of the infected animal. The adults are 5–15 cm long and 2–3 mm in diameter, and they are large, white worms which are easily recognisable (Fig. 5).

Ascarid eggs are easily identified in the faeces. Females are considered prolific, and lay spherical to subspherical eggs measuring approximately 75–85 µm in diameter. These eggs contain a single, brown cell which does not fill the whole egg. The brown shell is thick and the wall features concentric striations (Figs. 6 and 7). The external layer of the shell is irregular and pitted in the *Toxocara* genus, with a "thimble-like" surface, whereas the external layer of *Toxascaris* eggs is completely smooth (Figs. 8 and 9). The distinction is important since only *Toxocara* can infest humans.

Eggs are seen in large numbers shortly after the adult worms appear in the small intestine because the females are highly prolific, laying approximately 200,000 eggs per day.

Figure 3. *Toxocara* buccal lips. Electron microscopy.

Figure 5. *Toxocara canis* adults.

Figure 4. Anterior end of *Toxascaris leonina*.

Biology

Ascarids are not haematophagous, but do consume large amounts of glucose, amino-acids, vitamins, trace elements and minerals, such as calcium and phosphorous. The loss of these nutrients may explain the bone disorders observed in heavily infested puppies and kittens, and the risk of convulsive hypoglycaemic seizures.

The worms can form balls in the small intestine of young carnivores, which leads to signs of obstruction and diarrhoea or constipation. In rare cases, the gastro-intestinal tract may be perforated, leading to fatal peritonitis.

Ascarids have a monoxenous cycle (i.e., they parasitise a single host). The eggs laid in carnivores by the female worms are shed in the faeces and develop in the external environment for 3 to 4 weeks, before becoming infective (Fig. 10). They are particularly resistant and can survive at temperatures between -10 °C and +45 °C, and development is not arrested by drought or wet conditions, so they can thus remain infective to dogs and cats for 2–5 days.

When the eggs containing the *Toxocara* larvae are ingested by a young puppy or kitten (under 6 months old) they migrate through the intestine and eventually develop into adults. The larvae can also pass through the wall of the intestine and travel through the lymph vessels or the bloodstream to the liver and heart. They also move through the pulmonary arteries to the lungs, where they leave the vessels to enter the pulmonary alveoli. Next, they travel up the bronchi to the trachea, where they are swallowed and return to the intestine where they finally become adults and mate. This entero-pneumo-tracheo-enteral migration takes 5 weeks. Passage through the lungs explains the respiratory signs (coughing, with no hyperthermia) which precede or accompany the intestinal disorders.

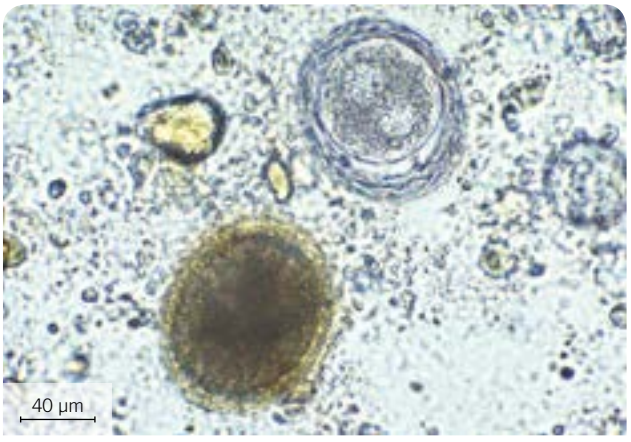


Figure 6. *Toxocara* (dark) and *Toxascaris* eggs in dog faeces. Coproscopy.

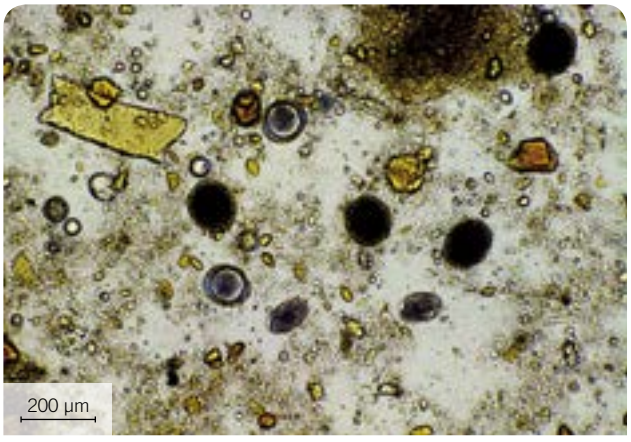


Figure 7. *Ancylostoma, Toxocara* and *Toxascaris* eggs in dog faeces. Coproscopy.

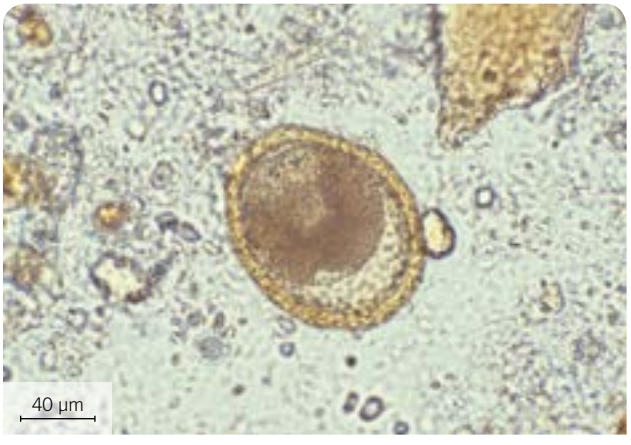


Figure 8. *Toxocara* egg in dog faeces. Coproscopy.

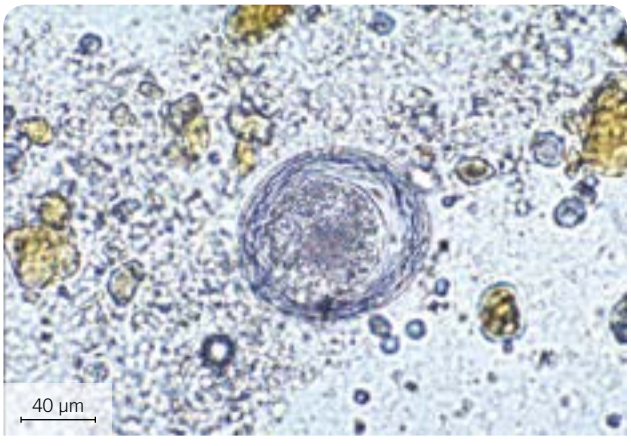


Figure 9. *Toxascaris* egg in dog faeces. Coproscopy.

This cycle only takes place in *Toxocara*, while *T. leonina* develops directly in the small intestine, without any migration.

When embryonated eggs of *Toxocara* are ingested by dogs or cats, 6 months or older, the larvae migrate to the lungs but may not penetrate the alveoli. They head for the heart through the pulmonary veins, are distributed throughout the body in the bloodstream and will become encysted in various organs while still alive. In male dogs, larvae die out, usually after about a year. In female dogs, the encysted larvae remain infective for several years.

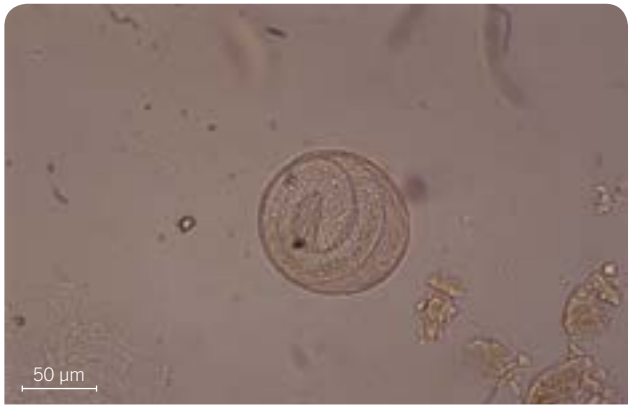


Figure 10. Embryonated *Toxocara* egg. Flotation from sand.

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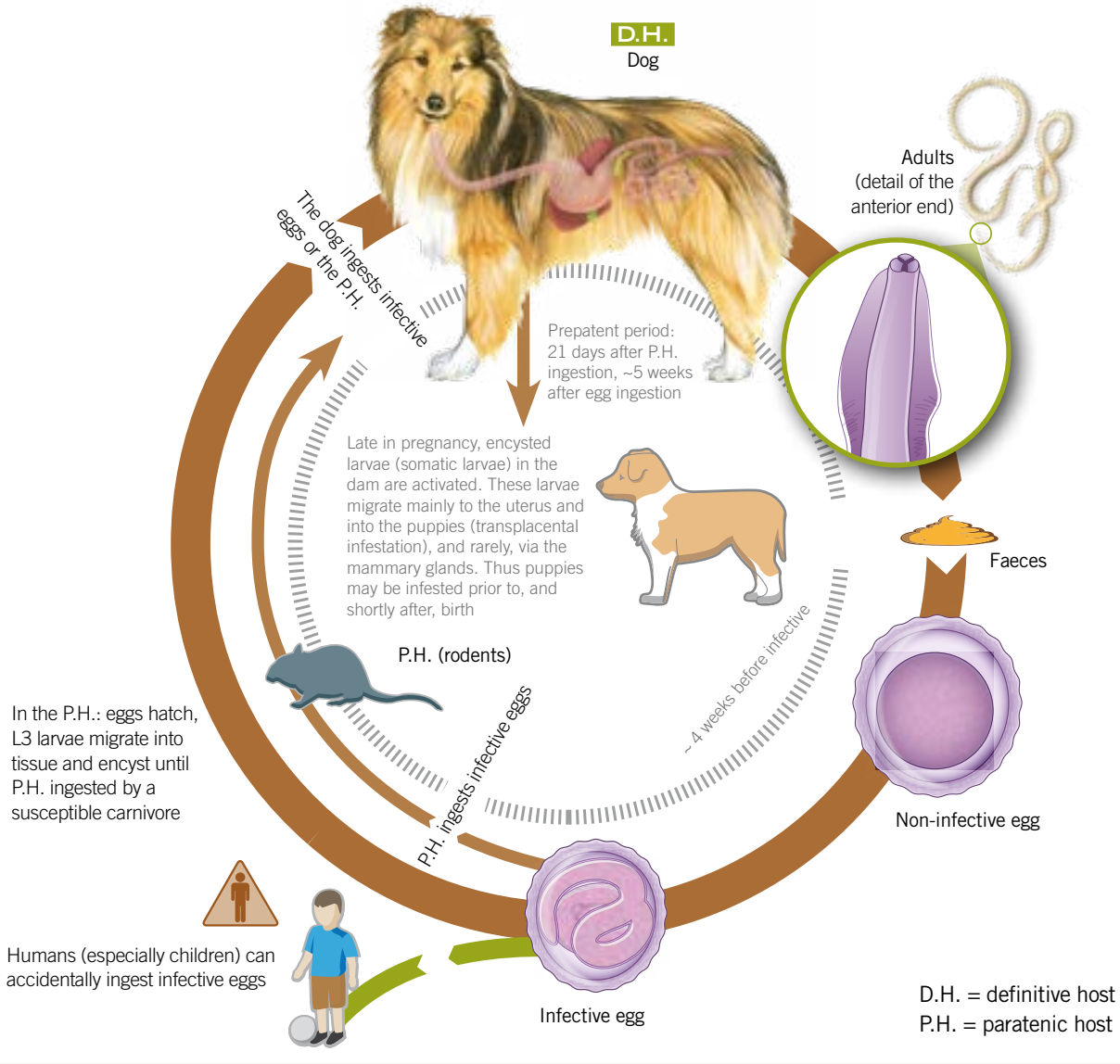
Life cycle of *Toxocara canis*



This phenomenon is progressive and depends on a number of factors including age, immune status and, possibly, the breed of animal.

Toxocara canis larvae can reactivate in bitches when they are on heat or before giving birth. This parasitic activity depends on the hormone cycle of the bitch and involves dormant larvae which are encysted in the mammary glands, uterus or muscle tissue. The larvae that are reactivated around oestrus will travel to the lungs and undergo the classic migratory cycle so adult worms will be present in the intestine after 5 weeks. Larvae that are reactivated between 15 days before birth and 15 days after birth will either develop into adult worms and infest the puppies through the uterus, or via the colostrum and milk. The larvae that infest puppies prior to birth will develop into adults when the puppies are 10 days old. Larvae ingested from the colostrum or milk will migrate through the puppies' bloodstream and lungs, before developing into adults in the intestine.

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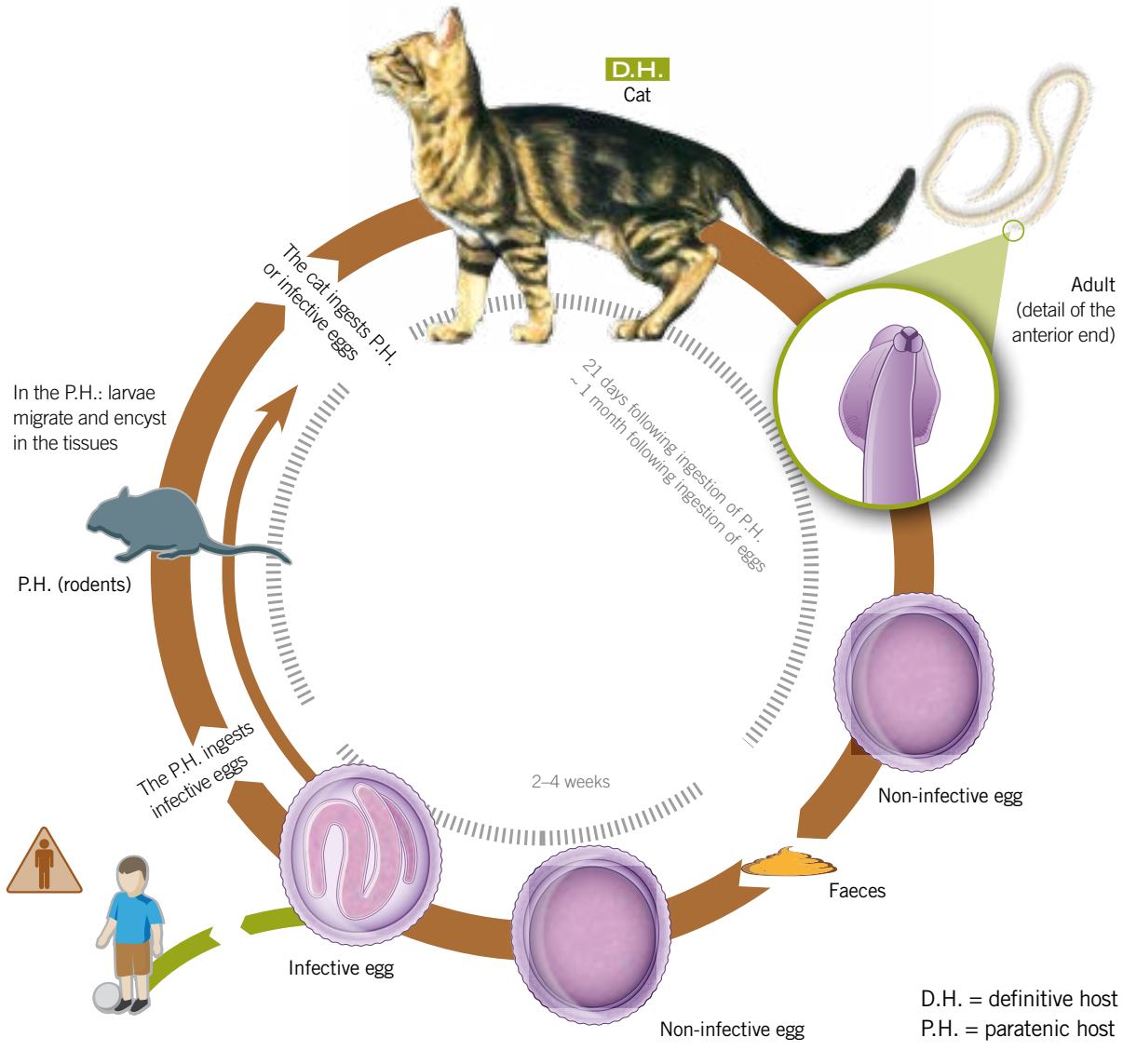
Life cycle of *Toxocara cati*

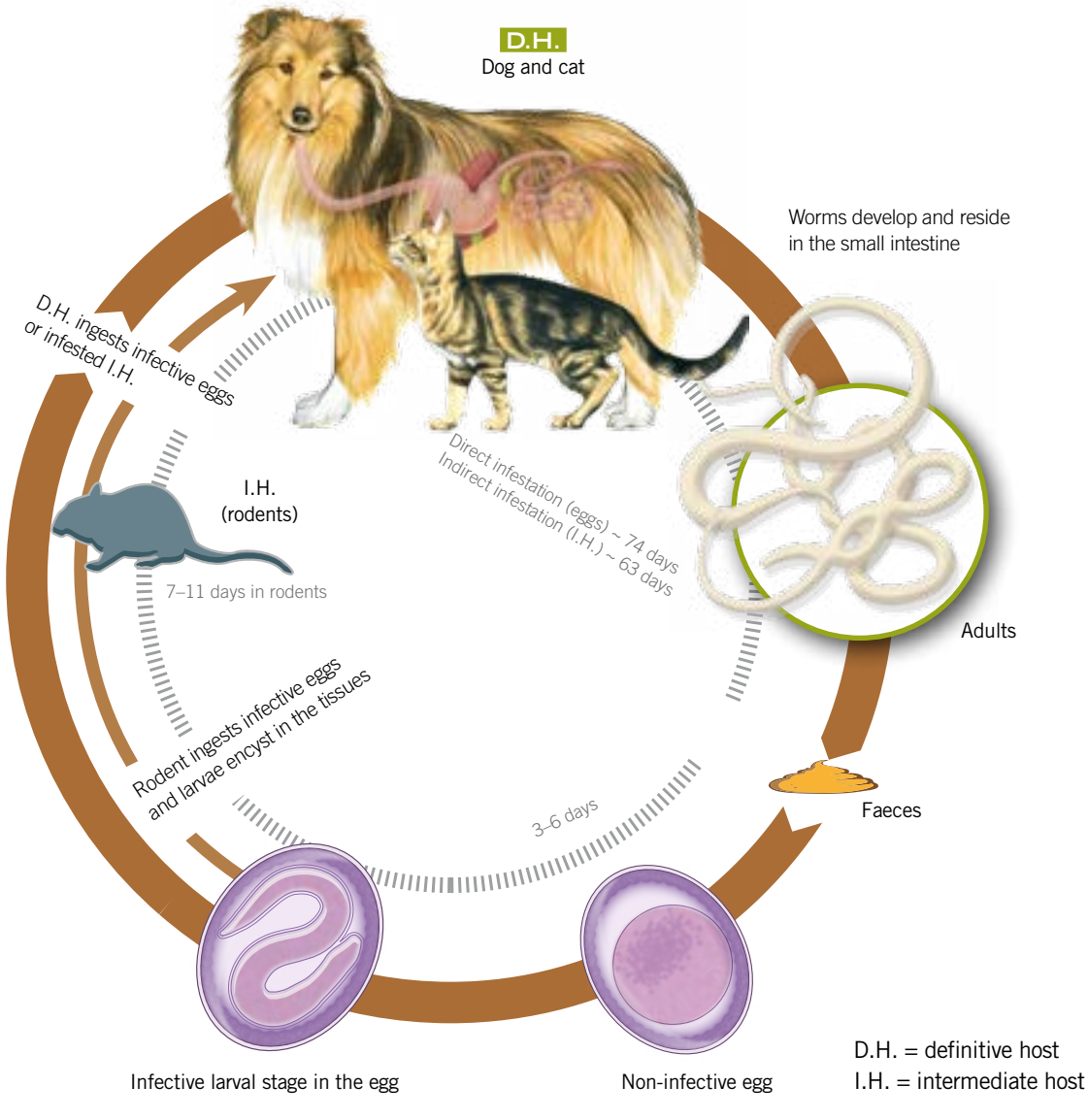


There is no *in utero* passage in queens, but *T. cati* larvae will infest kittens through their mother's milk from the second day after birth and for about 10 days thereafter.

If a rodent ingests an egg containing L3 larvae, it will harbour quiescent larvae in its tissues and will then act as a paratenic host. This role is very important in ascaridosis caused by *T. leonina*, but also for *T. cati* infestation.

Infestation with roundworms triggers an immune response which reduces the risk of infestation in adult carnivores. However, this response is incomplete and can be lost. Moreover, carnivores which were not infested at a young age will remain naive and fully receptive to the parasite.

Life cycle of *Toxascaris leonina*



Epidemiology

Parasite sources

Sources of roundworms include the environment, in which very resistant eggs are found, and bitches and queens that harbour dormant larvae in their tissues that can infest their young. Roundworms have a relatively short life span and they disappear naturally in 4 to 6 months. However, they are very prolific parasites which is why decontamination of the environment is so important.

Mechanisms of infestation of puppies and kittens

There are three types of *T. canis* and *T. cati* infestations in puppies and kittens: puppies can be contaminated before birth by larvae encysted in their mother's tissues. This will occur in successive litters, which will present with toxocarosis at the end of the first week of life. They can then be infested via their mother's colostrum (for approximately 10 days) and milk; and finally they can ingest embryonated eggs, which they find in the external environment. These eggs may have been laid by worms formed in other young animals or from the females.

Role of paratenic hosts

Eggs present in the environment can be ingested by animals other than dogs or cats, especially rodents (i.e., rats and mice). Larvae remain alive and active in the paratenic host, and migrate to various organs and encyst. Young cats that hunt and eat rodents may become infested. This mechanism of infestation is the main way in which *T. leonina* develops.

Susceptibility

Young carnivores, especially those under 6 months old, are particularly vulnerable. Dogs or cats over 6 months old are less often infested but can become so during a period of temporarily impaired immunity. As far as *T. leonina* is concerned, the age factor does not seem to be so important and infestation can be found in young and adult carnivores alike.

Clinical signs and lesions

Toxocarosis caused by *T. canis* or *T. cati* mainly affects young dogs and cats from birth to a year old.

Clinical signs

- **Respiratory disorders**: coughing is seen first, before other clinical signs occur (respiratory disorders correspond to the passage of the larvae from the pulmonary arteries to the alveoli and then the bronchi, before being swallowed and entering the gastro-intestinal tract to become adult worms).
- **General failure to thrive**: stunted growth in puppies and kittens, irregular appetite, emaciation, dull coat with small bald patches, arthralgia (possibly with rickets and bone deformation, particularly in large breed dogs).
- **Intestinal disorders**: diarrhoea (alternating with constipation) and a pot-belly appearance accompanied by vomiting, with worms in the vomitus. Roundworms may also be found in the faecal matter.

Toxocarosis enables other disorders to appear, especially intestinal conditions such as coccidioses. It may also reduce the efficacy of vaccinations due to its immunosuppressive effect.

Toxascaris infestation is usually well tolerated and asymptomatic. No respiratory signs are seen, as the parasite develops directly in the small intestine, but this development can be fatal for animals with massive infestations.

Balls of roundworms can cause intestinal obstruction, accompanied by bloating, bacterial disorders (autointoxication) and, occasionally, laceration of the intestinal wall, causing fatal peritonitis.

Hypersensitivity may occur after successive reinfestations, causing the death of larvae during pulmonary migration and the appearance of respiratory symptoms (asthma-type coughing). In these cases, there are no adult stages in the intestine and faecal examination will remain negative.

Treatment will usually cure carnivores but the sudden and brutal lysis of the worms in heavy infestations can release numerous antigens, which can potentially cause a considerable allergic reaction. Hypersensitivity can cause significant diarrhoea, and toxic shock with respiratory distress. This is why it is sometimes recommended to treat animals with half a dose first, before administering a full dose a week later.

Lesions

Roundworm infestations may cause localised congestive haemorrhagic enteritis and its ensuing lesions (Figs. 11 and 12). Many roundworms may be visible in the small intestine and granulomas of parasitic origin may be found in various organs, including the lungs.

Diagnosis

Clinical diagnosis is easy in young animals which have just been purchased, but it must be confirmed with tests. At the end of the prepatent period, eggs are shed in large quantities so examination of the faeces under a microscope will usually reveal roundworm eggs and enable the genus (*Toxocara* or *Toxascaris*) to be identified.

Control measures

Toxocarosis must be controlled because of its prevalence, its veterinary and economic impact on breeding facilities and kennels, and the risk of zoonotic transmission. Measures are taken in a healthy environment to avoid introducing a parasite carrier, whereas measures in a contaminated environment aim to reduce infestation rates.

Prevention in breeding facilities and kennels

Measures to be taken in a healthy environment (e.g., a kennel)

The introduction of parasite carriers should be avoided when a new animal is introduced to the kennel (for instance, after the purchase of a breeding animal), so faecal matter must be examined and, if results are positive, the animal must be given the appropriate treatment.

Biosecurity: people who enter the dog or cat kennels or breeding facility may bring infective elements with them from outside, or carry them from one enclosure to another (for example, on muddy shoes or boots). For this reason, one or more footbaths should be installed between enclosures and at the entrance to the kennels. Not many disinfectants are active against ascarid eggs: 3 % formalin, 2 % creosote mixtures, or a mixture of 3 % formalin and copper sulphate at 2 %. Bleach, phenol derivatives, iodophors and amphoteric amino acids are not sufficiently active.

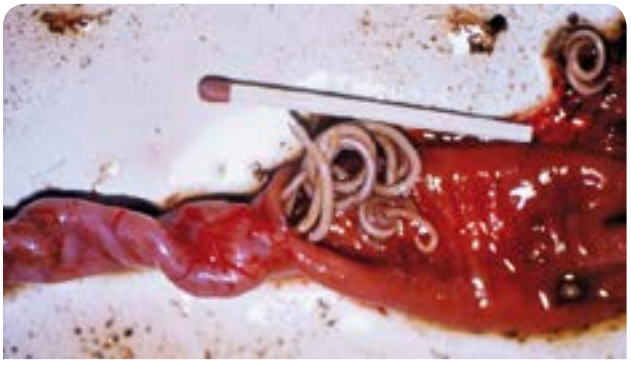


Figure 11. Toxocarosis in a puppy.

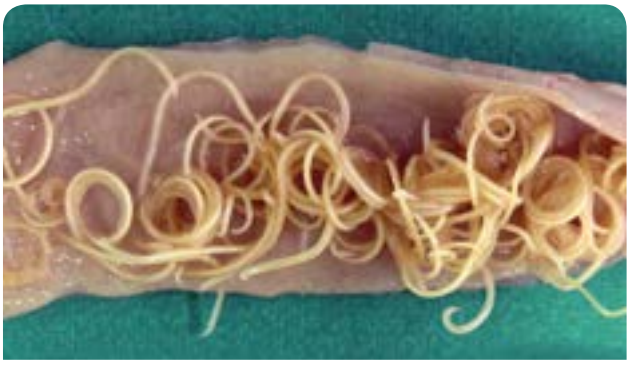


Figure 12. Toxocarosis in a cat.

Measures to be taken in a contaminated environment

General hygiene in the kennel. Parasite eggs are very resistant in the external environment (*Toxocara* eggs can survive for several years).

The following three measures must be put in place:

- **Limit contamination of the kennel environment** and, therefore, infection of the animals. Avoid overcrowding, isolate young bitches as soon as possible post-partum and bring them back for feeding purposes only.
- **Keep the kennel environment clean**:
  - Surfaces made of bare earth, clay or sand should be covered in gravel. Large-particle gravel will let the eggs drop through and develop but they will not be able to contaminate the dogs. Earth can be dug over to bury the eggs, but they will not be destroyed.
  - Hard surfaces (concrete, cement), kennels and cages: these must be hosed down once or twice daily to eliminate faecal matter and most of the parasitic elements. A high-pressure jet is more efficient than an ordinary hose. Scrubbing floor surfaces, including cracks and crevices, regularly is an excellent idea (once a week or every 10 days). This mechanical action is essential to keep the environment clean and to prevent parasites.
- **Disinfect the kennel environment**: disinfection must be preceded by cleaning and should be carried out regularly, at least once every 2 months, depending on the degree of parasitic infestation or other infectious problems.

Medical treatment

In a contaminated environment, hygiene measures should be combined with medical treatment to keep the infestation rate low. There are two separate types of treatments: those suitable for bitches and queens, and puppies and kittens, and those used for the other adults (non-gestating females, and males).

Treatment for reproductive females

- Deworming females during reproductive periods and at the start of gestation. Females should be dewormed when they are in oestrus; this will destroy the adult worms and partially destroy dormant larvae which reactivate when the animal is in oestrus and at the beginning of gestation. Standard anthelmintic nematicides can be used to destroy adult worms, but only dewormers which diffuse into the tissues (e.g., fenbendazole, flubendazole, oxfendazole, levamisole, emodepside, milbemycin, moxidectine, selamectin, eprinomectin) will stamp out reactivating or migrating larvae. Specific protocols exist for each anthelmintic drug.
- Postpartum deworming of females: bitches and queens should be treated 15 days after the birth, then every 2 weeks until the litter is weaned (8th to 12th week).

Treatment for puppies

Puppies and kittens should be dewormed at 15 days old (or at 10 days in cases of heavy infestation), and then every 15 days until they are weaned. They should then be dewormed once a month until they are 6 months old. In the case of a heavy infestation, the treatment can be divided, starting with a half dose, then a full dose 2 to 3 days after to avoid allergic reactions. The deworming rate for puppies should be linked to the assumed presence of various stages of migrating roundworm larvae, and therefore the possibility of worms appearing as soon as treatment is stopped. Deworming needs to start before weaning because of the possible contamination from the mother's milk. Repeated treatment with dewormers is necessary because the products are most effective against adult worms.

In a healthy breeding facility or kennel environment without prior history of infestation, or in puppies/kittens living in a clean household, deworming frequency can be reduced, with one treatment at 8 weeks and then one at 12 weeks, i.e., at the same time as the vaccinations.

Treatment for adults

A quarterly deworming is advisable for adult pets, although faecal examinations can be performed at suitable intervals, from monthly to three-monthly, as an alternative to repeated treatments.

Risk to humans

Both *T. canis* and *T. cati* are potentially zoonotic, but *T. leonina* does not present any danger to humans.

According to serological studies carried out in humans, the zoonotic potential of *T. cati* and *T. canis* are similar. *T. cati* is currently thought to be responsible for the majority of *larva migrans* cases, as cats have more access to places where children go. If infective eggs are ingested by humans they release a larva which will migrate for a time, before dying (visceral *larva migrans*). The zoonosis is incomplete (i.e., the parasite dies), but can be medically serious, especially if it migrates to the brain or the eyes. Children are at the highest risk because the eggs may be found in their environment: public parks and gardens, sand boxes, or private gardens when a puppy or kitten has been acquired.

Video 1.1
View of a *Toxocara canis* (roundworm) egg under the microscope.

Video 1.2
Embryonated eggs of *Toxascaris leonina* (roundworm) in 10 % formalin for 24 hours.

Strongyloidosis *

General comments

Strongyloidosis is an enteric disease caused by nematodes of the genus ***Strongyloides*** (threadworms) penetrating the skin and migrating in the body of the host. The disease can cause severe enteritis. The species which infests dogs, cats and humans is ***Strongyloides stercoralis***. It causes a true zoonosis, since humans can be a major source of infestation for dogs. Strongyloidosis can be diagnosed in cats even if it is less common than in dogs. *Strongyloides* species (other than *S. stercoralis*) have been described in cats. Other free-living Rhabditidae (*Pelodera*, *Rhabditis*) may cause skin lesions.

Synonyms

Threadworm infestation.

Geographical distribution

Found throughout the world but with a higher prevalence in countries with hot, humid climates. Canine strongyloidosis is sometimes seen in Europe, particularly in breeding facilities and kennels with low levels of hygiene. The disease has also been seen in areas where soils are damp and marshy, or where there is unauthorised camping and unsanitary conditions prevail.

Hosts

Horses are infected by *S. westeri*, pigs by *S. ransomi*, and ruminants by *S. papillosus*. Carnivores and humans are infested by *S. stercoralis*. Although cats can be infested, strongyloidosis caused by *S. stercoralis* is mainly a parasitic disease of dogs.

Importance

S. stercoralis is important as it is a zoonosis. It is possible to genetically distinguish between the parasite populations adapted to each host.

Morphology

Threadworms are small, slender nematodes measuring 2–9 mm in length (Fig. 1). Only the parthenogenetic females are parasitic and they produce eggs without males being present. These eggs are small, oval and clear and they measure approximately 30 × 40 µm and contain one stage 1 larva (L1) when they are laid (Fig. 2). This L1 will be shed and found in the faeces. It has a rhabditiform oesophagus and is approximately 300 µm long.

Biology

Threadworms are parasites of the small intestine. The ovoviviparous females shed eggs containing larvae, which will hatch in the soil and develop if the environment is sufficiently damp, muddy and warm. These rhabditiform larvae will

Figure 1. Adult nematode of the genus *Strongyloides* (threadworm).

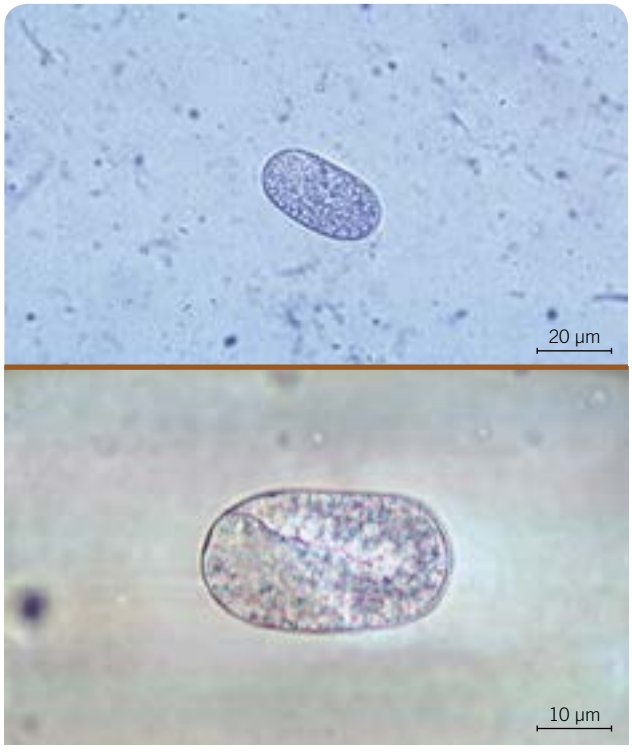
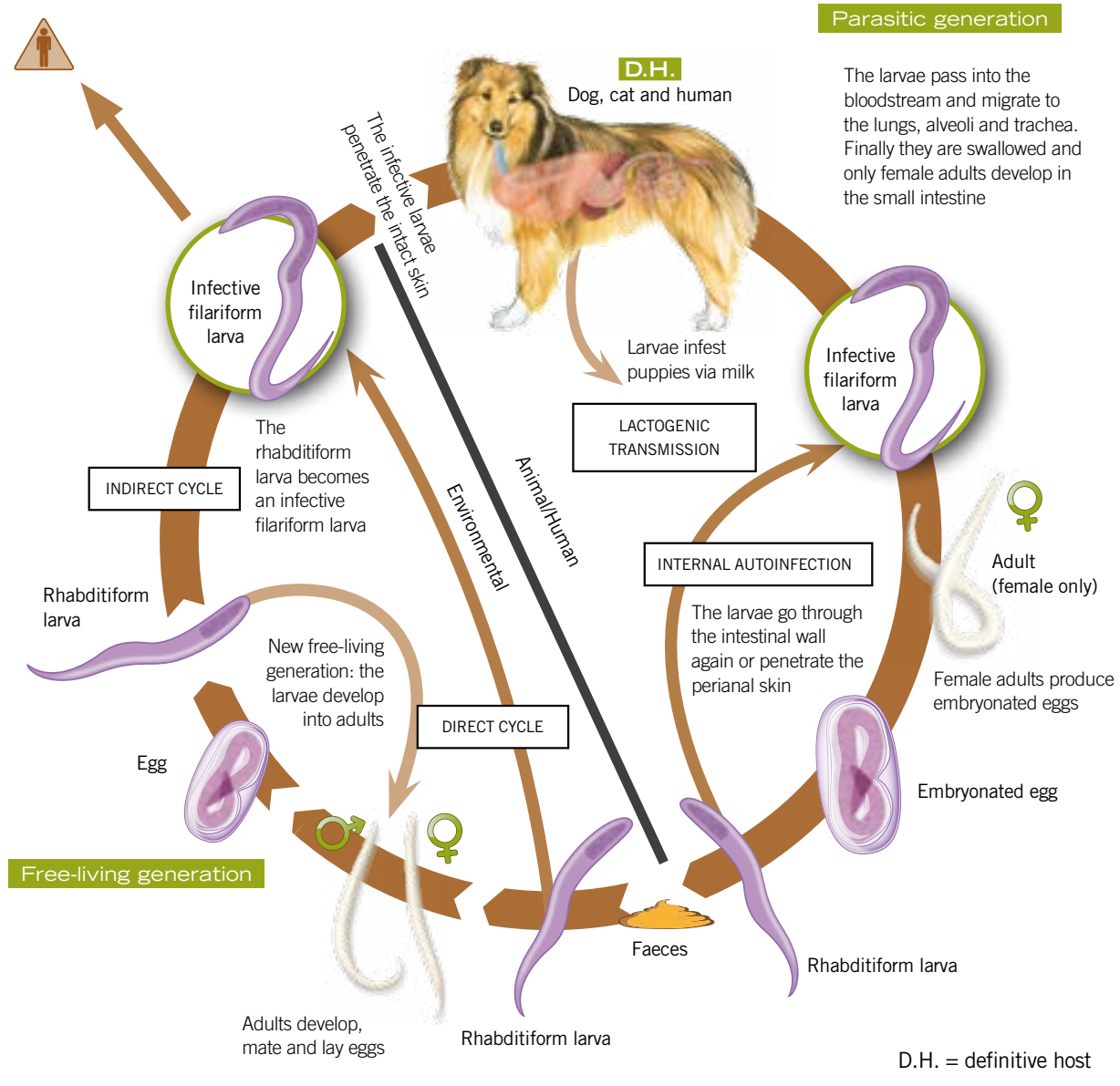


Figure 2. *Strongyloides* eggs.

develop through rhabditiform stages 2, 3, 4 and pre-adult and finally become free-living adult male and female worms. This happens very rapidly, in a single week if environmental conditions are favourable. After mating, the non-parasitic females lay eggs which hatch and develop into L1, L2 and finally L3 infective filariform larvae. This second generation stage 3 larva is the parasitic stage, and it consists solely of females which can penetrate the host by ingestion, but they more commonly enter via the skin. They reach the bloodstream and pass into the lungs via the right ventricle of the heart. From the trachea, they are coughed up and swallowed down into the small intestine where they become mature adults.

In immunocompromised hosts, the eggs laid by the parasitic females may hatch and develop inside the host, producing stage 3 larvae and new generations. These then invade other organs and the peritoneum, causing hyperinfective (sometimes called disseminated) stongyloidosis in humans, but not described in dogs.

Life cycle of *Strongyloides stercoralis*



The normal life cycle takes 4 to 6 weeks to complete. Stage 3 larvae continue their migration into the pulmonary arterioles and disseminate throughout the host's tissue. They then become encysted and remain dormant for several months but they can recommence migration during periods of stress, for example parturition, when they can then infest puppies through the mother's milk.

Epidemiology

Strongyloidosis is seen all year round in warm countries, and through the summer in temperate countries. It is a helminth infestation which can affect animals housed indoors as well as those which have access to the outdoors, and it affects young animals in particular. The free-living adults can develop in badly kept breeding facilities and kennels, with damp soils and surfaces.

Parasite sources

Infective strongyloid larvae are found on damp surfaces or in damp soil (they are sensitive to desiccation). The free-living adults can survive for a considerable period in the external environment, but only the parthenogenetic females are parasitic.

Mechanisms of infestation

Penetration is mainly through the skin; ingestion through the mouth is of secondary importance (most stage 3 larvae ingested are destroyed in the stomach). Stage 3 larvae can become encysted in muscles or mammary tissue and activity will recommence in females after gestation, causing infestation of young mammals through the milk (see *Toxocarosis*, page 18).

Susceptibility

Young animals are more susceptible. An immunocompromised state (malnutrition, immunosuppresive treatment, AIDS in humans) will encourage the parasites to multiply in the intestine (autoinfestation) and stage 3 larvae to invade the body, causing disseminated strongyloidosis.

Clinical signs and lesions

Invasive phase: appearance of papules on the ventral parts of the animal. These skin infestations can also be caused by other types of nematodes or rhabditoids (of the genus *Pelodera* or *Rhabditis,* for example).

Migration phase: coughing may occur when larvae migrate into the lung parenchyma.

Intestinal phase: severe enteritis accompanied by colic, diarrhoea and anaemia. Threadworms lead to profuse diarrhoea and often cause a febrile syndrome (pyrexia, tremors and lethargy). Strongyloidosis can easily be mistaken for bacterial enteritis, such as colibacillosis or salmonellosis.

Lesions

Development of acute catarrhal enteritis, sometimes with ulcers and haemorrhaging.

Diagnosis

Differential diagnosis must consider other causes of enteritis and weight loss in young carnivores. Definitive diagnosis is by the identification of eggs and larvae on faecal examination.

Control measures

Treatment

Treatment is usually more difficult than for other helminth diseases of the gastro-intestinal system, especially ancylostomosis. Ivermectin is usually recommended.

Prevention

Prevention is based on regular disinfection of breeding facilities and kennels and keeping them clean, as well as treating females before birth of their young.

It must be remembered that *S. stercoralis* is potentially zoonotic and that humans and other carnivores are mutual sources of parasites.

Risk to humans

Infective L3 larvae penetrate the human skin when it comes into contact with the soil. Several studies have shown an association between invasive strongyloidosis and HIV infection.

Trichuriosis

General comments

Definition

Trichuriosis (whipworm infestation) is a disease of the posterior part of the gastro-intestinal tract in mammals caused by the presence and development of nematodes of the genus ***Trichuris***. Whipworms are host-specific: in canids, infestation is caused by ***Trichuris vulpis*** (Fig. 1). In pigs, infestation is caused by *Trichuris suis* and in humans by *Trichuris trichiura*.

Although cats in Europe do not harbour specific *Trichuris*, some species of *Trichuris* do infest certain wild feline species in South and Central America, and infestation of pet cats by these species has been described.

Figure 1. *Trichuris vulpis* on the colon mucosa.

Synonyms

Trichocephalosis. The name *Trichocephalus* refers to the very fine (hair-like) anterior extremity of this parasite, while the posterior third is thicker in diameter. In fact, this name is actually more exact than *Trichuris*, which means the opposite. But international nomenclature has retained the first description, even though erroneous.

Geographical distribution

Worldwide.

Importance

This parasite causes colitis, and even significant anaemia when associated with *Ancylostoma* nematodes.

Morphology

Trichuris nematodes are clearly divided into two parts: a fine, thin, long anterior portion (measuring 2/3 of the total length), and a thicker, shorter posterior portion. *Trichuris vulpis* measures 3–5 cm in length (Figs. 2 and 3).

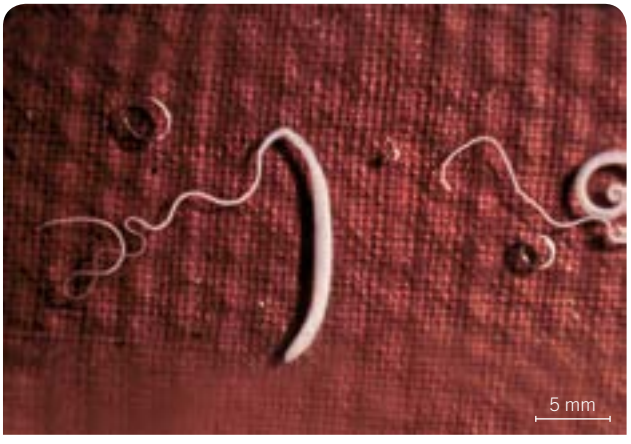


Figure 2. *Trichuris vulpis*.

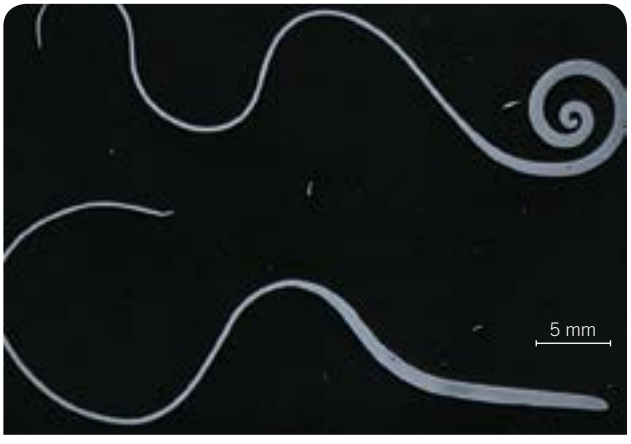


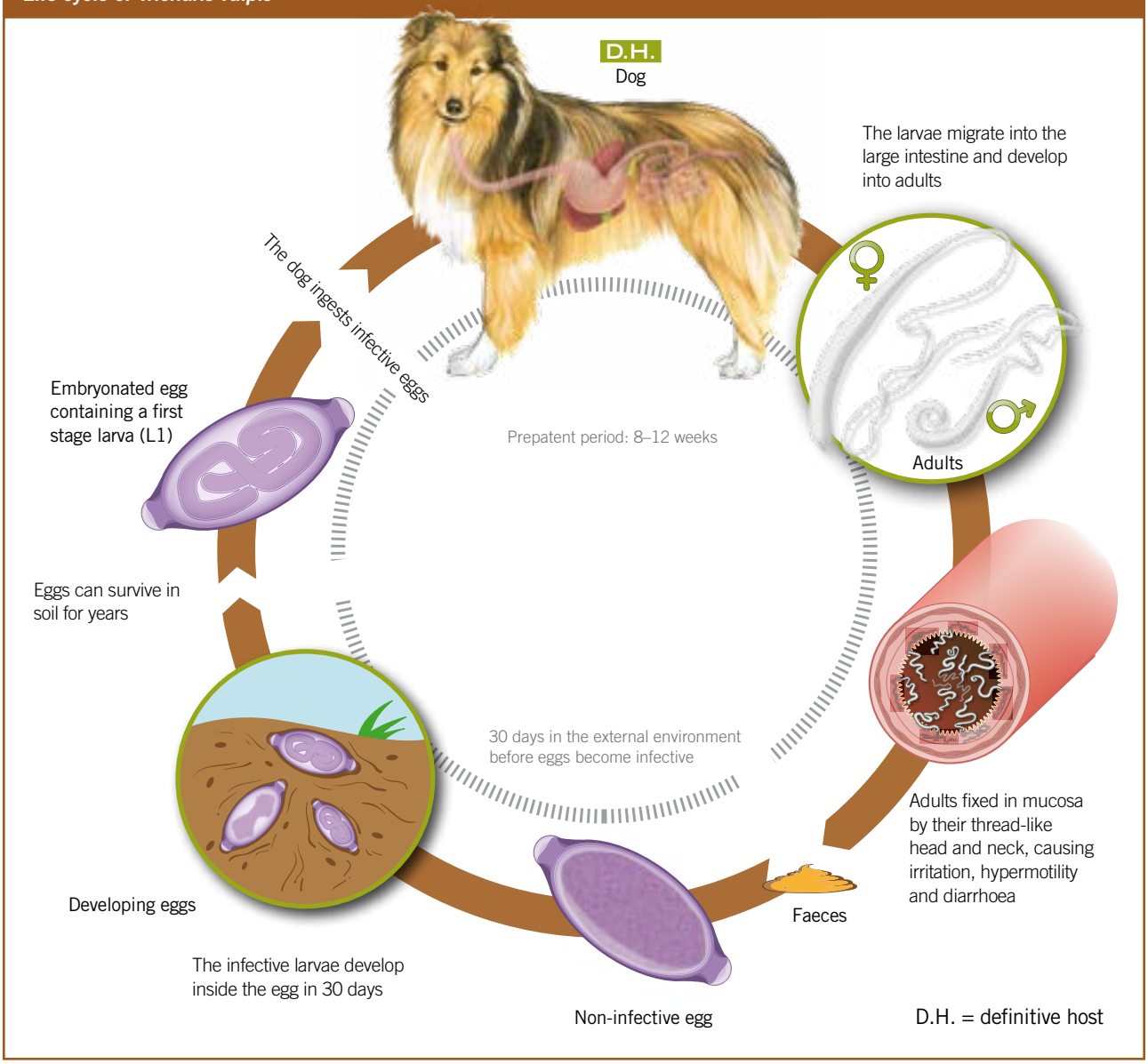
Figure 3. *Trichuris vulpis* male (at the top) and female (at the bottom).

Biology

Trichuris are parasites of the caecum and colon and they attach themselves to the mucosa with the anterior end, by embedding it in the tissue. After the eggs, containing larvae, are ingested and the larvae are released, complete development takes 10–12 weeks. This development occurs with no systemic migration, the larvae remaining in the wall of the intestine. The females are relatively prolific and lay barrel-shaped, yellow-brown coloured eggs with a thick, smooth shell with bipolar plugs at each end and measuring 60–70 × 25–40 µm. When the eggs are shed, they contain only a single cell but they develop into embryonated eggs in 1 month in the external environment and survive in the soil for several years, where they are not particularly sensitive to extreme weather conditions (cold, drought) or to ordinary disinfectants.

PDF

Life cycle of *Trichuris vulpis*



Epidemiology

Infestation is possible at any age by ingesting embryonated eggs; however, adult dogs are more commonly infested than young animals.

These eggs are formed 3 to 4 weeks after being shed and are very resistant, surviving in the soil for several years, which is why the risk of contamination can be long-lasting. This explains the incidence of trichuriosis in breeding facilities and kennels.

Certain types of floor surfaces, such as clay or mud, promote survival of the eggs.

Infested dogs are the only parasite reservoir, which explains why trichuriosis is often found in communal environments (rescue shelters, breeding kennels).

Clinical signs

Infestation causes congestive and haemorrhagic colitis, resulting in diarrhoea which is sometimes haemorrhagic. This may be the only clinical sign in mild infestations.

Anaemia may also be connected with whipworm, particularly in chronic infestations or due to a combination with another helminth, especially *A. caninum*.

Chronic infestation will cause considerable weight loss in dogs.

Lesions

This parasitic infestation causes catarrhal and haemorrhagic typhlitis, with inflammation of the colon and rectum (Figs. 4 and 5).

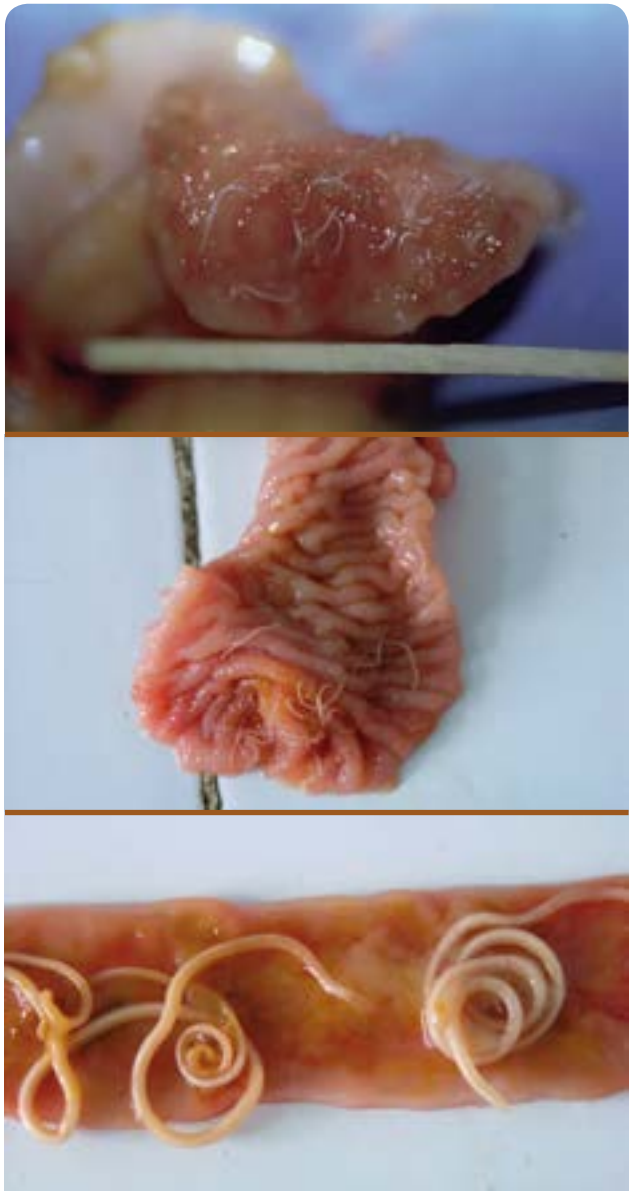


Figure 4. Trichuriosis lesions.

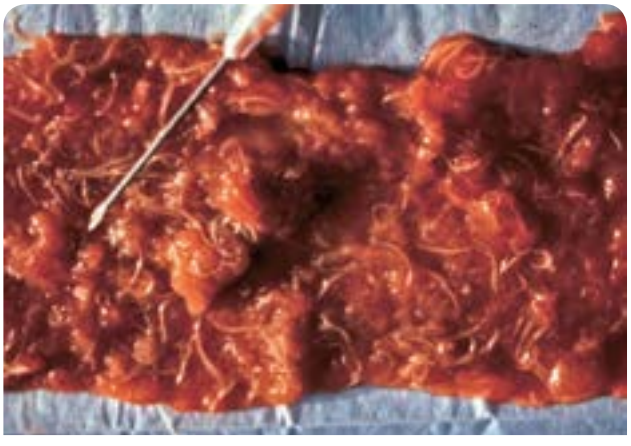


Figure 5. Trichuriosis lesions: ulcerative colitis, presence of numerous adults.

Diagnosis

Clinical signs are non-specific, so any faecal examination which will reveal the eggs is the best method of diagnosis (Fig. 6).

Control measures

Treatment

Trichuris are usually less sensitive to anthelmintics than other common nematodes (roundworms, hookworms).

In dogs, benzimidazoles are effective as long as they are administered for several consecutive days.

Oxantel is a tetrahydropyrimidine anthelmintic (related to pyrantel) which is specifically effective against *Trichuris* at a dose of 20 mg/kg (it is often combined with pyrantel and praziquantel).

Emodepside is also active against *Trichuris* in an oral dose of 1 mg/kg.

Macrocyclic lactones can be active, depending the molecule and the formulation. Milbemycin oxime is active in an oral dose of 0.5 mg/kg. Moxidectin, administered topically, is also effective at 2.5 mg/kg.

Prevention

Eggs can be eliminated by cleaning hard surfaces with high pressure jet hoses. Loose soil can eventually be dug over, thus burying the eggs out of reach. Covering loose soil surfaces with gravel or pebbles is also a solution, to limit contact between eggs and dogs.

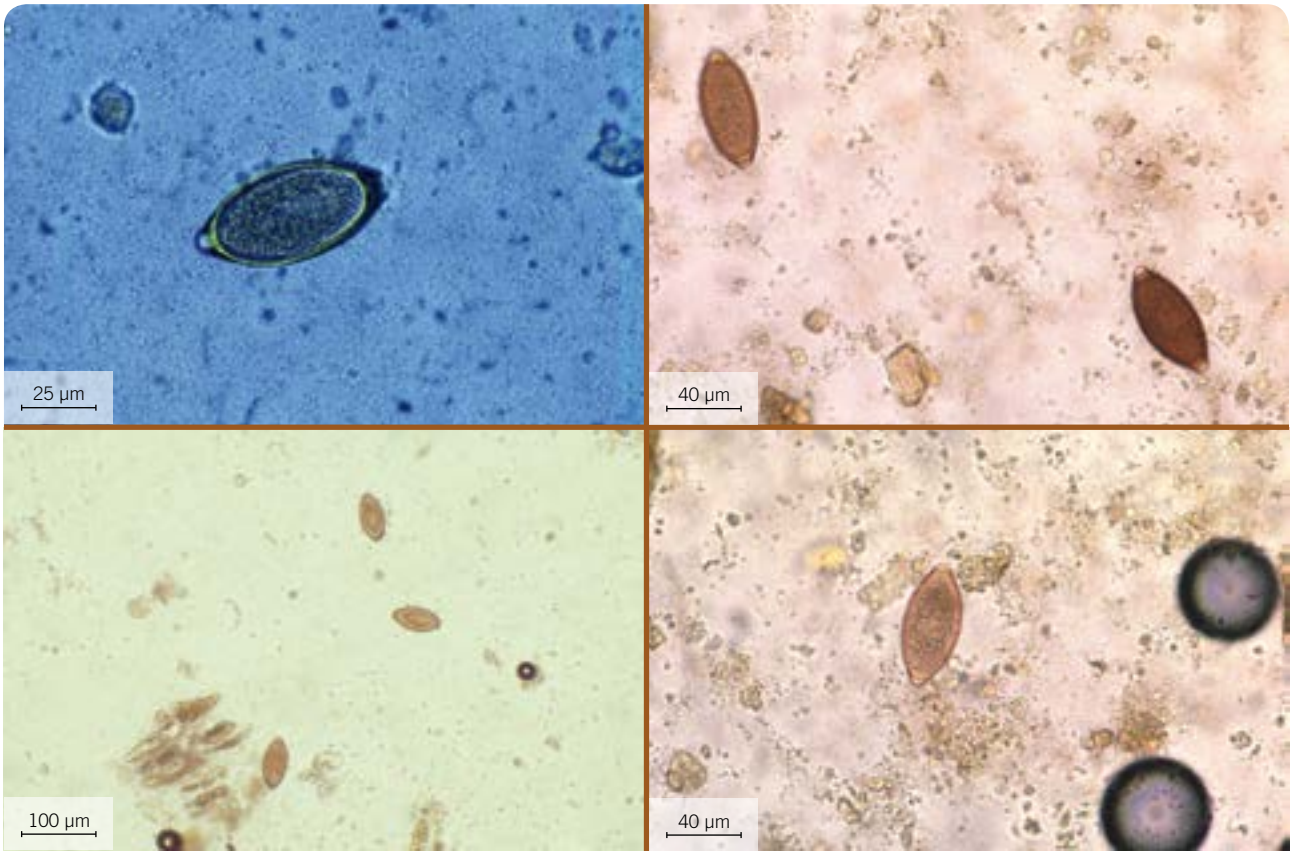


Figure 6. *Trichuris vulpis* eggs.

Capillariosis

The Capillariidae are thread-like nematodes, usually measuring 10–80 mm in length and with a diameter in the region of 50–100 µm.

Most Capillariidae have a monoxenous life cycle.

Capillaria (syn. *Aonchotheca*) ***putorii*** is a parasite of the small intestine in wild carnivores, especially Mustelidae (polecats, skunk, martens, minks, weasels, stoats and ferrets) which can occasionally infest cats. It is not pathogenic and infections are generally asymptomatic. The eggs can be seen on faecal examination (Fig. 1).

Other *Capillaria* are not intestinal parasites but their eggs can be shed in the faeces (see *Respiratory capillarioses*, page 150). They are:

- *Capillaria* (syn. *Eucoleus*) *aerophila,* which infests the trachea and bronchi of wild carnivores (foxes, in particular) and which can occasionally infest dogs and cats.
- *Capillaria boehmi,* a parasite of the nasal cavity and sinuses of canids.

Capillaria (syn. *Calodium*) *hepaticum* is a very particular member of the Capillariidae family, which can also be found in carnivore faeces. This nematode is a parasite of wild rodents that settles in the liver parenchyma. The eggs, contained in the uterus of the female, are not shed and remain within the final host. They are only disseminated if the host is ingested by a predator, such as a fox, but sometimes a cat or dog.

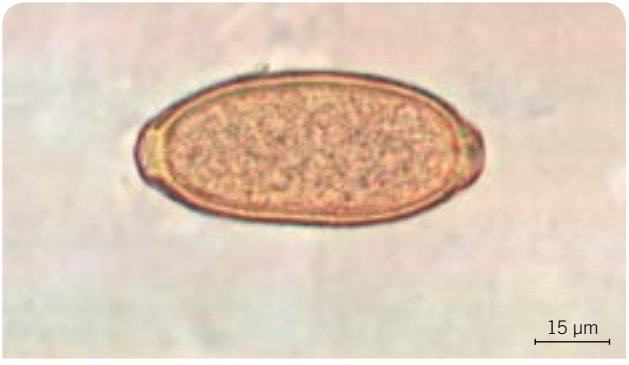


Figure 1. *Capillaria* egg.

CESTODOSES

Dipylidiosis

General comments

Teniosis, intestinal cestodosis, or tapeworm infestation, is a common intestinal disorder in dogs and cats. The presence of tapeworm segments in the perianal region and signs of perianal itching are common causes for consultation. Intestinal cestodoses are parasitic diseases caused by infestation with adult cestodes: flat, segmented tapeworms belonging mainly to the order Cyclophyllidea, or to the order Pseudophyllidea. Clinial signs are subtle or even absent.

Most cestode parasites of dogs and cats are host-specific, however some are common to both species, such as various Dilepididae, in particular ***Dipylidium caninum***. Wild carnivores (Mustelidae, Felidae and Canidae) can also harbour dog and cat cestodes, as well as those that are specific to their own species. Two genetically distinct populations of *Dipylidium caninum* have recently been described: one is frequently found in dogs, the other one in cats.

Dipylidium caninum can infest humans, who become contaminated through ingestion of the intermediate host, the flea.

Figure 1. Segments of *Dipylidium caninum*.

Domestic carnivores, such as cats and dogs, often excrete whitish elements about half a centimetre in length around the anal area. These are usually the ovigerous segments of *D. caninum*. The posterior gravid segments, which are elongated and called proglottids (Fig. 1), contain capsules full of eggs (oviferous capsules).

Dipylidium caninum is a long, white, tape-like worm, 15–70 cm long and 2–3 mm wide (Figs. 2 and 3). Other, much rarer Dilepididae can infest dogs and cats; these are *Diplopylidium* spp. or *Joyeuxiella* spp., very similar in morphology but smaller in size (Figs. 4–8). The intermediate hosts of the last two cestodes are reptiles (snakes and lizards) rather than arthropods. The oviferous capsules contain a single egg, as opposed to the dozens in *D. caninum* (Fig. 9).

Figure 2. Scolex of *Dipylidium caninum*.

Figure 3. Rostrum of *Dipylidium caninum*.

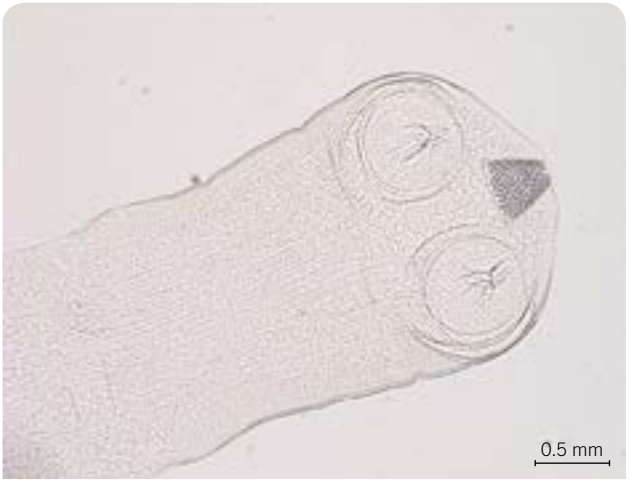


Figure 4. Scolex of *Joyeuxiella* sp.

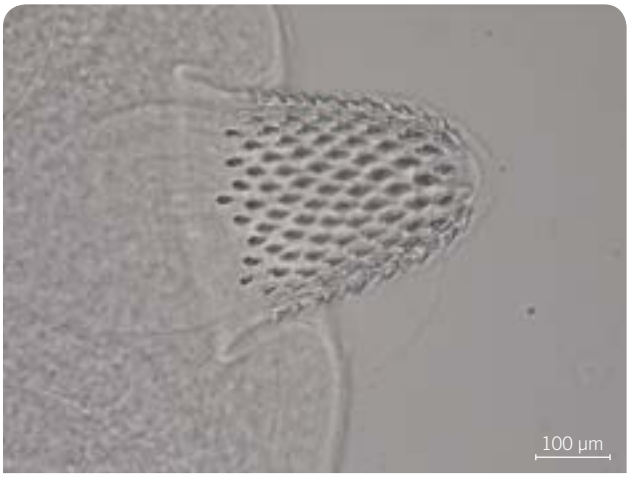


Figure 5. Rostrum of *Joyeuxiella* sp.

Figure 6. Segments of *Joyeuxiella* sp.

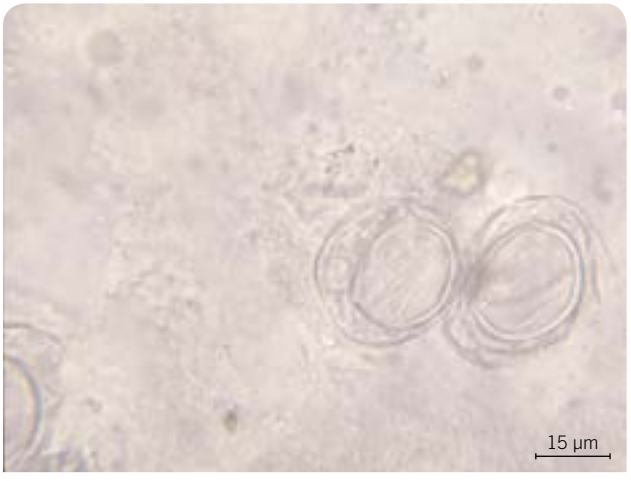


Figure 7. Eggs of *Joyeuxiella* sp.

Figure 8. Segments of *Diplopylidium* sp.

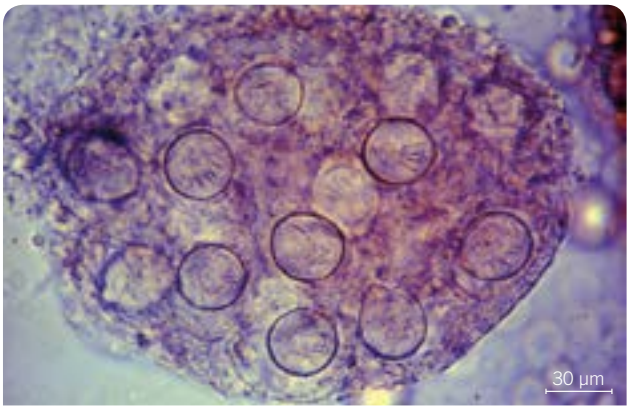


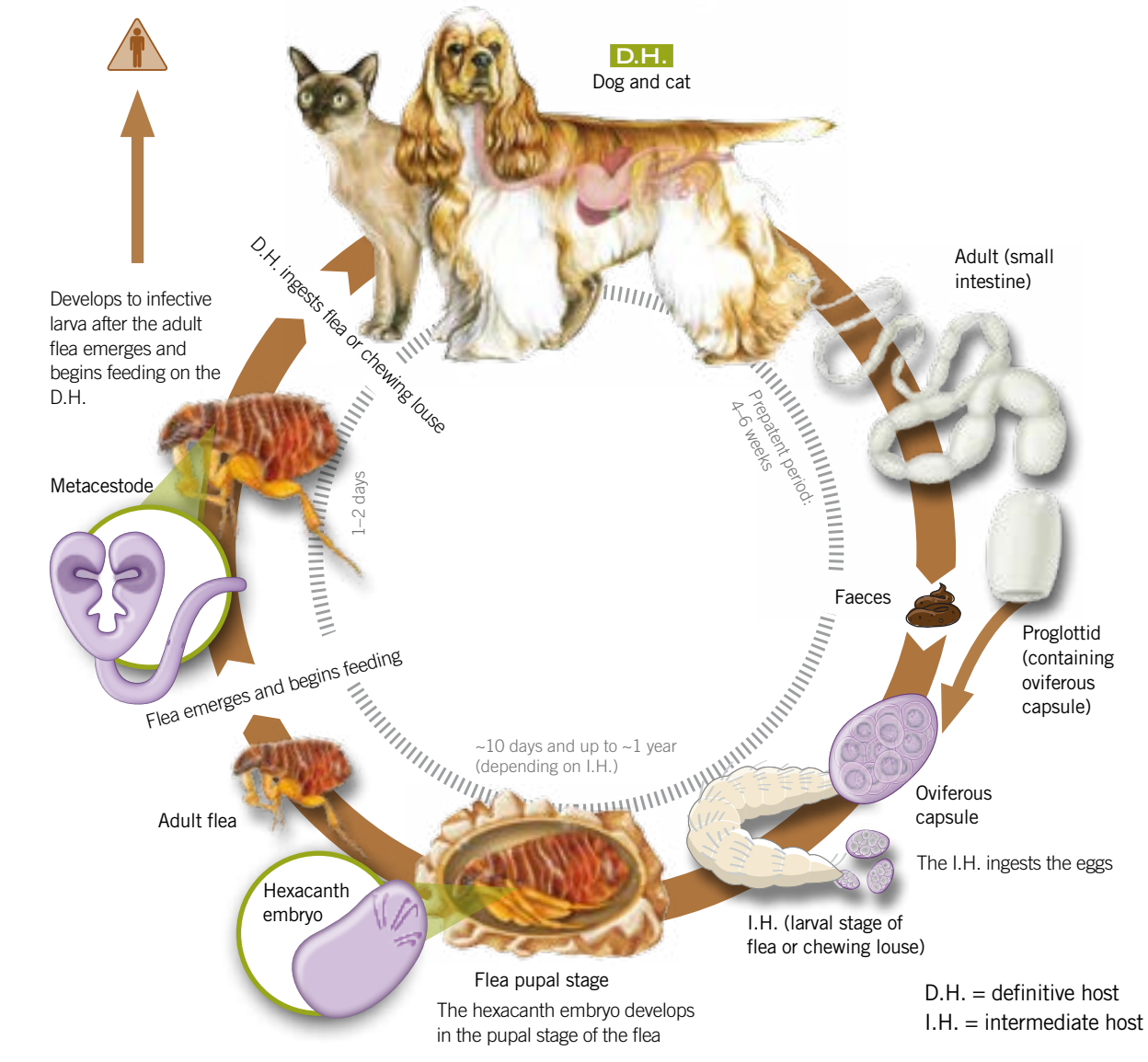
Figure 9. Oviferous capsule of *Dipylidium caninum*.

Biology

The life cycle of *D. caninum* is dixenous, i.e., it has two host species. The most common intermediate host is the flea, and the other one is the louse. Flea larvae actively ingest several types debris in their environment, including hairs, skin debris, and also faeces or *Dipylidium* proglottids, so the flea larvae can ingest the oviferous capsules in the gravid terminal segments. *D. caninum* eggs survive for between 1 and 3.5 months in the dried segments or in the capsules.

PDF

Life cycle of *Dipylidium caninum*



Cestode cysticercoid larvae then develop in the flea larva and remain viable, but not infective to carnivores, until the flea's pupal stage (Fig. 10). These larvae only become infective in adult fleas, i.e., approximately 36 hours after the fleas have infested their host (Figs. 11 and 12) and maturation is linked to temperature. The metacestode larvae must be ingested in a flea by the dog or cat during grooming in order to develop. They do not migrate, and form adult cestodes in 4–6 weeks in the small intestine.

Epidemiology

Dipylidium caninum has a worldwide distribution. Geographical distribution of *Joyeuxiella* and *Diplopylidium* is limited (Mediterranean region to Central Africa). D.H. are domestic or wild dogs and cats. Copepods and fish are intermediate hosts, as they are for *Diphyllobothrium*.

The sources of parasites are fleas or lice. Dogs and cats are infested by ingesting intermediate hosts which have themselves been parasitised.

Age does not affect susceptibility and a final host will never acquire immunity. Reinfestation is therefore possible throughout the life of the dog or cat, however, certain lifestyles can promote infestation: suburban or rural cats and dogs are often infested with fleas and therefore risk infestation with *D. caninum*.

Clinical signs and lesions

Dipylidiosis is generally mild, and clinical signs are often not apparent. Symptoms will depend on the level of infestation and the dog or cat's own susceptibility (for instance, allergic reactions can occur).

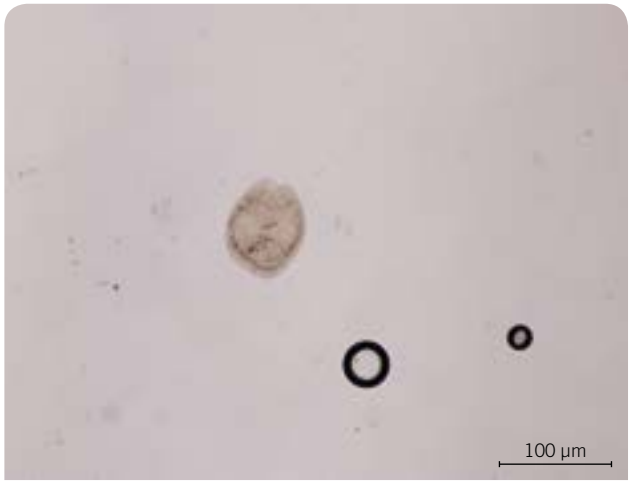


Figure 10. *Dipylidium caninum* non-infective larval stage.

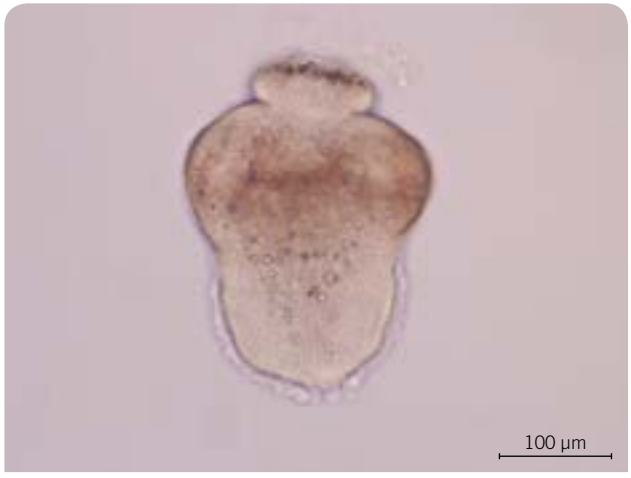


Figure 11. *Dipylidium caninum* early infective larval stage taken from an adult flea.

Figure 12. *Dipylidium caninum* infective metacestode taken from an adult flea.

Both general and localised clinical signs can be seen:

General signs

Adult cestodes can cause moderate loss of vitamins, mineral trace elements and carbohydrates, so emaciation can be seen in underfed or severely infested animals, or in growing young carnivores.

Neurological signs due to B group vitamin deficiency (B1, B6 and B12) and hypoglycaemia are both possible, but very rare. This manifests itself by epileptiform convulsions and seizures and, very rarely, blindness. These signs may also be linked to significant irritation of the autonomic plexuses of the neurovisceral system, or to a lack of glucose, as has been observed with toxocarosis in puppies and kittens.

Localised signs

These are generally the only ones seen.

Intestinal signs are irregular and diversely associated:

- Variable appetite, sometimes even increased.
- Loose or diarrhoeic faeces (due to congestive enteritis).
- Elimination of gravid segments (Fig. 13). These are generally easily visible and measure 10–12 × 5–8 mm. *Dipylidium* segments can move around the perianal region by themselves. They desiccate and shrivel up, resembling whitish, uncooked rice grains, 3–5 mm long. The segments can be found either in the perianal region or in the faeces. Owners sometimes mistake these grains for the oxyurids (pinworms) seen in children, but it should be noted that dogs and cats are never infested with pinworms.

Itching:

- Itching of the perianal region is common and characterised by licking and nibbling at the base of the tail. One of the most characteristic signs is rubbing or dragging the rear end on the ground. The itching is connected with mechanical irritation and congestion of the anal glands. Licking of the perianal region also causes eggs to be deposited on the animal's fur.
- Congestion of the anal glands accentuates itching. This is evidenced by the expulsion of a foul smelling, brownish liquid containing the disintegrated oviferous segments. Anal abscesses are a possible complication.
- General itching can be seen. Cestodosis reduces the skin sensitivity threshold in dogs, and abdominal or general pruritis can be associated with parasites of the gastro-intestinal system.

Lesions

Dipylidiosis in both cats and dogs is evidenced by chronic enteritis of the small intestine (Fig. 14) as the parasites are found in the duodenum and jejunum.

A proinflammatory, irritative effect is responsible for reactions in the gastro-intestinal system. Fixation of the scolex, the chain of segments rubbing against the mucosa of the gastro-intestinal system and the fragments of membrane removed by the parasite cause this enteritis. Mechanical action obstructs the anal gland orifices; and, very rarely, intestinal obstruction similar to that caused by ascarid pellets can occur.

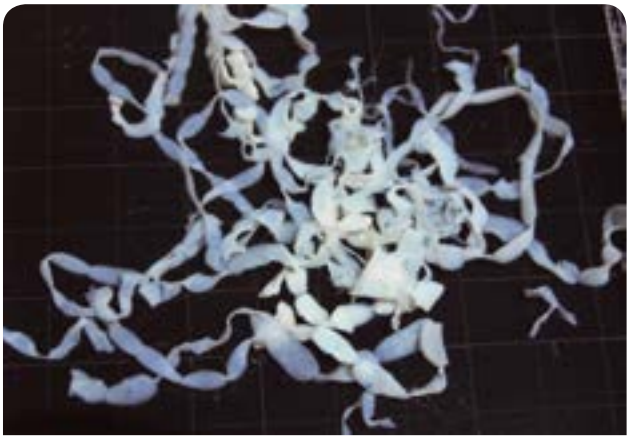


Figure 13. *Dipylidium caninum* segments.

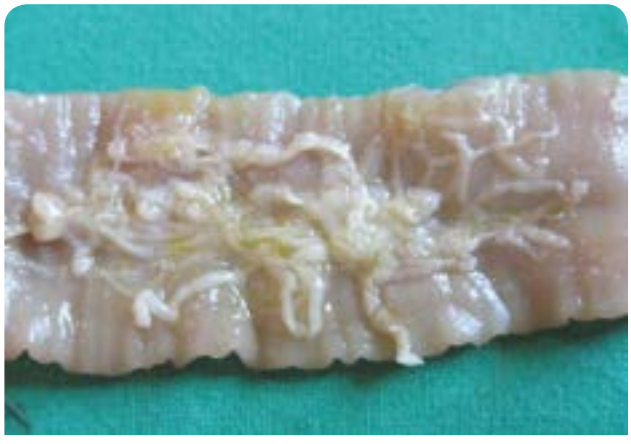


Figure 14. Adult *Dipylidium caninum* in cat intestine.

Diagnosis

Clinical diagnosis is impossible except when proglottids are visible. The reason for veterinary consultation is generally the presence of moderate intestinal disorders (variable appetite, diarrhoea, signs of perianal itching). Diagnosis of dipylidiosis is based on finding oviferous segments by macroscopic examination of faecal samples. Eggs can be found in the faeces if a segment is destroyed before it is expelled. These eggs can be isolated or grouped together inside the oviferous capsules.

Control measures

Various cestodicides can be used to treat *D. caninum*: benzimidazoles, niclosamide and praziquantel.

Oxfendazole in a drinkable suspension is used in dogs, at a dose of 11.3 mg/kg/day for 3 days. It is also active against cestodes of the genus *Taenia* at this dose.

Niclosamide can be used in a single dose of 80 mg/kg.

Praziquantel in a single dose of 5 mg/kg, in oral or injectable form, or at 10 mg/kg in transcutaneous formulations. This will have an effect on all cestodes present, including *Echinococcus*.

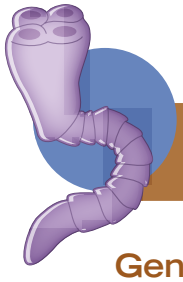
Treatment for *D. caninum* is advisable in dogs and cats with flea infestations and the insecticides used to treat fleas will limit the risk of infestation with *D. caninum*. Effective and ongoing prevention of the former prevents infestation by the latter.

Risk to humans

The accidental ingestion of a flea by a child, which is unusual, can result in a case of dipylidiosis. The symptoms are the same as those for dogs and cats: reduced appetite, abdominal discomfort, anal pruritus.

Video 2.1
Dipylidium caninum (tapeworm) eggs in an oviferous capsule (microscopic view).

Video 2.2
Moving *Dipylidium caninum* (tapeworm) proglottids on the anal area of a cat.



Mesocestoidosis

General comments

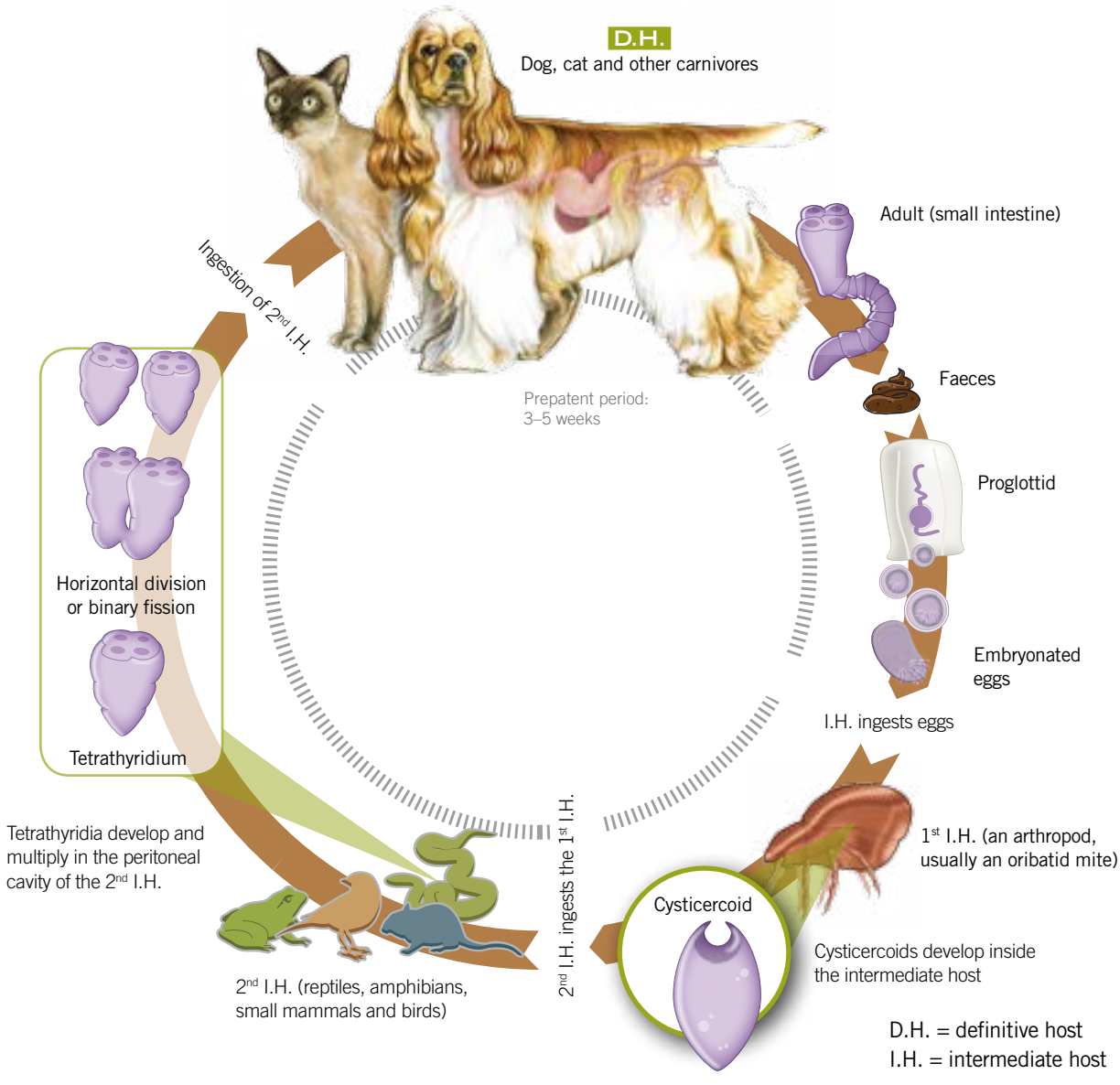
Mesocestoides are cestodes of the order Cyclophyllidea which use two intermediate hosts, whereas all other Cyclophillidea have only one. The first is an arthropod, which harbours the cysticercoid larvae. The second is an insectivorous vertebrate, which harbours an elongated larva called a tetrathyridium.

Morphologically these tapeworms are of average size, measuring 20–50 cm in length, and their segments are as wide as they are long and have rounded edges (Fig. 1).

Mesocestoides lineatus and ***Mesocestoides litteratus*** cause cestodosis in both dogs and cats.

PDF

Life cycle of *Mesocestoides* spp.



Biology

Carnivores become infested by ingesting the second intermediate hosts which harbour the second larval stage, the tetrathyridium. These hosts are amphibians, reptiles or birds.

In the case of *M. lineatus*, second intermediate hosts are amphibians and, in *M. litteratus*, they are birds.

Another species, *Mesocestoides corti* (syn. *Mesocestoides vogae*) is a parasite of carnivores in North America.

Normal larvae, found in the second intermediate hosts, are 5–7 cm long and a few millimetres wide. They are ribbon-like, with a depression enclosing the future scolex of the adult at one extremity.

The tapeworm will appear approximately 4–6 weeks after ingestion of the larvae. Carnivores excrete the oviferous segments containing the eggs, which are spherical in shape with a smooth, thin outer shell (Fig. 2). The hexacanth embryo is protected by a second inner shell, separated from the first by a vitelline layer.

Epidemiology

Mesocestoidosis is a relatively rare type of tapeworm infestation, which is sporadically seen in dogs and cats which hunt, and it is mainly a rural parasitosis.

Clinical signs

Mesocestoidosis is often well tolerated, only rarely causing intestinal disorders, as is the case with other cestodes. Changes to appetite, from anorexia to polyphagia, have been described. *Mesocestoides* parasites are unusual in that the larval stage (tetrathyridium) can develop in the peritoneal or pleural cavities of cats and dogs (see *Peritonitis due to Mesocestoides larvae*, page 194). After accidental ingestion of the first intermediate host (arthropod), or sometimes the second intermediate host, the larvae migrate through the wall of the intestine into the peritoneum, where they will transform and multiply after repeated budding (asexual reproduction). The larvae appear to be deformed, due to this multiple budding and they proliferate in a disorganised fashion, usually in the peritoneum of infested carnivores, and cause parasitic peritonitis and ascites. This proliferation produces numerous white, semolina-like grains, in an inflammatory exudate. Clinically, the infestation can be asymptomatic, or give rise to signs of peritonitis, and diagnosis is often made by accident, during surgery. This larval cestodosis seems to be more common in cats than in dogs.

In dogs, parasitic peritonitis with ascites can be caused by multiplication other cestodes, including the larva of *Taenia crassiceps*, a fox parasite.

Diagnosis

Clinical diagnosis is difficult. Ascites may be diagnosed by X-ray or ultrasound scan, and then confirmed on abdominocentesis, when the numerous white grains will be seen. This parasite infestation can often be diagnosed by laparotomy (for example, during ovariohysterectomy).

Control measures

Treatment for adult forms consists of standard cestodicides (such as praziquantel).

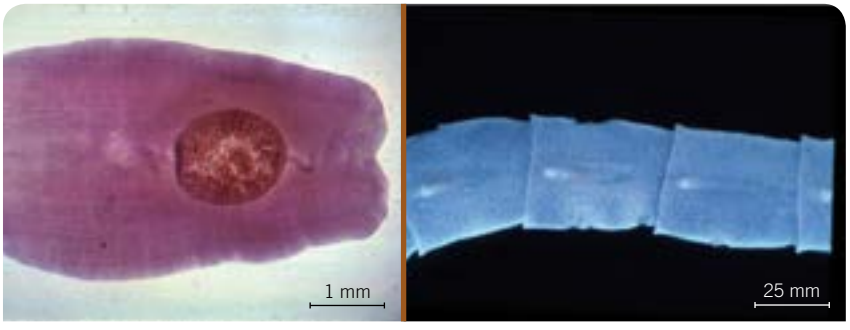


Figure 1. Segments of *Mesocestoides* sp.

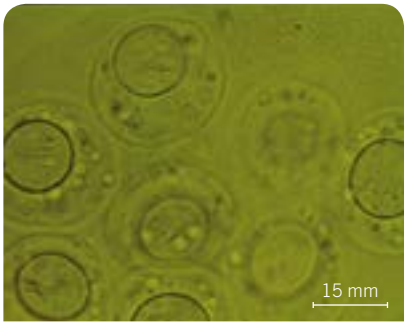


Figure 2. Eggs of *Mesocestoides* sp.

Taenioses *sensu stricto*

General comments

Dogs and cats can be infested with numerous types of cestodes. In addition to *Dipylidium*, in the Dilepididae family and the most common tapeworm in domestic carnivores, they can harbour several cestodes of the genus ***Taenia***, belonging to the Taeniidae family. *Taenia* are more common than other cestodes infesting dogs and cats (e.g., Mesocestoididae, Diphyllobothriidae or *Echinococcus*). *Taenia* tapeworms cause an intestinal cestodosis which is usually asymptomatic and well-tolerated.

Dogs are infested by ingesting the viscera of mammals carrying the larval stage of the parasite. There are two types of larvae: cysticerci and coenuri. Cats are infested by ingesting rodents, usually mice, containing the hepato-peritoneal cysticerci of *Taenia taeniaeformis*.

The disease caused by the larval forms in intermediate hosts is usually more severe. Historically, Latin terminology was used to name the larval stage at a time when corresponding adult forms were unknown, but these latin names are now obsolete.

The infestation of definitive host is usually called taeniasis, but following the nomenclature, it should be taeniosis.

Two types of *Taenia* tapeworms can infest dogs:

- ***Taenia* with cysticerci-type larvae:**
  - ***Taenia* with cysticerci-type larvae located in the liver and peritoneum:**
    - ***Taenia pisiformis*** in rabbits (domestic and wild).
    - ***Taenia hydatigena*** in herbivores or omnivores (sheep, cattle, goats and pigs)…
  - ***Taenia* with cysticerci-type larvae in muscle tissue:**
    - ***Taenia ovis*** in ruminants, especially sheep, but occasionally goats and deer.
- ***Taenia* with coenurus-type larvae:**
  - ***Taenia multiceps:*** in the central nervous system of ruminants (mainly in sheep).
  - ***Taenia serialis:*** in the subcutaneous tissue of rabbits.

The *Taenia* tapeworm which infests cats is ***T. taeniaeformis***, whose cysticerci-type larvae are located in the peritoneum or liver of infested rodents (mainly mice).

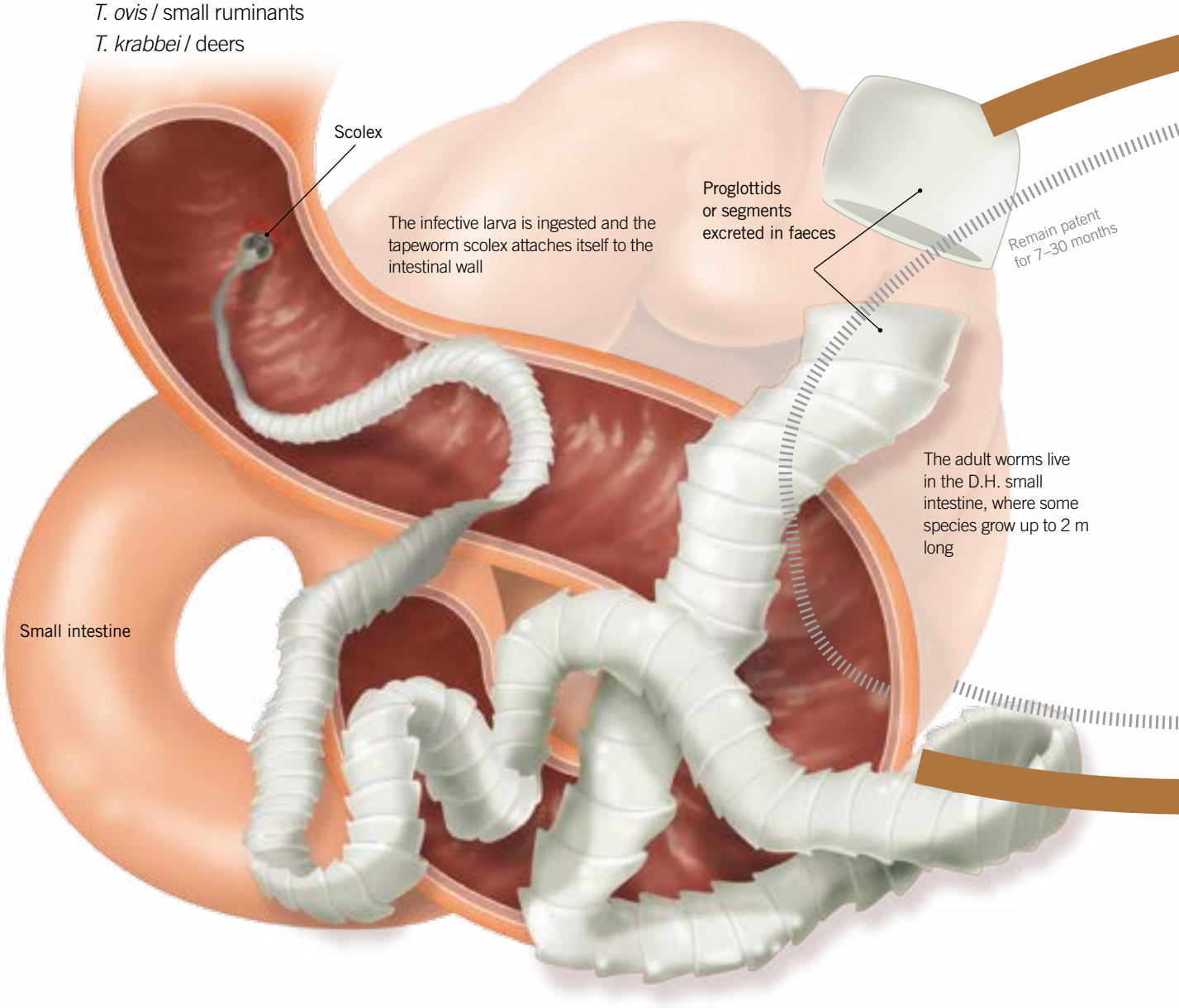
Geographical distribution

These parasites are widely distributed but, infestation is predominantly seen in rural areas, where farm dogs and hunting dogs become infested, because of the variable distribution of intermediate hosts (usually ruminants or lagomorphs). Cats living in rural areas, and cats living in urban areas that are able to get out and roam are most infested, because the life cycle passes through mice and rats.

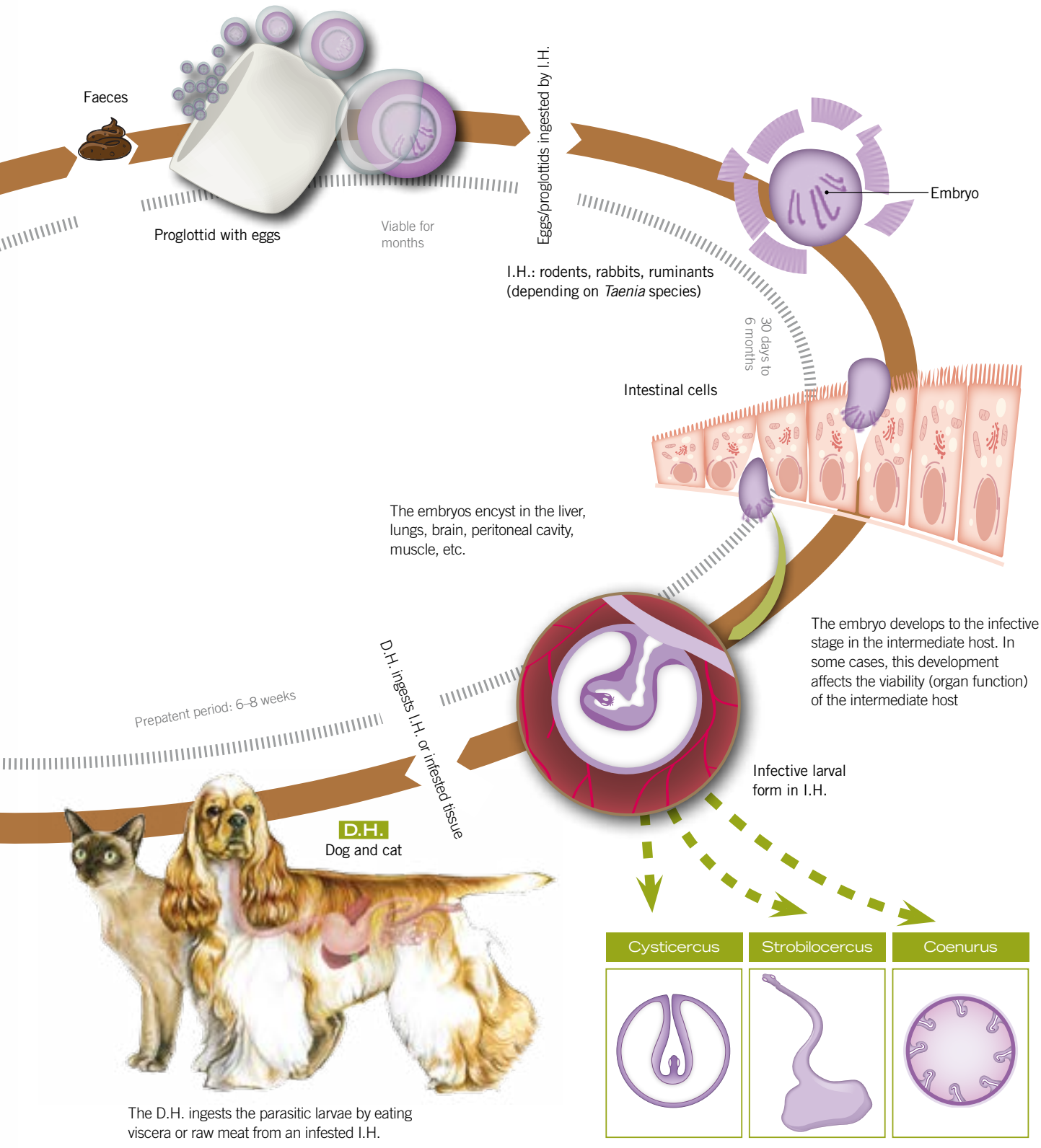
Muscular cysticercosis in ovines is only enzootic in the Southern Hemisphere (Australia, New Zealand, South America). It is unusual in Europe, although it is observed sporadically on certain farms. It has been identified in imported carcasses in slaughterhouses, or in imported live animals that have been slaughtered in Europe.

Life cycle of *Taenia* spp.

- In cats: *Taenia taeniaeformis* / rodents
- In dogs: *T. pisiformis* / lagomorphs
  T. hydatigena / small ruminants and pigs
  T. serialis / lagomorphs
  T. multiceps / small ruminants
  T. ovis / small ruminants
  T. krabbei / deers



D.H. = definitive host
I.H. = intermediate host



The D.H. ingests the parasitic larvae by eating viscera or raw meat from an infested I.H.

Figure 1. *Taenia* sp. scolex viewed from the top.

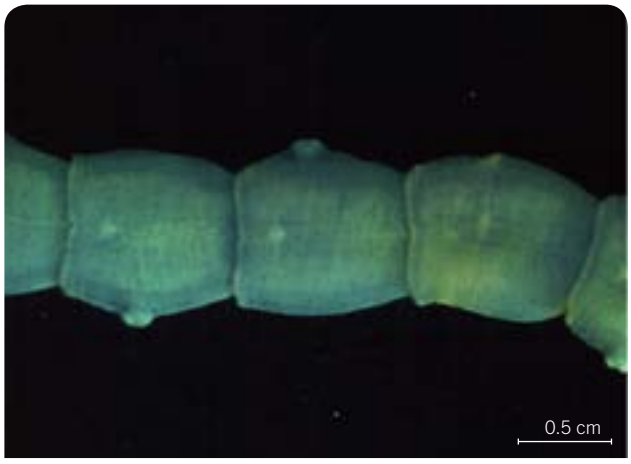


Figure 2. *Taenia* sp. proglottids.

Morphology

The *Taenia* cestodes that are seen in cats and dogs are flat, segmented tapeworms, 60 cm to 2 m long. The scolex has a rostrum with two rows of hooks in a typical claw hammer shape (Fig. 1). The white, oviferous segments are rectangular and measure 10–15 × 6–8 mm (Fig. 2). They enclose a longitudinally stretched uterus, laterally ramified, containing thousands of eggs consisting of a thick, concentrically striated wall, protecting a hexacanth embryo (with six hooks), and whose appearance is specific to this family of cestodes. The eggs are oval and measure approximately 30–45 µm (Fig. 3). Other cestodes eggs have two walls separated by a vitelline layer. The segments are shed in the faeces or pass out of the anus, due to their independent movement.

Biology

Dogs and cats ingest the cestode larvae when they eat infested prey (such as mice, in the case of cats), raw viscera or flesh. The *Taenia* tapeworm is formed approximately 6–8 weeks after ingestion of the cysticercus or coenurus.

The cysticerci or coenuri are digested in the gastro-intestinal tract and the cephalic invaginations are liberated, each with a scolex which will give rise to the future tapeworm segments. One cysticercus will produce only one cestode, whereas one coenurus will give rise to several dozen parasites.

The pathogenicity of adult cestodes is limited and they are usually well tolerated by domestic carnivores.

The oviferous segments are excreted in the faeces of the dog or cat host at the end of the prepatent period. These segments can exit the anus at times other than during the passage of faeces because they are independently motile. The segments disintegrate in the external environment, releasing thousands of eggs and these very resistant embryophores, which can live up to a year in the external environment, may be found in fodder and ingested by intermediate hosts. Their development then depends on the species and the intermediate host involved.

Hepato-peritoneal cysticercosis caused by *Taenia pisiformis*

Development of cysticerci takes 1 month in rabbits and the larvae attach in the peritoneum or on the surface of the liver. Cysticerci are often numerous and small (5 mm diameter), resembling a bunch of grapes. As with all cysticerci, they invaginate and form a single scolex which will eventually produce an adult cestode after ingestion by the final host.

Hepato-peritoneal cysticercosis caused by *Taenia hydatigena*

This parasitic disease is also caused by the development of cysticerci on the surface of the liver or in the peritoneum, and the adult form of this cestode is the cause of a taeniosis in dogs.

Development of a cysticercus takes 5 weeks and is preceded by a phase of migration under Glisson's capsule. This cysticercus looks like a water ball and measures several centimetres (4–7) in diameter.

Muscular cysticercosis in sheep, caused by *Taenia ovis*

Muscular cysticerci measure 2.5–4 × 4–9 mm in length, and are similar to those of *Taenia solium*, one of the three tapeworms infesting humans (the others being *Taenia saginata* and *Taenia asiatica*). They develop fully in approximately 83 days, but are infective from the 46th day. They degenerate rapidly after death of the ruminant, those found in the cardiac region taking approximately 3 months.

Nervous coenurosis in sheep

The *Taenia multiceps* larva develops in the intermediate host's central nervous system, mainly in sheep's brains. It is infective after about 2 months but continues to grow. The larva looks like a small balloon containing water, with a diameter of 10–20 cm, and it contains several white dots which are the cephalic invaginations of future *Taenia multiceps* scoleces. It compresses the nervous tissue, causing specific neurological signs in infested ruminants.

Subcutaneous coenurosis of rabbits

The *Taenia serialis* larva develops in the subcutaneous tissue of the intermediate hosts, domestic and wild lagomorphs. It is infective after about 2 months but continues to grow. The larva looks like a subcutaneous tumour, with a diameter of approximately 10 cm. On opening, the larva is a soft, large cyst containing several white dots which are the cephalic invaginations of *Taenia serialis* scoleces. It can induce particular clinical signs in rabbits by compressing muscles or articulations.

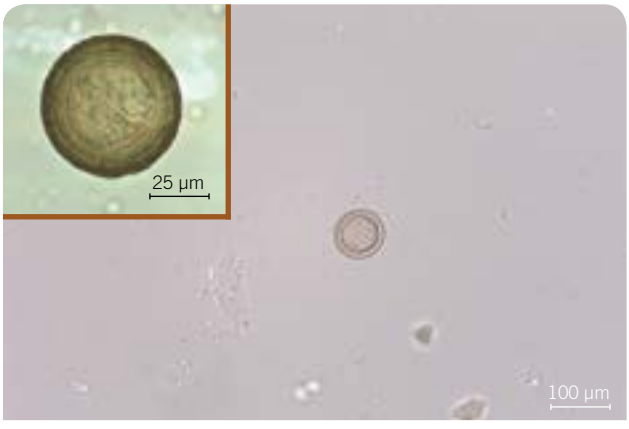


Figure 3. *Taenia* sp. eggs.

Epidemiology

Hepato-peritoneal cysticercosis in rabbits

This parasitic disease is found in the wild and in rural areas. In the latter, it occurs where rabbits are reared in hutches and have been fed with grasses soiled by dog faeces.

Hepato-peritoneal cysticercosis in ruminants

This parasitic disease is found in rural areas where dogs have access to the viscera of sheep which have been slaughtered or died from various causes.

Coenurosis in sheep

Nervous coenurosis is enzootic to sheep-producing regions, and is contracted by animals under 2 years old. It is currently very rare in Europe and mainly affects sheep , but cattle and goats can also be infested. Horses or humans can occasionally be infested. Once again, dogs must ingest raw sheep's brains, and therefore need access to dead sheep, for animals to become infested and for the life cycle to be continued.

Muscular cysticercosis in sheep

The final hosts of *T. ovis* are dogs or other canids. The adult tapeworm is 45–110 cm long, and the oviferous segments measure 15 × 3.5 mm. The prepatent period lasts 7–9 weeks. The segments are usually excreted by dogs and the eggs then ingested by sheep. Cysticerci are formed in approximately 5 weeks and dogs are infested by eating infected meat or offal. The cysticerci mainly attach in their host' heart and liver, but also in the muscles.

Subcutaneous coenurosis in rabbits

This is found in wild lagomorphs (hares, wild rabbits), or domestic, farm-bred rabbits (which are let out into grassy areas or fed on grass which may have been contaminated). Dogs become infested by eating rabbit or hare offal and other waste.

Hepatic cysticercosis in small rodents

Cysticerci take approximately 15 days to develop in mouse livers. Here, the cysticerci begin their development into adult tapeworms, already inside their intermediate host, where they are called strobilocercus larvae (Fig. 4). Adult *T. taeniaeformis* will rapidly appear in the small intestine of cats (Fig. 5) after ingestion of infected mice.

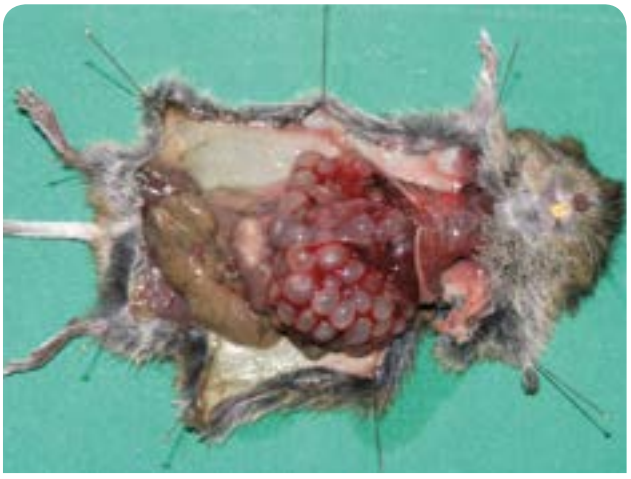


Figure 4. *Taenia taeniaeformis* larvae in a vole.

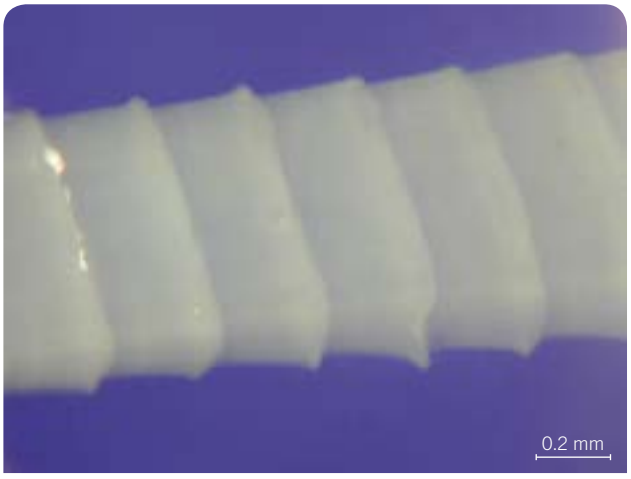


Figure 5. *Taenia taeniaeformis* proglottids.

Another *Taenia* may be found in domestic carnivores: *Taenia crassiceps*. Foxes are definitive hosts, and small rodents (mainly voles) are intermediate hosts for this species. The larvae are small cysticerci, attached in the peritoneum or under the skin of the microtid. They can then start to proliferate and fill the peritoneum. Infestation proceeds with repeated budding (asexual reproduction) and this proliferation produces numerous white, semolina-like grains, in an inflammatory exudate. Dogs may not only act as a definitive host, but also as an intermediate host in some cases. Proliferation of *T. crassiceps* larvae in dogs can cause peritonitis, but also a subcutaneous tumour-like process when larvae are located under the skin.

Clinical signs and diagnosis

Taenia infestation is generally well tolerated in both dogs and cats which sometimes present moderate intestinal disorders, such as colic or diarrhoea. Appetite can be variable, but it is usually increased. Anal pruritus can occur, with the animal dragging its rear end on the ground.

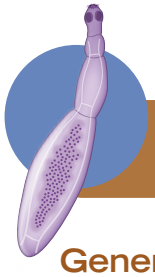
Diagnosis is made by identification of the distinctive segments in the faeces. Microscopic faecal examination for eggs will be negative if no segments have been fragmented in the gastro-intestinal tract.

Control measures

Treatment is based on regularly deworming sheep and farm dogs (the optimum interval corresponds to the prepatent period, i.e., every 6 weeks). In practice, and in sheep farming in mountainous areas, dogs are treated before they leave for the mountain pastures and after their return. In rural areas, dogs should be dewormed 4 times a year with a cestodicidal anthelmintic, preferably praziquantel because of the risk of *Echinococcus* spp. infestation.

Sheep and rabbit carcasses and viscera should not be fed to dogs. In mountain pastures, carcasses should be buried or disposed of out of the reach of dogs. With rabbit cysticercosis or coenurosis, the cycle must be broken: providing compound feed will prevent rabbits from ingesting grass contaminated by dog faeces.

As a general rule, dogs and cats should be dewormed quarterly with a product providing protection against all types of cestodes.



Echinococcoses

General comments

Hydatid echinococcosis, or hydatid disease, is an infectious but non-contagious disease caused by the larvae of a cestode, common in humans and a number of other animal species. It is caused by development of the cestode, ***Echinococcus granulosus sensu lato,*** in tissues and organs, mainly the liver and lungs. This cestode is an intestinal parasite in the dog.

Another species that can also be observed in dogs is ***Echinococcus multilocularis***, which mainly affects foxes, but it is the agent of multilocular or alveolar echinococcosis in rodents and humans.

Echinococcus granulosus cannot develop into the adult stage in the cat, which therefore plays no role in the epidemiology of hydatid disease. Cats can, however, be infested with *E. multilocularis*, which sheds some oviferous segments, although this is rare.

Hydatidosis is usually asymptomatic in animals and is characterised by the formation of a cyst, called a hydatid cyst, consisting of the larvae surrounded by inflammatory tissue caused by the host's reaction.

Dogs are infested by the adult cestodes. *Echinococcus granulosus* is a small cestode, 3–6 mm long, and consists of 4 to 5 segments, only the final segment being oviferous (Fig. 1). This final segment represents about half of the total length of the worm and contains an elongated, non-sack-like uterus. These two characteristics enable it to be distinguished from *E. multilocularis*, in which the oviferous segment contains a sack-like uterus (Fig. 2) and is less than half of the whole worm.

The eggs contained in the uterus are identical to all Taeniidae embryophores and cannot be distinguished from *Taenia* eggs. They are spherical, measuring 30–45 µm in diameter, with a single, thick shell with concentric striation. Each egg contains a hexacanth embryo with six hooks, a number of which are visible (Fig. 3).

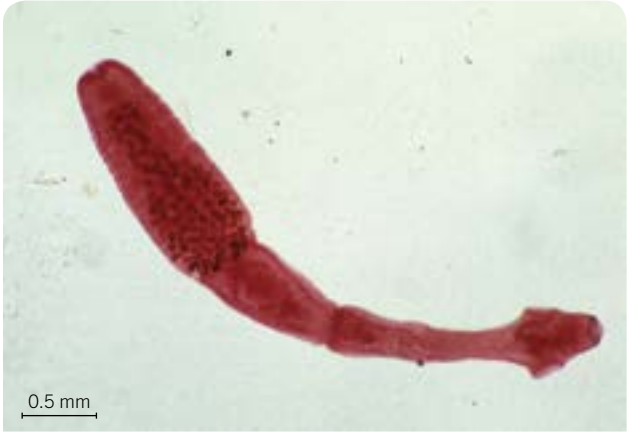


Figure 1. *Echinococcus granulosus.*

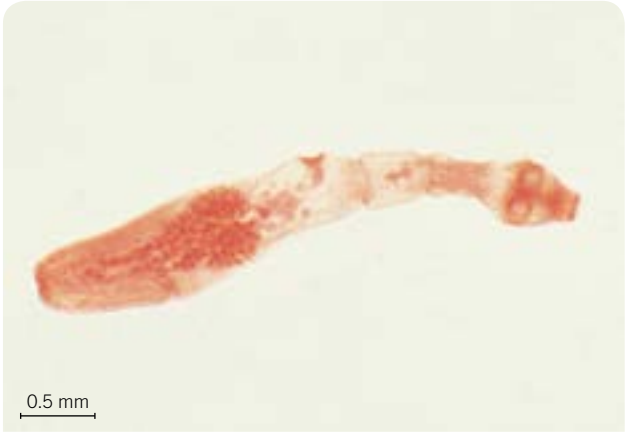


Figure 2. *Echinococcus multilocularis.*

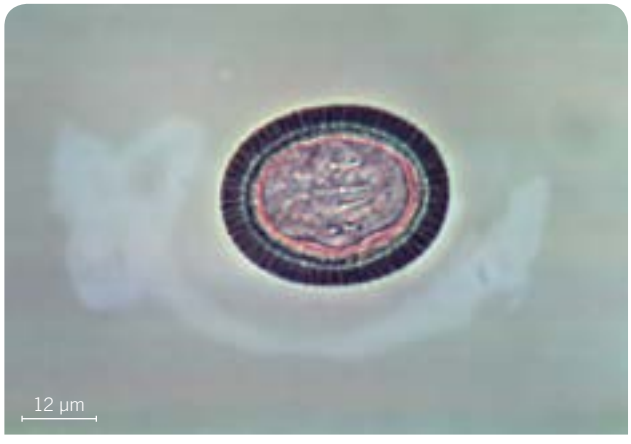


Figure 3. Taeniidae egg.

Synonyms

Infestation with *Echinococcus* larvae (in intermediate hosts)

Hydatid echinococcosis caused by the larva of *E. granulosus* and multilocular or alveolar echinococcosis caused by the larva of *E. multilocularis*.

Infestation with adult Taeniidae

Taeniosis (in dogs or other canids).

Hosts of *E. granulosus*

- Final hosts: canids. Of considerable epidemiological importance in dogs in Europe and Africa.
- Intermediate hosts: numerous wild and domestic species. The larvae infest goats, cattle, horses and pigs, and it has considerable epidemiological importance in sheep in Europe and Africa.

Hosts of *E. multilocularis*

- Final hosts: red and artic fox, wolf, raccoon, jackal, coyote, dog, cat.
- Intermediate hosts: rodents (mainly voles), humans, monkeys, dog, etc.

Humans can become infested and act as intermediate hosts. Human echinococcoses are major zoonoses which are both relatively common and clinically serious.

Geographical distribution

Hydatid echinococcosis is found worldwide and is highly enzootic in important sheep-farming regions and developing countries.

Multilocular echinococcosis is found in the cold and temperate regions of the Northern Hemisphere. There are two genetically distinct parasite populations:

- Strain M1: found in Arctic regions, the principal final host being the arctic fox (*Alopex lagopus*).
- Strain M2: originally found in wilderness regions of Central Europe, it has extended westwards and has now reached the eastern and northern regions of France. The principal final host is the red fox (*Vulpes vulpes*).

Significance

E. granulosus is important because it can cause zoonotic infestation. Several hundred surgical operations to remove hydatid cysts in humans take place in Europe each year.

Although much rarer, multilocular echinococcosis is medically much more serious, leading to death in about 30–50 % of cases.

Biology

E. granulosus

The life cycle of *E. granulosus* requires two hosts, the dog being the principal final host. Canids become infested by eating viscera containing the parasites. A hydatid cyst contains numerous oviferous capsules with several protoscolices per capsule so, for every cyst ingested, hundreds or even thousands of cestodes will appear in the dog's small intestine. The prepatent period is 6 to 8 weeks, depending on how early the strain matures, and dogs will then excrete the oviferous segments for several weeks (3 to 6 months). These segments measure 1 mm and are therefore difficult to see.

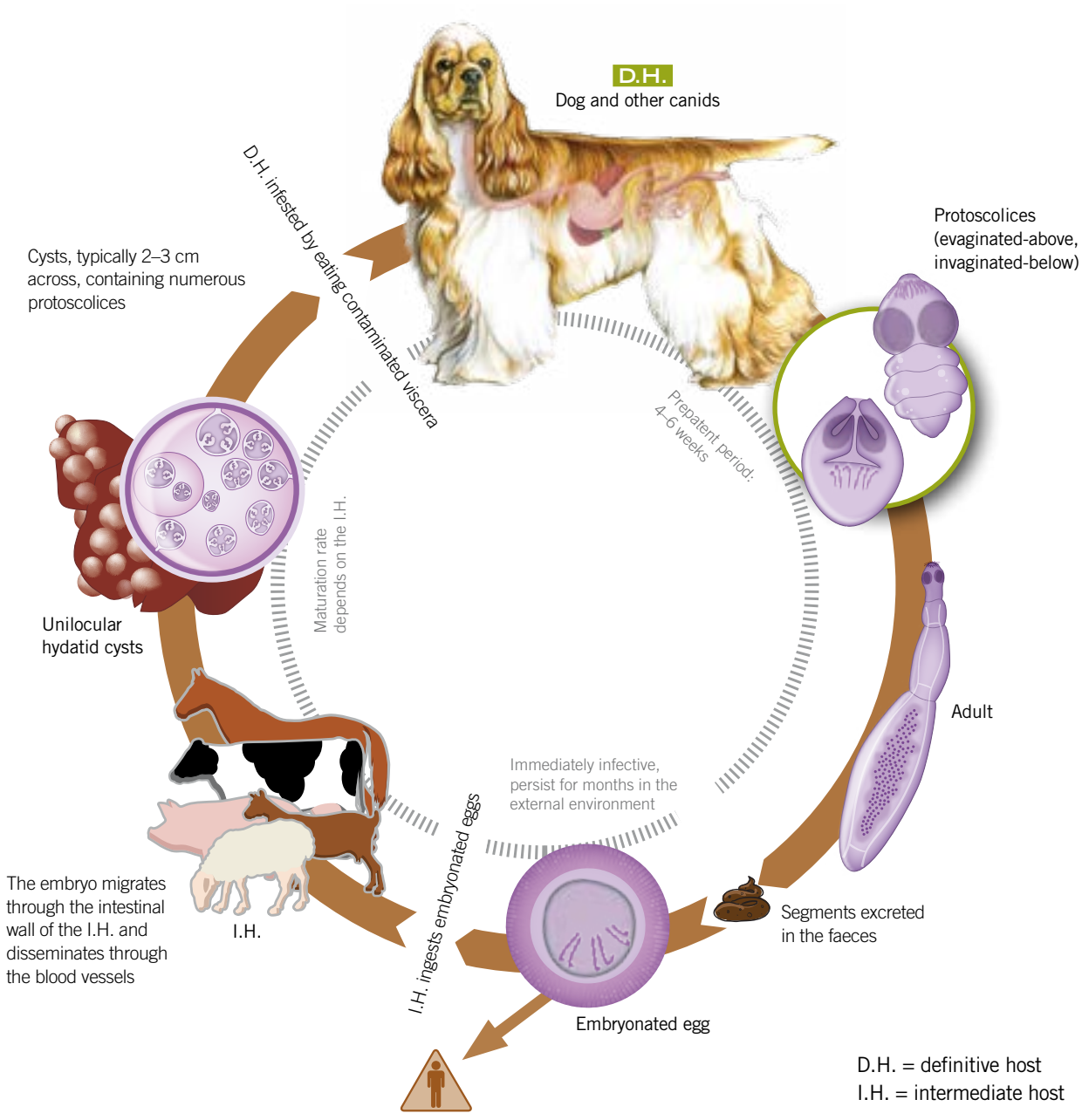
They eventually tear apart, and the eggs are disseminated into the soil. Coprophagous insects and birds, and the weather, are all factors affecting dissemination. Receptive intermediate hosts, which ingest one or more eggs, enable the cycle to continue. The embryo penetrates the intestinal wall and, carried by the blood, reaches various organs and tissues, mostly the lungs and liver. The larva, or hydatid, develops after several months. In sheep, the larvae will be infective to dogs within a year and they remain viable for a number of years.

E. multilocularis

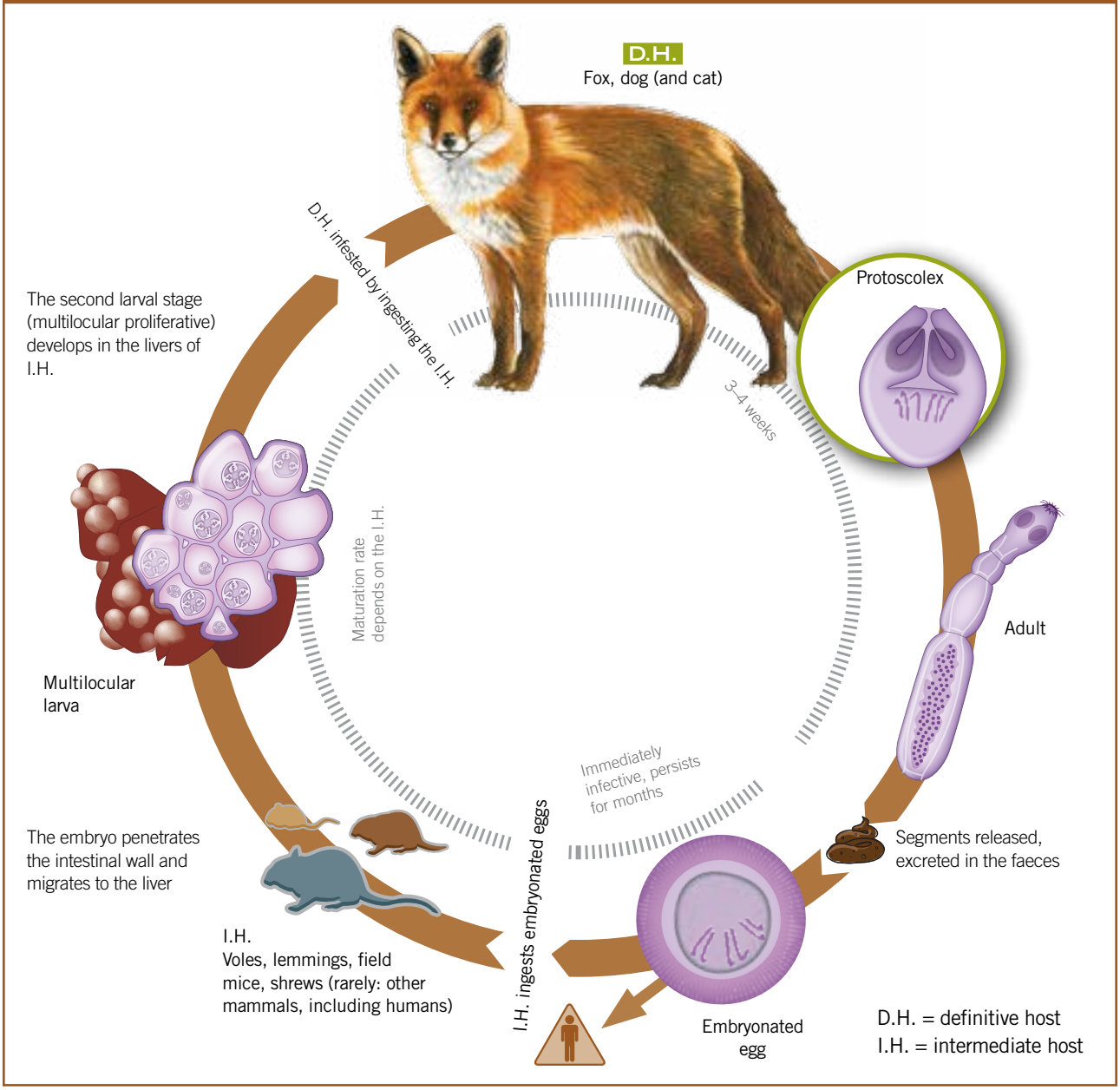
A canid (usually a fox) and a rodent (mainly a field vole) are required during the life cycle of *E. multilocularis*. Adults develop in 4 weeks in the final host, and only survive for 4 months, but infestations are often massive, involving several hundred cestodes. A lack of acquired immunity in the host allows repeated infestations.

E. multilocularis larvae take 1 to 3 months to develop in the hepatic parenchyma of the intermediate rodent host. One of the main parasite reservoirs in Europe is *Arvicola terrestris*, the water vole, but other *Microtidae* can be infested, such as the muskrat *Ondathra zibethica*, the red-backed vole *Clethyrionomys*, or the field vole *Microtus arvalis*.

Life cycle of *Echinococcus granulosus sensu lato*



Life cycle of *Echinococcus multilocularis*



Epidemiology

Hydatid echinococcosis

It is possible to distinguish epidemiological cycles in wild animals from those in domestic rural animals where infestation of urban human populations is possible. In Southern Europe, the sheep/dog cycle is predominant. This does not mean that dogs in an urban environment cannot be infested; they only need to access infested viscera, for example, in a dustbin after sheep have been illegally slaughtered.

Different cycles can be seen in different countries. For instance, a sheep/dog cycle exists alongside a dingo/kangaroo cycle in Australia. Cycles involving zebus in Africa, or buffalo in Asia, have been described. Sometimes pigs also play an important role. These cycles generally involve different host animals and genetically different parasite genotypes (and maybe even species). Ten genotypes of *E. granulosus* have been identified, but are still a subject of controversy.

Echinococcus granulosus: different genotypes or species

- G1 and G2: sheep genotypes, distributed worldwide, zoonotic; marsupial genotypes in Australia, zoonotic.
- G3: cattle and buffalo genotypes (first identified in India), zoonotic.
- G4: horse genotypes in Europe, either less zoonotic or not zoonotic. Proposed name *Echinococcus equinus*.
- G5: cattle genotypes, distributed worldwide, zoonotic. The name *Echinococcus ortleppi* proposed by some authors.
- G6: camel genotypes in Africa, and Near and Middle East, zoonotic. Proposed name *Echinococcus intermedius.*
- G7 and G9: pig genotypes, distributed worldwide, zoonotic.
- G8 and G10: northern genotypes, infesting *Cervidae* in particular, zoonotic, considered by some authors to be ancestral genotypes. Proposed as *Echinococcus canadensis* (G10) and *Echinococcus borealis* (G8).

Sources of parasites

- Intermediate hosts carrying cysts, mainly sheep, and especially those over a year old (the time required for infective larvae to form; 6 months in pigs, 10 months in sheep).
- Dogs or other canids that excrete oviferous segments (a long-lasting source: 6-month life span for the adult cestode. A dog can harbour several thousand echinococcal cestodes; the lack of acquired immunity means that reinfestation is possible).

Mechanisms of infestation in intermediate hosts

Ingestion of food or water contaminated with oviferous segments or embryophores. Possible contamination of humans if embryophores are present on the dog's coat.

Predisposing factors

Dog/sheep association in mountain pastures or on farms. Illegal slaughter of sheep and distribution of offal to carnivores.

Multilocular echinococcosis

This parasitic disease has a woodland element involving wild animal hosts in its epidemiological cycle. Occasional infestation of domestic dogs or cats as final hosts is still a possibility.

Sources of parasites

- Direct: foxes, and sometimes dogs.
- Indirect: rodents (Microtidae).

Mechanisms of infection

- Of the final host: by consuming rodents.
- Of the intermediate host: by ingesting plants or fruits contaminated by *Echinococcus* eggs (blackberries, blueberries, wild strawberries).

Susceptibility

Where human echinococcosis (alveolar or hydatid) is concerned, the parasite is destroyed in some individuals and allowed to develop in others, although there are more seropositive patients than there are individuals infested with a viable parasite.

Predisposing factors

- Behavioural habits of foxes which defecate near vole holes.
- Behavioural changes in infested rodents which become less reactive, and are therefore caught more easily.
- Some intermediate hosts die rapidly, but the life span of others is doubled and they become parasite reservoirs, hence the distinction between species of receptive voles which are called "permissive", and which play a determining role, and species which are susceptible but which do not play an important part in the epidemiology.
- Risk of increased cases of humans infection during periods of rodent proliferation, or because of increasing fox populations. The impact of rabies vaccination should therefore be measured.

Clinical signs

Echinococcus cestodes in the gastro-intestinal tract of the definitive host are tolerated well, although diarrhoea may be seen on rare occasions.

Clinical signs of hydatid echinococcosis (in intermediate hosts)

Hydatidosis in animals is usually asymptomatic, probably because of their relatively short life span, and is characterised by the formation of hydatid cysts consisting of larvae surrounded by the inflammation caused by host's adventitial tissue response (Figs. 4–7).

Echinococcal larvae are vesiculated, spherical, and vary in diameter from a few centimetres to several dozen. They are never visible in isolation, as they are surrounded by the encysted host tissue.

The larva consists of three parts: **a wall**, formed by a proligerous internal membrane and a cuticle; **the germinal elements**, the proligerous capsules and protoscolices; and **the hydatid fluid**. The protoscolices constitute the hydatid sand (approximately 4–6 ml per larva). The hydatid fluid is clear, like mineral water, hence its name, originating from the Greek *hydatis*, watery vesicle. The fluid is pressurised and irritant. Localisation of the larvae is very variable, but they are often found in the lungs (in cattle), or the liver (in sheep). A hydatid cyst varies in size from 2 to 20 cm, has a rigid structure (unlike coenurus and cysticercus) and is opaque. It is found in the tissue or parenchyma of organs and never on the surface, as with hepato-peritoneal cysticercus such as the larva of *Taenia hydatigena*. The fluid inside the cyst is under constant pressure. The cyst may become necrotic and calcify.

The appearance of infested organs may vary:

- Small number of hydatid cysts.
- Many cysts: polycystic echinococcosis.
- Multitude of small cysts on the serosa (hydatid pseudotuberculosis) or in the parenchyma: pseudomultilocular polycystic echinococcosis.
- The infested organ may be structurally affected. For instance, the liver may develop fibrous cell cords and parenchymatous hypertrophy (cirrhosis of the liver).

In humans, the parasite develops slowly but fully and the infested organ will exhibit chronic inflammation and mechanical obstruction. Resorption of toxins by the host is possible when the parasite is found in the liver.

Localised signs

When the parasite is in the liver: cholestatic jaundice, ascites, hepatic encephalopathy, intestinal disorders.

When the parasite is in the lungs: coughing, dyspnoea.

Other sites: kidney and nervous disorders, myalgia, bone fractures.

General signs

Weight loss. Possibility of cysts rupturing and causing anaphylactic shock.

Clinical signs of multilocular echinococcosis (in intermediate hosts)

- In rodents: numerous small cysts (2–5 mm diameter) joined by parasite filaments. The infested organ is hypertrophic and sclerotic, and this will eventually kill the host (Figs. 8 and 9).
- In humans: adventitia absent, numerous parasite vesicles several millimetres in diameter with an alveolar appearance, hence the name "alveolar echinococcosis". Centrifugal growth of the parasite whose proligerous membrane buds in all directions, leaving a necrotic cavity in the centre. Depletion of the parenchyma (discolouration) with proliferating hepatocytes resembling a neoplastic process.

Diagnosis

Diagnosis of echinococcosis in dogs

Clinical diagnosis is impossible; laboratory diagnosis is essential. It may not be possible to find eggs if no segments have been fragmented. It is impossible to distinguish *Echinococcus* eggs from *Taenia* eggs. Specialised labs can search for segments in the faeces, however strict precautions must be taken, given the zoonotic nature of this parasite and the severity of the human disease, which usually requires surgical removal of the cysts.

New diagnostic techniques have been developed, especially the detection of parasite antigens in faeces (coproantigens, for which ELISA kits are available), and specific DNA fragments in faeces, using gene amplification techniques (PCR).

Control measures

Treatment in the definitive host (dog, fox)

The cestodicide of choice is praziquantel, at 5 mg/kg, *per os*. Because infested dogs represent a potential source for humans, they must be treated under veterinary supervision.

Figure 4. *Echinococcus* protoscolex in hydatid cyst.

Figure 5. Free *Echinococcus* protoscolex.

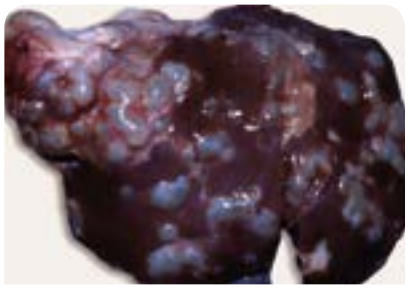


Figure 6. Hydatid larva in liver.


Figure 7. Hydatid larva in the lung of a cow.

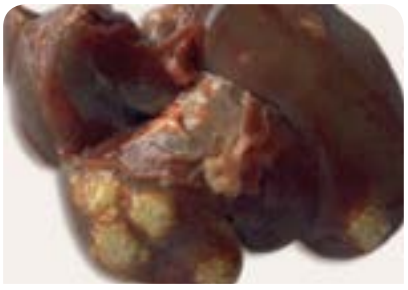


Figure 8. *Echinococcus multilocularis* larva in the liver of a microtid rodent.

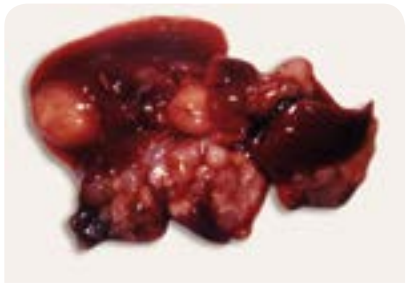


Figure 9. *Echinococcus multilocularis* larva in the liver of a microtid rodent.

Prevention

Preventative measures are directly derived from knowledge of the parasite's life cycle. Simple measures can eradicate hydatidosis, or at least limit its prevalence considerably (eradication has been successful in Iceland, Cyprus and Malta).

Hygiene measures that should be taken when a dog that may be infected visits the surgery: gloves should be worn, and hands should be washed and disinfected.

Prevention in the definitive host

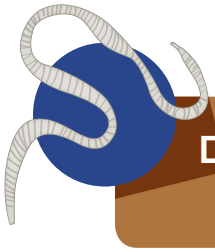
- Screening and treatment of infested dogs. Diagnosis used to consist of examining the faeces after deworming with arecoline, but it is now based on detecting antigens in the faeces. Treatment with praziquantel, followed by removal of faecal matter for 2–3 days. In theory, dogs at risk should be treated every 4 to 6 weeks; in practice, sheep dogs should at least be treated before departing for mountain pastures and upon their return.
- Control of stray dogs and preventing pet dogs from roaming far from home.
- Elimination of wild canids.
- Preventing dogs from accessing slaughterhouses.

Prevention in the intermediate host

- Slaughterhouse inspections and destruction of infected offal.
- Preventing the illegal slaughter of sheep and cattle.
- Educating local populations not to distribute raw offal to carnivores.
- Burial or destruction of sheep and cattle carcasses.
- Vaccination: recombinant vaccines are being tested for use in sheep and their use in highly enzootic regions would break the parasite life cycle. Preventative measures could then be put in place. This type of vaccine has been used for another type of cestodosis: muscular cysticercosis in sheep, caused by *Taenia ovis* larvae.

Prevention of alveolar echinococcosis in enzootic regions

- Wild red fruits and mushrooms should only be consumed once cooked.
- Precautions should be taken when handling foxes.
- Rodent populations should be controlled.
- Foxes can be treated using bait impregnated with praziquantel.



Diphyllobothriosis and spirometrosis

General comments

Diphyllobothriosis is a type of cestodosis caused by the development of a Pseudophyllidea cestode, ***Diphyllobothrium latum***, in the mammalian small intestine and is characterised by the appearance of intestinal disorders associated with anaemia. In other regions of the world (Asia, the Pacific), carnivores may be infested with another Pseudophyllidea, of the same *Diphyllobothrium* genus, or the genus ***Spirometra***, whose life cycle is similar to that of *D. latum*.

The *Spirometra* species that can be diagnosed in domestic carnivores are: *S. mansonoides* (North and South America), *S. erinacei europaei* (Europe), and *S. mansoni* (Asia, Pacific).

Synonyms

Bothriocephalosis, or "fish tapeworm", because ingestion of fish containing the second stage larvae causes infestation.

Hosts

Mammals that eat fish harbouring the second stage (plerocercoid) larvae in their abdominal cavity:

- Wild mammals: Ursidae, Mustelidae, Canidae, Felidae.
- Domestic mammals: dogs, cats and pigs.
- Humans: *D. latum* causes a zoonosis which can be medically significant.

The final hosts for the genus *Spirometra* are wild or domestic carnivores which are likely to ingest the second intermediate hosts (amphibians or fish). The second stage, pleroceroid larvae may cause *larva migrans* in humans because amphibian skin is sometimes used in traditional medicine for its healing properties. Subcutaneous larvae can also penetrate through cuts and sores and cause serious tissue inflammation containing living larvae. This larval cestodosis is called sparganosis.

Importance

Diphyllobothriosis is zoonotic and medically significant, because of the size of the adults (which can reach 12 metres in length), and the loss of vitamin B12 it can cause. This can lead to a state of pernicious anaemia.

Geographical distribution

Diphyllobothrium latum is the species found in the lake regions of Europe (Northern Italy, Switzerland, French Alps, Scandinavia).

There are other species of *Diphyllobothrium*, some of which use ocean-swimming or estuary fish as a second intermediate host (*D. pacificum* in Asia and the Pacific, *D. cameroni* and *D. yonoganense* in Japan, *D. cordatum* in Greenland, and *D. gilajacica* in Russia).

Cestodes of the *Spirometra* genus, especially *Spirometra mansoni*, are mainly found in Asia and the Pacific islands.

Morphology

Diphyllobothrium latum is a cestode whose segments are wider than they are long (10–20 mm wide x 2–3 mm long). The scolex has no hooks and or suckers but does have two longitudinal slits (bothria).

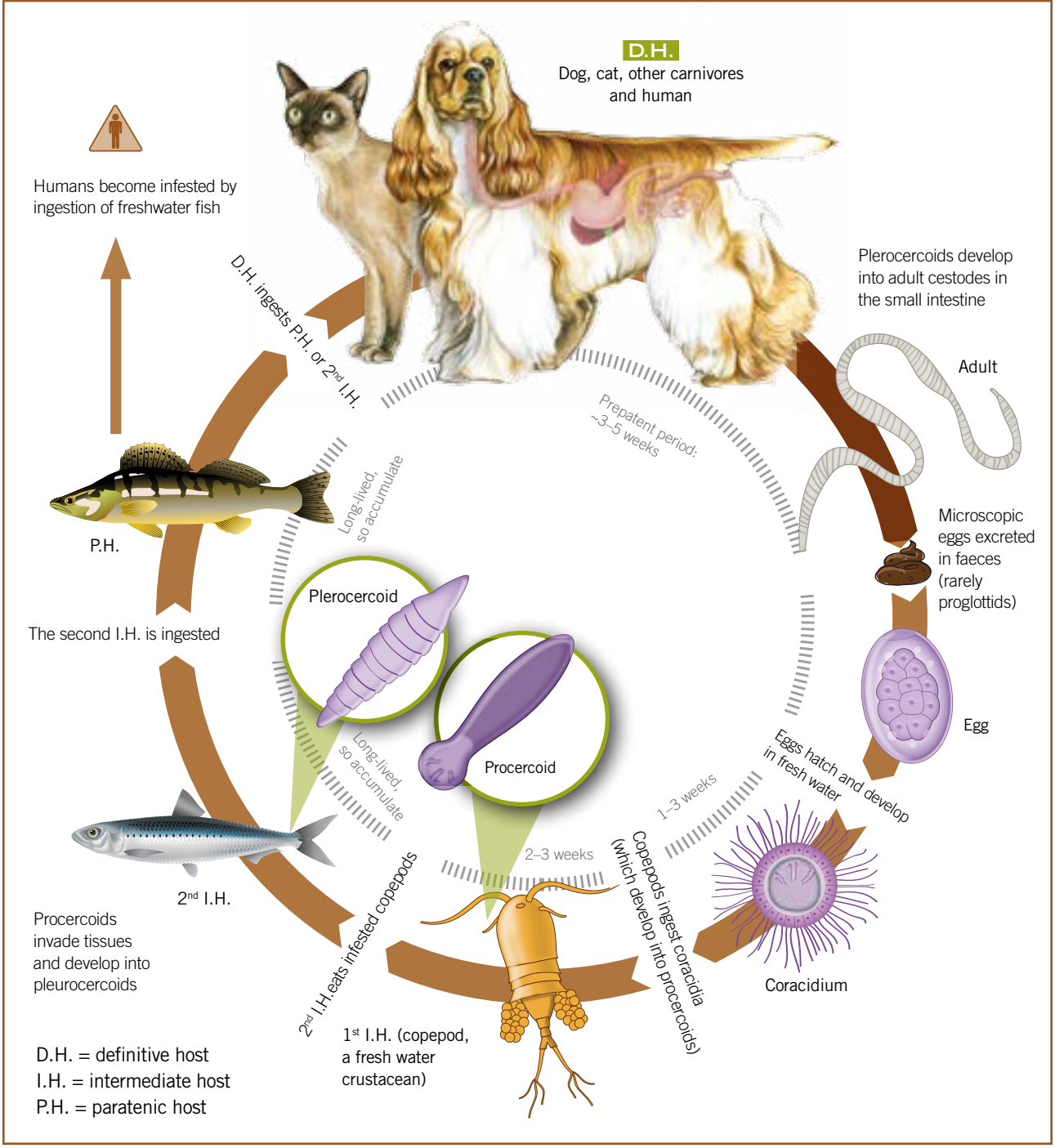
The segments have an egg-laying orifice, the tocostoma, a feature which is only found in Pseudophyllidea and not n Cyclophyllidea. Oviferous segments enclose a rosette-shaped uterus which is dark brown in colour.

Unlike Cyclophyllidea cestodes such as *Taenia* and *Dipylidium*, the eggs are laid and then shed in the host's faeces. They are very similar to trematode eggs (especially fluke eggs) and are oval with a smooth, thin shell (70 × 45 µm). They enclose a yellowish-coloured embryo which gives the eggs a granulated appearance. The eggs are operculated, allowing the embryo (coracidium) to exit eventually.

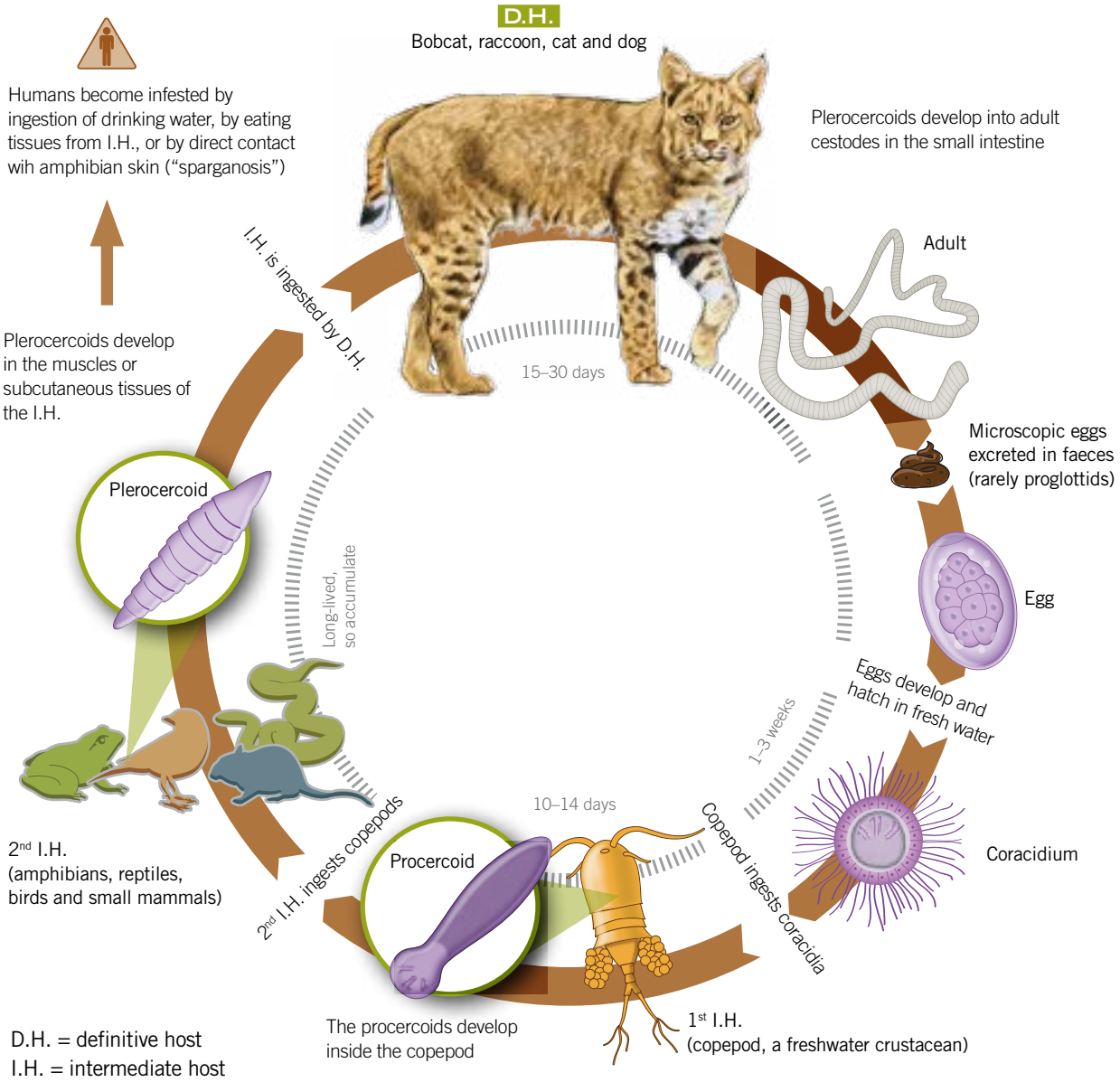
Biology

Bothriocephalic cestodes are found in the small intestine and take approximately 6 weeks to form after an infested intermediate fish host is ingested. The tapeworm sheds the eggs which are found in the faeces and, if the eggs fall into water, they hatch and liberate spherical, ciliated and mobile coracidium larvae. These are ingested by freshwater copepods (Cyclops), and develop into procercoid larvae inside them. The first intermediate host copepods are themselves ingested by second intermediate host fish, in which the plerocercoid larvae develop. The plerocercoid larva resembles an adult and measures 2–3 cm. It is located in the abdominal cavity of the fish. If that particular fish is hunted by another fish, the larva will reencyst and accumulate in the predator fish.

Life cycle of *Diphyllobothrium latum*



Life cycle of *Spirometra* spp.



The plerocercoid larvae can be found in many fish, such as the Esocidae or pike family, (the northern pike, *Esox lucius*), Percidae or perch family (the European perch, *Perca fluviatilis*) and Salmonidae (trout, *Salmo trutta* and *Oncorhynchus mykiss*; and the char or brook trout, arctic salmon and trout, *Salvelinus*).

Epidemiology

Final hosts always become infested by ingesting the second intermediate hosts harbouring the plerocercoid larvae (freshwater fish for *D. latum* and amphibians for *Spirometra*).

The final hosts shed the eggs (cestodes with tocostoma) which develop in an aquatic environment.

These are essentially wild cycle cestodes that are limited to regions where there are lakes and ponds.

Domestic mammals become part of the cycle by chance: for example, a dog that goes fishing with its owner, or walking around a lake.

Clinical signs

D. latum is probably the most pathogenic of the tapeworms. It causes intestinal disorders, abdominal discomfort and diarrhoea, but also vitamin B12 deficiency by inhibiting absorption of this vitamin, so infested animals or humans will present with pernicious anaemia that will only be eradicated with the death of the parasite. Intestinal infestations of cats and dogs with *Spirometra* spp. rarely cause disturbance.

Diagnosis

Diagnosis is made by examining the faeces for eggs, which are usually numerous (Fig. 1).

Control measures

Treatment

Diphyllobothriidae are less sensitive than other cestodes to the active ingredients in many anthelmintic products so praziquantel is the only effective treatment, but it must be used at 8 times the regular dose, i.e., 40 mg/kg instead of 5 mg/kg. It should be given orally.

Prevention

Preventing infestation in dogs and cats includes not feeding them fish or the viscera of fish caught in lakes, particularly mountain lakes.

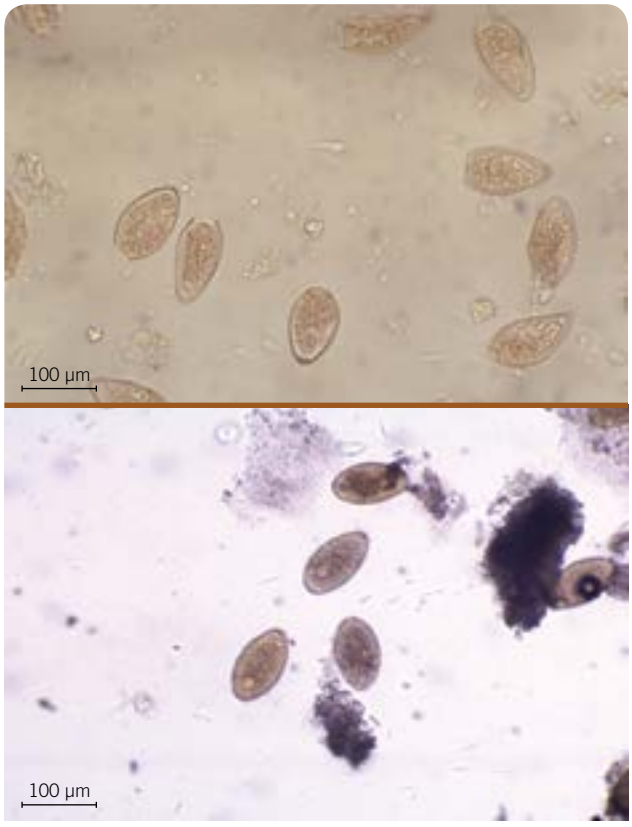
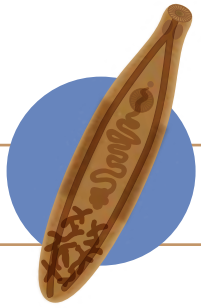


Figure 1. Diphyllobothriidae eggs.



TREMATODOSES

General comments

Carnivores, usually in tropical climates, can be infested by intestinal trematodes (flukes).

These are generally very small trematodes which are not very pathogenic. These parasites are often discovered incidentally, during faecal examination (Fig. 1).

Intestinal flukes which can infest carnivores, belong to different groups.

Order of distomes

Dicrocoeliidae

Trematodes with the ovary posterior to the testicles:

- ***Platynosomum fastosum***: 8 × 2.5 mm. A parasite of the gallbladder and pancreatic ducts which seems to have re-emerged in the southeastern United States and the Caribbean. It is a parasite of wild and domestic carnivores that are infested by ingesting the second intermediate host, a lizard.
- ***Eurytrema procyonis***: 8–16 × 7 mm. A common parasite of the pancreatic ducts in the raccoon (*Procyon lotor*). Infestation in of domestic carnivores is possible; infestations in cats have been reported in the New York region.

Heterophyidae

Very small trematodes (less than 2 mm long) with a broadened posterior end. Most have a sucker enclosing the genital pore (gonotyl) (Fig. 2). Aquatic molluscs and fish play a part in the life cycle, the latter being host to the metacercaria, or encysted stage. Heterophyids are parasites of the small intestine in mammals and birds.

Heterophyid eggs measure approximately 30–40 × 10–20 mm, with variations according to the species:

- ***Heterophyes heterophyes***: 1–2 × 0.5 mm. This small trematode is a parasite of the small intestine in humans and carnivores, found in the Far East and North Africa (e.g., Egypt and Tunisia).
- ***Apophallus donicus***: 0.5–1 × 0.2–0.4 mm. A parasite of the small intestine incats, dogs, foxes and seals that is found in Northern Europe and North America.
- ***Cryptocotyle lingua***: 0.5–2 × 0.2–0.9 mm. A bird parasite, but occasionally found in dogs, cats, seals and mink in the arctic regions of Asia, Europe and North America.
- ***Metagonimus yokogawai*** (Fig. 3): 1–2.5 × 0.5 mm. A parasite found in the Far East and Central Europe (Balkans), which infests carnivores, humans and fish-eating birds.

Troglotrematidae

Trematodes with a genital pore very close to the ventral sucker. Some are intracystic trematode parasites of lung parenchyma (*Paragonimus*). *Nanophyetus* is a parasite of the small intestine:

- ***Nanophyetus salmincola***: a parasite of the carnivore small intestine in North America (mainly on the Pacific coast), where the second intermediate hosts are salmon. It also carries a pathogenic bacterial species which infects wild carnivores and dogs, *Neorickettsia helminthoeca*.

Figure 1. *Mesostephanus* egg.

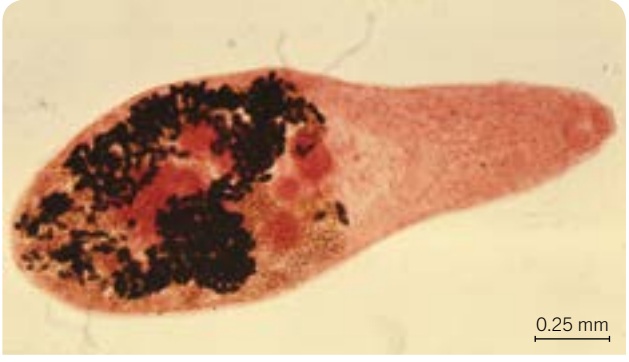


Figure 2. Adult of the genus *Heterophyes*.

Echinostomatidae

Trematodes whose anterior suckers enclose a necklace of 1 or 2 rows of spines. The second intermediate hosts are molluscs or fish. The eggs are large, similar to those of the fasciolid ruminant parasite: 90–150 × 60–80 µm:

- ***Euparyphium melis***: 3.5–12 × 1.5 mm. A parasite of the small intestine of aquatic carnivores, such as otters, and also cats and hedgehogs. It has been found in Europe.
- ***Echinochasmus perfoliatus***: 2–4 × 0.5–1 mm. A parasite of aquatic carnivores, and sometimes cats, dogs, pigs and humans. Found in Central Europe and Asia (Fig. 4).

Order of holostomes

Trematodes whose bodies are divided into two parts, the proximal part having two suckers (oral sucker and tribocytic organ), the sac-like extremity containing the sexual organs.

Diplostomatidae

Trematodes with an oblate anterior end:

- ***Alaria alata, Alaria marcianae, Alaria americana***: 3–6 mm long. The eggs are large: 100–125 × 60–80 µm. Parasites of the small intestine in cats, dogs and wild carnivores. The second intermediate host is an amphibian or a reptile. Paratenic hosts are possible (such as rodents or pigs).

Clinical signs and treatment

These parasites do not usually cause any intestinal disorders and they are discovered accidentally through faecal examination.

Intestinal disorders and pancreatic failure have been described very rarely, in massive infestations.

Various anthelmintic treatments have been tested, including the standard flukicides used in ruminants (nitroxynil, triclabendazole, albendazole) and praziquantel (used in humans against schistosomes). Praziquantel at 75 mg/kg, taken for 2–3 days seems to offer the best results.

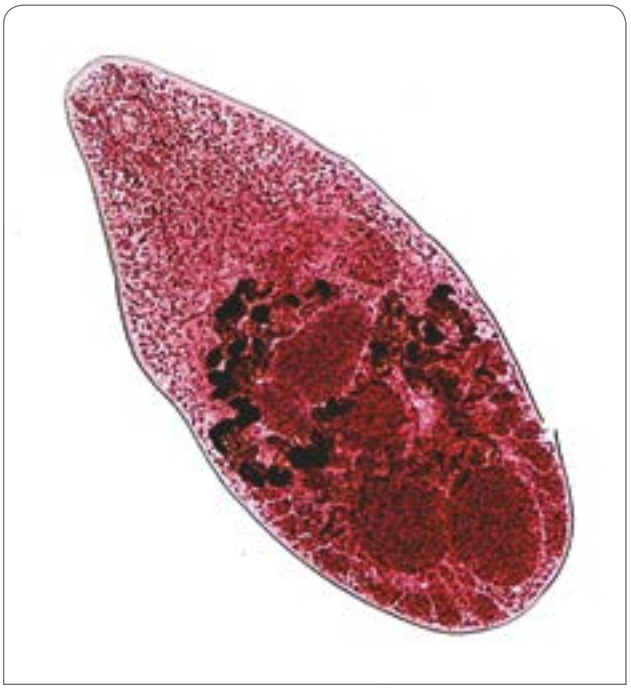


Figure 3. *Metagonimus yokogawai*. Courtesy of Guangxi University.

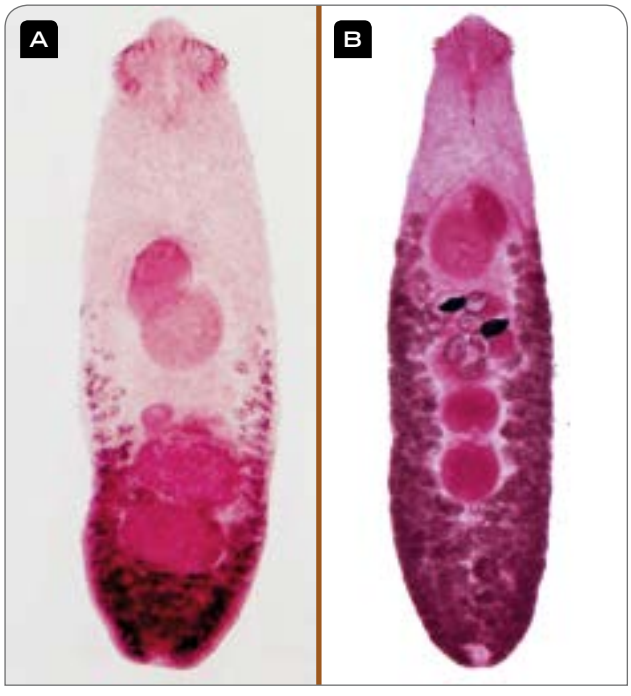


Figure 4. (A) *Echinochasmus liliputanus* and (B) *Echinochasmus perfoliatus*. Courtesy of Guangxi University.

Table 1. Overview of trematodes infesting domestic carnivores.

Location	Family or subfamily	Genus	Species	Risk to humans
Oral cavity	Clinostomatidae	*Clinostomum*	*abdoni, falsatum, kalappahi*	
	Cyathocolidae	*Mesostephanus* *Prohemistomum*	*milvi* *vivax*	+
Small intestine	Diplostomatidae	*Alaria* *Cynodiplostomum* *Fibricola* *Pharyngostomum*	*alata, marcianae* *azimi* *minor* *cordatum*	+
	Echinostomatidae	*Echinochasmus* (Fig. 4) *Echinostoma* *Artyfechinostoma* *Episthmium* *Stephanoprora* *Isthmiophora* *Echinoparyphium*	*brevivitellus, liliputanus, perfoliatus* *ilocanum, revolutum* *malayanum, sufrartyfex* *caninum* *denticulatoides* *melis (?)* *spp.*	+
	Gymnophallidae	*Gymnophalloides*	*seoi*	+
	Heterophyidae Apophallinae	*Apophallus (Rossicotrema)*	*donicus, muehlingi, venustus*	+
	Ascocotylinae	*Ascocotyle* *(= Phagicola = Parascocotyle)*	*angrense, arnoldoi, ascolonga, longa,* *longicollis, minuta, pachycystis*	+ *(longa)*
	Centrocestinae	*Centrocestus* *Pygidiopsis* *Pygidiopsoides*	*armatus, caninus, formosanum genata, longus,* *ormulus, summa* *spindalis*	+
	Cryptocotylinae	*Cryptocotyle*	*concavum, lingua, quinqueangularis*	+
	Euryhelminthinae	*Euryhelmis*	*monorchis, pacifica, squamula*	
	Galactosominae	*Galactosomum*	*fregatae*	
	Haplorchiinae	*Haplorchis* *Procerovum* *Stellantchasmus*	*microrchis, parataichui, pumilio, sprenti,* *taichui, yokogawai,* *calderoni, minutum, varium,* *amplicaecum, falcatus*	+
	Heterophyinae	*Heterophyes*	*aequalis, continua, heterophyes, nocens*	+
	Metagoniminae	*Metagonimus* *Dexiogonimus*	*minutus, takahashii, yokogawai* *ciureanus*	+
	Stictodorinae	*Stictodora*	*fuscata, lari, sawakinenesis*	+
		Acanthotrema *Diorchitrema*	*felis* *formosanus, pseudocirratum*	+
	Microphallidae	*Microphalloides*	*vajrasthirae*	
	Nanophyetidae	*Nanophyetus (Troglotrema)*	*salmincola*	
	Neodiplostomidae	*Neodiplostomum*	*seoulense*	
	Plagiorchidae	*Plagiorchis*	*massino, muris*	
Biliary and/or pancreatic ducts	Dicrocoeliidae	*Eurytrema* *Euparadistomum* *Platynosomum*	*procyonis* *buckleyi, heischi, pearsoni* *concinnum, illiciens, fastosum*	
	Opisthorchiidae	*Amphimerus*	*pseudofelineus*	
		Clonorchis	*sinensis*	+

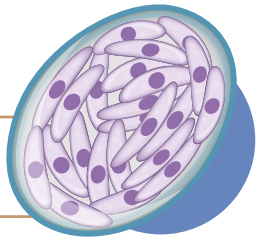
Table 1. Overview of trematodes infesting domestic carnivores (continuation).

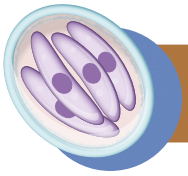
Location	Family or subfamily	Genus	Species	Risk to humans
Biliary and/or pancreatic ducts	Opisthorchiidae	*Metorchis*	*albidus, conjunctus, orientalis*	+
		Opisthorchis (Fig. 5)		
		Parametorchis	*complexus*	
		Paropisthorchis	*caninus*	
		Pseudamphistomum	*truncatum*	+
Nasal cavities	Orchipedidae	*Orchipedum*	*isostoma*	
	Troglotrematidae	*Troglotrema*	*mustelae*	
Lungs?	Microphallidae	*Microphalloides*	*vajrasthirae*	
Lungs	Troglotrematidae	*Paragonimus*	*africanus, amazonicus, caliensis, heterotremus, inca, kellicotti, mexicanus, miyazaki, ohirai, peruvianus, pulmonalis, siamensis, skrjabini, uterobilateralis, westermani*	+
		Euparagonimus	*cenocopiosus*	+
Blood vessels	Schistosomatidae	*Schistosoma*	*japonicum*	+

Table 2. Characteristics of common zoonotic trematodes infesting carnivores.

Animal infestation	Parasite	Intermediate hosts (direct source)	Human infestation	Distribution
Echinostomatidosis	*Echinochasmus*	Freshwater fish	Intestinal flukes	China, Korea, Japan Egypt Russia, Hungary, Denmark, Italy
	Echinostoma spp.	Fish, tadpoles, snails, clams, mussels, frogs		Brazil Egypt Asia, Philippines, Australia Russia, Europe
	Echinoparyphium	Snails, tadpoles, frogs		Indonesia
	Artyfechinostomum	Snails		India, China, Indonesia, Thailand, Malaysia, Philippines
	Isthmiophora	Tadpoles, loaches, clams		Europe, USA
Heterophyidosis	*Haplorchis*	Fresh/brackish water fish (Gambusia, carp, mullets)	Intestinal flukes	Asian Pacific (Philippines)
	Heterophyes			Southeast Asia Mediterranean (Greece to Tunisia) and Middle East Peru
	Metagonimus			Southeast Asia: from Japan and Korea to India, Spain, Central Europe
Opisthorchiidosis	*Clonorchis*	Fish (Cyprinidae, carps.)	Liver flukes	Southeast Asia
	Opisthorchis spp.			Europe, Russia, India
	Metorchis			North and Central America
	Pseudamphistomum			Europe, Siberia, India
Paragonimosis	*Paragonimus* spp.	Crabs, crayfish, shrimps	Lung flukes	China, Japan, Southeast Asia West tropical Africa Ecuador, Peru, Venezuela, Mexico

PROTOZOOSES





Cryptosporidiosis

General comments

Cryptosporidiosis caused by ***Cryptosporidium parvum*** is an infectious protozoan disease, which has been observed in a number of mammalian species, including dogs and cats. It causes diarrhoea which can be acute in young animals and older individuals with impaired immune systems. Several species have now been described, including *Cryptosporidium felis*, which affects cats. Mammalian cryptosporidiosis can be zoonotic, and some genotypes have a higher zoonotic potential than others. Cryptosporidiosis has not been well described in carnivores, unlike in other animals such as ruminants.

Taxonomy

Cryptosporidium are Apicomplexa protozoa belonging to the class Coccidea (*sensu lato*) and to the family **Cryptosporiidae**, characterised by a homoxenous life cycle and extracytoplasmic intracellular development (just below the cell membrane).

Morphology

The life cycle of *C. parvum* takes place in enterocytes in the distal parts of the microvilli in the small intestine, mainly the ileum. The various stages of development can be seen in the brush borders of these enterocytes after staining with Giemsa.

The average size of the subspherical oocysts is 5 × 4 µm and they contain a very visible oocyst residuum, and four vermiform sporozoites which are difficult to discern by light microscopy.

Biology

These parasites become established in the brush border of the enterocyte without penetrating the cytoplasm. They then become enclosed in a vacuole alongside the rest of the cell, by a feeder and attachment organelle, which explains the particular positioning of the *Cryptosporidium*: they are intracellular because they are surrounded by a cell membrane, but they are also extracytoplasmic, because they are separated from the cytoplasm.

The parasite then undergoes schizogony, gametogony and sporogony inside the enterocyte and the sporulated oocysts are then shed into the intestinal lumen. These oocysts consist of two types:

- Thin-walled oocysts which can shed their sporozoites *in situ* to start a new cycle.
- Thick-walled oocysts, which are shed into the environment in the faeces.

Incubation has been shown to take from 2 to 5 days in experimental infection.

Epidemiology

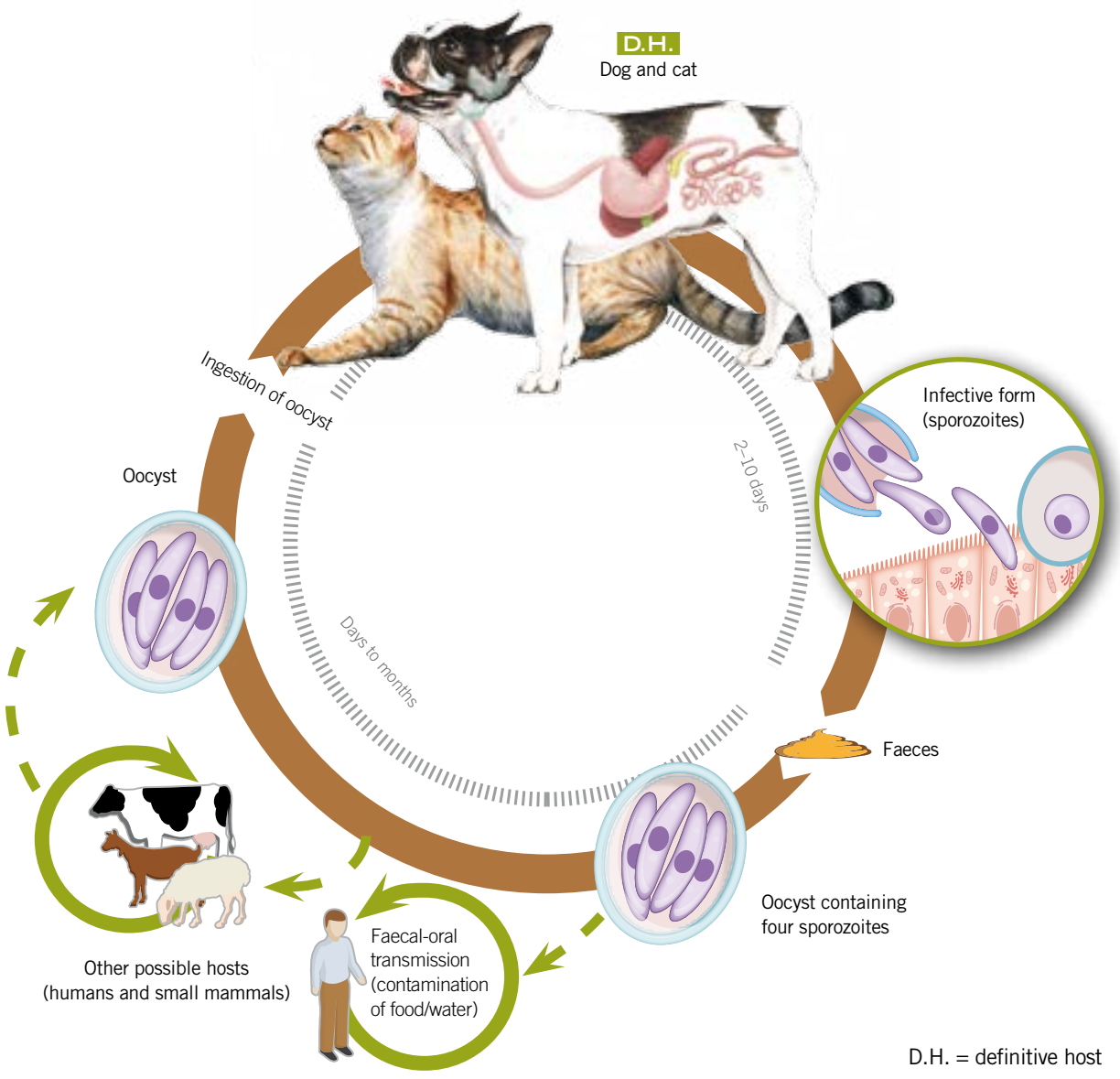
Cryptosporidiosis due to *C. parvum* is a disease which mainly affects young animals in their first weeks of life and, to a lesser degree, older animals with impaired immune systems. Several cases have been described in dogs after bouts of canine distemper, and in adult cats following *C. felis* infection.

The faeces of infected animals provide new sources of parasites. Numerous species of mammals can be cryptosporidia carriers, particularly ruminants which can excrete large numbers of oocysts.

Oocysts shed in the faeces are immediately infective and particularly resistant in the external environment, so they can survive for a long time, especially in water. They are sensitive to desiccation, heat (destroyed in 30 minutes at 65 °C), and cold (destroyed in 24 hours at -18 °C) but disinfectants have little effect: only 5 % ammonia and 10 % formalin solution have proven effective, and bleach may even promote excystation.

Outbreaks have been observed in animals kept in communal housing, such as breeding facilities and kennels.

Life cycle of *Cryptosporidium* spp.



Clinical signs and lesions

Cryptosporidiosis is often asymptomatic in dogs and cats. Persistent or intermittent diarrhoea is seen in clinical cases (often in puppies and kittens), causing weight loss, emaciation and a change in the general condition of the animal. The disease can be fatal in particularly severe cases, and in animals with impaired immune systems.

The clinical signs will be more marked if the animal is also suffering from other diseases, such as an enterotropic virus (coronavirus) or bacterial infection (*Colibacillus*, *Salmonella*). Gastritis due to *Cryptosporidium felis* infection seems to be increasing in cats.

Lesions

Lesions are mainly found in the small intestine, particularly the ileum. Histological examination shows destruction of the epithelial cells at the top of the villi, and replacement of these cells by cuboidal cells. Atrophy and fusion of the villi have also been noted.

Diagnosis

Diagnosis based on clinical signs is impossible. Intermittent or persistent diarrhoea in animals in poor condition can be the only guide to diagnosis and confirmation is based on the detection of oocysts in faecal matter. This cannot be done using the standard coproscopic techniques used in carnivores because of the size of the oocytes. Screening requires either specific colouration, such as a modified Ziehl-Neelsen stain, or flotation in a sucrose solution (Figs. 1 and 2).

Control measures

Treatment

In the absence of specific data on the treatment of cryptosporidiosis in carnivores, treatment of clinical signs by supportive therapy, including fluid replacement, is probably good practice.

Two drugs have also shown some success in other animal species and are worth trying in the most serious cases: these are paromomycin at a dose of 100 mg/kg/day *per os* for 7 days and halofuginone lactate at a dose of 100 mg/kg/day *per os* for 3 days. Nitazoxanide is currently used in the treatment of human cryptosporidiosis in children in the United States and a regimen has also been approved to treat animals (see *Antiprotozoals*, page 359).

Prevention

Prevention is limited in carnivores. On farms, and in breeding facilities and kennels, prevention must be based on keeping housing clean and dry (daily removal of faecal matter), and disinfecting surfaces. Oocysts are very resistant and are only destroyed by high-pressure water vapour (130 bars) and by ammonia-based disinfectants. Some physical alterations to the premises may be necessary, especially concreting of communal areas to enable better disinfection.

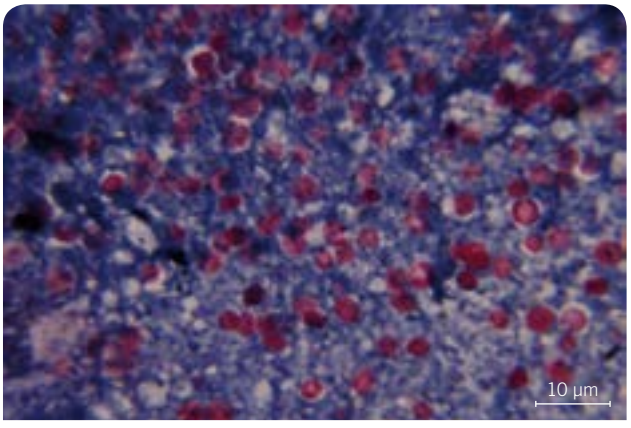


Figure 1. *Cryptosporidium* oocysts (in red) (Ziehl-Neelsen staining).

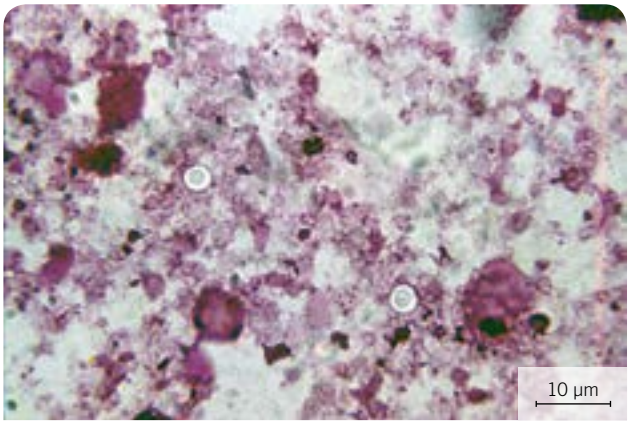
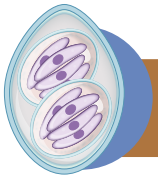


Figure 2. Two *Cryptosporidium* oocysts (white) (Gomori staining).



Coccidioses

General comments

Canine and feline coccidioses are infectious diseases caused by intestinal Apicomplexan protozoa of the class Coccidea. Coccidioses are characterised by the development of generally severe enteritis with diarrhoea which can sometimes be haemorrhagic. Coccidioses are caused by coccidia which are specific to dogs - ***Isospora canis***, ***Isospora ohioensis***, ***Sarcocystis* spp.** specific to dogs, ***Hammondia heydorni*** and ***Neospora caninum***, or to cats - ***Isospora felis*** and ***Isospora rivolta***, ***Sarcocystis* spp** specific to cats, ***Hammondia hammondi*** and ***Toxoplasma gondii***.

Coccidiosis is a protozoan disease with worldwide distribution. Coccidiosis caused by *Isospora* spp. is seen in young animals, where it is common and frequently underestimated.

Coccidiosis caused by *Sarcocystis* spp. is linked to the ingestion of raw or undercooked meat.

Taxonomy

Coccidia are Apicomplexan protozoa of the subphylum Sporozoa, belonging to the class Coccidea (Coccidia *sensu lato*) and to the suborder Eimeriorina. Dog and cat coccidia belong to three different families:

- The Isosporidae family, subfamily Isosporinae; *I. canis*, *I. ohioensis*, *I. felis* and *I. rivolta*, characterised by a homoxenous life cycle.
- The Isosporidae family, subfamily Toxoplasmatinae; *H. heydorni* and *H. hammondi*, *T. gondii*, *N. caninum* and *Besnoitia* spp., with a heteroxenous life cycle. An asexually reproductive phase is followed by a sexual reproduction phase in the definitive host dog or cat. There is a variety of intermediate hosts, including birds and rodents for *Hammondia* reptiles and mammals for *Besnoitia*; many birds and mammals (including humans) for *T. gondii* and many mammals (but not humans) for *N. caninum*.
- The Isosporidae family, subfamily Sarcocystinae; *Sarcocystis* spp. which have a heteroxenous cycle and only the sexual reproduction phase takes place in the definitive host dog or cat.

Morphology

These protozoa appear in different forms: intracellular forms called schizonts (meronts) and gamonts (gametocytes) can be seen in intestinal cells.

Free forms also exist in the intestinal lumen. Some have a very short life, and are therefore rarely observed: these are the sporozoites, merozoites and microgametes. Others are more resistant and are found in the faeces: these are the oocysts, with the exception of *Sarcocystis* spp., which shed sporocysts. Diagnosis of the type of coccidiosis is based on finding one or the other on faecal examination.

Biology

Coccidia, which cause coccidiosis in dogs and cats, multiply in the intestinal tract and invade the mucous membranes. They may even migrate to extraintestinal locations, as is the case with genus *Isospora*. Multiplication of the parasites generally takes place in the small intestine, with the exception of *I. ohioensis* where it generally takes place in the large intestine (caecum and colon).

The pathogenesis of coccidiosis is not well understood, but it seems to be connected with the destruction of the intestinal epithelium by the parasite and the host's inflammatory reaction, which causes oedema and thickening of the mucous membranes. These lesions then reduce the absorption by the intestine. There is also a generally toxic effect, which would explain cases of neurological dysfunction.

Epidemiology

The epidemiology of coccidiosis depends on the species of coccidia involved because their life cycles are different.

Coccidiosis caused by *Isospora* spp.

Coccidiosis caused by *Isospora* is both widespread and common. It generally affects young animals living in a communal environment (breeding facilities, kennels, pet shops), or those that have just been acquired from one of these sources.

The initial symptoms appear at around 3 weeks of age and are often observed after stress (weaning, sale, transport). Clinical signs are more severe in animals with impaired immunity. Protective immunity is triggered by a primary infection, so clinical coccidiosis is less common in adult animals.

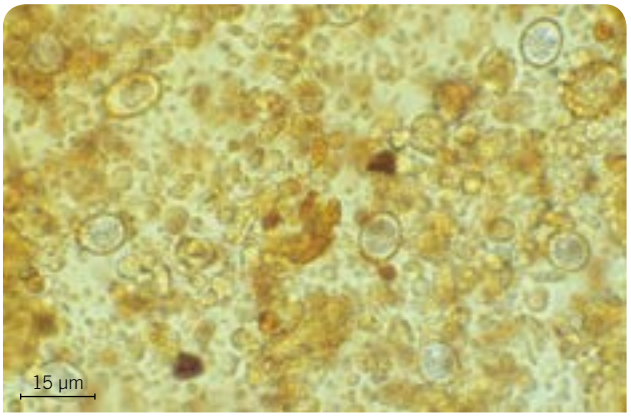


Figure 1. Oocysts of *Isospora felis*. Coproscopy.

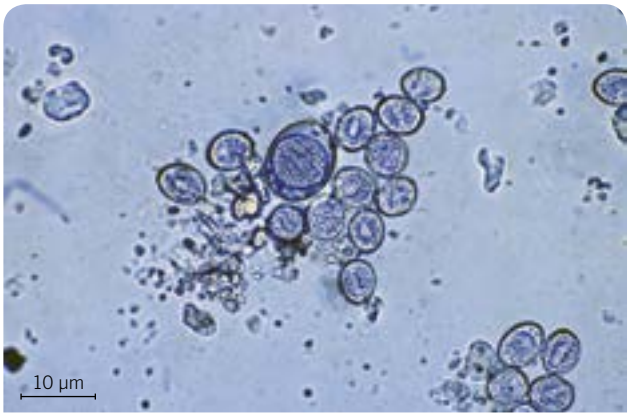


Figure 2. *Isospora* oocysts (*Isospora canis* [large] - *Isospora ohioensis* [small]). Coproscopy.

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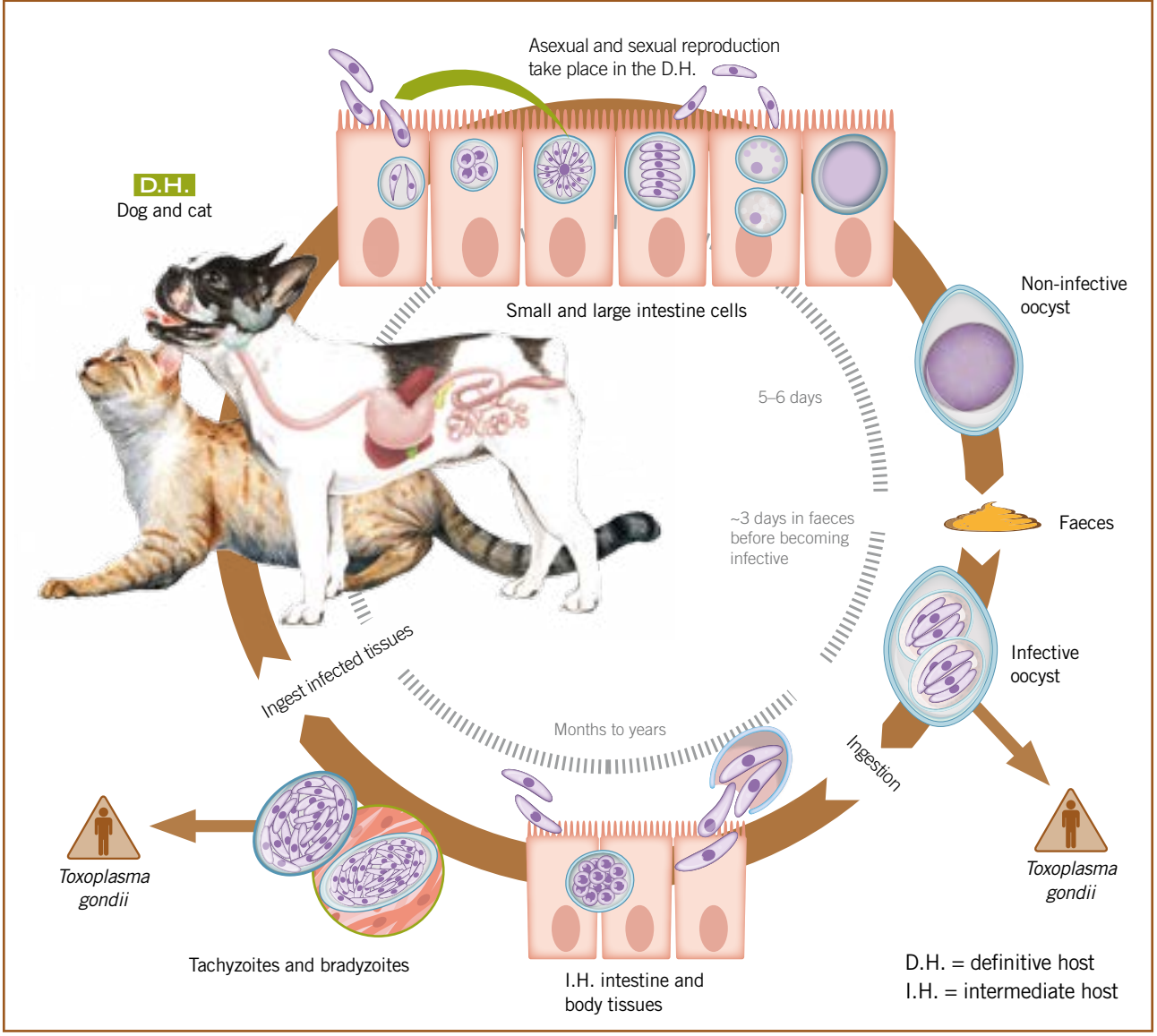
Life cycle of heteroxenous coccidia (*Besnoitia, Hammondia, Toxoplasma* and *Neospora* spp.)

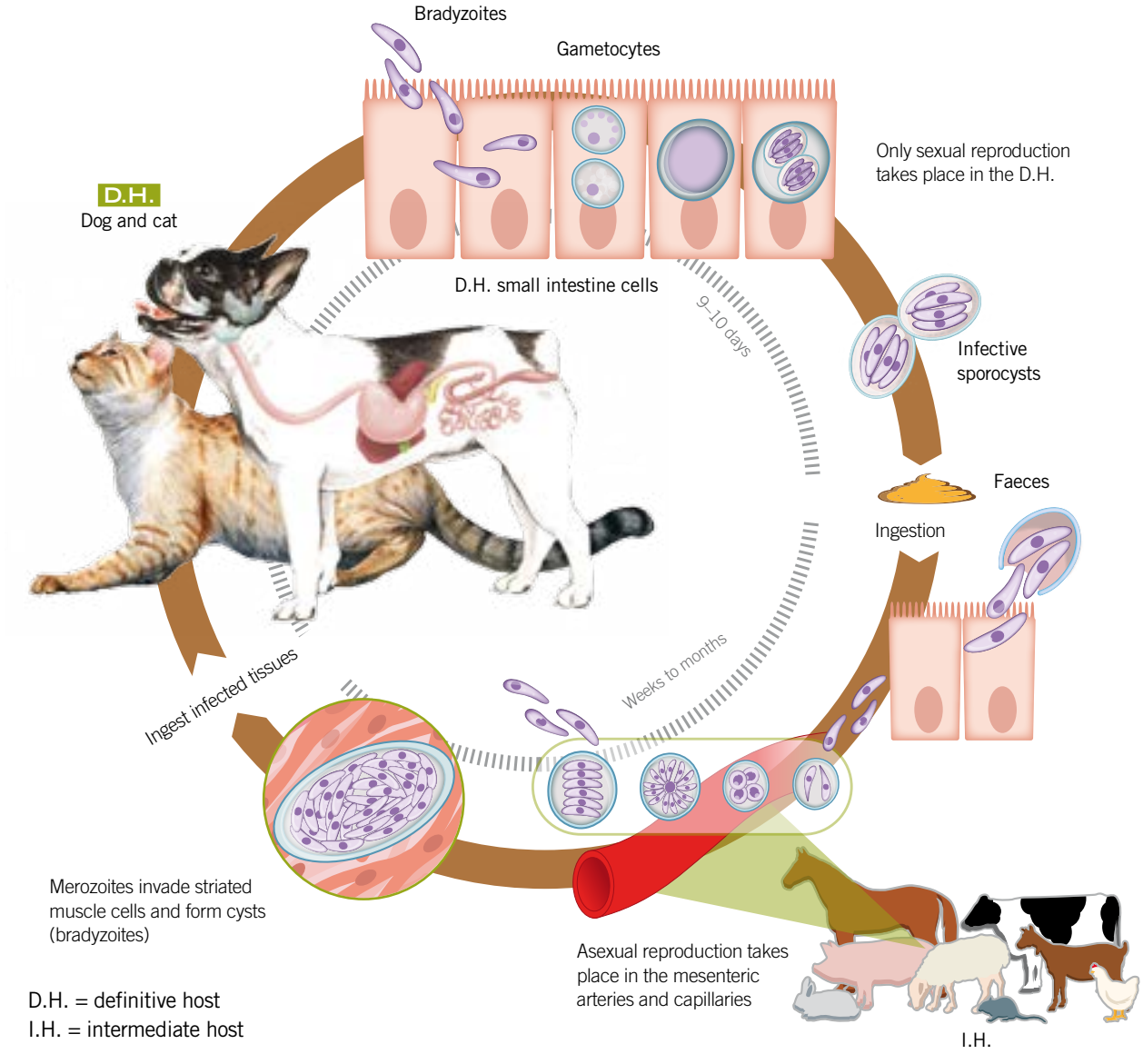


Carrier dogs and cats are sources of parasites and they shed oocysts in their faeces for a limited period of time (Figs. 1 and 2). The parasitic forms may survive for a long time in various organs, enabling persistent infection, so breeding animals are a potential source of infection. A carnivore developing a concurrent disease may also re-excrete oocysts and many mammals, such as mice, can become paratenic hosts after ingesting sporulated oocysts. These hosts retain a latent form. Infection of new carnivore hosts occurs after they ingest the viscera of these paratenic hosts.

Oocysts shed into the external environment must undergo sporulation in order to become infective. Sporulation takes at least 24 hours, but is sometimes longer, depending on the humidity and temperature. Sporulated oocysts consist of a smooth wall enclosing two sporocysts, each containing four sporozoites. They are very resistant and can survive in the environment for 1 to 2 years, but they are sensitive to desiccation, heat (destroyed in 30 minutes at 60 °C), ultraviolet light, and cold (destroyed in 3 months at 0 °C and in 7 days at 25 °C). Many disinfectants have little or no effect: only ammonia, and to a lesser degree, Cresyl, is effective.

PDF

Life cycle of *Sarcocystis* spp.



Coccidiosis caused by *Sarcocystis* or *Hammondia* spp.

This can affect cats and dogs of any age after ingestion of raw or undercooked meat (Fig. 3). Immunity does not develop very well after primary infection and there is no cross-immunity between species so reinfection is always a possibility.

Sources of the parasite are (a) indirect: carrier dogs or cats which excrete sporocysts or oocysts in their faeces for a short period of time (Fig. 4); or (b) direct: intermediate host carriers of bradyzoite cysts (Miescher's tubules, or sarcosporidian cysts and *Hammondia* cysts) in their skeletal and myocardial muscles (Fig. 5).

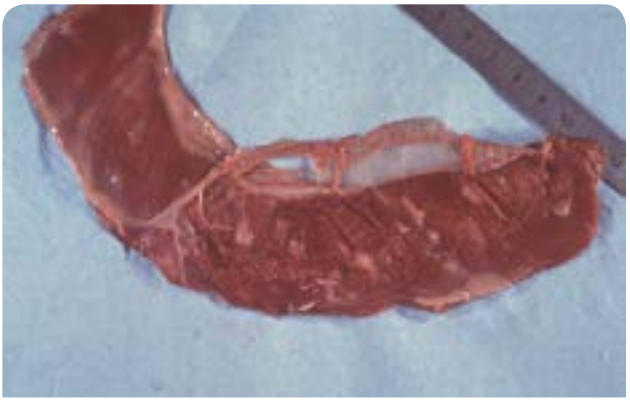


Figure 3. Lesions of sarcocystosis in small ruminant muscle tissue.

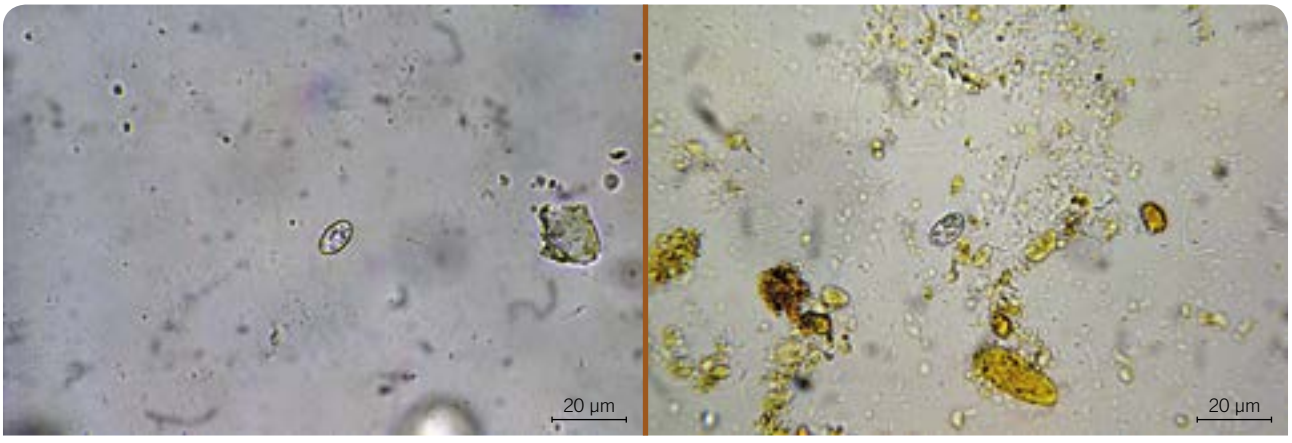


Figure 4. *Sarcocystis* oocysts. Coproscopy.

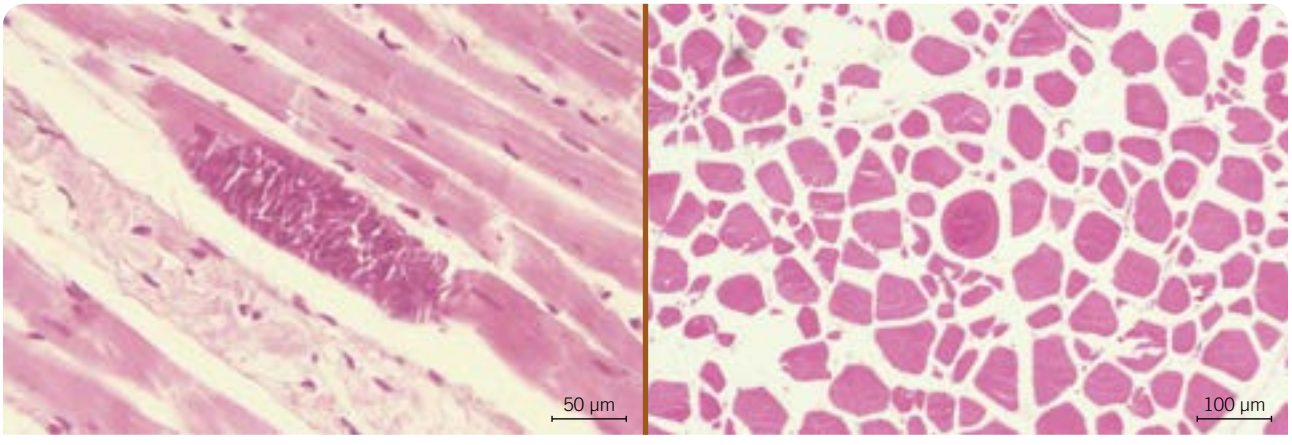


Figure 5. *Sarcosystis* cyst in muscle tissue. Haematoxylin-eosin stain.

Table 1. *Sarcocystis* and *Hammondia* species with cats or dogs as definitive hosts.

Species	Definitive host	Intermediate host
Sarcocystis cruzi	Dog	Cattle
Sarcocystis ovicanis	Dog	Sheep
Sarcocystis moulei	Dog	Goat
Sarcocystis cameli	Dog	Dromedary
Sarcocystis levinei	Dog	Water buffalo
Sarcocystis miescheriana	Dog	Pig
Sarcocystis berframi	Dog	Horse
Sarcocystis horvathi	Dog	Chicken
Hammondia heydorni	Dog	Cattle and other ruminants
Hammondia hammondi	Cat	Mice and other small rodents
Sarcocystis hirsuta	Cat	Cattle
Sarcocystis gigantea (syn. *S. tenella*)	Cat	Sheep
Sarcocystis porcifelis	Cat	Pig
Sarcocystis cuniculi	Cat	Rabbit
Sarcocystis leporum	Cat	Rabbit

Each of these coccidia has a specific intermediate host.

Sarcocystis sporocysts that are shed into the external environment are directly infective and measure from 11–20 × 8–16 µm, depending on the species. They contain four sporozoites, as well as a sporocyst residuum.

Hammondia oocysts resemble those of *Toxoplasma*; they are spherical and measure 13 × 11 µm. They need to undergo sporulation to become infective and this takes at least 24 hours, and often longer, depending on humidity and temperature. The oocysts and sporocysts are very resistant in the environment, like those of *Isospora*.

Coccidiosis caused by *Besnoitia besnoiti*

The definitive host of *Besnoitia besnoiti* was supposed to be the cat, where the sexual reproduction phase takes place, forming oocysts measuring 14–16 × 12–14 µm which sporulate on the ground.

Cattle are the only known intermediate host (Fig. 6) and the asexual multiplication phase (the tachyzoite stage) takes place in their endothelial cells, then cysts are produced in their fibroblast cells. *Besnoitia* cysts have the peculiarity of being hypertrophic, particularly in the ocular conjunctiva, and they can reach 200–600 µm in diameter which is just visible to the naked eye (Figs. 7 and 8).

Bovine besnoitiosis is considered to be an emerging disease in Europe. This parasite is transmitted to cattle by vectors (flying insects).

Figure 6. Cow showing signs of besnoitiosis (elephantiasis).

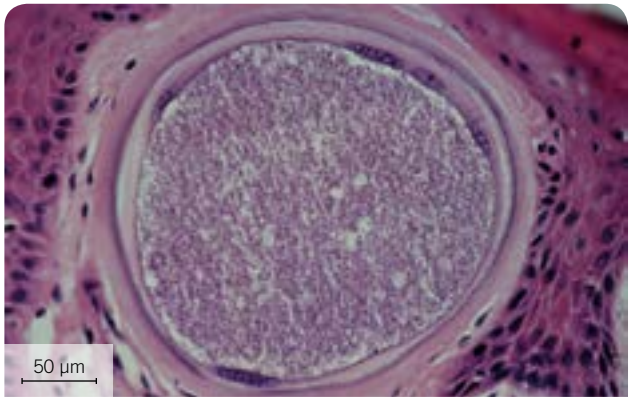


Figure 7. *Besnoitia* cyst. Subcutaneous location. Biopsy. Haematoxylin-eosin stain.

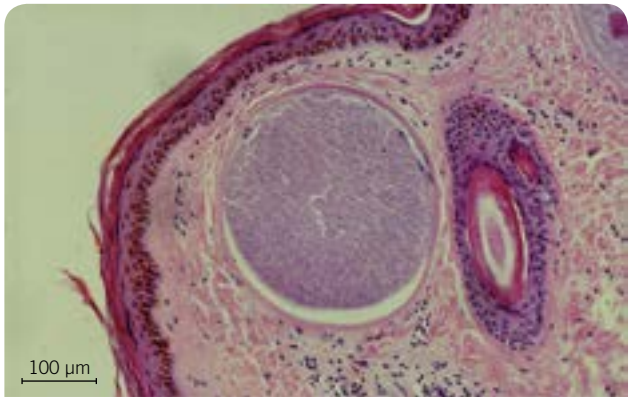


Figure 8. *Besnoitia* cyst. Subcutaneous location. Biopsy. Haematoxylin-eosin stain.

Coccidiosis caused by *Neospora caninum*

A whole chapter is devoted to this coccidial disease, because of its specificity to dogs (see *Neosporosis*, page 182).

Coccidiosis caused by *Toxoplasma gondii*

A whole chapter is devoted to this coccidial disease, because of its specificity to cats (see *Toxoplasmosis*, page 175).

Clinical signs

Coccidiosis caused by *Isospora* spp.

This coccidiosis presents in a variety of forms, from asymptomatic coccidiosis to a subacute, or severe, type of coccidal disorder.

The asymptomatic form is more common in well-kept breeding facilities and it corresponds to a primary infection with a low parasite burden for the first weeks of life, during which time immunity is acquired.

The subclinical form reduces growth rate. The acute form is characterised by foul-smelling, mucoid-to-haemorrhagic diarrhoea, sometimes with abdominal pain, accompanied by a change in the general condition of the animal. Other signs include anaemia, dehydration, anorexia and weight loss. A febrile syndrome and encephalitic disorders may also be seen. Death may occur in a few days in extreme cases, but improvement is usually seen in 7 to 10 days.

The chronic form is characterised by pasty, foul-smelling diarrhoea. The general condition of the animal gradually changes and there is significant weight loss which may result in stunting.

Coccidiosis caused by *Sarcocystis* and *Hammondia* spp.

These types of coccidiosis are usually asymptomatic, although episodes of diarrhoea can sometimes be seen.

Coccidiosis caused by *Neospora caninum* (in dog) and *Toxoplasma gondii* (in cat)

These two coccidia are not very pathogenic in their definitive hosts. Infection is asymptomatic and only the excretion of oocysts is conspicuous (Figs. 9 and 10), however, both dogs and cats may present with toxoplasmosis, as can all the other intermediate hosts (Fig. 11).

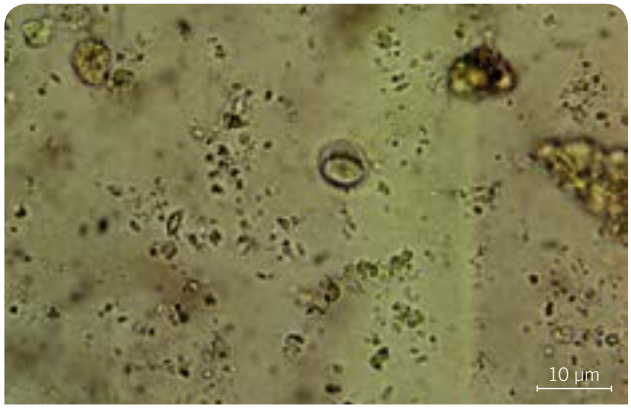


Figure 9. *Toxoplasma* oocyst. Coproscopy.

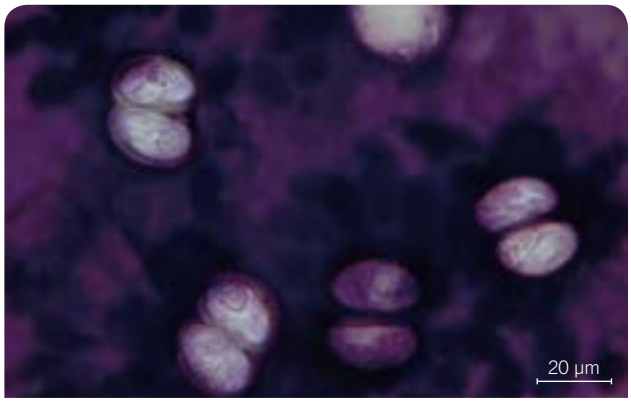


Figure 10. Sporulated oocysts of *Toxoplasma*. Confocal microscopy.

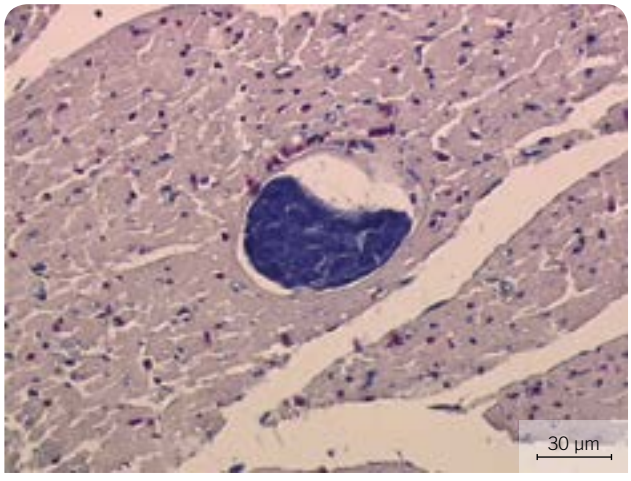


Figure 11. *Toxoplasma* cyst in brain tissue. Histology. Haematoxylin-eosin stain.

Diagnosis

Clinical diagnosis is impossible: only the presence of frank or gelatinous blood in the faeces of a young animal will suggest coccidiosis. Differential diagnosis must include canine parvovirus infection in young dogs, and panleukopenia (feline distemper) in kittens.

Confirmation is based on the detection of oocysts or sporocysts in the faeces (Fig. 12), which is relatively simple using coproscopy after enrichment with a dense liquid.

The presence of oocysts in the faeces is not always associated with clinical coccidiosis, so faecal examination always needs to accompany careful consideration of the clinical signs.

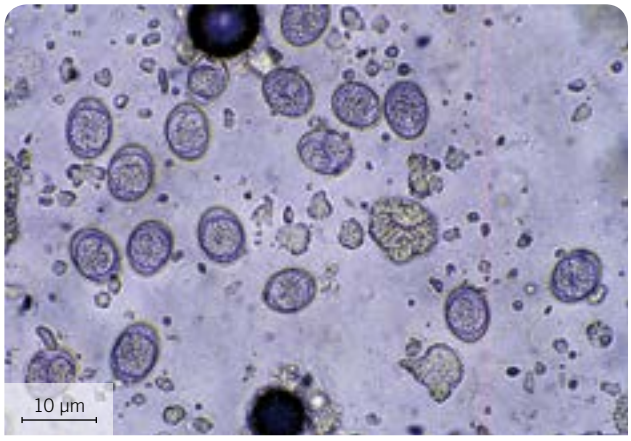


Figure 12. *Isospora* oocysts. Coproscopy.

Table 2. Characteristics of oocysts and sporocysts that can be found in dog and cat faeces. It should be noted that *Hammondia*, *Neospora* and *Toxoplasma* oocycts cannot be differentiated.

Species	Parasitic elements shed in faeces	Average size	Infective forms (after sporulation)
Isospora canis (dog)	Oocyst with one rounded end (basal) and one pointed end (conical)	38 × 30 µm	Oocyst with two sporocysts, each containing four sporozoites
Isospora ohioensis (dog)	Oocyst with one rounded end (basal) and one pointed end (conical)	23 × 19 µm	Oocyst with two sporocysts, each containing four sporozoites
Isospora felis (cat)	Oocyst with one rounded end (basal) and one pointed end (conical)	38–51 × 27–39 µm	Oocyst with two sporocysts, each containing four sporozoites
Isospora rivolta (cat)	Oocyst with one rounded end (basal) and one pointed end (conical)	21–28 × 18–23 µm	Oocyst with two sporocysts, each containing four sporozoites
Sarcocystis spp.	Sporocyst containing four sporozoites	12 × 8 µm to 20 × 16 µm depending on the species	Directly infective sporocyst
Hammondia spp.	Subspherical oocyst	13 × 11 µm	Oocyst with two sporocysts, each containing four sporozoites
Neospora caninum (dog)	Subspherical oocyst	13 × 11 µm	Oocyst with two sporocysts, each containing four sporozoites
Toxoplasma gondii (cat)	Subspherical oocyst	12–15 × 10–13 µm	Oocyst with two sporocysts, each containing four sporozoites

Control measures

Treatment

Outcome is normally favourable after administration of a symptomatic treatment. The specific treatment traditionally consists of sulphonamides, and the most active is considered to be sulfadimethoxine. It is used at a dose of 30 mg/kg/day *per os* for 10 to 14 days, and is sometimes combined with baquiloprim. A combination of trimethoprim and sulfadiazine at a dose of 15 mg (of sulfadiazine)/kg/day *per os* for 6 days can also be used.

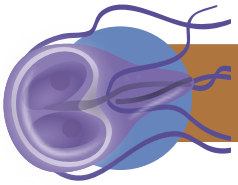
Toltrazuril and diclazuril, newer drugs that were originally used in poultry and ruminant farming, are often used by dog breeders.

Toltrazuril is labelled for use at a dosage of 9 mg/kg, in combination with emodepside (0.45 mg/kg) orally, to treat *Isospora* infection as well as gastrointestinal nematodes in dogs.

Diclazuril can be used at a dosage of 2.5 mg/kg *per os*.

Prevention

Prevention is limited in carnivores. The housing in breeding facilities and kennels should be kept clean and dry (through daily removal of faecal matter), and surfaces, such as floors and walls should be disinfected regularly. Sporulated oocysts are highly resistant but they can be destroyed by high-pressure water vapour (130 bars) and ammonia-based disinfectants. Other measures may also be adopted, such as concreting surfaces in communal areas to allow better disinfection.



Giardiosis

General comments

Giardiosis is a protozoan infection of the small intestine, characterised by the development of enteritis with chronic diarrhoea, often appearing steatorrhoeic. Protozoa of the genus ***Giardia*** (formerly *Lamblia*) infect amphibians (*G. agilis*), reptiles (*G. muris*), birds (*G. muris*), and mammals (*G. muris* in rodents, ***G. duodenalis*** (syn. *G. intestinalis*) in numerous mammals, including humans).

Giardiosis is a protozoan infection which occurs worldwide because the parasitic reservoir consists of a great number of healthy carriers.

It is an infection shared by animals and humans, although the genotype adapted to each species is quite distinct. This intestinal parasite is common in cats and dogs but underestimated in veterinary medicine, because of its difficult diagnosis, which is still based on faecal examination.

Taxonomy

Giardia duodenalis is a flagellated protozoan (phylum Sarcomastigophora, subphylum Mastigophora), belonging to the order Diplomonadida (bilaterally symmetrical due to the incomplete longitudinal division of the parasite) and to the family Hexamitidae (having eight flagella).

Morphology

This protozoan has two active stages: trophozoites, measuring 6–8 × 12–15 µm and equipped with a sucking disk which enables them to adhere to the surface of intestinal epithelial cells; and the quiescent, cyst stage, which is shed in faecal matter, and is resistant and infective (Fig. 1).

The trophozoite stage is rarely seen, except on examination of fresh faeces.

The cysts are ovoid and contain two to four nuclei, as well as the residue of the flagella and mid-body parts, giving the impression of an S shape in the centre. These elements found in the cyst correspond to two incompletely formed trophozoites and the cysts measure 7–10 × 8–12 µm. Cysts ingested by carnivores will each liberate two trophozoites.

Biology

In dogs and cats, the parasites are found mostly in the lower two thirds of the small intestine (duodenum, jejunum and anterior ileum). However, this distribution varies according to the individual and to their diet. Very rarely, *Giardia* can invade the entire mucosa, but no cases have been reported of the parasite infiltrating the biliary tract in carnivores as in humans.

Giardia live fixed to the surface of the brush border of intestinal cells, mainly at the base of the villi. The sucking process is maintained by movement of the flagella and also by a mechanism of specific cell recognition. Fixation is associated with a protein (lectin) in the plasma membrane which binds to the glycosylated residues of the host cell.

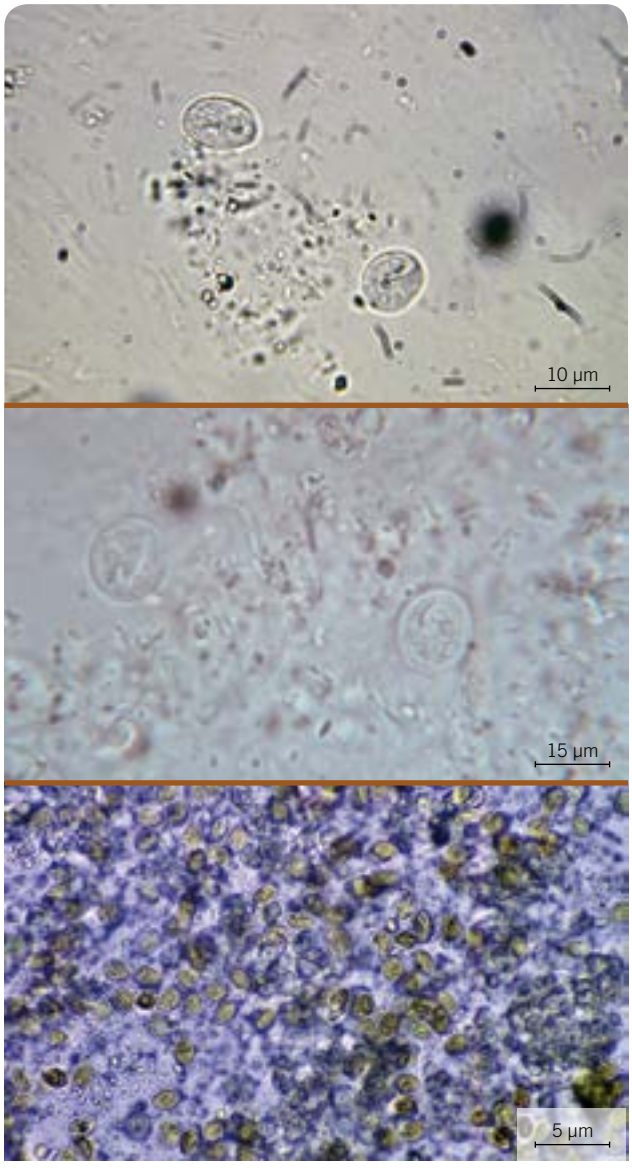


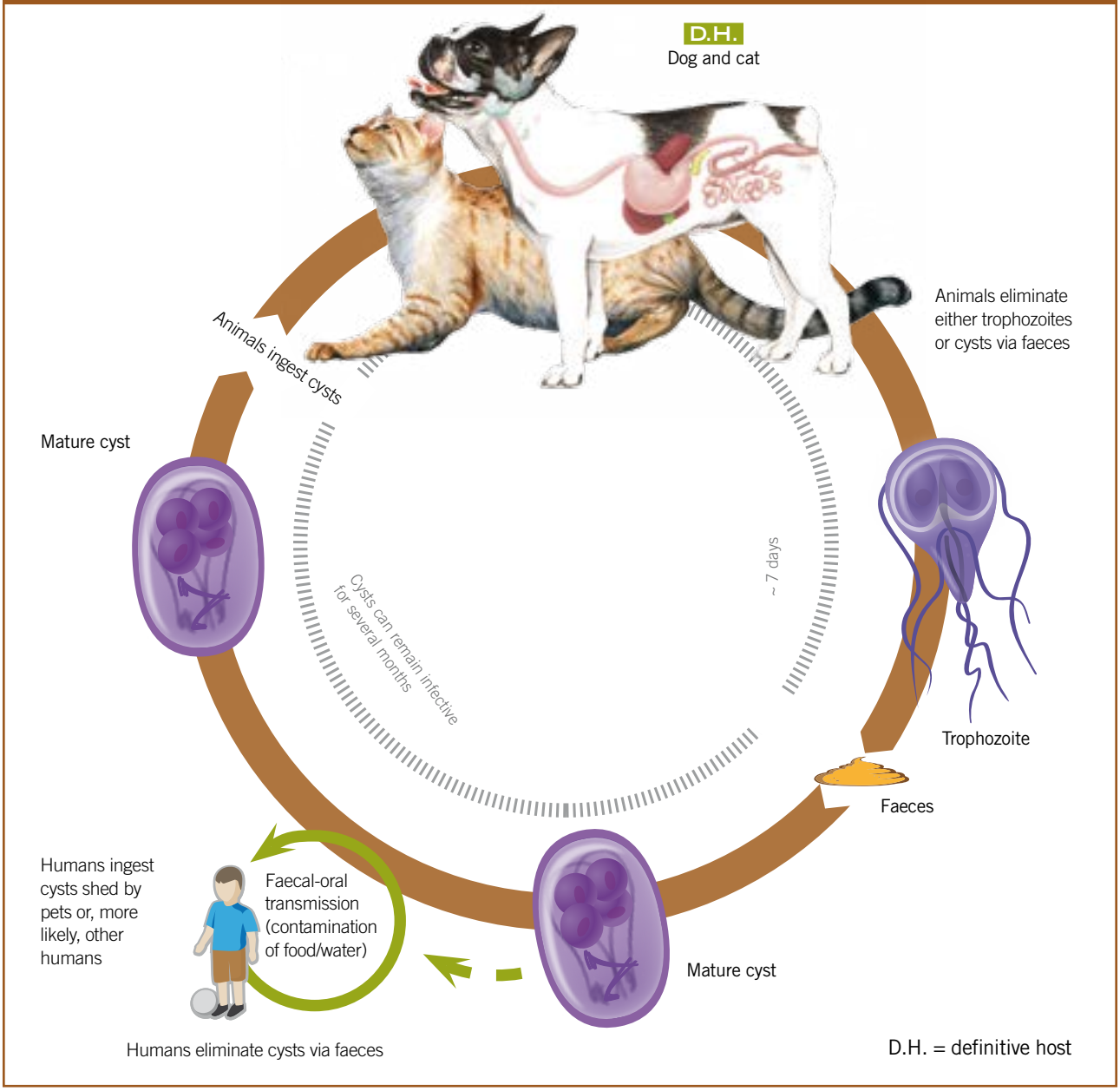
Figure 1. *Giardia* cysts (no staining, except Lugol's iodine in the bottom image). Coproscopy.

Giardia feed by a process of pinocytosis, engulfing nutritional elements mostly through the dorsal membrane. They multiply by binary division of the trophozoites in the small intestine, where they carpet the surface of the intestinal epithelium.

Life cycle

The life cycle of *Giardia duodenalis* is simple, with alternating phases of trophozoite multiplication and cyst formation. Infection occurs when the cysts are digested by gastric or duodenal enzymes, then the two trophozoites contained in the cyst mature and the cyst will then liberate them into the duodenum. Experimentally, this stage takes only 10 to 30 minutes. The trophozoites then actively multiply by simple longitudinal binary fission in 5 to 40 hours. In humans, it has been shown that the speed of trophozoite growth depends on the *Giardia* strain and on the immune and nutritional status of the host. No sexually reproductive stage is known.

Life cycle of *Giardia duodenalis*



Cysts form gradually during passage from the small to the large intestine, by a mechanism which is not yet well understood. pH, concentration of bile salts, and certain fatty acids all play a part. The cyst contains two incompletely formed trophozoites (two to four scarcely visible nuclei, fragments of crescent-shaped ventral disks).

Various characteristics of the host and the parasite are involved and the main host factors seem to be: pre-existing malabsorption, deficient nutritional status and various physical and chemical changes which alter conditions for the parasite's development in the gastro-intestinal tract. Atrophy of the villi is partly caused by the host's inflammatory and immune responses. The virulence varies according to the strain.

- *Giardia* carpet the brush border and mechanically interfere with absorption by the intestine (lactase and sucrase activity is disrupted in humans).
- They also cause mucus hypersecretion which disrupts exchange at the enterocyte level and shortening of the villi reduces the surface area for exchange.
- Enterocyte renewal is accelerated, which may cause defective glucose and amino acid transport (immature cells have inadequate enzyme systems). *Giardia* also interfere with fat absorption by inhibiting pancreatic lipase.
- The parasites may release toxic substances which can affect the metabolism of the brush border and inhibit a number of enzyme systems. A direct cytopathic effect of reduced sucrase and alkaline phosphatase activity in enterocytes has been demonstrated. Cytopathic effects have been observed in some cell lines (Vero and Hela) *in vitro*.
- *Giardia* may disrupt bile secretion and promote bacterial proliferation, though the diarrhoea observed in *Giardia* infection is caused by an absorption disorder rather than increased secretion.

Young animals are usually more sensitive than adults, which can spontaneously limit the infection and this disease is more common in individuals in a state of altered immunity.

Immunity is based on both humoral and cell mechanisms, and antibodies and effector cells may cooperate to eliminate the parasite. Specific antibodies have been found in humans and mice, IgA and IgG in particular. They are transmitted through the mother's milk and determine the resistance of unweaned mice from mothers which have been infected. The local lymphocyte reaction also seems to play a role in eliminating the parasites, and causes epithelial lesions (atrophied villi).

Epidemiology

Healthy human or animal carriers are sources of the parasite and infection takes place when cysts are ingested. They are relatively fragile in the external environment and are sensitive to desiccation and to ordinary disinfectants. Cysts tend to accumulate in moist environments (such as vegetable gardens) and are conveyed by contaminated water or foodstuffs (raw vegetables, for instance). They can resist for several weeks in a moist environment (2 months at 8 °C, 1 month at 21 °C, only 4 days at 37 °C).

It is likely that any period of weakened immunity will facilitate clinical expression of the disease following infection, or cause a latent infected state to turn into a full-blown disease, as it does in humans.

Giardiosis is common in dogs and cats in Europe and in the United States, affecting animals of all ages, with a higher prevalence in young animals from weaning to 2 years old. According to a number of epidemiological studies, it can be found in approximately 10 % of faecal examinations in carnivores that have diarrhoea and are taken to the vet for examination. Epidemiological studies in breeding kennels indicate that the parasite is present in nearly 100 % of cases, and that the prevalence of infection in dogs is up to 50 %. These figures are identical or slightly higher than in helminth infections, which makes *G. duodenalis* one of the most common intestinal parasites in domestic carnivores.

Clinical signs

Carnivores that have ingested cysts will usually present clinical signs one week later, but the incubation period varies greatly from one animal to another, and some show no signs of infection and become carriers. There are two forms of the disease: a rare, acute form, and a common chronic form.

The acute form is characterised by watery diarrhoea which is resistant to treatment, colic and bloating, and a change in the overall condition of the animal. There is usually no fever.

The chronic form is characterised by pasty, foul-smelling diarrhoea and steatorrhoea which causes faeces to be yellowish and fatty. Frequency of emission is often increased, from one to five or six times a day. Abdominal pain is perceptible on palpation. The overall condition of the animal will gradually deteriorate and weight loss will occur as the animal generally retains its appetite but will be polydipsic.

Lesions

Giardiosis lesions vary greatly in severity and location. The intestinal villi are the site of massive lymphocyte infiltration and a mixed inflammatory reaction involving macrophages, granulocytes and lymphocytes can also be seen. Parasites are sometimes found in the lamina propria and necrosis at the apex of the villi has been described in dogs.

Diagnosis

Clinical diagnosis is difficult, as only the steatorrhoea and chronic diarrhoea which develops over a number of days or weeks, punctuated by phases of remission, will indicate giardiosis.

Diagnosis must differentiate between giardiosis and bacterial enteritis, which is usually accompanied by fever, and exocrine pancreatic insufficiency, which presents a very similar clinical picture in young dogs.

Confirmation is based on identification of *Giardia* cysts in the faeces. Elimination of the cysts may be variable, which is why a second test should be carried out about a week after a negative faecal examination. Elimination of cysts is generally massive in clinical giardiosis, and they are easily identified using coproscopy after enrichment, but the number of cysts is much lower in asymptomatic carriers.

Giardia cysts are more or less rounded, approximately 8 × 12 µm in size, therefore not easily visible with the ×10 objective lens used for helminth eggs. They are quite light in colour, with a thin, smooth shell, and enclose a number of elements which are sometimes difficult to see, corresponding to two to four nuclei and fragments of flagella. Stains which are fixed by the cyst walls can be used so that internal structures can be seen more easily. It is then possible to distinguish the cysts on first observation of the slide at a low magnification (×100 = obj. 10). Lugol's iodine solution is useful and and is made up of 10 g sublimated iodine, 50 g potassium iodide and water qs 100 mL. A drop of iodine on the edge of the slide is all that is required and this will make *Giardia* cysts take on a very clear orange hue (Fig. 2). Merthiolate-iodine-formaldehyde (MIF) is another iodine-based stain which is used in human medicine and can also be used to detect parasites. Iodine does not colour coccidial oocysts or sporocysts, and facilitates differential diagnosis of *Giardia* cysts.

These cysts can also be seen on duodenal smears during endoscopy, but this method requires more equipment and is more difficult than faecal examination. It also does not necessarily provide more accurate results.

Summary of coproscopy methods

The flotation technique, using a high-density liquid, is the most commonly used. Most dense solutions are suitable because *Giardia* cysts are not very dense. Simple flotation solutions, such as magnesium sulphate with a specific gravity (s.g.) of 1.28 or zinc sulphate (s.g. 1.33) are suitable. Centrifuge equipment is not necessary. A method which is simple and which can be used to detect all intestinal parasites consists of mixing 1 g of faecal matter with 10 mL of the dense solution in a standard haemolysis tube and placing a coverslip over the tube so that it is in contact with the liquid. After about 10 minutes, any protozoan cysts or helminth eggs become stuck to the underside of the coverslip which can then be placed on a slide for observation under the microscope. If a centrifuge is used, place a coverslip on the tube, centrifuge for 5 minutes at 1500 rpm, then recover the coverslip.

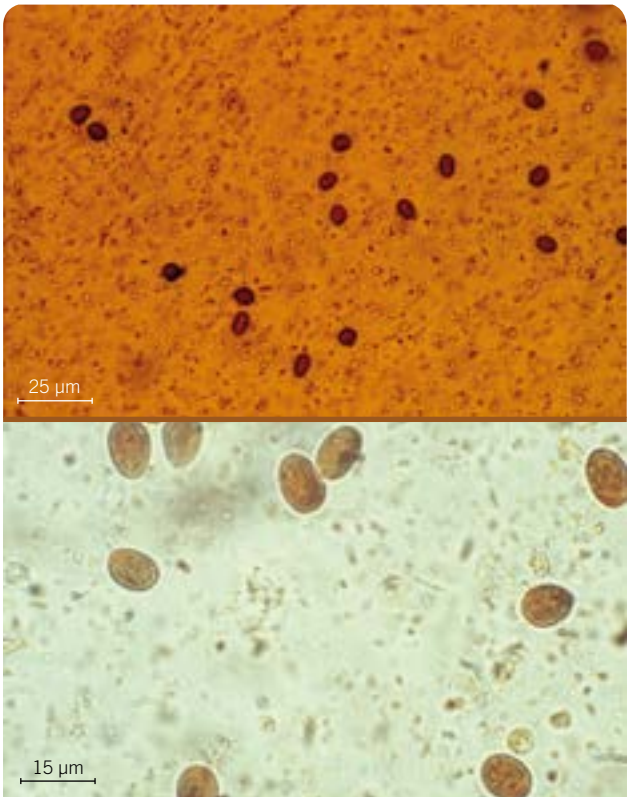


Figure 2. *Giardia* cysts (Lugol's iodine stain).

Immunological diagnosis is possible:

- By direct immunofluorescence assay: monoclonal antibodies which allow *Giardia* cysts to be detected by immunofluorescence assay are available. This technique is as effective as the flotation method of faecal examination for detection of *Giardia* cysts in humans but a fluorescence microscope is required, which limits this test to specialised laboratories.
- Using an ELISA kit to detect the coproantigens present in faecal matter from infected individuals.

Control measures

Treatment

The outcome is favourable after a course of symptomatic treatment (mucosal protective agents, antispasmodics) and specific therapy. The latter will almost certainly consist of either metronidazole, or certain benzimidazoles.

Metronidazole may be administered at a dose of 20 mg/kg *per os* twice a day for 10 days. According to the studies published, efficacy varies from 100 % down to 67 % and adverse reactions, such as nausea, vomiting and ataxia, have been described.

A number of benzimidazoles have been shown to be 90 to 100 % effective and use of these drugs could be advantageous because of their excellent safety, even at high doses. Fenbendazole (50 mg/kg), oxfendazole (11.3 mg/kg) and febantel (15 mg/kg) administered for 5 consecutive days all demonstrated their efficacy.

Treatment failure, or the persistence of cysts in faeces is mainly attributable to almost immediate reinfection. Indeed, dogs undergoing treatment continue to ingest cysts which develop very rapidly (in 2 to 3 days), so when the dogs are placed in clean kennels (washed and disinfected), treatment is much more effective. Recontamination in dogs belonging to private owners is much less common, so treatment results are good and relapses are rarer.

Prevention

Prevention is limited in carnivores but possible with human giardiosis, through measures to ensure that drinking water is clean. In cat or dog kennels, treatment is based on keeping cages clean and dry (by frequent removal of faecal matter) and disinfecting floors. Cysts are very sensitive to quaternary ammonium (the majority of common commercial disinfectants), but appear to be fairly resistant to chlorine (bleach). During epidemics in communal housing, the carriers must be treated as well as the sick animals, so they must all be screened.

Giardiosis is probably the most common intestinal protozoan disease in carnivores but its prevalence is underestimated due to the limited number of faecal examinations carried out in veterinary clinics. The technique is actually easy, and does not necessarily require expensive equipment, such as a centrifuge. Finding evidence of *Giardia* cysts is facilitated by staining with iodised solutions.

An inactivated vaccine has been marketed in some American countries over the past few years but there have been contradictory reports on its efficacy.

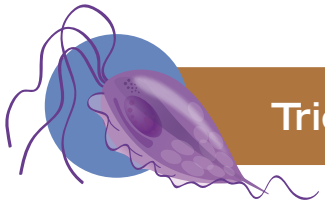
Risk to humans

Many arguments insist on the zoonotic nature of some strains/genotypes of *G. duodenalis* but not all:

- *Giardia* from humans can be transmitted to various animal species in the laboratory.
- There is a strong antigenic similarity between isolates from animals (cats, beavers, sheep, muskrats, dogs) and those from human patients.
- Morphometry is identical.
- There are significant genetic similarities between isolates of animal and human origins, as restriction fragment length polymorphism (RFLP) studies using DNA imprints or sequencing have shown.
- Electrophoretic profiles were very close to identical in most of the enzyme groups studied, but there were variations between geographical isolates.
- Various epidemiological studies have shown that animals, especially carnivores, have acted as parasite sources for humans.

However, genetic studies tend to show that different populations of the parasite *G. duodenalis* are more or less adapted to each type of host. Studies of *Giardia* DNA and isoenzymes from humans and dogs discovered different characteristics.

It should be accepted that *Giardia* of animal origin may infect humans.



Trichomonosis*

General comments

Trichomonads are flagellate protozoan members of the order Trichomonadida. Trichomonads are animal parasites or commensals and they reside in mucous membrane-lined, microaerophilic, non-sterile organ cavities such as the gastrointestinal and reproductive tracts. They are spindle- to tear-drop shaped, highly motile flagellates, similar in size to *Giardia* and they exist only as trophozoites (no cyst stage), they divide by binary fission, and are transmitted directly between hosts. Trophozoites bear a characteristic number of anteriorly directed flagella and a single, posteriorly directed flagellum that arises at the anterior end and courses along the body, creating the undulating membrane which is a characteristic feature. The axostyle, a rigid, rod-shaped organelle, runs through the trophozoite and protrudes from the posterior end (Fig. 1).

Epidemiology

Tritrichomonas foetus was first molecularly identified as a cause of chronic large bowel diarrhoea in cats in 2003. *T. foetus* has been demonstrated to colonise the distal ileum and colon in experimentally or clinically infected cats, resulting in lymphoplasmacytic and neutrophilic colitis and chronic foul-smelling diarrhoea. *T. foetus* has now been described in cats in many countries where the prevalence of infection varies from 10 % to 59 % and assumed to be transmitted from cat to cat via the faecal-oral route. Trichomonads can survive for several days in moist faeces, although they do not persist for more than a few hours in clean, dry, and aerobic conditions. Although *T. foetus* is sexually transmitted in cattle, there is

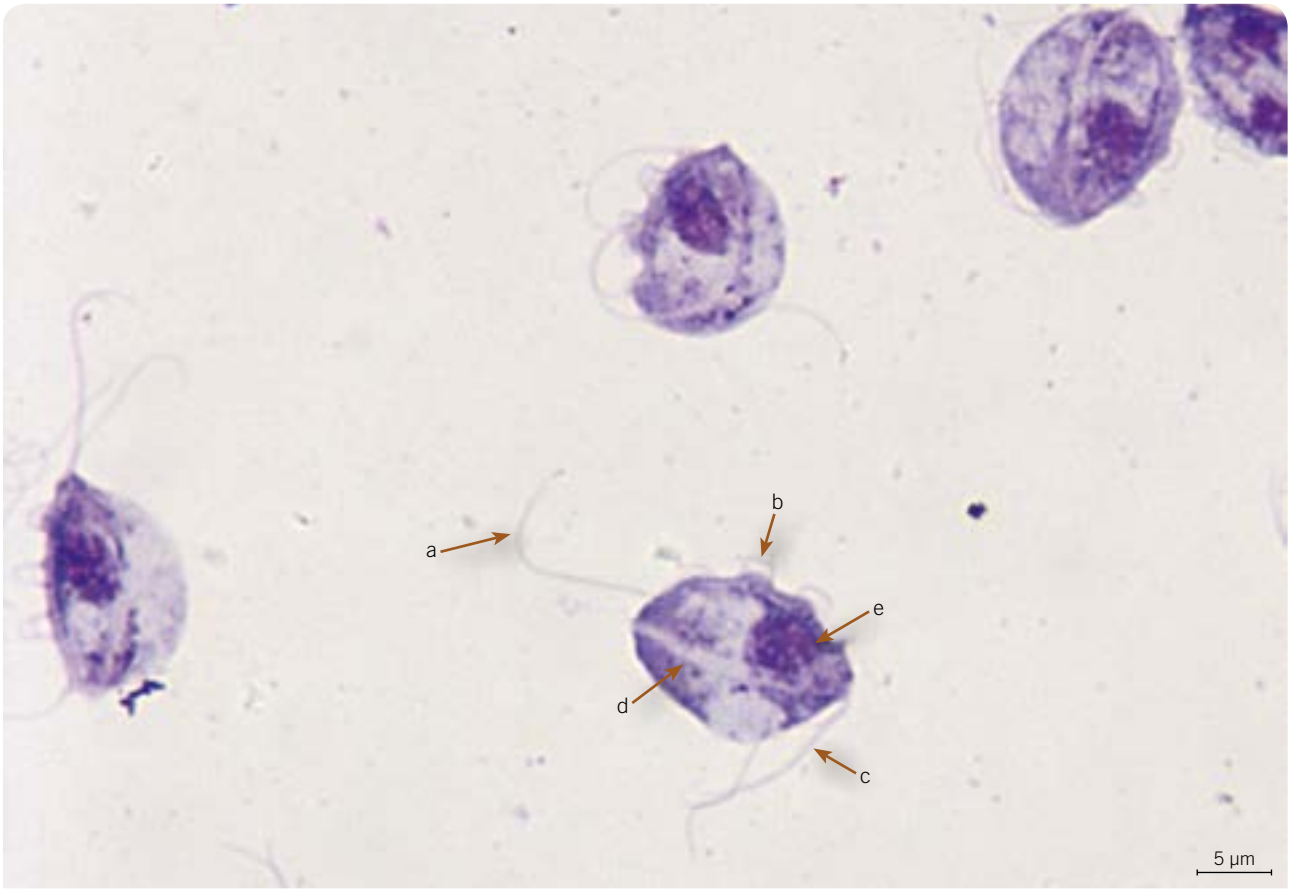


Figure 1. Cytological preparation of cultured feline *Tritrichomonas foetus*. Stained with Wright-Giemsa and photographed at ×100 magnification. (a) Anterior flagella, (b) undulating membrane, (c) posterior flagellum, (d) axostyle, (e) nucleus.

* From chapter originally written by Jody Gookin for the book Parasitoses and Vector Borne Diseases of Cats. Ed. Merial, 2015. Pictures from Jody Gookin.

little evidence for venereal transmission of *T. foetus* in cats. Studies conducted on the reproductive organs of purebred cats where a high prevalence of intestinal *T. foetus* infection was identified, found no light microscopic, immunohistochemical or molecular evidence of *T. foetus* colonisation. Infection of the uterus with *T. foetus* was reported in one cat, although it was unclear whether *T. foetus* was a primary or opportunistic pathogen. From these data, it is unlikely that reproductive tract infection with *T. foetus* plays a significant role in transmission of the disease or is a frequent cause of reproductive tract pathology in breeding catteries.

Relationship between feline and bovine *Tritrichomonas foetus* and porcine *Tritrichomonas suis*

There has been considerable debate regarding the relationship between feline and bovine isolates of *T. foetus* and porcine *T. suis*. *T. foetus* is a well-recognised cattle pathogen and is sexually transmitted from bull to cow. In the cow, *T. foetus* infects the vagina, cervix and uterus and may cause mild infection or more severe sequela including vaginitis, early abortion, and occasional pyometra, resulting in permanent infertility. The porcine trichomonad *T. suis* colonises hollow organs, including the nasal cavity, stomach, small and large intestines and caecum in pigs. It had previously been described as a pathogen possibly associated with rhinitis in pigs but additional research has now shown it to be a harmless commensal in that host. The trichomonosis first described in cats in the late 1990s was identified as *T. foetus* from a limited molecular comparison between bovine and feline isolates.

Based on a plethora of comparisons, including cross-infectivity studies, virulence assays, molecular analysis, geographical distribution, morphological characteristics and immunological analysis, there appears to be no significant difference between the bovine *T. foetus* "genotype" and porcine *T. suis*. Infecting cattle with *T. suis* resulted in similar pathology to infection with the bovine parasite, and pigs have been found to be easily infected with the cattle trichomonad. The conclusion is that these "sister species" are synonymous.

Recent studies have demonstrated significant genetic differences between the *T. suis*/*T. foetus* "cattle genotype" and the *T. foetus* "feline genotype". Sequencing the ITS-1 - 5.8 DNA gene through ITS-2 of both cattle and feline isolates revealed a single-nucleotide polymorphism that distinguishes feline isolates from those of cattle and pigs. Molecular sequencing also revealed 10 distinct genotypic polymorphisms between the *T. suis*/*T. foetus* "cattle genotype" and the *T. foetus* "feline genotype" for a total of 1.03 % difference between genotypes from these hosts, with the greatest difference recorded for the cysteine protease (CP) genes. From this and other data, it has been concluded that the feline and cattle isolates may be divided into two distinct genotypes so it has been proposed that the feline trichomonad be renamed *T. blagburni* based on molecular analyses, host specificity and pathology.

Unlike in the cross-transmission studies between pig and cattle trichomonads, experimental infection of cattle with a feline *T. foetus* isolate resulted in a similar, but not identical, course of colonization of the vagina, cervix and uterus but less endometrial pathology than caused by bovine *T. foetus*. When cats were infected with a bovine *T. foetus* isolate, the researchers reported that the bovine isolate was less pathogenic for cats than the feline isolate.

Pathogenesis

Great progress has been made in the past 15 years in determining the molecular identity and genetics of feline *T. foetus* and in developing diagnostic tests and, to some extent, effective treatment for this infection. However, very little is known about how these organisms actually cause diarrhoea. Based on what is known about the pathogenic mechanisms of bovine *T. foetus* in the reproductive tract and what can be observed in cats infected with feline *T. foetus*, multiple pathogenic factors are likely. Pathogenic factors associated with *Trichomonas* infection include interaction with endogenous bacterial flora, adherence to host epithelium, and production of cytotoxins and enzymes. Infecting specific-pathogen-free cats with cultures of feline *T. foetus* results in chronic colonisation of the terminal ileum, caecum, and colon, and large bowel diarrhoea similar to that observed in naturally infected cats. In cats infected naturally, *T. foetus* is found in the superficial mucus and in contact with the surface epithelium of the caecum and colon. Uptake of *T. foetus* antigens by the colonic surface epithelial cells can also be demonstrated. Histologically, this is associated with the infiltration of lymphocytes, plasma cells, and neutrophils into the colonic lamina propria.

Clinical signs

Feline *T. foetus* infection is characterised by waxing and waning diarrhoea that often contains fresh blood or mucus. Diarrhoea is semi-formed to a "cow pat" consistency is and malodorous. In most cases, infected cats maintain good health and body condition which presumably reflects confinement of the infection to the colon. However, some kittens develop faecal incontinence and overt swelling and inflammation of the anal region from faecal scalding (Fig. 2). Cats with diarrhoea and concurrent *T. foetus* infection are generally young but can range widely in age. Older infected cats may be clinically healthy or may have a long history of diarrhoea since they were a kitten. Cats originating from catteries (i.e., pedigrees) or shelters appear to be at increased risk of infection, presumably because of the dense housing conditions and increased likelihood of faecal-oral transmission. The predominance of infection among young cats may also reflect increased susceptibility to infection due to environmental stresses or immunological immaturity. There does not appear to be any gender predilection or consistent reports in support of any specific breed predilections for *T. foetus* infection in cats. A consistent feature of *T. foetus* diarrhoea is that faecal consistency improves and trichomonads disappear on administration of antimicrobial drugs, but diarrhoea containing trichomonads reappears shortly after treatment is discontinued. Misdiagnosis of *Giardia* is common in cats infected with *T. foetus*. Cats diagnosed with *Giardia* based on a direct faecal smear examination and that fail to respond to appropriate antimicrobial therapy, should be thoroughly re-evaluated for the possibility that the observed trophozoites were actually *T. foetus*.

Diagnosis

T. foetus infection is diagnosed by identifying the organism on a faecal smear, after culturing the faeces in media that promote the growth of *T. foetus*, or by PCR performed on DNA extracted from a faecal sample. *T. foetus* cannot be detected by routine faecal analysis, such as centrifugation/flotation, and the organisms do not survive refrigeration. Suitable faecal samples may be obtained by (1) collecting a freshly voided specimen, devoid of contaminating litter, (2) inserting a faecal loop per rectum into the proximal colon, or (3) passing a catheter into the proximal colon for the instillation and recovery of several mL of sterile saline.

Figure 2. Faecal incontinence and anusitis in a kitten with *Tritrichomonas foetus* colitis and chronic diarrhoea.

In the saline flush technique, approximately 10 mL of sterile saline is injected through the catheter into the colon, and then gently aspirated. A drop of the recovered solution can then be examined for trichomonads directly under the microscope or placed in a faecal culture pouch. Alternatively, the solution can be sedimented in a centrifuge at approximately 2000 × g for 5 minutes and the resulting faecal pellet submitted for PCR analysis.

Faecal samples should always be fresh, free of contaminating litter, and kept unrefrigerated before testing. If a stool sample is to be transported to the veterinary clinic, trichomonad survival in the faeces can be extended by removing any adherent litter and diluting the sample with saline to prevent desiccation (3 mL 0.9 % saline per 2 g faeces). Analysis of the sample will begin to lose diagnostic sensitivity after 6 hours. Samples obtained from non-diarrhoeic or dry stools are not suitable for *T. foetus* testing and rarely yield positive results, even if the parasite is present. Administration of antibacterial drugs at the time the sample is collected also appears to reduce the likelihood of finding *T. foetus*, so antimicrobial therapy of any type should be discontinued for several days before collecting samples for testing.

It is important to recognise that no available diagnostic tests have 100 % sensitivity for *T. foetus*. If test results are positive, the cat is considered to be infected with *T. foetus* but, if results are negative, the possibility of infection cannot be excluded, particularly in cases where there is a high index of suspicion for *T. foetus*. Veterinary surgeons should strongly consider repeated testing in these cases.

Direct faecal smear

For a direct faecal smear examination, commonly known as a "wet mount", a small amount of faeces is diluted with saline solution and examined under a coverslip using a light microscope equipped with a 20× or 40× objective. Lowering the microscope condenser will increase contrast and enhance visualization of any trichomonads (Fig. 3). Trichomonad trophozoites are almost identical in size to *Giardia* and must be carefully distinguished. *Giardia* trophozoites have a concave shape similar to a rose petal and sluggish motility reminiscent of a "falling leaf" but Trichomonads are shaped like a teardrop and possess an undulating membrane that courses the entire length of the body. They are also vigorously motile. Where there is difficulty distinguishing trichomonads from *Giardia* spp. trophozoites, a *Giardia* antigen test can be performed on the faeces. The presence of trichomonads will not cause a positive *Giardia* antigen test result. However, it is important to recognise that co-infection of cats with *Giardia* spp. and *T. foetus* is common, so a positive *Giardia* antigen test result does not rule out the possibility of a co-infection with *T. foetus*.

A direct smear examination is the easiest way to diagnose trichomonosis; however it is also the least sensitive. *T. foetus* can also be difficult to distinguish from non-pathogenic intestinal trichomonads, such as *Pentatrichomonas hominis* based on light microscopic examination of live organisms only. Feline trichomonads are generally presumed to be *T. foetus* in cats. *P. hominis* can be distinguished from *T. foetus* by species-specific PCR testing if necessary.

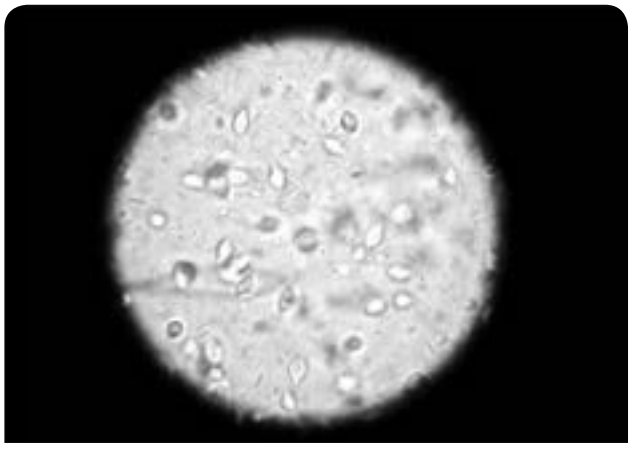


Figure 3. Faecal wet mount taken through the ocular lens of a light microscope. Copious tear-drop shaped trichomonads can be observed at various depths in the saline solution.
Courtesy of Bronya Redden.

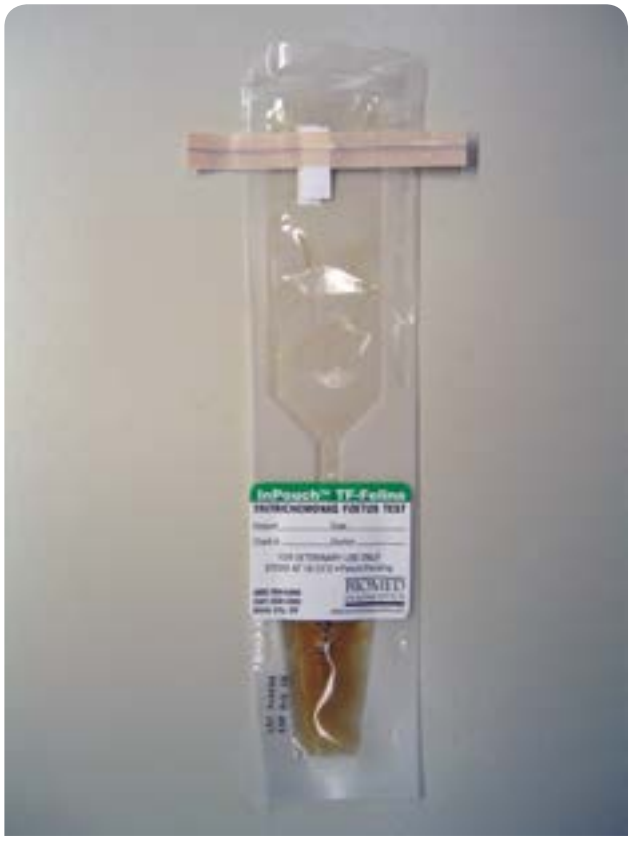


Figure 4. Pouch system for the culture of *Tritrichomonas foetus*.

Faecal culture

If repeated direct microscopic examination is negative for trichomonads, faeces may be cultured using commercially available pouches (such as the In Pouch TF-Feline system from Biomed Diagnostics) (Fig. 4). Faecal culture using In Pouch TF is more sensitive than direct faecal smear examination for *T. foetus* diagnosis. The pouches are made of clear plastic and contain a proprietary culture medium and antibiotics that suppress unwanted bacterial growth. For diagnosis of feline *T. foetus*, the pouches should be inoculated with 0.05 g (approximately the size of a rice grain) of faeces and incubated in an upright position at either 37 °C / 98.6 °F or room temperature (25 °C / 77 °F). Trichomonads multiply quickly at 37 °C and many organisms can be observed by light microscopy within 72 hours.

If the pouch is incubated at room temperature, fewer trichomonads will be present, and it may take up to 12 days after inoculation to obtain positive results. The faecal sample must contain live organisms to obtain positive results from faecal culture, and optimum growth conditions for the organisms must be maintained during the test period so the specimen and pouch must be handled with care to avoid a false negative result. It is strongly recommended that these cultures are performed in the clinic, rather than by an external diagnostic laboratory, so that the trichomonads do not die during shipment of the pouch.

Trichomonads can be observed inside the pouch by placing the pouch on the stage of a light microscope directly under a ×20 or ×40 objective. *Giardia* spp. cannot survive in the In Pouch TF medium for longer than 24 hours, so any trophozoites proliferating within the pouch can be assumed to be trichomonads. Both feline *T. foetus* and feline *P. hominis* can survive in the In Pouch TF medium. *P. hominis* can be distinguished from *T. foetus* by species-specific PCR testing if necessary.

Colonic mucosal biopsy

Trichomonads can be observed in crypt lumen or in the mucus lining the surface epithelium of the colon by routine light microscopy. However, because trichomonads are lumen-dwelling and extremely fragile, their preservation in intestinal biopsy specimens is highly unreliable. Diagnosis of *T. foetus* is differential, and a minimum of six tissue sections are required to achieve 95 % confidence that trichomonads will be identified. Immunohistochemistry, fluorescence *in situ* hybridisation, and chromogenic *in situ* hybridisation techniques have been described as means to enhance detection of *T. foetus* in histological specimens. In cases where trichomonads are observed in tissue, DNA can be extracted from formalin-fixed paraffin-embedded specimens and used in PCR to identify the trichomonads as *T. foetus*.

Control measures

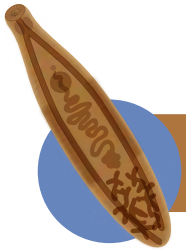
Trichomonads are generally susceptible to treatment with 5-nitroimidazoles because the anaerobic metabolic pathways that these organisms use reduce these drugs to cytotoxic nitro radical anions that disrupt protozoan DNA. Feline *T. foetus* is presumed to be resistant to metronidazole, as treatment failure is common with this drug. This has prompted investigation of related 5-nitroimidazoles, such as tinidazole and ronidazole to treat the infection. Tinidazole at high doses fails to consistently eradicate the infection from experimentally infected cats and has not been very useful for treatment of naturally infected cats. Ronidazole, a nitroimidazole similar to metronidazole, is the only antimicrobial which has demonstrated convincing efficacy in the treatment of *T. foetus* infection.

Studies investigating the pharmacokinetics of ronidazole in cats suggest that 30 mg/kg *per os* once a day for 14 days is likely to be most effective in resolving diarrhoea and eradicating *T. foetus* infection. Signs of ronidazole neurotoxicity include lethargy, inappetence, ataxia, and seizures, so cats must be monitored closely while receiving ronidazole. If signs of toxicity are observed, owners should be advised to discontinue treatment as continuing treatment after the onset of toxicity could result in life-threatening complications. Ronidazole should be avoided in cats with systemic illnesses that could confuse recognition of adverse drug effects, and it should not be given to pregnant or nursing queens or their unweaned kittens. If treatment with ronidazole has to be discontinued due to clinical signs of toxicity, the cat should be re-tested for *T. foetus* infection. Many of these cats will have received sufficient ronidazole to clear the infection. Most cats with *T. foetus* infection show significant improvement in faecal consistency, or resolution of diarrhoea, during the course of treatment with ronidazole.

Other therapies for the treatment of *T. foetus* in cats are limited. Many approaches to diarrhoea control have been tried without success, including changes to the diet, use of different antimicrobials, and supplementation with nutraceuticals and probiotics. However, there have been no controlled studies of any of these therapies. It has been suggested that frequent changes in diet and indiscriminate use of antimicrobials prolong the time it takes for cats to resolve the diarrhoea on their own. Vets should be careful of embracing any particularly successful antimicrobial drug as treatment for *T. foetus* infection because many drugs merely suppress detection of the organism rather than eradicating it.

If left untreated, it is estimated that diarrhoea in most cats (88 %) with *T. foetus* infection will resolve spontaneously within 2 years (median 9 months; range 5 months to 2 years). However, most of these cats will still be infected, based on positive PCR test results for *T. foetus* and may therefore be sources of infection for other cats. The role of these "asymptomatic carriers" in disease transmission is unclear, but these cats can suffer a full recurrence of diarrhoea that is teeming with trichomonads as much as 6 years after onset of their clinical "remission". Any cat carrying *T. foetus* should be therefore be considered a potential source of infection, and screening for these cats appears to be warranted for the sake of preventing disease transmission. No studies have been carried out, and there is currently no evidence to suggest any long-term adverse health effects of asymptomatic *T. foetus* infection in cats.

Hepatic parasitoses



Opisthorchidoses

General comments

Carnivores can be infested by trematode flukes, which are parasites of the liver and bile duct, particularly in Asia. These flukes belong to the family Opisthorchiidae, hence the name of the disease, opisthorchidosis. Infestation is usually asymptomatic and the significance of these diseases is mainly due to their prevalence in certain areas (where more than 80 % of dogs and cats are infested) and to the subsequent public health risk, as most species are zoonotic. Carnivores ensure the survival of these Asian flukes and are the main reservoir of the parasites.

Opisthorchis felineus and ***Opisthorchis viverrini*** measure 10–18 × 2 mm, and are reddish when fresh, with a non-branching caecum and lobed testes located at one end (Fig. 1). They shed eggs measuring 26–30 × 10–15 µm that have a characteristic operculum (Fig. 2).

They are parasites of the bile ducts in domesticated and wild carnivores (such as raccoon dogs), as well as humans. They are mainly found in Southeast and Central Asia, but cases have been reported in Eastern Europe, as far west as Germany.

The first intermediate hosts are aquatic snails, mainly of the genus *Bythinia (B. leachi)*, and many fish (cyprinids: tench, carp, pike, etc.) are second intermediate hosts for the infective metacercariae.

Clonorchis sinensis: this small fluke, called the Chinese fluke or the Oriental liver fluke, measures 5–12 × 1–2 mm and is commonly found in carnivore bile ducts in the Far East. The fluke lays small eggs with opercula measuring 25 × 15 µm. As is the case with *Opisthorchis*, the first intermediate hosts are aquatic snails, mainly of the genus *Bythinia*, and many fish, especially cyprinids, are second intermediate hosts.

Metorchis albidus and ***Metorchis bilis***: these small parasitic flukes measure 5.5–4 × 1–2 mm (Fig. 3) are found in Central Europe, where they live in the gall bladder of wild carnivores (foxes, wildcats), and sometimes in domesticated cats. They lay eggs measuring 30 × 15 µm. As in the two previous flukes, the first intermediate host is an aquatic snail, and the second intermediate host, which harbours the metacercariae, is a cyprinid fish.

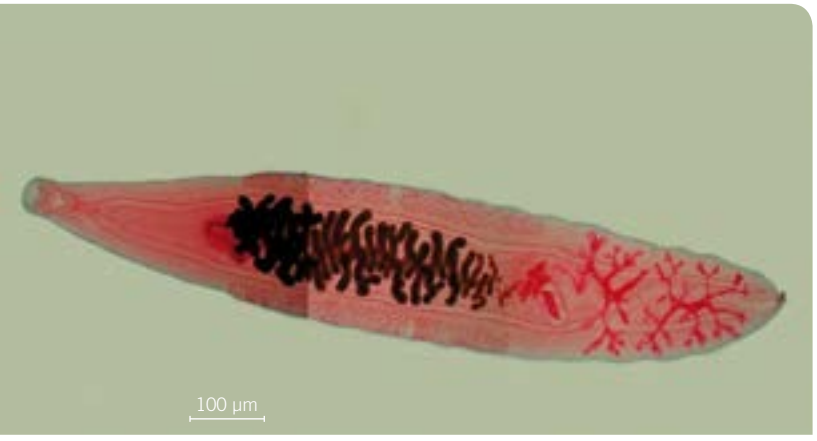


Figure 1. *Opisthorchis felineus.*

Figure 2. Egg of *Opisthorchis felineus.*

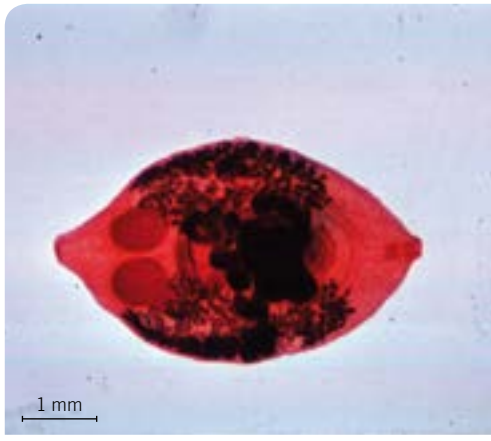


Figure 3. Adult *Metorchis.*

Epidemiology

Opisthorchiidae are parasites of ichthyophagous mammals and are not very host-specific, so they infest not only domesticated and wild carnivores, but also pigs and humans.

They are very common in Asia, where several million humans and carnivores are infested each year, and several outbreaks have also been reported in Europe.

More than 7 million humans in Central Asia (mainly in China and Russia) are infested by *C. sinensis*. 113 species of fish have been listed as hosting the metacercariae and nine species of snails as the first intermediate hosts.

In Southeast Asia, more than 7 million Thai people and 2 million Laotians are infested by *O. viverrini*.

In Russia, more than 2 million people are infested by *O. felineus*. Indigenous outbreaks were reported in East Germany in 1996 and confirmed in 1999, with 32.5 % of foxes infested (6.7 % by *O. felineus* and 28.1 % by *M. bilis*).

Clinical signs and diagnosis

The disease is usually asymptomatic in infested carnivores but, liver failure and gastro-intestinal disorders can be seen in heavy infestations, and the disease can develop into cirrhosis and jaundice.

After several years, chronic infestation can lead to liver cancer, which has a poor prognosis.

Diagnosis is made by faecal examination, revealing the characteristic eggs (Fig. 4).

Control measures

Treatment is with praziquantel, at 75 mg/kg *per os* in 3 individual doses on the same day, which is 25 times the normal cestodicide dose (5 mg/kg).

Prevention in domesticated carnivores entails simply preventing the consumption of raw or undercooked fresh-water fish.

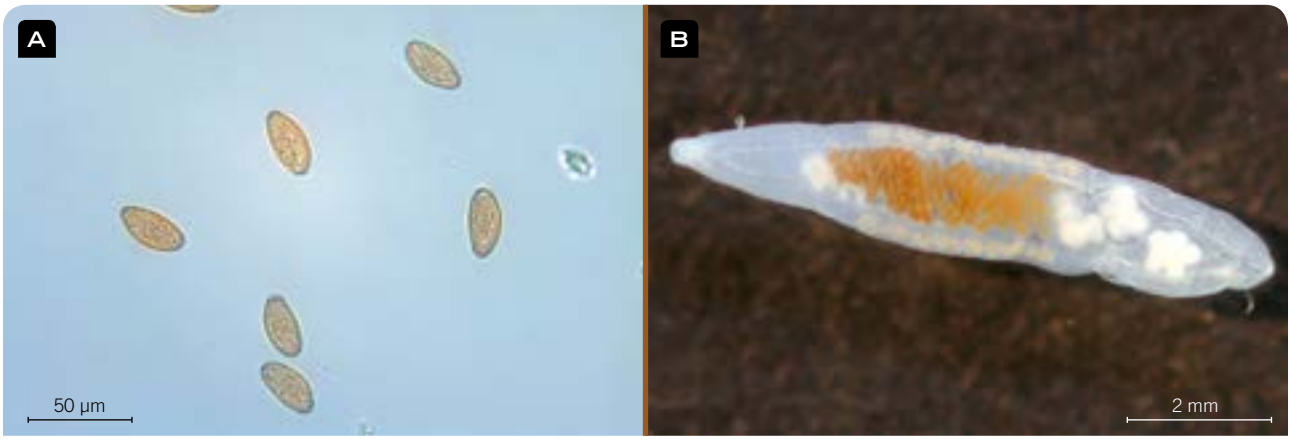
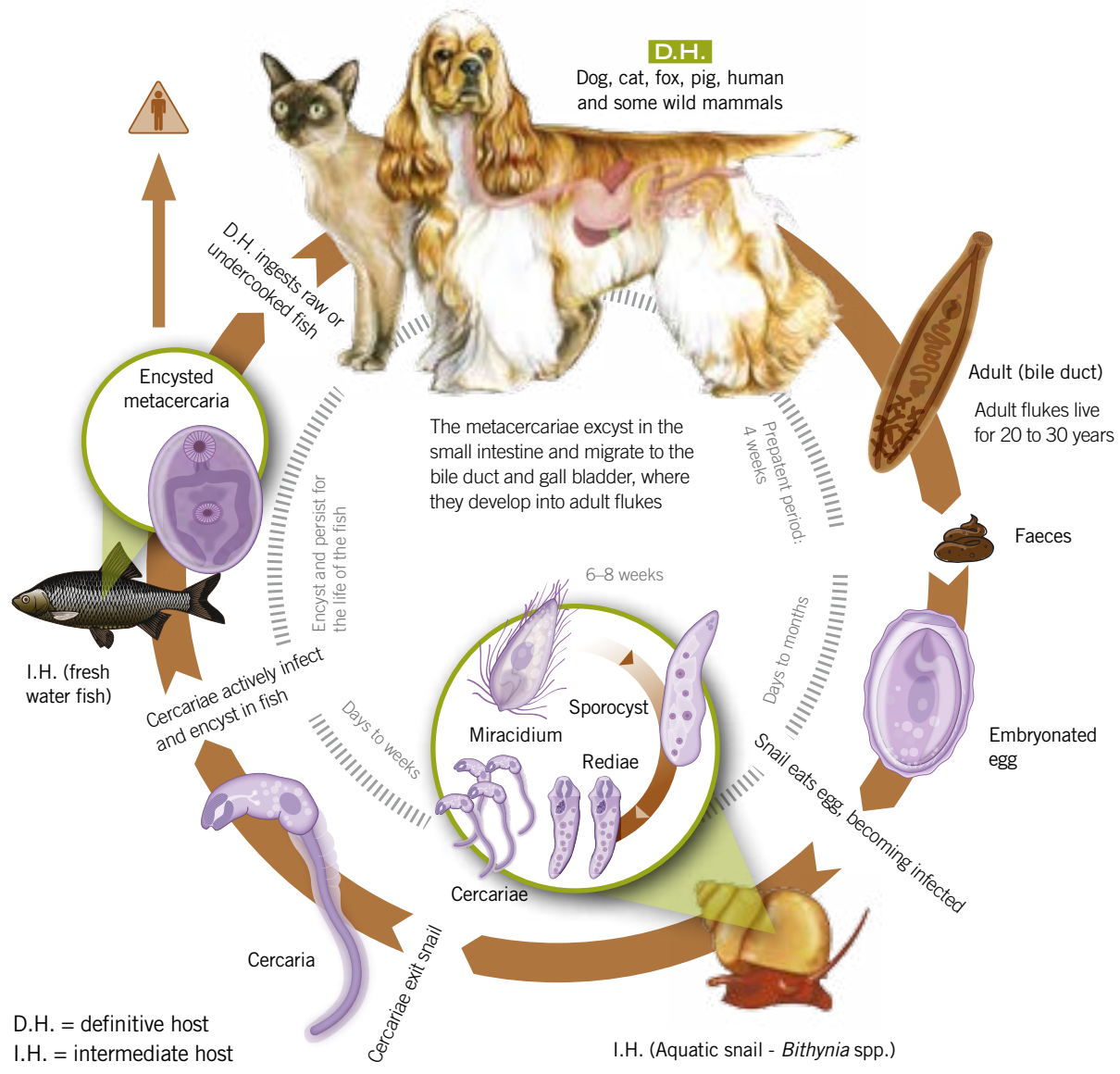


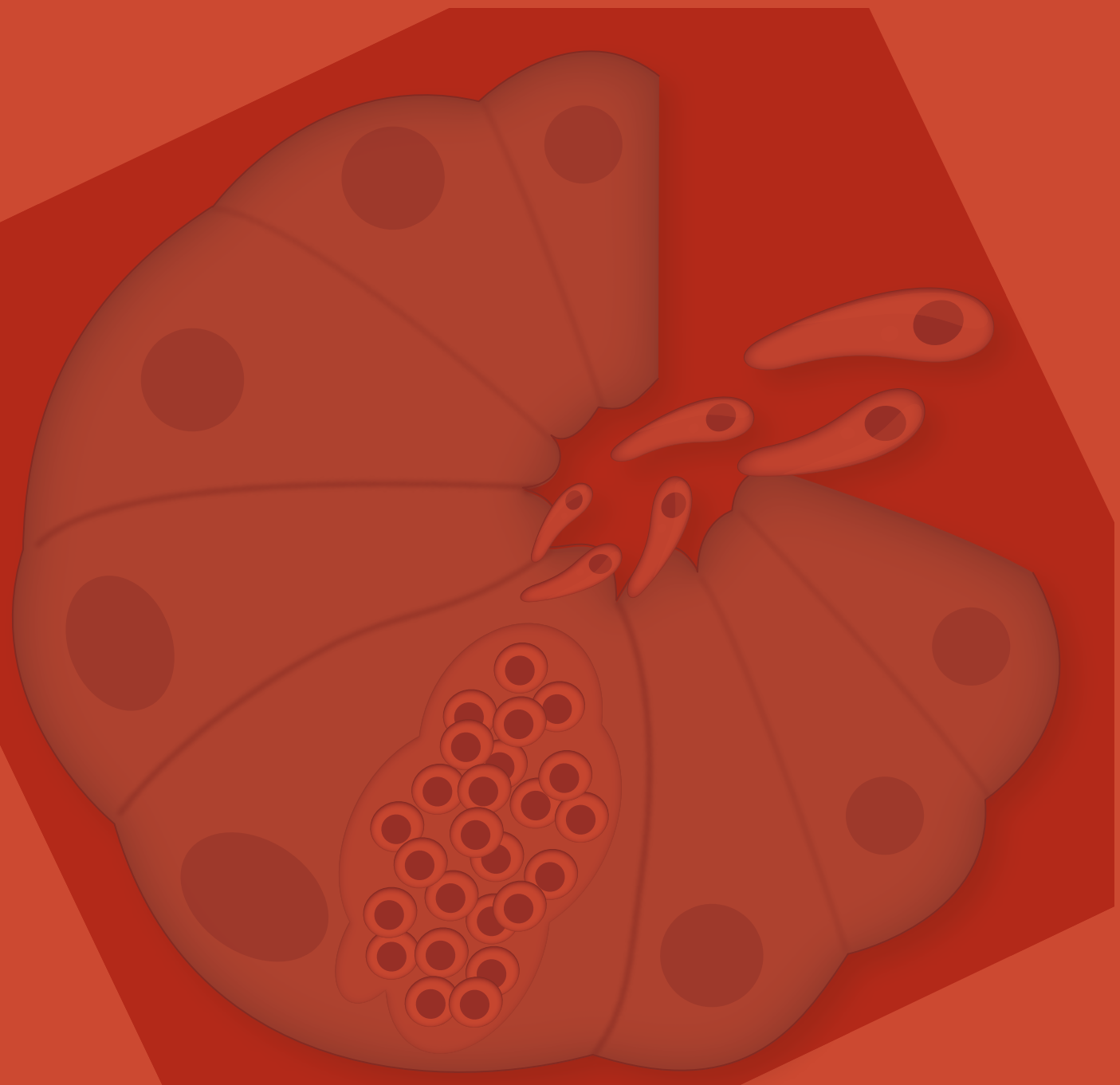
Figure 4. *Opisthorchis* (A) eggs and (B) adult.

PDF

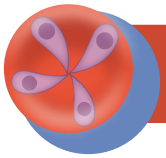
Life cycle of *Opisthorchis/Metorchis* spp.



INTERNAL NON-GASTROINTESTINAL PARASITOSES



Blood parasitoses



Piroplasmoses in dogs

General comments

Piroplasmoses, also known as babesioses or theilerioses, are a group of infectious, inoculable protozoan diseases caused by multiplication of Apicomplexa protozoa (these have an apical complex made by a group of organelles at one pole) belonging to the genus *Babesia* or *Theileria* in various mammalian hosts. *Babesia* infect the erythrocytes, whereas *Theileria* undergo exoerythrocytic merogony in other cells of the body before invading the erythrocytes. These organisms are transmitted by various hard tick species and affect a wide range of mammals, including humans, ruminants, horses and carnivores, each species being specific to its vector and host.

Babesiosis and theileriosis are diseases with diverse clinical forms, the most characteristic of which is a combination of fever, anaemia and haemolysis.

Taxonomy

Piroplasms (order Piroplasmida) are protozoa of the phylum Apicomplexa, class Sporozoasida, characterised by infectious intracellular organisms or parasitic sporozoites, which use the organelles of the apical complex to penetrate their host's cells.

The Haemosporidia, a subclass of this phylum, contain sporozoites that parasitise blood and lymphocytes and are transmitted by haematophagous arthropods. These differ from Eucoccidea, which infect epithelial cells, mostly those of the gastro-intestinal tract (coccidia *sensu lato*).

Haematozoa can be split into two categories: the Haemosporidae, which are located in a parasitophorous vacuole in the host cell and synthesise pigments (such as the genus *Plasmodium*) and the Piroplasmidae or piroplasms, which are free in the host cell and do not synthesise pigments (*Theileria*, *Babesia*).

Dogs and cats can be infected by large *Babesia* species (diameter larger than the radius of the erythrocyte, i.e., 3–5.5 µm) and several small piroplasm species (diameter smaller than the radius of the erythrocyte, i.e., <3 µm).

Table 1 summarises the various piroplasm species which can infect pets and lists their geographical distribution and vectors.

Distinct genetic populations can be distinguished within each species. This geographical and genetic diversity may explain the variety of clinical signs that can be observed, and their varying severity, as well as the relapses and vaccination problems that are linked to imperfect cross-protection.

Morphology

Several morphological forms of piroplasm can be seen in the erythrocyte:

- A typically pear-shaped form (hence "piriform" and "piroplasm") characterised by one thinned, tapered end, and another rounded end (Fig. 1). Large *Babesia* are 3.5–5.5 µm

Table 1. Geographical distribution and vectors of the various *Babesia* and *Theileria* species infecting dogs worldwide.

Species	Type	Vectors	Geographical distribution
Babesia canis (formerly *Babesia canis canis)*	Large	*Dermacentor reticulatus*	Europe
Babesia vogeli (formerly *Babesia canis vogeli)*	Large	*Rhipicephalus sanguineus* *Haemaphysalis* ticks?	Worldwide
Babesia rossi (formerly *Babesia canis rossi*)	Large	*Haemaphysalis elliptica* (formerly *Haemaphysalis leachi*)	Africa
Unknown *Babesia* sp. (sometimes called *Babesia coco*)	Large	?	USA (East coast)
Babesia gibsoni	Small	*Haemaphysalis longicornis* in Asia elsewhere?	Mainly Asia, USA, South America, Australia, Europe
Babesia conradae	Small	*Rhipicephalus sanguineus*?	USA (California)
Babesia vulpes (formerly *Babesia* microti-like, *Theileria annae*)	Small	*Ixodes hexagonus*	Europe

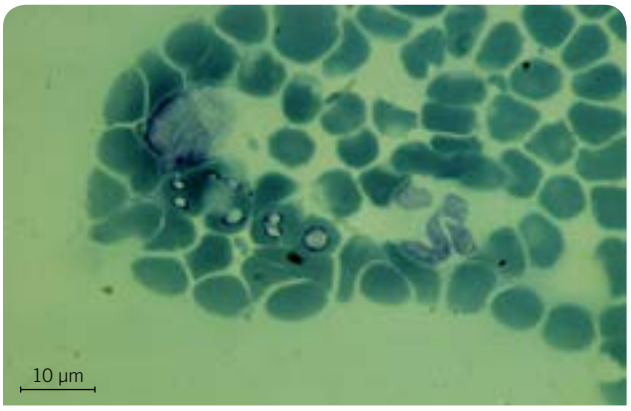


Figure 1. *Babesia canis*. Round intraerythrocytic forms. Blood smear, Stevenel's blue stain.

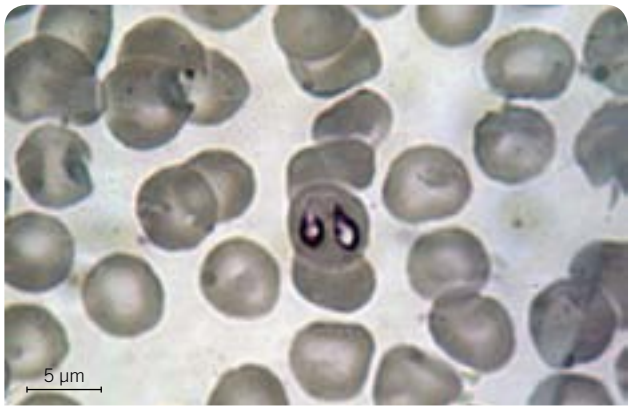


Figure 2. *Babesia canis*. Intraerythrocytic bigeminated forms. Blood smear, MGG stain.

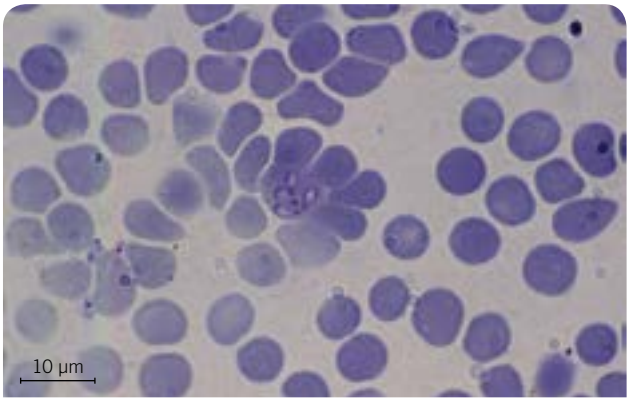


Figure 3. *Babesia canis*. Intraerythrocytic bigeminated forms, showing four elements resulting from binary divisions. Blood smear, MGG stain.

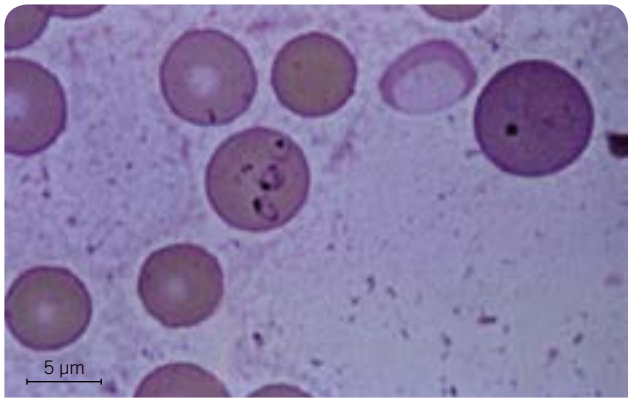


Figure 4. Blood smear, MGG stain, showing small round intraerythrocytic element (diameter smaller than the radius of the erythrocyte), indicating a small *Babesia*, type *Babesia gibsoni*.

long and their diameter is greater than the radius of an erythrocyte. Small *Babesia* and *Theileria* spp. are less than 3 µm long.

- A bigeminal form, consisting of two pear-shaped elements, joined by their anterior ends and meeting at an acute angle (Fig. 2). There is also a "plurigeminal" form, with four or more elements within the erythrocyte (Fig. 3).

All of these forms have a large central vacuole and a dense periphery made of chromatin, so the centre is optically clear and the periphery strongly coloured on staining (Stevenel's blue, MGG). The apical complex is a group of organelles situated at the anterior end of the cell. It contains numerous enzymes and is used by the parasite to penetrate the cell. This can be seen most clearly in large *Babesia*, while morphological characteristics are less evident in small *Babesia* and *Theileria* spp. (Figs. 4 and 5).

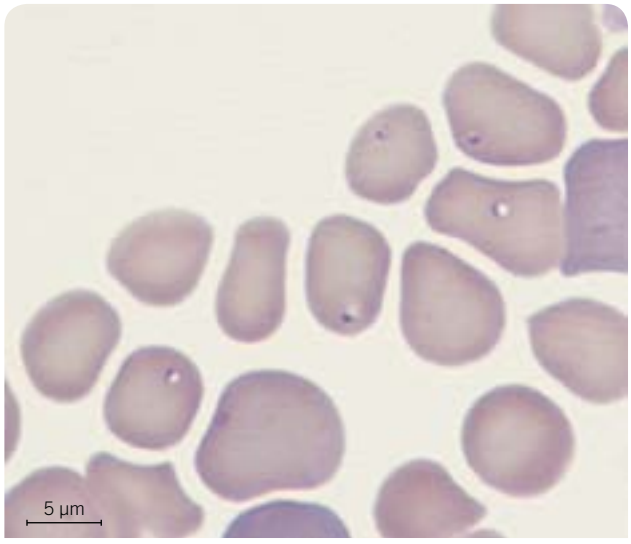


Figure 5. Blood smear, MGG stain, showing several small comma-shaped intraerythrocytic elements (diameter smaller than the radius of the erythrocyte), indicating small piroplasms. Courtesy of Luis Cardoso and Gad Baneth.

Biology

Babesia are strictly intraerythrocytic parasites. In cases of babesiosis and acute piroplasmosis, they can be seen in the peripheral blood (in the cutaneous capillaries), and the parasite may also be observed in the venous blood of some viscera (spleen).

Theileria spp. have an exoerythrocytic cycle in lymphocytes, histiocytes, erythroblasts and other cells of the internal organs, and an intraerythrocytic cycle similar to that of *Babesia* spp.

Protozoan piroplasms feed on the contents of the erythrocyte by pinocytosis.

Life cycle

The life cycle of the Piroplasmidae is fundamentally dixenous, involving hard ticks as definitive hosts to ensure sexual reproduction, and mammalian intermediate hosts to ensure asexual multiplication of the parasite.

In the dog (intermediate host)

- Piroplasms are transmitted by tick bites: sporozoites in the salivary glands of the tick are inoculated into the host at the end of the blood meal.
- While *Babesia* spp. directly invade erythrocytes, *Theileria* spp. reach a variety of different cells, where they undergo a schizogonous process of asexual replication to produce multinucleated intracellular schizonts. At the end of this process, the parasites differentiate to form uninucleated merozoites that will enter the erythrocyte.
- In the erythrocyte, the parasite develops into a trophozoite which undergoes asexual multiplication: binary division (sometimes multiple divisions) leading to the formation of two (or more) pyriform elements; these are released by haemolysis and, after a very short free-living phase, these parasitise other erythrocytes.
- This process may last for several days in the animal, after which it is normally limited by the immune system. Ring-shaped elements thought to be gamonts then appear. These are the only elements which can ensure that the life cycle continues in the event of another tick bite.

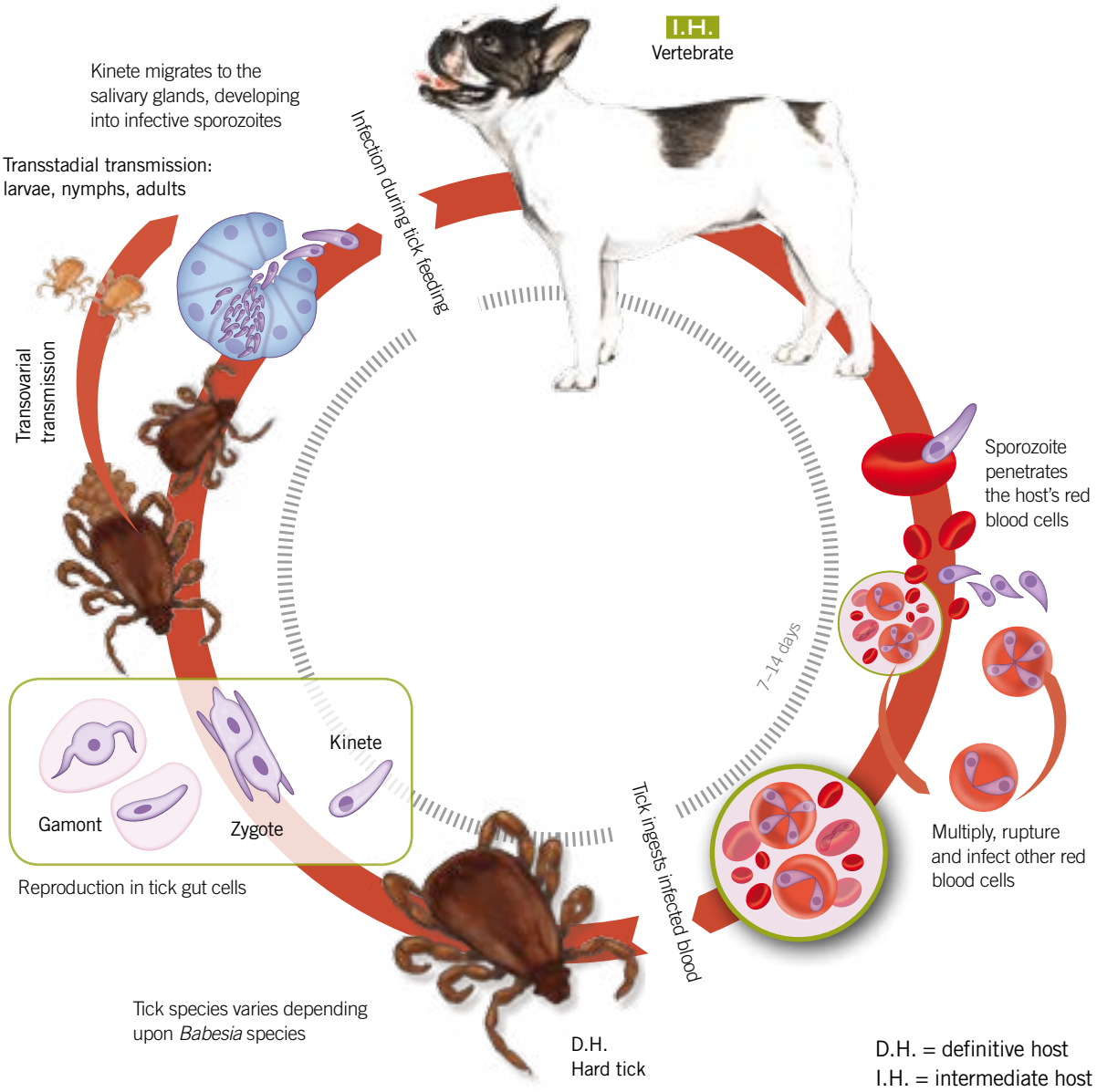
In the tick (definitive host)

- The tick absorbs parasitised erythrocytes during a blood meal: the pear-shaped elements die, and only the gamonts which have been freed into the gastro-intestinal tract continue to develop. These form elements with fine extensions, called starred or radiated bodies, which fuse together in pairs as gametes then form mobile zygotes or ookinetes, in cells of the gut wall. Sexual reproduction therefore occurs in the tick, which is the vector and definitive host.
- The ookinetes divide to form mobile elements or sporokinetes which infect various organs in the tick (gut, Malpighian tubules, etc.). Some sporokinetes of *Babesia* spp. infect the ovaries due to simple anatomical contiguity between the gastro-intestinal tract and the genital apparatus, allowing vertical transmission to the next generation. This is not the case for *Theileria* spp., in which transovarial transmission is impossible.

The following aspects of this life cycle should be noted:

- Piroplasms can persist from one stage to the next and retain their infectivity. This is called transstadial transmission.
- The tick that gets infected is never the infectious tick: ticks are characterised by the fact that each stage takes only a single blood meal, after which there is a moult (or the death of the female after laying).
- Persistence of parasites in the genus *Babesia* is ensured by the tick, as an infected female tick will transmit the parasite to its progeny and ensure the persistence of the parasite into the following generation. This is called transovarial transmission.
- Inoculation with infectious sporozoites takes place after the start of the blood meal (48 to 96 hours after tick attachment) because the sporozoites need blood in order to move, so removing ticks as soon as possible after attachment helps avoid disease transmission.
- In the case of *Dermacentor reticulatus* and *Haemaphysalis* spp., only the adult tick infests dogs (the immature stages parasitise small mammals) transmission is therefore ensured only by the adults, and the immature stages sustain the parasite and its infectivity. *D. reticulatus* is the tick vector of canine babesiosis due to *B. canis*. It is widely distributed throughout temperate Europe and requires transovarial transmission. *H. elliptica* is the vector for *B. rossi* in Africa, and *H. longicornis* is the vector for *B. gibsoni* in Asia.

Life cycle of *Babesia* spp.



- In the case of *Rhipicephalus sanguineus* (a monotropic triphasic tick, having three different individual hosts of the same species), all stages infest the dog: transmission is possible from the larval stage and at all subsequent stages, over several generations; however, nymphs and female adults are potentially the best vectors for the parasite. This tick is the main vector of canine babesiosis in the Mediterranean area.

Epidemiology

Geographical distribution and epidemiological characteristics are linked to the biology of each tick vector (Fig. 6).

Babesiosis due to *B. canis* is widespread in France, Northern Italy, Spain, Belgium, Germany, Austria, Switzerland and Eastern Europe (Hungary, the Czech Republic, Slovenia), but distribution follows a heterogeneous mosaic pattern. Babesiosis is a seasonal disease (peak in spring and autumn); the cold of winter and drought in summer reduce the activity of the ticks that cause it. However, this may change drastically with the appearance of the endophilic tick *R. sanguineus*, which is found in kennels and can be active all year round.

Babesia and *Theileria* are fairly specific parasites: *B. canis* is a parasite of canids. Frequently observed in young dogs, babesiosis particularly affects "outdoor dogs" (hunting/farm dogs, etc.) that are exposed to tick bites.

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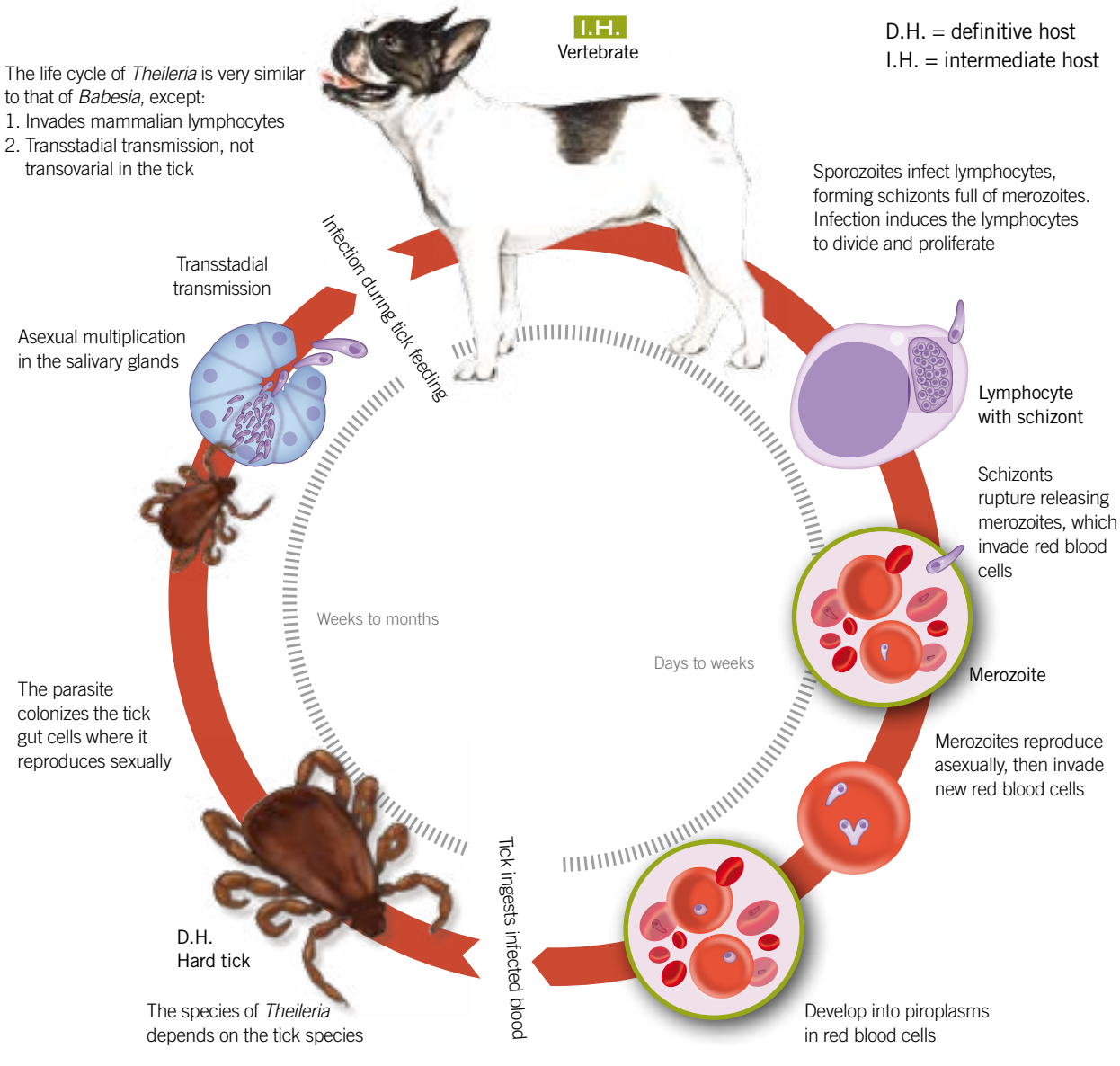
Life cycle of *Theileria* spp.



Clinical signs and lesions

In the majority of cases, the incubation period for all piroplasms is approximately 1 week. However, it may be shorter (2–3 days) or longer (10–15 days), depending on the immune status of the host.

Clinical signs of *Babesia canis* infection

Expression, duration and severity of the clinical manifestations of babesiosis vary greatly, and several forms can be seen.

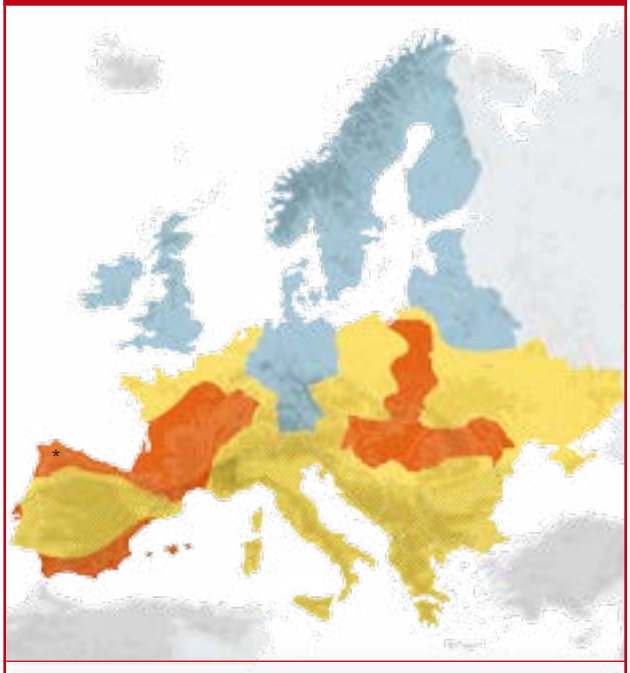
The classic (acute form) is the most common (>50 % of cases). It is defined by:

- General symptoms: owner is aware of a significant and sudden change in the behaviour of the animal. The dog is depressed, prostrate, ignores all stimuli and is totally anorexic.
- Pyretic syndrome: sudden onset of persistent hyperthermia (a minimum of 40 °C for at least 48 hours) combined with polypnoea and tachycardia.
- Haemolytic syndrome characterised by:
  - Anaemia (pale mucosae, sometimes subicteric).
  - Haematological changes: frequent, moderate or significant reduction in red blood cell numbers with the presence of erythroblasts (indicating regenerative anaemia), occasional leukopaenia, systematic and sometimes significant thrombocytopaenia (up to 100 x 10^9/L) and a change in blood count (lymphocytosis). However, results may seem contradictory (leukopaenia, leukocytosis, etc.). In fact, haematological changes noted in diseased animals within the first 24 hours are thrombocytopaenia, eosinopaenia and lymphopaenia; then, beyond 24 hours, thrombopaenia and persistent eosinopaenia, anaemia, lymphocytosis and monocytosis.
  - Urea, creatinine, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) values may be elevated, indicating the onset of hepatic and renal insufficiency.
  - Urinary changes: permanent bilirubinuria (strong yellow to orange coloured urine, which may be almost black ("rusty urine").

The chronic form is little defined and includes several different clinical manifestations:

- An animal which has previously been diagnosed and treated for piroplasmosis presents approximately 15 days later with a new bout of parasitaemia, identical to the first. This second bout shows clear clinical improvement on treatment, but it may be followed by further bouts; these successive relapses are attributable to an immune escape mechanism or to immune system insufficiency or malfunction, allowing the *Babesia* to multiply after the piroplasmicide has been eliminated from the host's system. The theory of parasite chemoresistance, which is frequently put forward to explain such phenomena, is possible but yet to be proven in the laboratory.
- An animal presenting with chronic anaemia with poorly-defined aetiology, which always tests negative, and whose clinical state seems to improve following the administration of piroplasmicides. These clinical states do not seem to be attributable to genuine piroplasmosis, because clinical improvement may be due to the action of piroplasmicides on other pathogenic agents and the piroplasm is almost never identified.

Figure 6. Canine babesiosis distribution in Europe.



Derived from Halos, 2014.
Courtesy of Luís Cardoso, Robert Farkas, Domenico Otranto, Kurt Pfister, Xavier Roura, Smaragda Sotiraki, Donato Traversa, Richard Wall.

Other forms, which vary greatly in their clinical expression, are described below:

- Locomotor forms: unsteady gait, lower back and joint pain, paresis, paralysis, ataxia.
- Cerebral and ocular forms: convulsions, nystagmus, anisocoria, behavioural changes and coma. This form is sometimes followed by complete recovery without relapse.
- Intestinal and respiratory forms.
- Renal forms; oliguria, anuria, haemoglobinuria, accompanied by renal insufficiency syndrome.
- Vascular, cutaneous and mucosal forms: oedema, diffuse haemorrhaging, purpura, stomatitis, haematoma (attributable to severe thrombopaenia), cutaneous ulcers and necrosis of the extremities.

Development of these diverse forms is very variable:

- Recovery without relapse is possible, even without treatment, either because the animal has an effective natural resistance or because it is infected by a strain that is not very pathogenic.
- Death from shock or acute renal insufficiency following the onset of jaundice.
- All situations between these two extremes are possible: rapid or slow recovery after treatment, more or less frequent relapses, apparent recovery followed by a new bout due to immunosuppression after surgical intervention (such as hysterectomy) or some other infection, etc.

Lesions

- Splenomegaly: congested, hypertrophied spleen, dark red in colour due to the process of extravascular erythrophagocytosis.
- Bilateral nephritis: congestion, necrosis and subcapsular haemorrhages, glomerulonephritis and tubular degeneration.
- Hepatic centrilobular degeneration.
- Vasculitis, haemorrhaging, pulmonary and subcutaneous oedema, ascites, and capillary embolism causing ischaemia and necrosis. These can affect various tissues and organs, such as the skin, lungs, kidneys, liver, brain, spinal cord, etc.

Clinical signs of small piroplasm infection

After an incubation period of a few days, the clinical presentation is similar to the typical form of babesiosis due to *B. canis*, but more intense.

The animal presents with hyperthermia (generally over 40 °C) lasting for at least a week and associated with anorexia and depression. Bilirubinuria is present, sometimes accompanied by haemoglobinuria. Anaemia is confirmed by a marked paleness of the mucosae. Intestinal problems, such as vomiting and diarrhoea, may be seen. Death may occur within a few days (acute renal insufficiency, shock, hypovolaemia, haemorrhaging).

Significant changes in blood and urine values indicate renal insufficiency: hyperazotaemia, hypercreatininaemia, proteinuria and haematuria. Clinical reports on dogs infected by *B. vulpes* (*T. annae*) reveal abnormally high serum concentrations of urea and creatinine, with elevated concentrations of inorganic phosphorus, hypoalbuminaemia, hypercholesterolaemia, proteinuria, a high protein/creatinine ratio. The presence of hyaline and granular casts on microscopic examination of urine sediment suggest a glomerular component to the disease.

Severe hyperchromic and regenerative anaemia (raised reticulocytes, Howell-Jolly bodies counts), moderate leukocytosis and thrombocytopaenia are almost constant characteristics of infection with small piroplasms in dogs. Azotaemia is also seen in many cases. Anaemia is attributable to erythrophagocytosis (extravascular haemolysis, an autoimmune process confirmed by the presence of anti-erythrocyte membrane antibodies).

Diagnosis

Diagnosis is based on epidemiological elements (seasons and areas of tick activity, age of the animal, etc.) and clinical signs (combination of pyretic and haemolytic syndromes, etc.). However, piroplasmosis must sometimes be differentiated:

- From other causes of anaemia, such as poisoning by rodenticides, canine monocytic ehrlichiosis, immune-mediated haemolytic anaemia, etc.
- From other causes of fever, depression, anorexia, etc.
- From other piroplasmosis: it is important to differentiate piroplasmosis caused by a small form from piroplasmosis caused by a large form because this will affect the choice of treatment.

Diagnosis must therefore be confirmed:

- Directly, by staining a blood sample and identifying the parasite visually.
- Diagnosis based on identifying the parasite visually presents a number of challenges:
  - The occurrence of false negatives where the sample is insufficient, or blood is taken from the central rather than the peripheral circulatory system, or is taken after parasitaemia has ceased.
  - Confusion with other intracellular agents: *Ehrlichia canis*, a parasite of the monocytes, which is seen as morulae, or *Mycoplasma canis*, small catenary chain elements.
  - The time necessary to take, stain and read the blood smear is sometimes incompatible with the need for an immediate diagnosis during the consultation. There is no correlation between the extent of the parasitism seen on the smear and the clinical severity of the disease.
- Serology is possible, showing antibodies by various methods (indirect immunofluorescence, ELISA). However, these methods are of little use in practice, as they only reveal a serological trace confirming contact between the animal and the parasite; only kinetics (two serological analyses taken several days apart) allows the existence of developing babesiosis to be confirmed, but no standardised serological method for clinical diagnosis is available as yet, and this method would also be incompatible with the need for a rapid diagnosis.
- Diagnosis by specific PCR on blood samples is now possible. This is the most sensitive method and it allows the pathogen to be identified at the species level, either by RFLP techniques or direct sequencing of the amplification products.
- Post mortem diagnosis is based on the observation of renal, hepatic and splenic lesions and the parasite itself (liver, spleen and kidney prints using a glass slide and Giemsa stain).

Prognosis

Prognosis is good when piroplasmosis is diagnosed and treated rapidly. However, it becomes poor when piroplasmosis has been developing for several days (jaundice, hypothermia, prostration, haematuria, etc.) or in an old animal that has already presented with several bouts of piroplasmosis, and is suffering from another pathology, renal insufficiency in particular.

Control measures

Treatment

Initial treatment is specific and based on the use of active piroplasmicides. Imidocarb is the most widely used and is highly effective against large *Babesia* is less effective against small piroplasms. Symptomatic treatment (perfusion, transfusion, resuscitation) is essential, especially in diseases caused by a small form.

Specific treatment of large piroplasm infection

Imidocarb, an aromatic diamidine, is presented as an injectable solution to be administered intramuscularly or subcutaneously at a minimum dose of 3 mg/kg (0.25 mL/10 kg) and up to 7 mg/kg (especially in the event of a relapse).

This injection may cause local reactions (the infection is painful, but significantly less than phenamidine and does not warrant dilution) and general effects (there is nearly always vomiting in the few minutes after the injection, and sometimes colic, diarrhoea, drooling). Intravenous administration is strictly prohibited. These secondary effects subside rapidly on administration of atropine.

Unfortunately, the parasite has been known to escape medication, causing relapses within 10 days which require a second injection of the product. However, the persistence of imidocarb is such that a single injection is usually sufficient, except in the case of these relapses. The use of this product in pregnant or lactating bitches is contraindicated.

It is important to explain the "normal" evolution of the disease to the owner: clinical improvement must be significant within a maximum of 36 hours, with animal's temperature, behaviour and appetite returning to normal. The animal must be taken back to the clinic if hyperthermia, prostration, anorexia or urinary changes persist or reappear, and especially if other manifestations (such as vomiting) occur, as these may indicate persistence of the parasite (known as parasite escape) or suggest complications, such as acute renal insufficiency (increased creatininaemia and uraemia with proteinuria or anuria), shock, haemorrhage, etc.

Persistence of the parasite, or its reappearance in the peripheral blood after a piroplasmicide injection, is a relatively common phenomenon; it is not attributable *a priori* to true chemoresistance of the piroplasm to the piroplasmicide, but to a failure of the host's immune system. In fact, specific therapy for blood protozoa in general has been shown experimentally to only be effective if it acts in synergy with the immune system:

the likelihood of recovery is significantly different when treatment is carried out following the same protocol as would be used for immunocompetent or immunosuppressed animals. The inability of certain dogs to acquire cellular mediated immunity to seems to explain this phenomenon.

It is essential to combine symptomatic treatment of susceptible individuals (old animals, or those with a history of piroplasmosis, or suffering from another pathology, etc.) with specific treatment: blood transfusion diuretics, isotonic sodium chloride solutions administration of corticoids to control glomerulonephritis due to deposition of immune complexes (for example, prednisolone 1–2 mg/kg/day for 1 week).

All treatment (specific and symptomatic) must be accompanied by a biological and clinical follow-up appropriate to the state of the animal (blood count, blood urea and creatinine levels, proteinuria, etc.).

Treatment of small piroplasm infection

It is more difficult to treat babesiosis caused by small forms than large forms. The piroplasmicides available appear to be less effective against these small forms.

Various combined strategies for treating small piroplasms, especially *B. gibsoni* have been described in dogs. However, relapses after administration of some combinations of anti-babesia drugs are common and pose significant challenges to veterinary surgeons. In Asia, atovaquone (ATV)-resistant strains of *B. gibsoni* are an additional challenge. Combinations of drugs appear to be a better choice for treating infection by small piroplasms. Different protocols for specific treatment are shown in Table 2.

Table 2. Protocols for the control of large and small piroplasms in dogs.

Type of piroplasm	Active ingredient	Dose and route of administration	Schedule and duration of treatment
Babesia large form	Imidocarb	For classic expression of the disease: 3–5 mg/kg For severe expression and relapse: 7 mg/kg IM or SC	A single injection is usually enough A second injection may be administrated in the event of a relapse
Babesia small form *Theileria*	Diminazene + Imidocarb + Clindamycin	3.5 mg/kg IM 6 mg/kg 30 mg/kg	A single dose on the day of presentation A single dose 24 hours after the diminazene was administered. Twice a day for 40–60 days
	Atovaquone + Azithromycin	13.3 mg/kg PO 10 mg/kg PO	3 times a day for 10 days Once a day for 10 days
	Clindamycin + Metronidazole + Doxycycline	25 mg/kg PO 5 mg/kg PO 30 mg/kg PO	Twice a day for 7–10 days Twice a day for 7–10 days Twice a day for 40–60 days

IM: intramuscular; SC: subcutaneous; PO: *per os* (oral).

Prevention

- Tick control: both on the animal, by repeated use of acaricides, and in the external environment (particularly in kennels in the case of *R. sanguineus*: cleaning the surrounding area and good general hygiene help eliminate this endophilic tick).
- Screening asymptomatic animal carriers in order to remove them from the list of potential blood donors.
- Chemoprophylaxis used to be recommended, but the real level of protection provided by this measure has never been clearly demonstrated. It consists of imidocarb at a dose of 6.6 mg/kg (i.e., double the labelled therapeutic dose), which should protect the animal for at least 3 weeks. The use of doxycycline at 20 mg/kg/day to prevent *B. canis* infection has also been advocated, but neither of those measures have demonstrated any efficacy against *B. gibsoni* and chemoprophylaxis does not replace the habitual use of acaricidal products.
- Vaccination against *B. canis* is possible in some countries. This is based on administration of natural soluble antigens from *B. canis*, and added to an adjuvant (saponin). The vaccine reduces the severity of the clinical signs. Vaccination includes checking that the animal is in a good clinical condition and is at least 5 months old, and it should only be used in dogs that have not been previously infected.

  It is contraindicated in pregnant females. It is advisable to administer this vaccination before the epidemiological risk periods (spring and autumn) to avoid simultaneous immunisation and disease, and to administer a top-up every 6 months in high-risk zones. The primary vaccination consists of two subcutaneous injections at least 3 weeks apart, but not more than 6 weeks. The animal is only protected some days after the second injection. The vaccine does not confer cross-protection against small piroplasms.

  In the absence of suitable direct preventative measures, the use of acaricidal products with adequate speed of kill and persistence is recommended. It is important to check the efficacy of a product against the specific tick species that transmits the disease in any given area.

Piroplasmoses in cats*

General comments

Piroplasmoses in cats are infectious diseases caused by the multiplication and pathogenic activity of various species of intraerythrocytic parasites whose taxonomic classification is subject to frequent modification.

Cats are usually less susceptible to piroplasmosis caused by parasites of the *Babesia/Theileria* genera than dogs. Feline babesiosis, although less common than canine, exists all over the world: ***Babesia cati*** in India, ***B. felis*** in India and Africa, ***B. herpailuri*** in South America, and recently ***B. canis presentii*** in the Mediterranean basin. ***Cytauxzoon felis*** (with the lynx as a reservoir) is present on the American continent.

The diseases caused by *B. felis* in South Africa and *C. felis* in North America are medically serious.

Apart from these two species, which are characterised morphologically and genetically, descriptions of *B. felis*, *B. cati* and *B. herpailuri* are often clinical. These species could only be described in this way following more extensive study.

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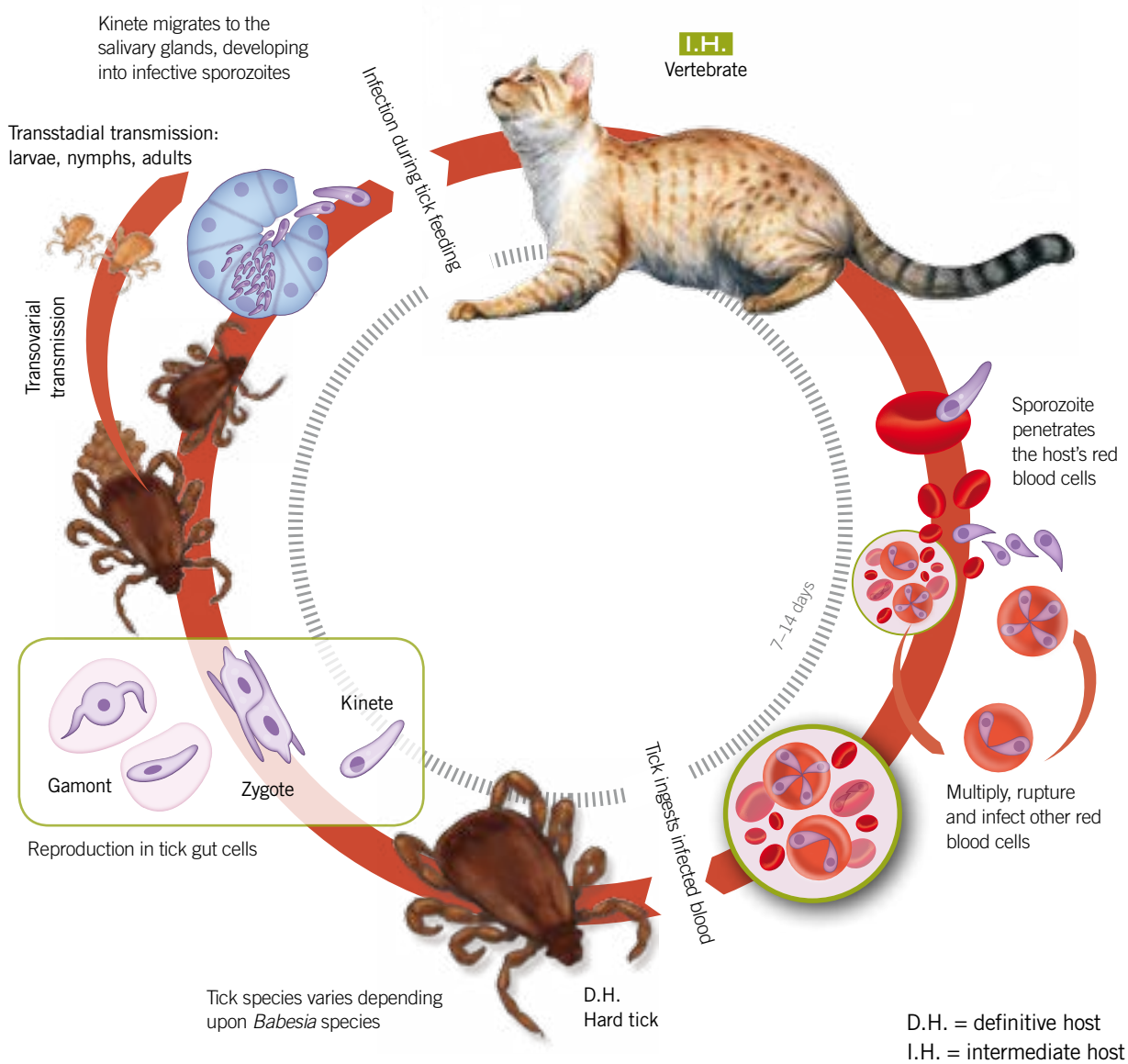
Life cycle of *Babesia* spp.



* Chapter inspired by the original chapters written by Prof. Adam Birkenheuer (chap. 3–02), Gad Baneth (chap. 3–03), Luis Cardoso and Banie Penzhorn (chap. 5–03) in Guide to Parasitoses and Vector Borne Diseases of Pets. Ed. Merial, Lyon, 2013.

Taxonomy

Cytauxzoon felis was first identified in 1973 as a cause of mortality in domestic cats in Missouri, USA. Since then, other *Cytauxzoon* species have been identified (i.e., *C. manul* in Pallas's cats, *Cytauxzoon* spp. in Iberian lynx and a *Cytauxzoon* sp. in European domestic cats).

The Babesia species which infect cats are all "small babesia".

Epidemiology

As with all piroplasms, no arthropods other than ticks are involved in transmission. Contamination relies on the bites of infected ticks, although transfusional transmission is theoretically possible.

Cytauxzoonosis is most commonly identified in young outdoor cats that have a history of tick attachment. There appear to be "geographical hot spots" where the incidence of *C. felis* infections is quite high, and often more than one cat in a household or neighbourhood will become infected. The majority of cases occur between April and

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Life cycle of *Cytauxzoon* spp.

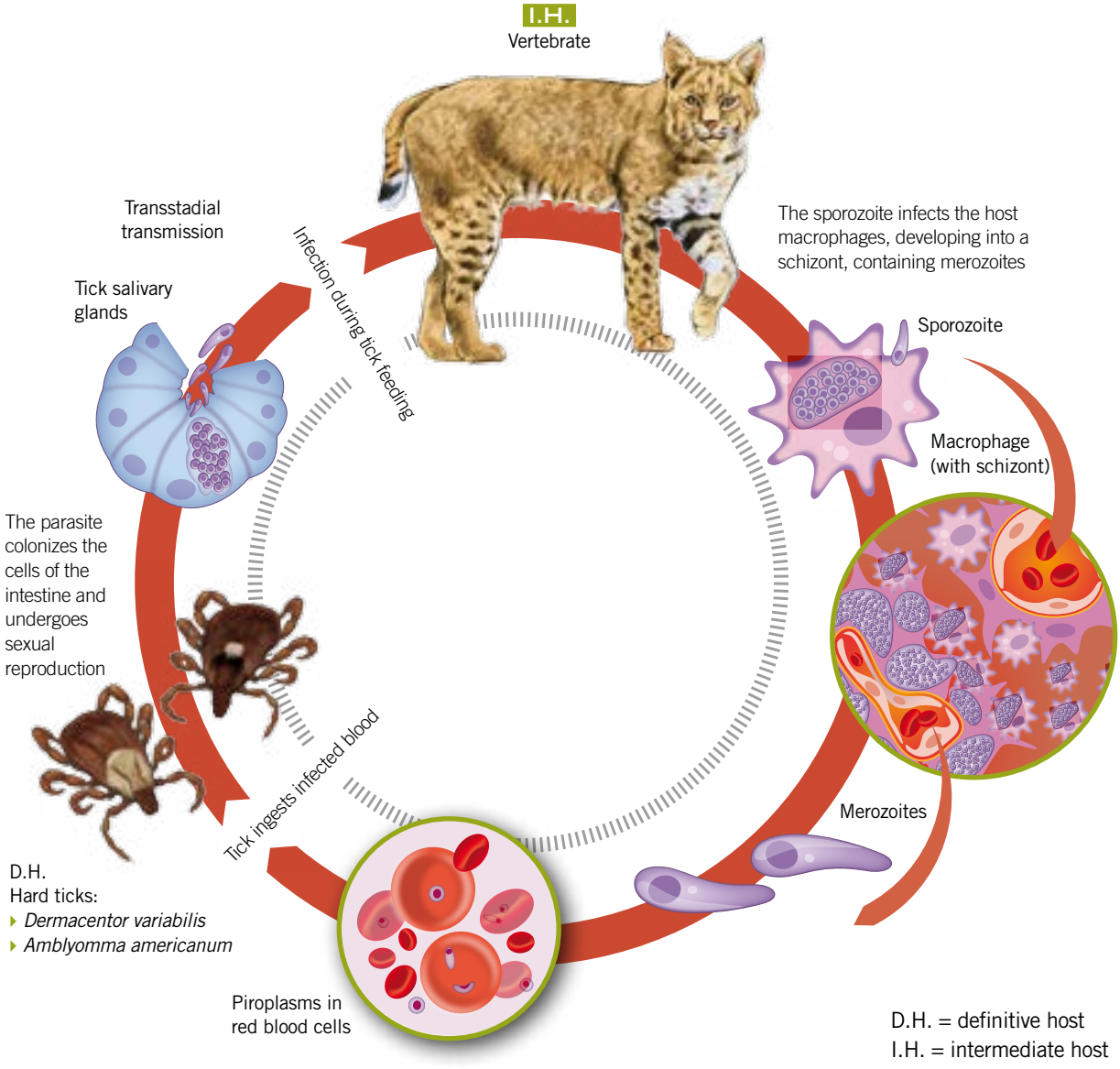


Table 1. Geographical distribution and vectors of the various piroplasm species infecting cats worldwide.

Species	Vectors	Geographical distribution
Babesia felis	?	South Africa
Babesia cati	?	India
Babesia lengau	?	Africa
Babesia herpailuri	?	South America
Babesia canis presentii	*Rhipicephalus sanguineus?*	Israel
Cytauxzoon felis	*Amblyomma americanum, Amblyomma cajennense?*	North and South America

October, which corresponds well with peak tick activity. It has been speculated that cytauxzoonosis in the Americas represents a "species jump" from bobcats to domestic cats. The prevalence of *C. felis* in bobcats ranges from 0 to 79 %, depending on their geographical location. Bobcats are not believed to develop severe disease symptoms, but this is based on an extremely small number of experimental infections. The specific cause of mortality in bobcats, particularly kittens, is poorly defined. The reason for the "species jump" and subsequent emergence of *C. felis* in domestic cats remains unknown. Changes in distribution of the tick vector (*A. americanum*) and host-parasite adaptation, making domestic cats a viable reservoir host for *C. felis* appear to be plausible causes. Domestic cats should no longer be considered dead-end hosts, and may in fact be the most important reservoir host for new infections in other domestic cats (Fig. 1). In some regions where cytauxzoonosis is enzootic, the prevalence in domestic cats may be as high as 30 %. An endemic focus of *Cytauxzoon* spp. infection in domestic cats has been described recently in the Northwest region and infection in feral cats was 30 %. However, no associations between breed, gender, age, presence of ticks and/or fleas, clinical status, laboratory findings such as anaemia, FIV and/or FeLV status and mortality rate were found.

Most cats affected by babesiosis due to *B. felis* seem to be young adults under 3 years old. No breed or sex predisposition is evident, but Siamese cats may be over-represented among purebred cats.

Clinical signs

Cytauxzoonosis

Acute cytauxzoonosis is characterised by disseminated parasitic thrombosis, a severe systemic inflammatory response and multi-organ dysfunction or failure. It is assumed that sporozoites infect a cell of myeloid origin, although the specific myeloid lineage of this cell (CD34+ blast, monocyte, macrophage, dendritic cell or Langerhans cell) remains unknown. Although it is known how merozoites develop from schizonts in infected cells, the specific mechanisms by which sporozoites target cells, and whether or not schizont-infected cells replicate, remain a mystery. The ability to transmit disease by serial passage of small volumes of infected tissue suggests that there is either a subset of schizont-infected cells that replicate, similar to *Theileria* spp., or there is "lateral transmission" of schizonts from one myeloid cell to another. Serial passage of sporozoites or continued sporogony in the vertebrate host seems least likely. Understanding the mechanisms behind the infection of myeloid cells, and the source of

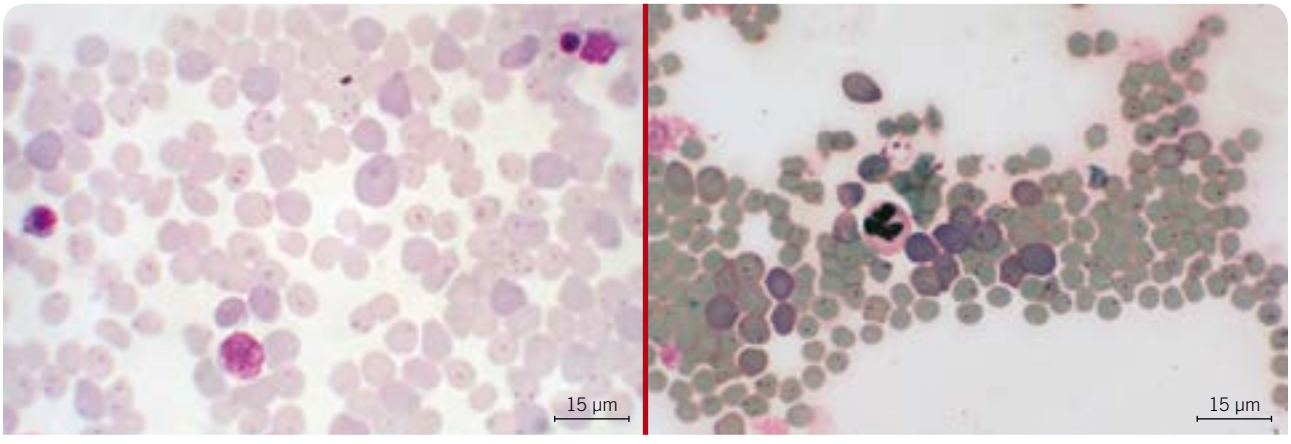


Figure 1. *Babesia felis* in a blood smear from a cat in George, South Africa. MGG stain. Courtesy of Tanya Schoeman.

the millions of schizont-infected myeloid cells, would provide insight into new treatments.

Systemic inflammatory response syndrome (SIRS) criteria frequently found in cats with cytauxzoonosis include:

- Rectal temperature ≤37.8 °C or ≥39.7 °C.
- Heart rate ≤140 or ≥225 beats/min.
- Respiratory rate ≥40 breaths/min.
- WBC count ≤5.0 × 10^3 cells/µL or ≥19.5 × 10^3 cells/µL, or ≥5 % band cells.

Infected cats present raised concentrations of pro-inflammatory cytokines, including tumour-necrosis factor α and interleukin-1 β8. The course of the disease is short and, without treatment, many cats succumb within 5 days of the onset of clinical signs.

Acute cytauxzoonosis is characterised by acute febrile illness. The results of physical examination are often non-specific. Cats usually have a high fever, but hypothermia may be observed in moribund animals. Cats are usually depressed, and vocalization (a so-called death yowl) is common in advanced cases. Dyspnoea is a prominent clinical feature or the main symptom in some cats. Lymphadenopathy and splenomegaly are common but not universal findings, and some cats will be jaundiced. On presentation, laboratory test abnormalities may include non-regenerative anaemia, leukopaenia, thrombocytopaenia, hyperbilirubinaemia and bilirubinuria, raised liver enzyme levels (often not as severe as would be expected considering the degree of hyperbilirubinaemia), hyperglycaemia and hypoalbuminaemia. Thrombocytopaenia and neutropaenia are the most common complete blood count findings. Coagulation test results are consistent with disseminated intravascular coagulation secondary to consumption of platelets and coagulation factors. Despite significantly increased prothrombin and activated partial thromboplastic times, and very reduced platelet counts, clinical bleeding is rare. Many of the typical laboratory abnormalities become more pronounced as the disease progresses (Fig. 2).

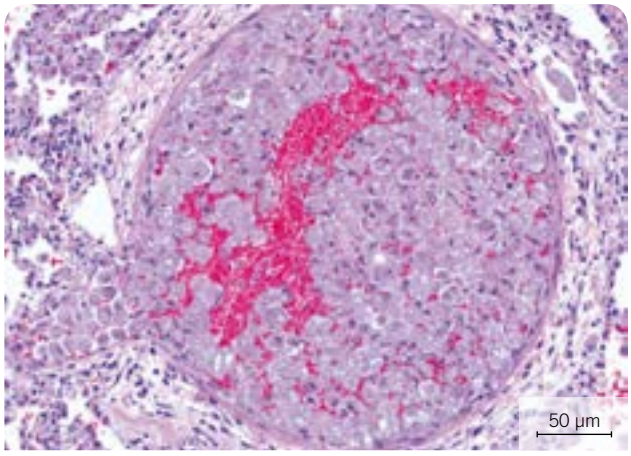


Figure 2. Schizont of *Cytauxzoon felis* shown in a cross section of infected tissue stained with H&E. Courtesy of Adam Birkenheuer.

Babesiosis due to *Babesia felis*

Unlike babesiosis in dogs, feline babesiosis is generally not associated with pyrexia. Anorexia, lethargy and weight loss are often the first signs observed by owners. The most common clinical signs are anorexia, listlessness and anaemia, followed by icterus. Less common signs are weakness, constipation and pica. Parasitaemia is variable and can be very low or extremely high. The strong correlation between central and peripheral parasitaemia indicates that sequestration is not a feature of the disease. Macrocytic, hypochromic, regenerative anaemia is the most consistent haematological finding, although not present in many infected cats. When present, anaemia can become severe in advanced cases, is haemolytic, presumably resulting from both intravascular and extravascular erythrolysis. Other changes in the cell blood count are inconsistent and may indicate concurrent illness or infection.

The most significant clinicopathological changes are raised hepatic cytosolic enzyme activity and increased total bilirubin concentration. Serum alanine transaminase is significantly elevated in most cases, but alkaline phosphatase and gamma-glutamyl transferase are generally within normal limits. This provides evidence of primary hepatocellular damage or inflammation in feline babesiosis. The hyperbilirubinaemia is probably due to haemolysis, but secondary hepatocellular damage is probably an additional contributing factor. Renal damage is not a consistent feature of the disease.

Diagnosis

Diagnosis of piroplasmosis in cats is based on epidemiology (region and season of tick activity) and clinical presentation (combination of haemolytic and pyretic syndromes), as it is in dogs. Confirmation relies on blood smears, stained with MGG to reveal parasitic elements. The presence of parasites in the blood is synchronous with hyperthermia phases.

Serology with indirect immunofluorescence is not diagnostically useful since antibodies are only detectable after approximately a week, and their presence only confirms contact between host and parasite. The possibility of cross-reaction with *B. canis* cannot be ruled out.

Diagnostic techniques involving PCR from a blood sample now allow the various species of Babesia to be distinguished and even studied. However, this is more for epidemiological interest, and to screen parasite carriers, than to carry out an urgent diagnosis.

Rapid diagnosis is critical for cytauxzoonosis. Microscopic identification of *C. felis* remains the diagnostic test of choice. An initial search for organisms can be performed by examining thin, stained smears of peripheral blood and in-house "quick stains" are usually adequate. The feathered edge should be examined with low power (×100) first, to identify schizont-infected myeloid cells. These cells are easily confused with clumps of platelets. High power (×500–×1000) inspection will reveal a very large single cell (50–250 µm diameter) with an eccentric nucleus containing a single prominent nucleolus. The cytoplasm contains a parasite syncytium with a variable number (tens to thousands) of basophilic particles stained magenta. These are the developing merozoites. Organisms in red blood cells are most easily identified on a ×1000 magnification. If organisms are not identified on peripheral blood smears, fine needle aspiration and cytology of liver, spleen and lymph nodes can facilitate a rapid diagnosis in suspected cases. Identification of schizont-infected myeloid cells confirms acute cytauxzoonosis and merozoite-infected red blood cells are a supportive, but sometimes incidental, finding. PCR testing can be sensitive and specific but should not be considered as a replacement for in-house microscopic diagnosis.

Treatment

Cytauxzoonosis

Treatment should be initiated within hours of admission and should be started empirically in suspected cases in enzootic areas, even if a definitive diagnosis is not available.

A combination of atovaquone (15 mg/kg *per os* t.i.d. with a fatty meal to facilitate drug absorption) and azithromycin (10 mg/kg *per os* q.24.h.) is the current treatment of choice for cytauxzoonosis. This combination should be administered as a matter of urgency. A nasoesophageal feeding tube should be inserted on admission, to facilitate medication and nutrition.

Two doses of imidocarb diproprionate 2–4 mg/kg IM at an interval of 15 days has not proven to be as effective. Side-effects include pain at the injection site and cholinergic reactions. The use of diminazene aceturate has been proposed.

Supportive care is the basis of all therapy for cytauxzoonosis. This includes maintaining hydration and blood volume, supplementing oxygen in patients with respiratory compromise, administering anti-coagulant/platelet drugs and providing nutritional support. Clinical recovery is slow, and most patients deteriorate during the first 24–48 hours, gradually improving over the next few days. Minimising handling and stress is recommended. Cats with severe respiratory compromise should be checked for pleural effusion by ultrasound, and therapeutic thoracocentesis should be performed if necessary.

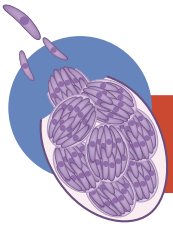
With recent advances in treatment, the prognosis for acute cytauxzoonosis should be considered fair.

Babesiosis

Primaquine phosphate at 0.5 mg/kg is highly effective, but often causes vomiting when administered orally, and has proven lethal at doses exceeding 1 mg/kg. Despite its drawbacks, primaquine remains the drug of choice. Repeated or long-term therapy may be required. Doxycycline may potentially improve treatment of this disease (10 mg/kg PO, in 1 or 2 doses, for at least 10 consecutive days). Concurrent symptomatic treatment is essential. Although response to therapy is usually good, and premunity is assumed to develop over time, mortality from feline babesiosis is estimated to be approximately 15 %.

Prevention

There is no vaccine against cytauxzoonosis or other feline babesiosis, so any method of reducing the risk of tick exposure has to be considered and appropriate use of acaricides is essential if cats are allowed outside.



Hepatozoonosis*

General comments

Hepatozoonosis is an infectious, non-contagious disease caused by development of the protozoan ***Hepatozoon canis*** in various cells in the host, such as endothelial cells and white blood cells (phagocytes, monocytes and polynuclear granulocytes) and transmitted by the tick *Rhipicephalus sanguineus*.

Hepatozoon species are apicomplexan parasites with a hematophagous arthropod final host and a vertebrate intermediate host. They are transmitted by ingestion of the final host, containing mature oocysts, by the intermediate host.

H. canis is a parasite of canids (dogs, foxes) and felids (domestic and wild cats). It has recently been demonstrated that feline infection is primarily caused by a morphologically and genetically distinct species from canine infection. Another species found in America, ***H. americanum***, is more pathogenic than the later one.

It is a parasite of muscular tissue, causing myositis and significant locomotive difficulties. More than 340 species of *Hepatozoon* have been described to date in amphibians, reptiles, birds, marsupials and mammals. The genus has no apparent zoonotic properties.

Hepatozoonosis is prevalent in all zones where the tick *R. sanguineus* can be found in Southern Europe, North Africa, the Middle East (Israel), and Asia.

Hepatozoonosis is often asymptomatic, but can present in a rare clinical form which leads to death. The disease is often expressed clinically through other infections, such as canine monocytic ehrlichiosis (*E. canis*) or leishmaniosis.

Taxonomy

Hepatozoon spp. are Apicomplexa protozoans in the order Eucoccidiida, subclass Coccidia, suborder Adeleorina of the haemogregarine complex (Haemogregarinidae family).

They are taxonomically closer to intestinal coccidia than to *Babesia*, *Theileria* or *Plasmodium*. Transmission of *Hepatozoon* spp. to the dog occurs through ingestion of a tick containing the parasite. There has been no documented transfer of this parasite by saliva. In this respect, *Hepatozoon* spp. differ from other protozoa transmitted by ticks, and from bacterial pathogens transmitted by the tick's salivary glands.

Morphology of observable stages in dogs

- **Schizonts:** macroschizonts (20 µm in diameter), so called because they contain 1–4 macromerozoites, and microschizonts (paradoxically larger, measuring up to 150 µm in diameter) containing several hundred microschizozoites. The macro- and microschizonts can be seen in the cytoplasm of many cells of the mononuclear phagocyte system (vascular endothelium of the spleen, bone marrow, liver, and lymph nodes), myocardium, and lungs.
- **Gamonts:** characteristically rectangular with rounded corners, 8–12 µm × 3–6 µm, with grainy cytoplasm. Gamonts are found in the cytoplasm of polynuclear neutrophils, monocytes and, very rarely, erythrocytes (Figs. 1 and 2).

Biology

When an infected tick is ingested by a dog, *Hepatozoon* sporozoites are released by oocysts in the intestine and penetrate the wall of the gastro-intestinal tract. Sporozoites invade the mononuclear cells and are disseminated haematogenously or via the lymph to the target organs. Meronts containing macro- or micromerozoites are formed in the dog's tissues during the process of merogony. Mature merozoites are released and invade the leukocytes in which gamonts are developing. Hepatozoon gamonts are found in the neutrophils (rarely in the monocytes) of the surrounding blood.

Ingestion of gamonts present in the dog's blood by the tick is followed by fertilisation (fusion of gametocytes) and formation of ookinetes which leave the tick's gastro-intestinal tract and enter the haemocoel. They then become infectious oocysts after undergoing sporogony, and each oocyst produces several sporocystes containing 16 sporozoites.

The nymph is usually contaminated during a blood meal, rarely the larva. The subsequent adult stage is responsible for onward transmission to the dog. Since the sporozoites are not situated in the salivary glands, the dog is not infected by a bite but rather by ingesting the infested tick. There is no transovarial transmission of the parasite to the tick, as is the case with *Babesia*.

The main vector of *H. canis* is the brown dog tick *R. sanguineus*, whilst *Amblyomma maculatum* is the tick vector for *H. americanum*. *R. sanguineus* is found in hot and temperate regions of the world, indicating a wide potential distribution of *H. canis*. However, the distribution of *A. maculatum* is limited to parts of America. The two species of

* Chapter inspired by the original chapters written by Prof. Gad Baneth in the books Guide to Vector Borne Diseases of Pets. Ed. Merial, Lyon, 2013; and Parasitoses and Vector Borne Diseases of Cats. Ed. Merial, Lyon, 2015.

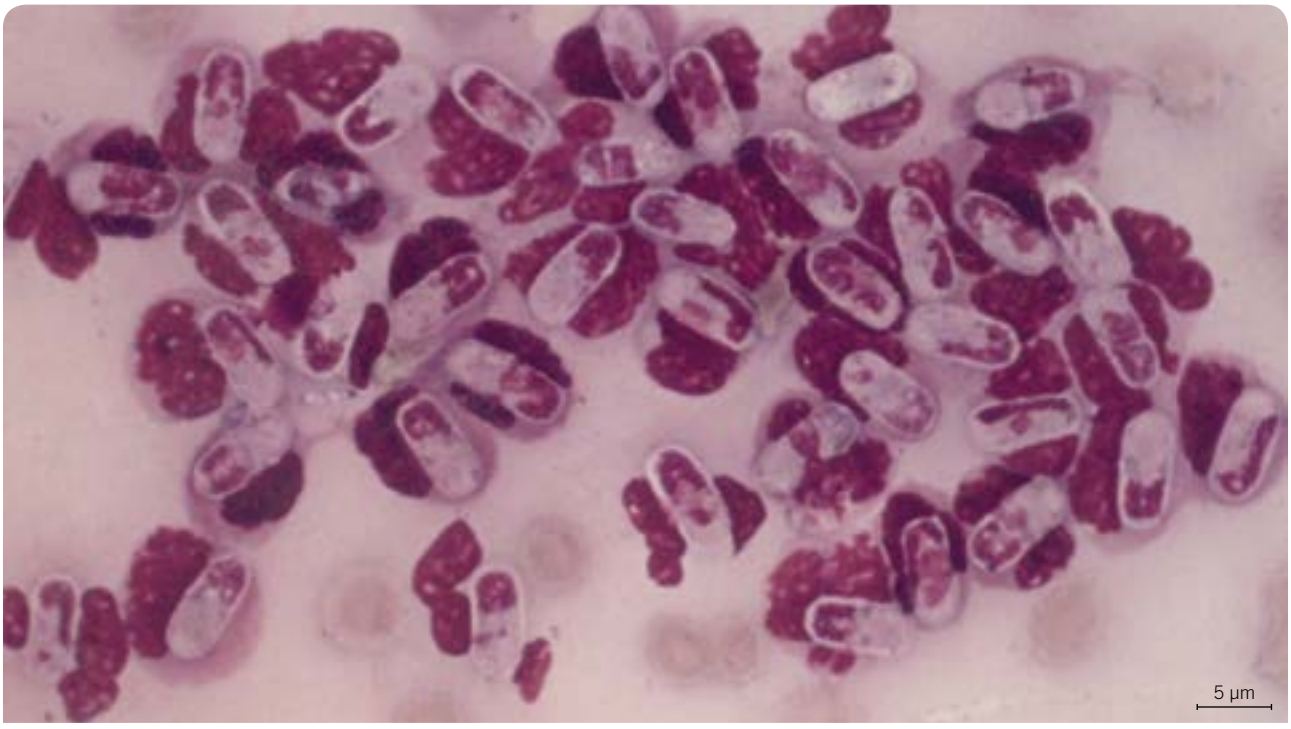


Figure 1. Heavy infection of polynuclear cells by *Hepatozoon canis* gamonts. MGG stain. Courtesy of Gad Baneth.

Hepatozoon which infect the dog are transmitted by nymphs or adult ticks. Vertical transmission of *H. canis* to the dog has been reported in puppies raised in a tick-free environment but born to an infected mother.

Epidemiology

Hepatozoon canis was first reported in India in 1905 and has since been found in Southern Europe, Africa, the Middle East, the Far East and South America. *H. americanum* was initially considered a strain of *H. canis*, until it was described as a different species in 1997. *H. americanum* is an emerging infectious agent in the USA. It seems that it spread from the North and East of Texas, where it was reported for the first time in 1978, to Louisiana, Alabama, Oklahoma, Georgia, Tennessee and Florida.

Exposure to *H. canis* can be high in the parts of the world where this pathogen was first discovered. According to the results of surveys conducted in several parts of the world, between 20 and 30 % of dogs are seropositive in enzootic areas but usually only 1 % of these dogs are parasitaemic, indicating that the rate of exposure is significantly higher than the level of parasitaemia suggests.

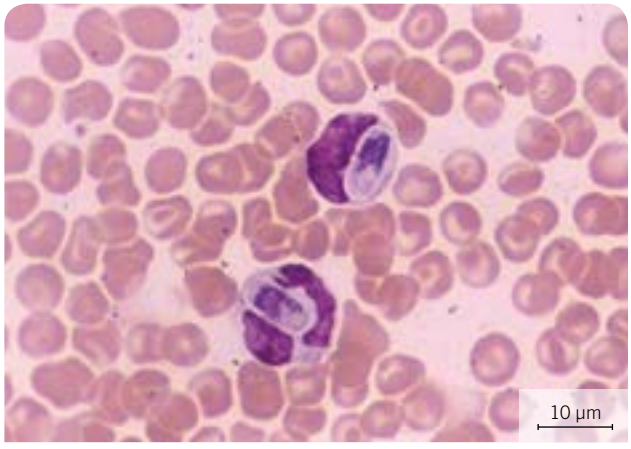
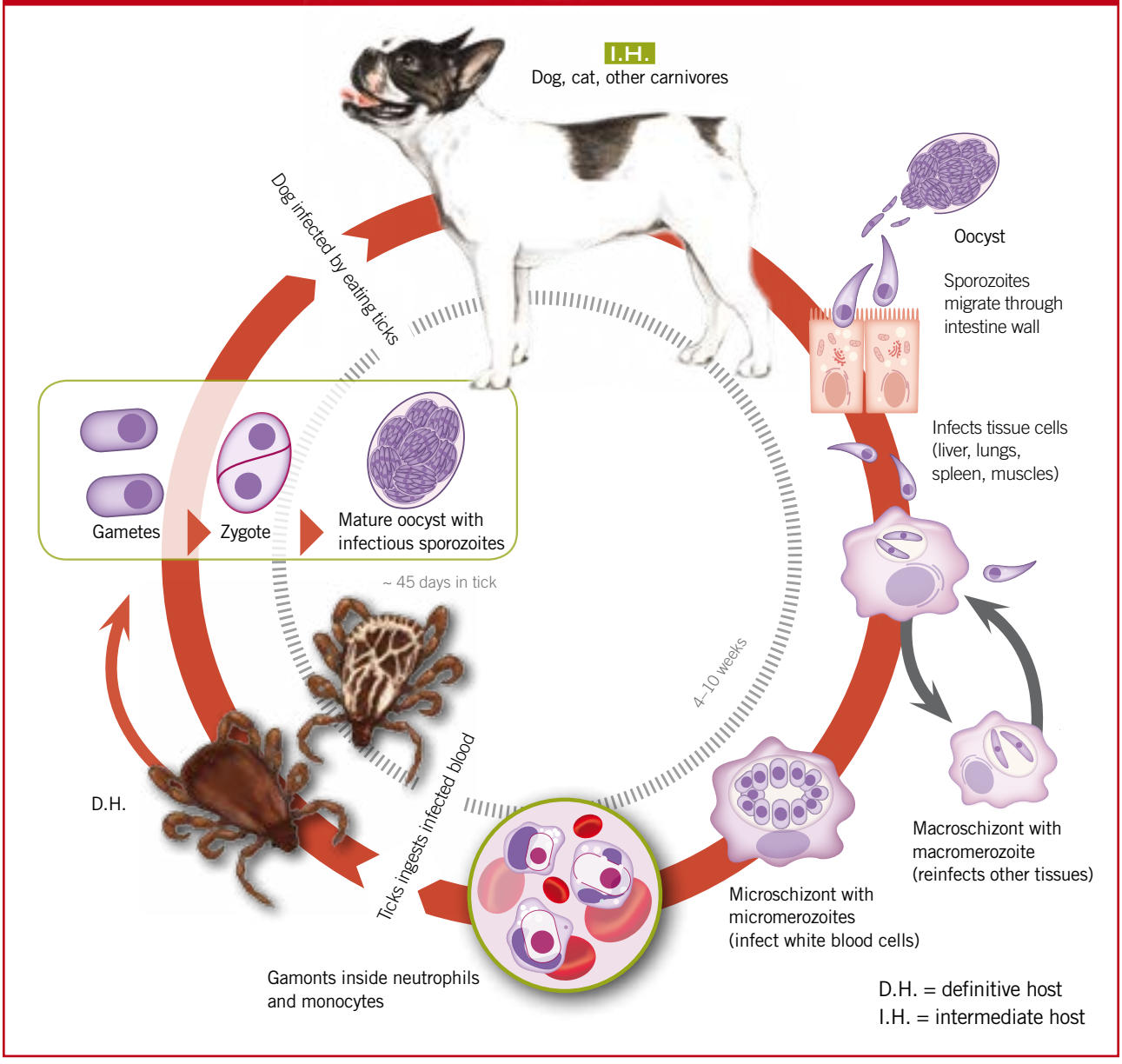


Figure 2. Infection of two polynuclear cells by *Hepatozoon canis* gamonts. MGG stain.

Hepatozoan protozoa are found particularly in outdoor dogs in the summer.

The existence of asymptomatic forms conceals the actual prevalence of the disease. When clinically expressed, hepatozoonosis is found in combination with other serious intercurrent diseases, such as leishmaniosis, ehrlichiosis, cardiac dirofilariosis, distemper, etc. in 40 % of cases.

Life cycle of *Hepatozoon* spp.



Clinical signs, lesions and diagnosis

Infection by *Hepatozoon canis*

Clinical signs

The incubation period is unknown; experimentally, it ranges from 3 days to 1 week.

Infection by *H. canis* can vary from the apparent absence of clinical signs to a disease which constitutes a severe threat to the survival of the animal. The most common form is benign, generally associated with a low level of parasitaemia (1.5 % of leukocytes infected). The severe form of the disease is associated with a high level of parasitaemia, often with nearly 100 % of neutrophils infected. It is characterised by lethargy, febrile syndrome, weight loss leading to cachexia, anaemia and hyperglobulinaemia.

Dogs with a high level of parasitaemia often exhibit considerable neutrophilia (up to 150,000 leukocytes/µL blood).
Other clinical signs may also be observed:

- Diffuse pain, sometimes acute and persistent: lower back pain, joint pain, hypersensitivity to palpation of the legs and spine. This pain causes abnormal locomotion (spinning, crossing of forelegs, collapse, etc.) and lameness. The animal may also refuse to move or to perform certain exercises.
- Excessive excretion by various systems and glands: abundant nasal discharge, diarrhoea, vomiting.
- Polyadenopathy, splenomegaly.
- Other clinical signs include epistaxis, epilepsy, polydipsia, weight loss.

Intercurrent infection by other infectious agents and immune deficiency are often associated with canine hepatozoonosis. The immune responses induced by simultaneous infections can weaken the immune system and interfere with the dog's defences against new infection by *H. canis* or allow a latent infection to emerge. Parvovirus, distemper, *Ehrlichia canis*, *Toxoplasma gondii*, *Babesia canis*, and *Leishmania infantum* have been reported in dogs affected by hepatozoonosis.

Lesions

Tissue biopsies and necropsy of dogs infected with *H. canis* show that it mainly parasitises haemolymphatic organs, including lymph nodes, spleen and bone marrow. *H. canis* schizonts are also associated with hepatitis, pneumonia and glomerulonephritis.

Proliferative and bilateral bone formation has been described in some long bones, reminiscent of hypertrophic pneumic osteoarthropathy or Alamartine-Ball-Cadiot syndrome (periosteal reactions, forming exostoses which offer a credible explanation for the pain but are apparently unrelated to the duration or severity of the disease).

Diagnosis

H. canis infection is usually diagnosed by microscopic detection of intracellular gamonts in MGG-stained blood smears (Fig. 3). Gamonts found in neutrophil cytoplasm have an ellipsoidal structure measuring approximately 11 µm × 4 µm.

Concentrating white blood cells by centrifuging the blood in a microhaematocrit tube allows increasingly sensitive detection, so that it becomes possible to find *H. canis* schizonts/meronts in infected tissues (Fig. 4). These schizonts/meronts contain micromerozoites, organised in circles around a central core, and these cysts must not be confused with those of other protozoan infections, such as *Toxoplasma* or *Neospora*.

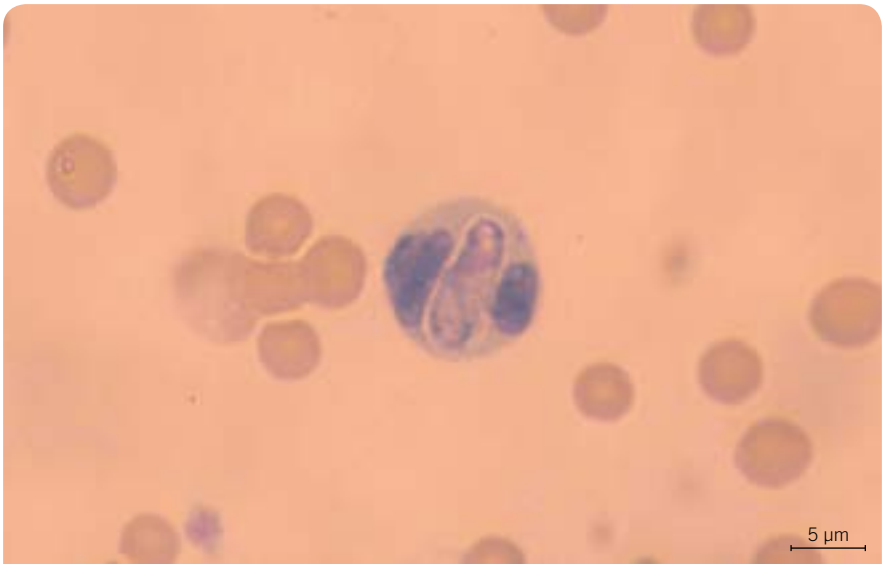


Figure 3. Blood smear showing suspected hepatozoonosis in a cat, MGG-stained. Infection of a polynuclear cell by a *Hepatozoon canis* gamont.

Indirect immunofluorescence testing, using gamont antigens, has been used to detect antibodies against *H. canis* in epidemiological studies in Israel and Japan. An ELISA test is also available in Israel.

IgM and IgG antibodies against *H. canis* are experimentally detected at 16 and 22 days after infection respectively, just before gamonts can be detected in the blood, on the 28th day after infection. It has been suggested that antibodies reacting to the gamont antigens could be formed against antigens from earlier stages of *H. canis*. The serum of dogs infected by *H. canis* only shows a low level of reactivity to *H. canis* antigens.

Infection by *Hepatozoon americanum*

Clinical signs and lesions

Unlike the generally benign disease caused by *H. canis*, in most cases infection by *H. americanum* results in a more serious disease which leads to deterioration of the general condition of the animal and to its death. Most dogs diagnosed with *H. americanum* experience fever, gait abnormalities, muscular pains due to myositis, generalised muscular atrophy and mucopurulent ocular discharge. Pain may be generalised, or localised in the loins, neck and joints. Gait abnormalities in the dog include muscular stiffness, paresis of the hind legs, ataxia and difficulty getting up. Marked neutrophilia is one of the significant haematological characteristics of this disease, and the leukocyte count ranges from 30,000 to 200,000 per mL of blood. Biochemical abnormalities of the serum include increased alkaline phosphatase activity and hypoalbuminaemia.

Diagnosis

H. americanum gamonts are rarely found in the blood and parasitaemia does not generally exceed 0.5 %. Consequently, diagnosis of infection by *H. americanum* is confirmed by muscle biopsy, demonstrating the existence of parasites in the cysts and granulomas (Fig. 5). Histopathology of the skeletal muscles of infected dogs reveals pyogranulomatous myositis and cysts with thick cystic walls arranged in concentric circles (cysts measuring 250–500 µm in diameter). These cysts are sometimes described as resembling an onion skin due to the structure of the membranes surrounding the centre. Radiography of the long bones or pelvis frequently shows significant periosteal proliferation. These radiological elements can prove useful for screening animals with a suspected *H. americanum* infection.

A serological test using tick sporozoite antigens has been developed and this test is as sensitive as a muscle biopsy for diagnosing *H. americanum* infection.

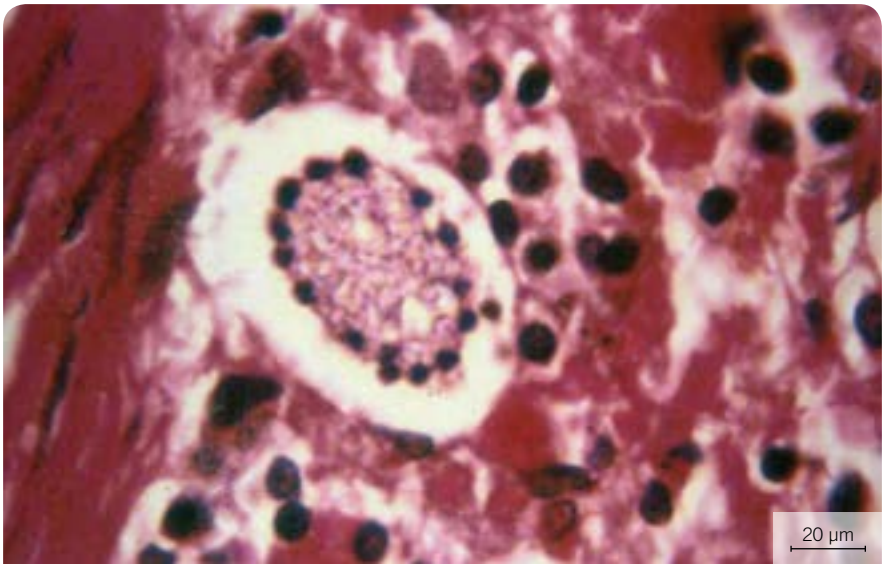


Figure 4. *Hepatozoon canis* schizont. H&E staining of a muscle biopsy.

Control measures

Imidocarb dipropionate at a dose of 5–6 mg/kg every 14 days until gamonts are no longer present in blood smears has long been considered the drug of choice to treat canine hepatozoonosis, along with toltrazuril, an anticoccidial drug (5 to 10 mg/kg/day *per os* for at least 10 consecutive days). Orally-administered doxycycline (10 mg/kg/day for 21 days) is also used in combination with imidocarb. Elimination of *H. canis* gamonts from the surrounding blood is a long process, taking up to 8 weeks, and treatment failure is often reported.

H. americanum infection is treated with a combination of oral trimethoprim-sulfadiazine (15 mg/kg every 12 hours), pyrimethamine (0.25 mg/kg every 24 hours), and clindamycin (10 mg/kg every 8 hours) and treatment must be followed for several weeks. After remission of clinical signs, this treatment can be prolonged by oral administration of decoquinate (an anticoccidial) at 10–20 mg/kg mixed in food, every 12 hours for 2 years. Relapse is common, whether or not the treatment period is respected. Symptomatic treatment of infected dogs with anti-inflammatories relieves pain and fever effectively.

Prevention is by reducing dogs' exposure to ticks and using acaricides.

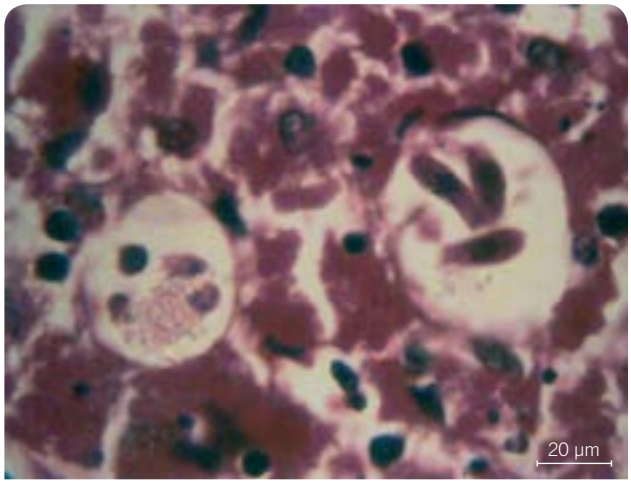


Figure 5. *Hepatozoon americanum* schizonts. H&E staining of a muscle biopsy.

Trypanosomoses *

General comments

Trypanosomoses are inoculable infectious diseases caused by the multiplication and pathogenic activity of various species of flagellate trypanosomes:

- ***Trypanosoma cruzi***, the agent of American trypanosomosis in South America, Central and Southern USA. *T. cruzi* can also be found in the Americas, from the USA to Chile and Central Argentina. This parasite is thought to be enzootic in the Southern half of the USA, as well as in California.
- ***T. congolense*** and ***T. brucei brucei***, agents of African trypanosomoses found in Sub-Saharan Africa.
- ***T. evansi***, agent of trypanosomosis, or surra, in hot regions of the world.
- Carnivores are naturally resistant to *T. vivax*.

These diseases affect canids (domestic and wild) and, far more rarely, felids.

Taxonomy

These parasites are flagellated protozoan parasites of the blood and lymph (and of other tissues in the case of *T. cruzi*) transmitted by haematophagous arthropods: bugs of the genera *Rhodnius* and *Triatoma* for *T. cruzi*; tsetse flies (*Glossina* spp., vectors present only in Africa) (Fig. 1); and tabanids and stomoxes for *T. evansi*.

Biology

Life cycle of *Trypanosoma cruzi*

Trypomastigotes are deposited in the faeces of an infected triatomine insect (reduviid/"kissing bug") near the feeding site; trypomastigotes then enter the vertebrate host through the bite wound or penetrate intact mucous membranes.

Trypomastigotes remain in circulation to disseminate throughout the body and colonize macrophages or myocardiocytes. Within the host cell, trypomastigotes transform into the asexually reproducing stage, the amastigote. Asexual reproduction of amastigotes by binary fission occurs rapidly and, once the host cell is full, amastigotes transform back into trypomastigotes before rupturing the host cell and re-entering circulation. The new trypomastigotes then either enter another host cell or remain in circulation. Triatomine insects acquire infection by ingesting blood containing circulating trypomastigotes. Trypomastigotes convert to epimastigotes within the insect, replicate by binary fission, and then convert back into trypomastigotes prior to exiting in the faeces of the vector.

Life cycle of African *Trypanosoma* spp.

During a blood meal on the mammalian host, an infected tsetse fly injects metacyclic trypomastigotes into the skin tissue and the parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes which are carried to other sites throughout the body, entering other body fluids (e.g., lymph, spinal fluid), and continue to replicate by binary fission. The entire life cycle of African trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. The parasites transform into procyclic trypomastigotes and multiply by binary fission in the fly's midgut, then they leave the midgut and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue to multiply by binary fission. The cycle takes approximately 3 weeks in the fly.

Clinical signs

Clinical presentation depends on the species of trypanosome responsible for the disease.

American trypanosomosis or Chagas disease (*T. cruzi*) has both acute and chronic forms. The incubation period for the acute disease in dogs appears to be 5 to 42 days; symptoms of acute heart disease are usually reported after 2 to 4 weeks in experimental infections. Like humans, some dogs may not develop clinical signs until the chronic stage, which

Figure 1. *Glossina* sp. (tsetse fly) in Africa. Courtesy of Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Pretoria.

* *Trypanosoma cruzi* (from carnivores)

occurs after a few years; the exact length of this period is not known. The acute, less common form is mainly found in the young dog and is rapidly fatal due to cardiac insufficiency: collapse is sometimes preceded by breathlessness and fatigue, anorexia and diarrhoea. The chronic form is more common and affects mainly adult or older dogs. It is characterised by adenomegaly, ascites combined with hepatomegaly and splenomegaly due to right-sided heart failure, and by various nervous manifestations (meningoencephalitis and ataxia, suggesting distemper). It is linked to the development of dilated cardiomyopathy with ventricular arrhythmia. The megaoesophagus and megaviscera reported in humans are not reported in the dog.

African trypanosomosis induces either an acute, rapidly fatal form, seen especially with *T. congolense* in non-native dogs, or a rarer chronic form. Dogs are considered to be very susceptible to infection with *T. congolense* and they develop more severe disease symptoms than other animals.

The following signs have been reported in acute cases of 2 to 3 weeks' duration: anaemia and jaundice, adenomegaly, intestinal disorders (acute ulcerative stomatitis), ocular disorders (photophobia, keratitis, uveitis and corneal oedema),

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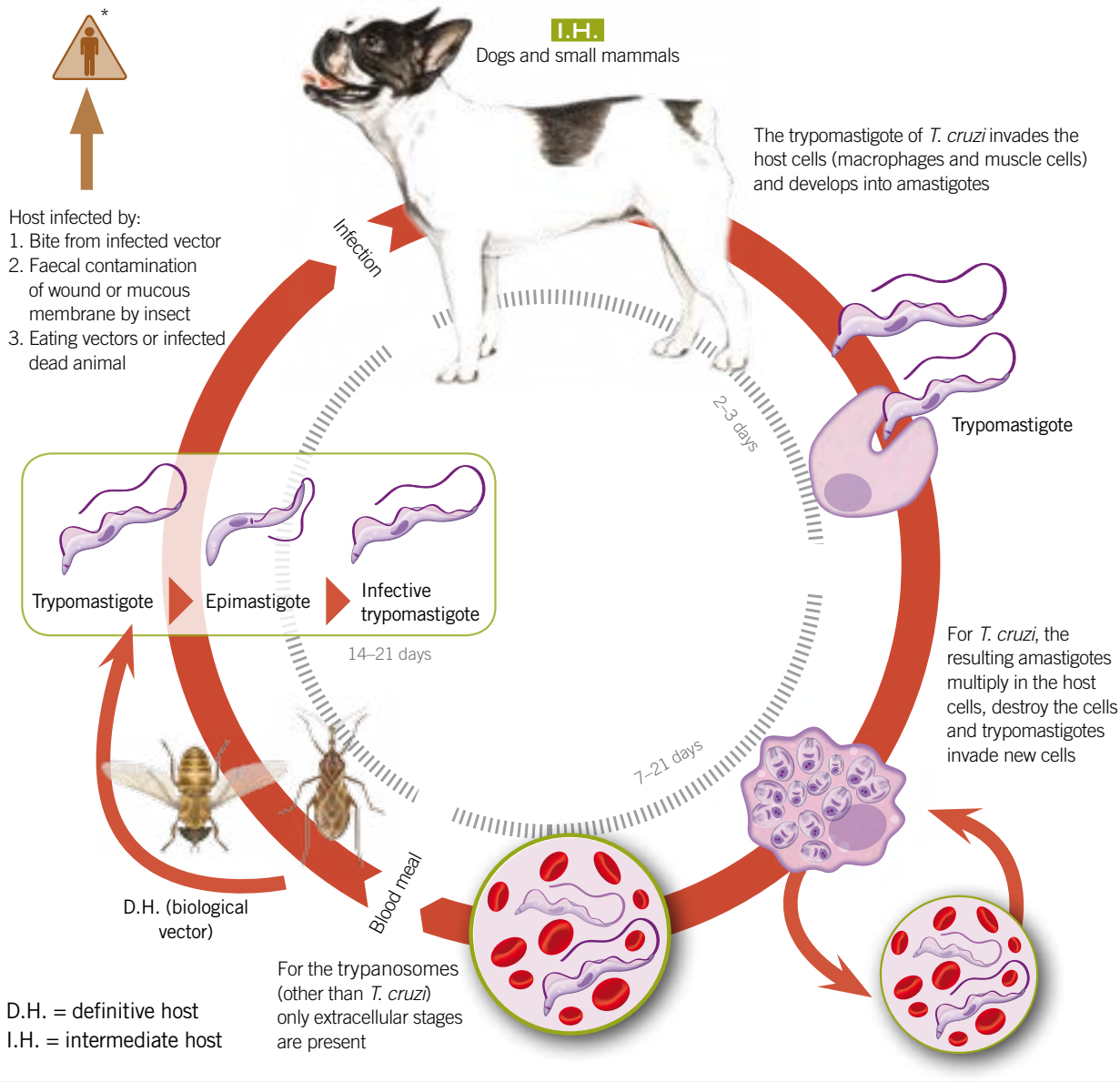
Life cycle of *Trypanosoma* spp.



* Zoonotic trypanosomes: *T. cruzi* (from different mammals including dogs), *T. brucei rhodesiense* (from ruminants)

and periorbital and facial oedema. Terminal convulsions have also been reported. Native dogs frequently present immunity to coinfection and are asymptomatic chronic carriers or have very slow-developing diseases.

T. evansi infects animals and causes a disease known as surra. Surra may be acute or chronic, depending on the strain of *T. evansi* and the location. Infected dogs present anaemia, adenomegaly, cachexia, and hyperthermia, eventually leading to death if untreated. Infected dogs primarily present with lymphadenopathy, malaise, weight loss, hyperthermia and anaemia. Conjunctivitis, blepharitis and uveitis have also been described.

Diagnosis

Diagnosis relies on:

- History: the origin of animal and any visits to enzootic areas.
- Clinical presentation which is not very distinctive, especially in dogs coming from regions where other parasitoses are enzootic. Some can cause similar clinical presentations, such as leishmaniosis, ancylostomosis, spirocercosis, babesiosis, dirofilariosis, etc.
- Additional clinical examinations, such as radiography, electrocardiology, and cell-counts (note that eosinophilia is absent).
- Observation of the parasite:
  - Observation of trypomastigotes in samples of peripheral blood and stained smears (Fig. 2). *T. cruzi* (15–20 µm long), *T. congolense* (12–15 µm), *T. brucei brucei* (shown in Fig. 3, 25–30 µm), *T. evansi* (25 µm on average); however, the parasite is not constantly present in the bloodstream but appears in successive waves, so repeat examinations are necessary and enrichment (centrifugation in a microhematocrit tube, or use of a thicker blood film) will sometimes be required. In the case of fatal acute forms of African trypanosomosis, trypanosomes are always seen and are numerous at the time of death.
  - Oedema and ascitic fluid from lymph node biopsies and possibly *T. cruzi*.
  - Sample cultivation or inoculation of the sample into a laboratory animal.
  - Various serological examinations: indirect immunofluorescence, haemagglutination, PCR, etc.

Control measures

There are few studies on drug treatment efficacy in trypanosomosis in dogs. Treatment consists of using molecules which are often unavailable in non-enzootic areas.

Diminazene aceturate has been proven to be an effective treatment for *T. evansi* in dogs, at a dose of 7 mg/kg on the first day and 3.5 mg/kg on the following day. Other trypanocides for use in ruminants may be used (for example, isometamidium).

Benznidazole (5–10 mg/kg *per os* every 24 hours for 2 months) is the drug of choice for treating *T. cruzi* in dogs. Vomiting is the main side effect. Many infected dogs are euthanized once a diagnosis is made because treatment is unavailable. Additional therapy should target the cardiac dysfunction.

Restricting contact with vectors is central to limiting *Trypanosoma* spp. transmission. Ectoparasiticide treatment with demonstrated repellent properties against Diptera and efficacy against other insects should limit the risk of transmission, even if their efficacy against *Glossina* spp. or triatomine insects has never been tested.

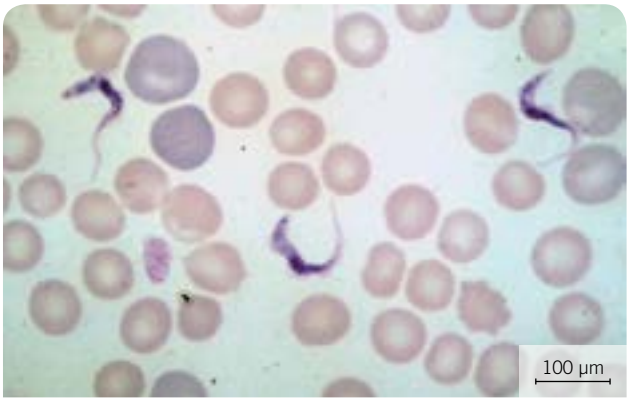


Figure 2. *Trypanosoma* (trypomastigote form) in the blood of an infected animal. MGG-stained blood smear.

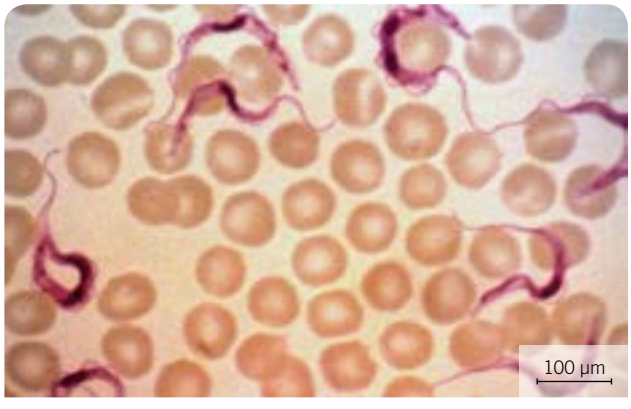


Figure 3. *Trypanosoma brucei* in a blood smear. Courtesy of Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Pretoria.

Respiratory and circulatory parasitoses

Cardiopulmonary dirofilariosis

General comments

Heartworm disease is a helminth infestation transmitted by Culicidae bites and caused by the presence and development of a filarial nematode, ***Dirofilaria immitis***, (heartworm) in the pulmonary arteries and right ventricle of carnivores, mainly dogs. It is characterised by the development of cardiac insufficiency with the progressive appearance of cardiorespiratory problems, sometimes associated with other clinical signs. It is medically important due to this progressive and irreversible cardiac insufficiency, and progression can be faster in heavy infestations.

Hosts

Cardiopulmonary dirofilariosis mainly affects dogs and other canids, but other carnivores such as cats, mustelids (including ferrets), sea lions, seals, etc. may also be infested. Cats in highly enzootic areas can also be infested, although they are less susceptible than dogs so, when the risk of infestation is high, the prevalence noted in felines can be considered to be 5 times less than that found in the canine population. Cats must be protected in the same way as dogs in enzootic areas, where domestic ferrets are widespread.

Geographical distribution

Dirofilariosis is widely distributed worldwide though prevalence differs from one country to another, and even from one region to another, depending on the density of mosquito vectors and their seasonality.

Dirofilariosis is particularly common in tropical areas (Africa, Asia, Australia, Central and South America, Pacific islands) where between 20 % and 60 % of dogs can be infested, but it is also found in Canada, Japan, and most other US states (although particularly in the south: Florida, Louisiana).

It is also found in Southern Europe, particularly in the Mediterranean area (Spain, Italy and Greece) and in the far South of France (Provence), but it is sporadic here. It is enzootic in Corsica, Sardinia and some Eastern European countries (Romania), although it is less common than the subcutaneous filarial nematode, *D. repens* in Northern Europe.

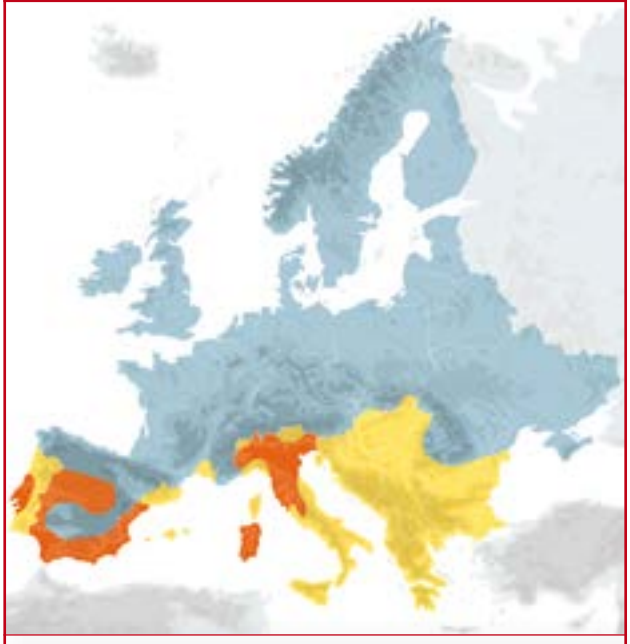
Dogs travelling to and from enzootic areas may become infested, especially during the hot season, when vectors are abundant, so diagnosis is sometimes made in areas where there is no cardiac dirofilariosis, such as the Northern half of France, Germany, Belgium, and the United Kingdom (Fig. 1).

Taxonomy and morphology

The adults are Filaroidea nematodes belonging to the Onchocercidae family, characterised by a thin, elongated body and simple anterior end, with no capsule or gall bladder. The posterior end is also simple (with no copulatory bursa), and is twisted and curly in males.

Adult males are 12–18 cm long and 1 mm in diameter, and females are up to 30 cm long and the same diameter, and both are a whitish colour. The females lay microfilariae, measuring approximately 300 µm long x 6 µm in diameter, in the blood.

Figure 1. Distribution of dirofilariosis (heartworm disease due to *Dirofilaria immitis*) in Europe.



Derived from Morchón, 2012.
Courtesy of Luís Cardoso, Robert Farkas, Domenico Otranto, Kurt Pfister, Xavier Roura, Smaragda Sotiraki, Donato Traversa, Richard Wall.

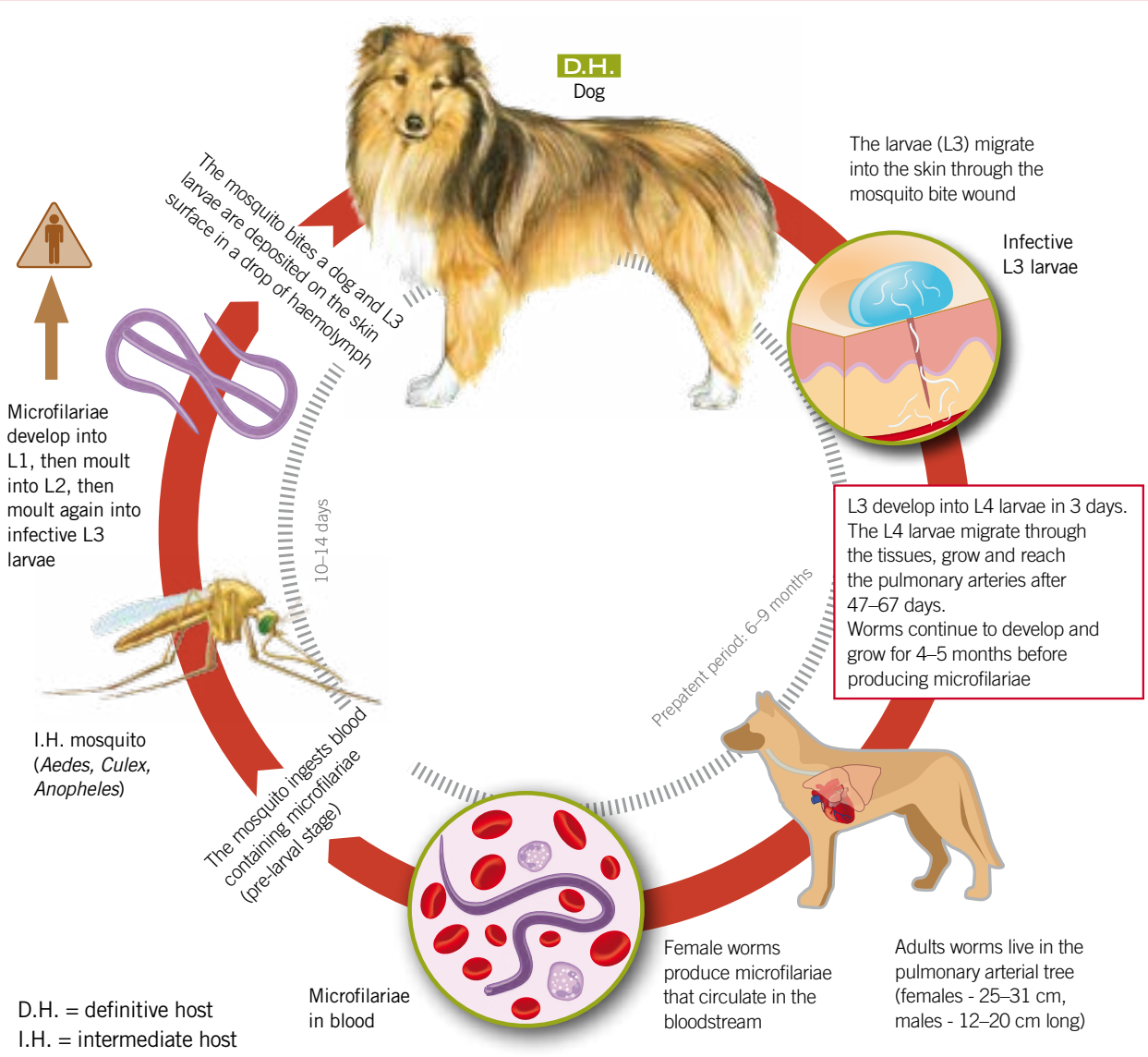
Biology

The adults live in the right ventricle and pulmonary artery of their definitive carnivore host and pre-adults live in the pulmonary arteries. When infestation is heavy, worms may be found in the posterior vena cava, causing acute haemolysis known as caval syndrome. Erratic localisation has been described (in the anterior eye chamber, subcutaneous connective tissue, spinal cord, etc.). There is usually a crepuscular peak of microfilaraemia at the moment when the mosquito vectors are the most active but this peak varies from region to region.

The microfilariae are ingested by the intermediate host, a female mosquito (*Aedes, Culex, Anopheles*), during a blood meal (Figs. 2 and 3). They become L1 larvae in the Malpighian tubules, taking on a short, squat, "sausage" shape before changing into L2 around day 4. Around day 10, they change into L3 larvae and pass into the mosquito's body cavity and enter the labium (or buccal cavity). An infestation of more than 10 larvae usually kills the insect. When the mosquito bites a definitive host, the labium and proboscis fold up and the L3 are transferred to the site of the bite and penetrate the wound.

PDF

Life cycle of *Dirofilaria immitis* in dogs



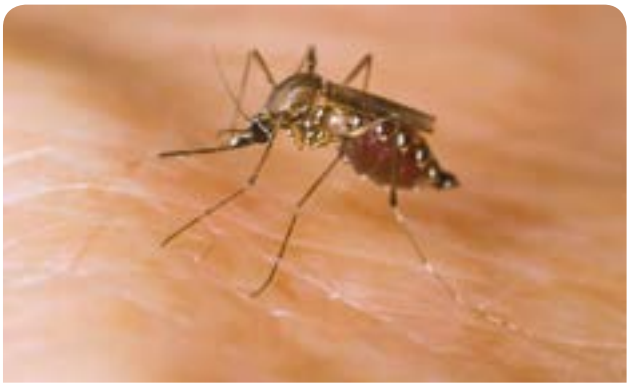
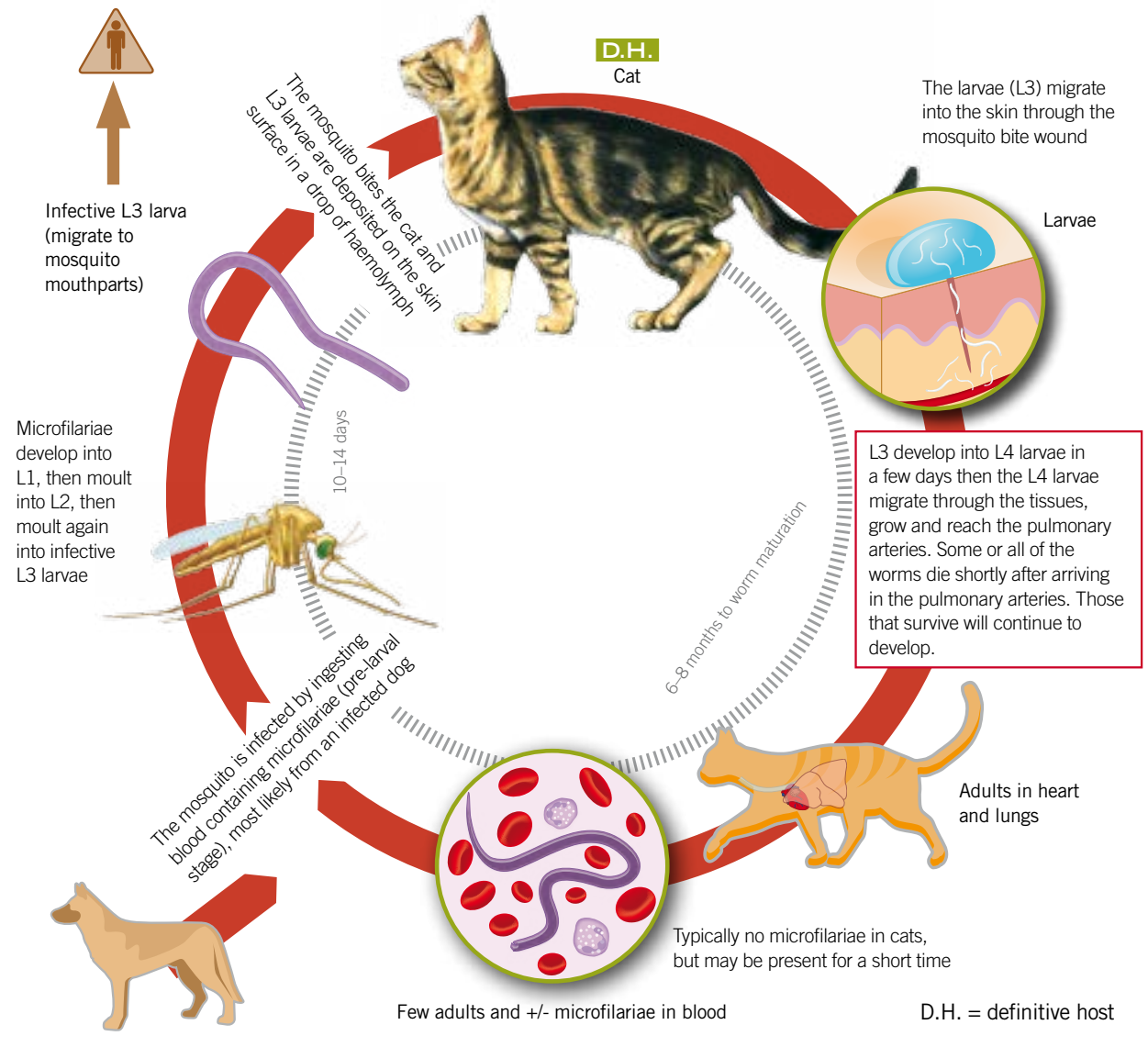


Figure 2. Female mosquito of the genus *Aedes*, a vector of *Dirofilaria immitis*.

Figure 3. Mosquito of the genus *Culex*, a vector of *Dirofilaria immitis* in Europe.

PDF

Life cycle of *Dirofilaria immitis* in cats



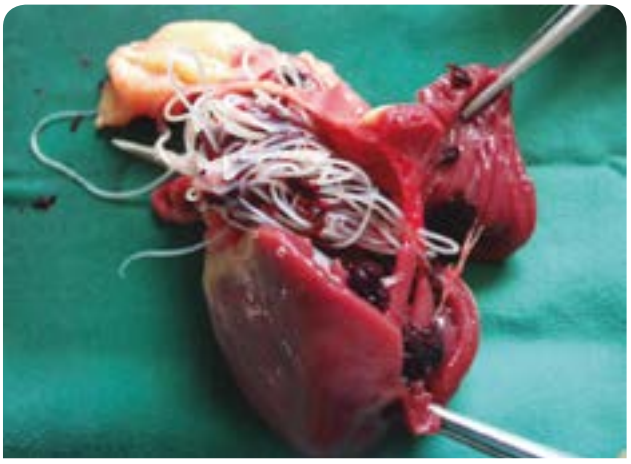


Figure 4. Adults of *Dirofilaria immitis* in the right heart of a dog. Courtesy of Thierry Bord.

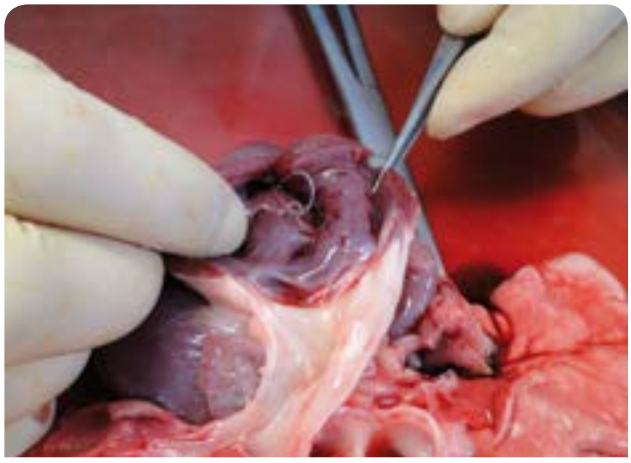


Figure 5. Section of right ventricle with adult *Dirofilaria immitis*.

They then travel to the subcutaneous connective tissue and stay there for approximately 2.5 months (60–80 days), moulting into L4 larvae around day 10 and maturing into pre-adults around day 60. These pre-adults are approximately 2–5 cm long and they enter the right heart via the circulating blood (Figs. 4 and 5). These pre-adults lodge in the pulmonary arteries on around day 80 and stay there for approximately 7 weeks, before returning to the ventricle by retrograde migration, becoming adults and then mating. Some remain in the pulmonary arteries in cases of heavy infestation. The prepatent period is very long, taking approximately 5–6 months, and sometimes longer.

Adult filariae live for a long time, around 4–5 years, but females become much less prolific after 3 years. In the absence of protective immunity, infestations accumulate over the following months or even years, explaining the very heavy infestations that can sometimes be seen, but also the progressive appearance of clinical signs due to the accumulation of parasites in the heart.

Any microfilariae transmitted from a bitch to her young via the placenta, or between dogs by transfusion, will continue to circulate but will not develop. Only L3 transmitted by mosquito vectors will develop into adult filariae.

Epidemiology

Dirofilariosis is a vector-borne parasitosis and may be seasonal, depending on the latitude.

Source of parasites

- **Reservoir**: microfilaraemic dogs are the source of microfilariae for mosquito vectors (10–20 % of dogs infested are amicrofilaraemic). The importance of stray dog populations, and wild canids (foxes, coyotes) in maintaining the disease in certain areas must not be underestimated.
- **Direct source**: this consists solely of the female mosquito vectors (*Culex, Aedes, Anopheles*), with nearly 70 species susceptible to the parasite and therefore considered potential vectors.

The vectorial capacity of any species of mosquito varies greatly from one region to another: in the Tropics, *Aedes aegypti* is often the main vector but in the Mediterranean it is often *Culex pipiens*.

Other biting arthropods (fleas, lice, ticks) are never involved in the transmission of *D. immitis*, but they may be vectors of other filariae (*Acanthocheilonema* genus).

Mechanism of infestation

Infestation occurs solely through inoculation of L3 larvae from a female mosquito bite.

Predisposing factors

All factors which increase the chance of mosquito bites increase the risk of infestation: dogs living and sleeping outside, for example. Depending on the latitude, the risk of infestation may be continuous throughout the year (as is the case in tropical countries) or seasonal (in Europe, where transmission mainly occurs between spring and autumn).

Clinical signs

Dogs which harbour few worms generally do not present with clinical signs, as these are linked to heavy infestations or to repeated infestations where there is an accumulation of parasites. Dogs may be grouped into four clinical classes. Incubation may be long (several years).

Cardiorespiratory signs

These are linked to the adult filariae and are the result of irritative mechanical action, and antigenic reactions. They cause chronic pulmonary hypertension, which leads to continuous cardiac effort to maintain sufficient pulmonary perfusion.

- **Stage 1**: excessive fatigability, reduced appetite. This initial phase is known as clinical stage 1. At this stage, the heart compensates for pulmonary hypertension, and clinical signs are still subtle but cardiac insufficiency due to decompensation gradually develops. This stage is afebrile.
- **Stage 2**: this stage corresponds to moderate dirofilariosis. The animal presents with coughing and dyspnoea on exertion, shortage of breath at rest, and often accompanied by anaemia.
- **Stage 3**: this stage corresponds to severe dirofilariosis. The animal presents with tachycardia and dyspnoea as well as coughing at rest, ascites, chronic renal insufficiency, and often exhibits cyanosis of the mucosae. These clinical signs get worse, the animal loses weight and its general condition is affected. This stage ends indeath from respiratory distress or violent pulmonary embolism caused by nematode fragments.
- **Stage 4**: complications linked to heavy infestation. This is caval syndrome, and corresponds to the arrival of worms in the posterior *vena cava*. The animal presents with anaemia and haemoglobinuria, and is in a state of shock linked to the sudden and intense haemolysis caused by the haemodynamic disruption.

Heart murmurs can be heard on auscultation at all stages.

Other clinical signs

These are potentially linked to the microfilariae, which cause thromboembolism and associated localised immune and inflammatory reactions. These embolisms may also be the result of the fragmentation of adult worms.

- **Cutaneous signs**: pruritus, hair loss, necrosis of the extremities (ears, tail).
- **Nervous signs**: the most commonare paresis, motor incoordination, occasional convulsive crises, phases of aggression, and temporary loss of consciousness.
- **Haemorrhagic signs**: melaena, epistaxis, haemoptysis.
- **Ocular signs**: uveitis.
- **Renal impairment**: chronic renal insufficiency.

Lesions

- **Macroscopically**: cardiomegaly, hepatic congestion, ascites, pneumonia (haemorrhages and congestive areas in the diaphragmatic lobes) (Fig. 6). Filariae can be seen in the ventricle, wrapped around the heart strings.
- **Microscopically**: villous endarteritis with hyperplasia of the walls in the pulmonary arteries, and the appearance of reactive microvilli; interstitial pneumonia with inflammatory granulomas containing microfilariae; cardiac dilation and hypertrophy; glomerulonephritis.

Figure 6. Typical pot-bellied appearance of a dog with heartworm disease and displaying ascites related to right-sided congestive heart failure.

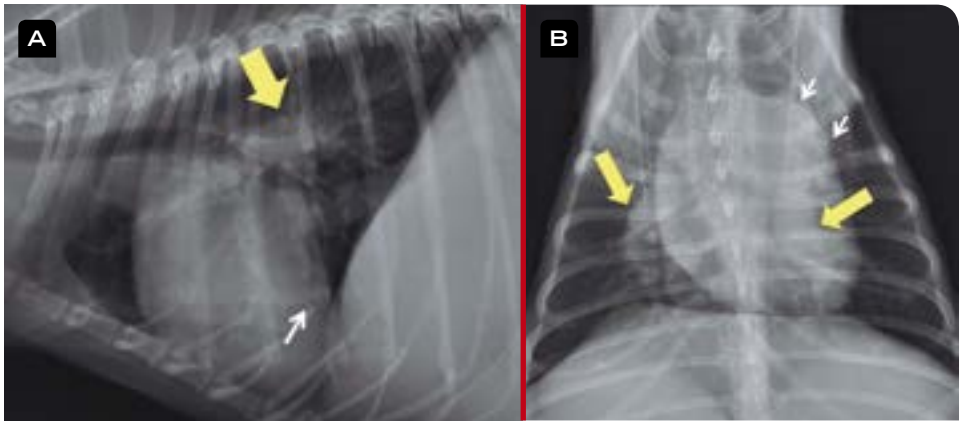


Figure 7. Radiography of the thorax of a dog showing right-sided cardiac hypertrophy due to cardiopulmonary dirofilariosis. (A) On the lateral view, interstitial densification (large arrow) and interlobar fissure are visible (small arrow). (B) On the ventral view, both pulmonary arteries (large arrows) and pulmonary trunk (thin arrows) are enlarged. Courtesy of Medical Imaging Department, Alfort Veterinary School.

Diagnosis

In enzootic areas, clinical suspicion is easy: the dog is tired, and can present with shortage of breath and coughing, and clinical signs get worse with effort (after going for a walk, for example).

Examination of the blood shows regenerative anaemia. Hypereosinophilia is possible but not symptomatic. Auscultation and electrocardiograms (ECG) do not identify particularly characteristic elements but confirm the diagnosis of right-sided cardiac insufficiency.

Radiography

Radiography shows cardiomegaly and distinct arborisation of the pulmonary vessels, which is relatively characteristic. Thoracic radiography is performed on dogs which present with cardiac or respiratory disorders, such as tachypnoea, dyspnoea, excessive fatigability, and abnormal cardiac auscultation (breaths). In some cases thoracic radiography will reveal the spread of organ lesions and suggest dirofilariosis or angiostrongylosis. Radiographs are taken at 1–1.20 m to limit deformation, and at the end of inhalation to avoid over-taxing the diaphragm or lungs, with an exposure time of less than 6 cm/s. Two exposures are taken: a lateral and a dorsoventral view. For the first, the dog is in a lateral position, limbs extended, and thorax parallel to the table, and the ray is centred on the fifth rib. The dorsoventral view is taken with the dog in sternal recumbency (sphinx position). The right ventricle appears normal to dilated (inverted D on frontal radiography and increased contact area with the sternum on the lateral view) due to the progressive development of right-sided cardiac insufficiency (Fig. 7). The pulmonary artery has a normal to dilated trunk and tracheal bifurcation may be raised. Pulmonary arteries are enlarged, deformed,

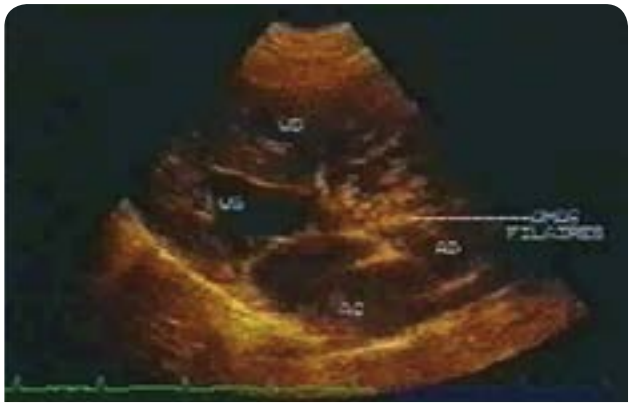


Figure 8. Echocardiograph following suspected dirofilariosis, allowing definitive diagnosis from the section of worms seen in the pulmonary arteries.

or interrupted and arborisation may be reduced or absent. The pulmonary parenchyma sometimes presents areas of densification (eosinophilic pneumonia). Lesions, characteristic of right–sided cardiomegaly, suggest dirofilariosis, especially when combinedwith abnormalities of the arterial trunk and parenchyma. Right–sided cardiomegaly and abnormalities of the pulmonary parenchyma are observed in cases of angiostrongylosis.

Echocardiography

Unlike radiography, echocardiography enables cardiopulmonary dirofilariosis to be diagnosed through visualisation of the parasites (Fig. 8). Non-specific lesions can be seen: dilation of the main pulmonary artery (right branch); right ventricular dilation (first hypertrophic then atrophic). Signs specific to the parasite: adult filariae present in the right ventricle and pulmonary trunk (cross-sectional visualisation of many round elements, 2–3 mm in diameter, which move with the contractions of the heart); thrombus in the pulmonary vessels.

Laboratory diagnosis

The infestation is confirmed by identification of blood microfilariae or by screening circulating filarial antigens.

Detection of microfilariae

Microfilariae are found by blood smear, observation of a drop of fresh blood, or after enrichment (blood filtration on a Millipore membrane, Knott technique, or via a thick smear), then by MGG staining (Figs. 9 and 10).

Sensitivity depends on the technique, level of infestation and sometimes the time. Microfilariae seem to circulate more at twilight. Some dogs are described as amicrofilaraemic because they do not have microfilariae, for various reasons: infestation by male worms only, or by old worms, treatment with ivermectin, or destruction by the immune system.

Identification of microfilariae is necessary because several species are found in dogs. Although differential diagnosis of microfilariae of the *Dirofilaria*, *Acanthocheilonema* or *Cercopithifilaria* genus is simple, differentiating between *D. immitis* and *D. repens* is more difficult. This is why histochemical staining, which shows alkaline phosphatase activity, is used. These areas of phosphatase activity differ depending on the species of microfilaria involved (Fig. 11 and Table 1).

Figure 9. Microfilariae observed by direct examination between slide and coverslip of a drop of peripheral blood (auricular puncture with a stylus).

Detection of circulating antigens

The rapid heartworm antigen tests currently available detect circulating proteins, secreted mainly by adult female *D. immitis*. These kits use different serological techniques (ELISA or agglutination) which are very sensitive, and it is possible to screen for a single female parasite. This particular test is specific to *D. immitis* because species-specific monoclonal antibodies are used. The kits can be used for dogs, but not for cats because the antigens detected are shed by adult female filariae and the parasites often remain in an immature state in felines. Serological tests that highlight antibody responses are available for all species, including felines.

The earliest that heartworm antigens and microfilariae can be detected is about 5 and 6 months post infestation, respectively.

Focus on feline heartworm disease

The scientific community has only recently focused on feline heartworm disease and recognised the differences in host response, pathogenesis and clinical presentation fin heartworm infection in cats and dogs.

Cats are usually infested by two to four heartworms (the range is 1 to 8), the prepatent period is 7–9 months and worms survive for just 2 to 4 years.

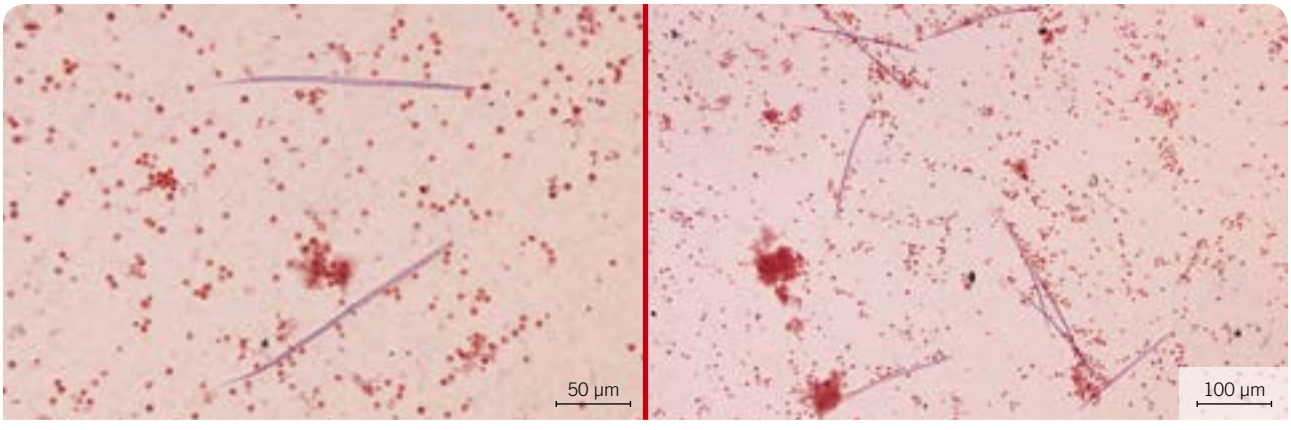


Figure 10. Microfilariae observed after concentrated MGG staining by the Knott method.

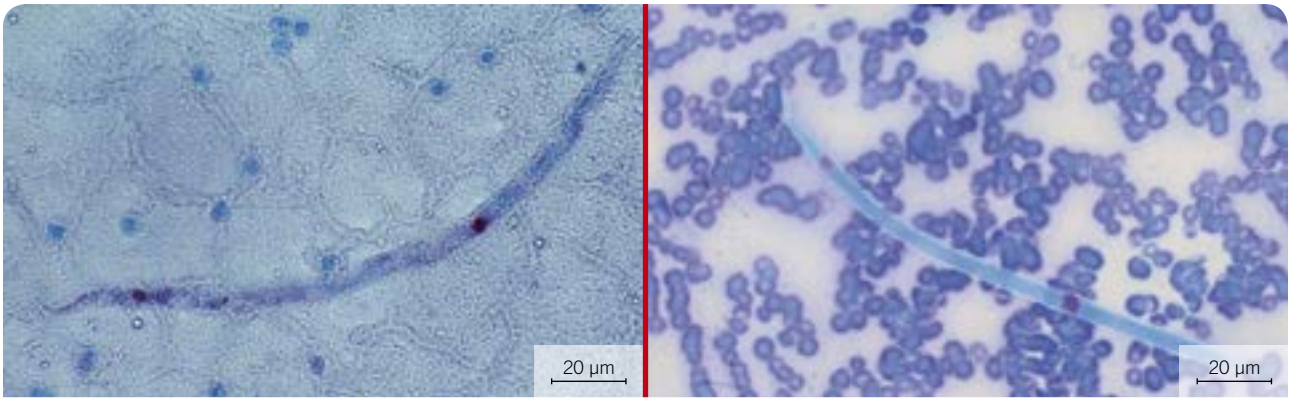


Figure 11. Histochemical staining of *Dirofilaria immitis* microfilaria. Phosphatasic acid activity (red stain) concentrated in the excretory pore (anterior 1/3) and anal pore (posterior).

Table 1. Differential diagnosis of microfilariae (µF).

	Dirofilaria immitis	*Dirofilaria repens*	*Acanthocheilonema* sp. and *Cercopithifilaria* sp.
Size	290–330 µm × 5–7 µm	300–370 µm × 6–8 µm	• *A. reconditum*: 260–280 × 4–5 µm • *A. dracunculoides*: 190–250 × 4–6 µm • *C. grassii*: 550 × 10–12 µm (µF dermotropic)
Direct examination between slide and slip cover	µF moving little in the field of view	µF moving little in the field of view	µF moving very quickly in the field of view
Examination after staining (MGG or eosin after filtration)	Straight body, regular anterior end, sub-rectangular cephalic space, frayed and straight caudal end	Regular anterior end, sub-square cephalic space, long and frayed tail, sometimes slightly curved	Irregular anterior end, short tail, hook-shaped caudal extremity
After histochemical staining and evaluation of acid phosphatase activity	One (red) spot at each end, corresponding to the anterior excretory pore and posterior anal pore	Area of posterior activity corresponding to the anal pore	For *A. reconditum* microfilariae: Diffuse activity; for *A. dracunculoides*: two zones of activity similar to those observed with *D. immitis* but smaller in size

Clinically, infection can be asymptomatic, chronic or acute. Clinical signs are usually more obvious in two particular stages of the cat infection: when young adult *D. immitis* arrive in the pulmonary arteries (4 to 6 months after infection) and die, and later, when adult die (Fig. 12).

Natural heartworm infection in cats is basically pulmonary in nature, so the most common clinical signs have a respiratory origin, but digestive signs are also frequent.

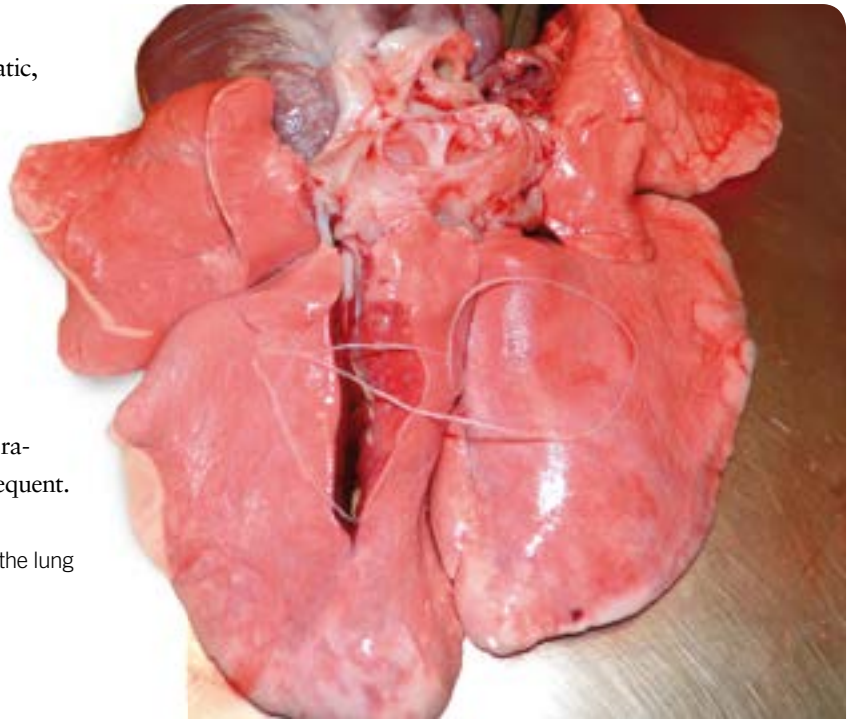


Figure 12. Immature adult *Dirofilaria immitis* in the lung of an infested cat.

The most common respiratory sign is dyspnoea, although coughing, tachypnoea and, more rarely, sneezing, can also be seen. Vomiting which is not associated with eating is the most common gastrointestinal sign but other symptoms, such as diarrhoea, are occasionally seen. Feline heartworm disease is now recognised as a significant pulmonary syndrome, defined as Heartworm Associated Respiratory Disease (HARD). Clinical signs associated with HARD are anorexia, lethargy, weight loss, coughing, rapid heart rate, vomiting, diarrhoea, blindness, convulsions, collapse and sudden death. In acute cases, cats can die so quickly that owners are usually not able to report any clinical signs before sudden death occurs.

In those very rare cases where worms are located in the right heart, a systolic cardiac murmur, caused by tricuspid valve insufficiency and galloping cardiac rhythm, is a common finding on auscultation. Signs that are common in dogs, such as congestive heart failure, are not often seen in cats and some infected cats recover from the infection spontaneously, with or without symptomatic treatment. However, reversion to the dangerous acute phase when adult parasites die is always possible, even in cases of chronic infection. The death of adult worms is associated with an intense pulmonary inflammatory reaction in response to thromboembolism that is itself responsible for pulmonary infarction and haemorrhage. Circulatory collapse and respiratory failure usually follow. Clinical signs at this stage can include dyspnoea, cyanosis, hypothermia, ataxia, haemoptysis and syncope.

In a study carried out in Italy to assess the duration, outcome (self-cure or death) and life expectancy of heartworm-infested cats, nine of the 43 infested cats died during the study and 34 self-cured. During the study, 27 cats showed no symptoms, three died suddenly 38 to 40 months after diagnosis and six died during the follow-up period, 8 to 41 months after diagnosis. According to the authors, the probability of death increased with age at diagnosis.

Control measures

Treatment

Treatment of asymptomatic infested dogs is not advised: measures to prevent re-infestation are more useful and the few filariae present will gradually disappear. A monthly prophylactic larvicidal dose of avermectins/milbemycins (moxidectin, selamectin or ivermectin) sterilises the female filariae and reduces their longevity.

Adulticide treatment

This is based on arsenic derivatives, including melarsomine.

- The protocol (for stages 1 and 2) is two intramuscular injections at a dose of 2.5 mg/kg at 24-hour intervals.
- In the case of animals presenting with serious clinical signs (stage 3), a single injection is administered followed by two further injections, 24 hours apart, the following month. There is some risk of pulmonary embolism from dead filariae, so the animal must be kept completely at rest.

Melarsomine sometimes has undesirable side effects, such as vomiting, diarrhoea, and nervousness for 24–48 hours after injection.

Treatment efficacy can only be confirmed by ELISA 4–6 months later, due to the persistence of circulating parasitic antigens.

Microfilaricidal treatment

This must be administered 1 month after the adulticide treatment, because microfilariae can survive for up to 18 months in the capillaries. It consists of avermectins/milbemycins which are equally effective against microfilariae, which disappear 3–4 weeks after treatment.

Some adverse effects linked to death of the microfilariae are possible, as are some allergic reactions: prostration, diarrhoea, ataxia. The administration of corticoids is beneficial in such cases.

Other antiparasitic drugs, such as diethylcarbamazine and levamisole, have been employed in the past but they are no longer used because of their often limited efficacy and the significant risk of side effects.

Adjuvant treatment

Absolute rest (hospitalisation) is important here. Antithrombotic treatment (preventing platelet aggregation), using aspirin at a dose of 5 mg/kg/day for 4 days before the adulticidal treatment and continuing for 3 weeks, has been recommended but is controversial, and some sources prefer to use heparin.

Surgical treatment

Surgically removing the filariae is possible in heavy infestations (dogs in stage 3 or 4) (Fig. 13). This is done by catheterising the jugular vein using a specially-adapted pair of forceps known as alligator forceps. This technique enables the parasitic load to be reduced and hemolysis linked to haemodynamic disruption to be stopped.

It is carried out only rarely, in emergency cases or in patients in very advanced stages, by some Japanese, Australian and American teams.

Prevention

Vector prevention

Preventative measures against mosquitoes, whether on a collective level (notably in the tropics), or individually, reduce the transmission of *D. immitis*.

Chemoprophylaxis

This is based on the use of avermectins/milbemycins. Doses depend on active ingredients and dosage forms.

Several oral formulations, classic or palatable ("chewable") are available. Ivermectin is administered monthly in this form, at a dose of 6 µg/kg, oral moxidectin is administered at 2 µg/kg, and oral milbemycin oxime at 0.5 mg/kg.

Spot-on formulations have also been developed, using selamectin (6 mg/kg), moxidectin (2.5 mg/kg), or milbemycin oxime at a dose of 0.5 mg/kg. These are also applied monthly and they destroy all developing larvae less than 6 weeks old, which have been inoculated by mosquitoes.

Long-acting injectable formulations have also been developed: the first is a product based on moxidectin, and its efficacy persists for several months due to its residual effect; it lasts for 6–12 months, depending on the concentration of moxidectin.

These active ingredients are sometimes combined with other antiparasitic agents to broaden their spectrum of activity (ivermectin + pyrantel, eprinomectin + praziquantel + fipronil + (S)-methoprene (spot-on for cats), moxidectin + imidacloprid (spot-on), milbemycin oxime + afoxolaner or spinosad, etc.).

Preventative treatment is not applied before leaving for the enzootic area, but 1 month after arrival, because this allows all the larvae inoculated by mosquitoes in the months preceding the treatment to be killed. The effect of tablets and spot-on are not persistent. Treatment should continue until 1 month after the last exposure to mosquitoes.

Prophylaxis continues throughout the mosquito season in enzootic areas, and sometimes throughout the whole year, and it begins when puppies are 3 months old.

Annual testing is integral to ensuring that prophylaxis is achieved and maintained. It also means that infestations can be diagnosed sooner and more timely treatment can be provided to minimise pathology and the potential selection of resistant subpopulations.

Prophylaxis for dirofilariosis is also used for cats and ferrets in highly enzootic areas.

Risk to humans

Humans can be infested but the human disease constitutes a parasitic impasse; larvae migrate then die encysted in nodules (usually in the lungs). Several hundred cases are reported worldwide.

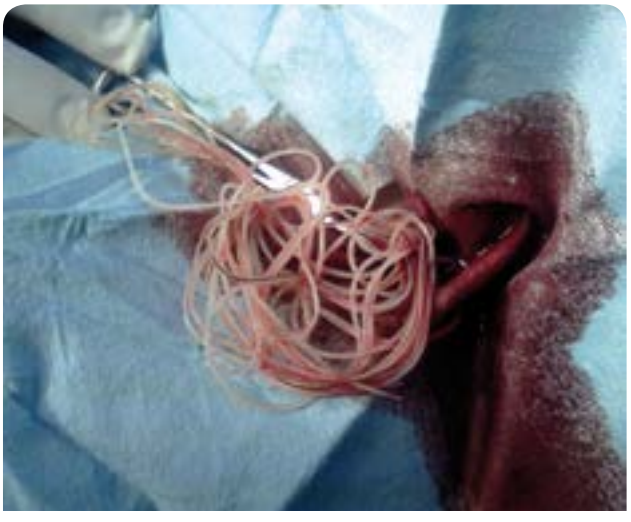


Figure 13. Adult *Dirofilaria immitis* surgically removed from infested right ventricle. Courtesy of Laura Rinaldi.

Video 3
Live worms in the autopsy of a dog that died of heartworm disease.

Canine angiostrongylosis

General comments

Angiostrongylosis is a helminth infection linked to the presence and development of a nematode, ***Angiostrongylus vasorum***, in the right heart and pulmonary arterial system in canids.

This small nematode belongs to the order Strongylida, suborder Metastrongyloidea, which includes parasites of the respiratory and circulatory system.

The first anecdotal reports of canine angiostrongylosis emerged in France in the early 19th century and explain the name "French heartworm" given to the worm. The common name is canine lungworm.

Species affected

The disease affects canids, especially dogs, and foxes, but also jackals, fennecs and other wild canids.

Geographical distribution and importance

Distribution is worldwide. In Europe, it has been reported in the UK, Ireland, Denmark, Switzerland, France, Italy, Portugal, Spain, Austria, Germany and Russia. It is also present in North America.

The clinical importance of the disease is related to the progressive development of cardiac insufficiency. Clinical sings of angiostrongylosis are similar to cardiopulmonary dirofilariosis.

Morphology

Angiostrongylus are very thin, reddish nematodes, 0.25–0.3 mm in diameter. Males are approximately 10–15 mm long and females, 18–25 mm long (Figs. 1 and 2). Females have a whitish uterus which is wound around a red intestine (haematophagy), hence the name barber's pole worm.

Biology

The life cycle of *A. vasorum* is dixenous.

In the canid (definitive) host

Adults are localised to branches of the pulmonary arteries and arterioles, where they cause arteritis and disrupt pulmonary perfusion. The heart then has to work harder, and right-sided cardiac insufficiency gradually develops. These parasites feed in the bloodstream and female worms begin to lay eggs from 38–60 days after infection. The embryonated eggs (70–100 × 40–60 µm) hatch rapidly in the pulmonary capillaries and the first stage larvae (L1) penetrate the alveoli.

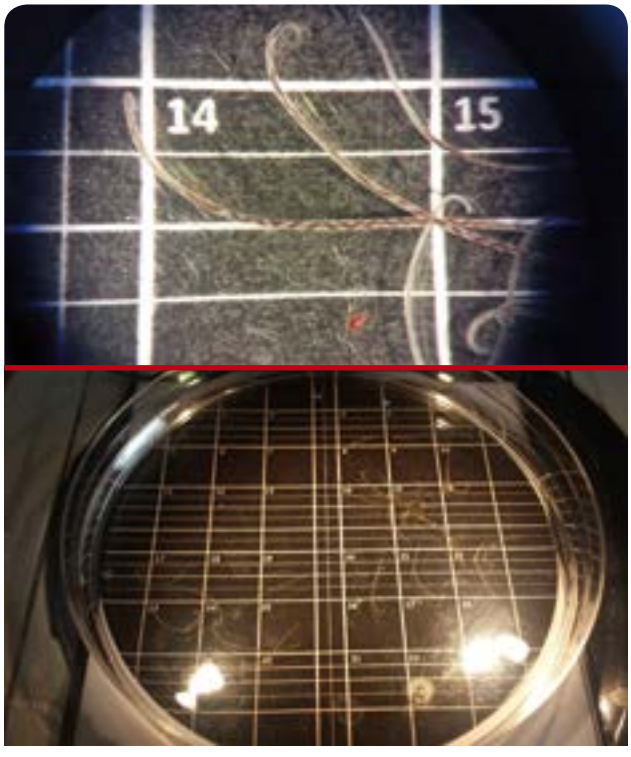


Figure 1. Binocular lens view of adult *Angiostrongylus vasorum* from a necropsy sample.

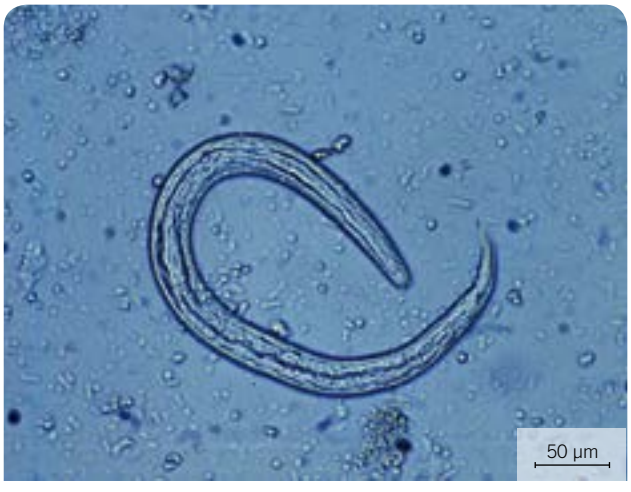


Figure 2. L1 larva of *Angiostrongylus vasorum*. Microscopic coproscopy.

They then migrate through the pulmonary system to the pharynx, are coughed up, pass through the digestive tract and are finally excreted in faeces. Once infestation is established, patency is very long – possibly lifelong if left untreated.

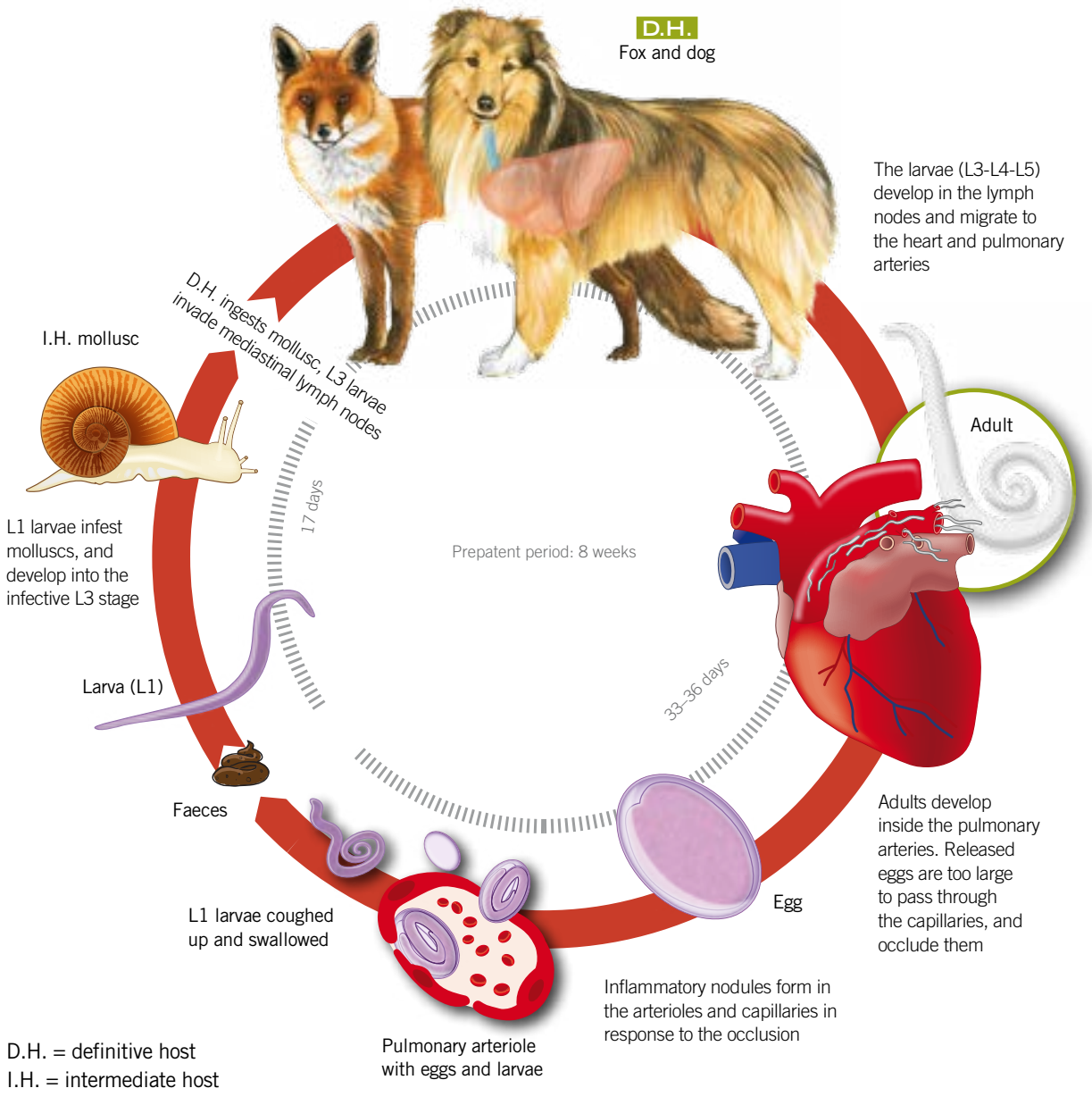
In the gastropod (intermediate) host

L1 can survive on the ground for up to 3 weeks. When they are ingested by a terrestrial gastropod (mainly slugs, but also snails), they develop into L3 in a few weeks (particularly in the gastropod's pedal mass) and these larvae remain infective for at least 6 months. The main intermediate host in Europe is the red slug (*Arion rufus*), in which larvae develop in 16–25 days. Dogs are then infested by eating the mollusc, a paratenic host or the L3 released by a dead gastropod, which can survive for a few days in the external environment.

Following ingestion of the infested gastropod or paratenic host by the dog, the infective L3 larvae are released into the digestive tract, cross the intestinal wall and moult into

PDF

Life cycle of *Angiostrongylus vasorum*



L4 larvae in the mesenteric lymph nodes on the 4th day. They then migrate through the lymphatic system towards the right heart, into the arteries and pulmonary arterioles, where they evolve into pre-adults and adults. Ovigerous females appear from the 33rd day and excretion of L1 larvae in the faeces starts around 44 days after infestation (the prepatent period in dogs is approximately 8–10 weeks). Prepatent period in cats: 6–8 weeks. Parasite lifespan: up to 2 years.

Like other metastrongylids, the life cycle of *A. vasorum* includes many species of slugs and snails as intermediate hosts:

- Slugs: *Arion rufus, Arion ater, Arion lusitanicus, Deroceras* spp.
- Snails: *Helix aspersa, Helix pomatia* and the *Cepaea, Euparypha, Sucuinea, Lymnacea, Physa, Planorbis* species, etc.

Paratenic hosts may be involved, as is the case with feline aelurostrongylosis and a Danish study has demonstrated that the green frog, *Rana temporaria*, could be a paratenic host.

Epidemiology

The prevalence of this parasitosis is little understood. It develops sporadically but is not rare. Foxes could play a role as reservoirs: an epidemiological survey conducted in Hungary found that 5 % of foxes were infested by *A. vasorum* and a similar survey in Italy found a prevalence of 34 % in this species.

A serological survey to evaluate the prevalence of antibodies and antigens in the blood of a random sample of dogs (4,030 animals) in the south of the UK showed that 1.32 % of the dogs were antigen-positive, and 3.2 % were positive for specific antibodies. The number of cases described in the UK and Ireland has been increasing over the last 5 years, which could be related to the increasing population of foxes and their changing habits, as they become suburban or even urban. Existing clusters of infection have been described in South East England and South Wales. A survey of more than 1,400 vet practices across the country found that practioners reported more than 20 cases of angiostrongylosis per year in those areas. Vet practices in the affected areas are 15 to 16 times more likely to see clinical angiostrongylosis cases than anywhere else in the UK.

Elsewhere, the disease is commonly described in hunting dogs which have been left to roam in forests and have probably eaten slugs.

The source of parasites is wild or domestic canids, which expel the larvae in their excrement.

The mechanism of infestation is the ingestion of an intermediate host, or perhaps a paratenic host (rodents, batrachians).

The predisposing factors are those which promote contact with intermediate hosts: hunting dogs appear to be especially at risk.

Clinical signs and lesions

Clinical signs

Angiostrongylosis usually causes a chronic illness.

- In the early stages, exercise intolerance, increasing breathlessness at rest, fatigability, and tachycardia are commonly seen and the animal often presents with a bad hacking cough, accompanied by expectoration. Blood counts usually indicate hypereosinophilia (with 10–30 % eosinophils) and this initial phase may last several months.
- The late stage corresponds to the development of right-sided cardiac insufficiency, associated with pulmonary disorders:
  - Emaciated, prostrate, anaemic dogs.
  - Dyspnoea and cough.
  - Abnormal pulmonary and cardiac auscultation (pulmonary emphysema, respiratory frequency).
  - Modified electrocardiogram.
  - Pulmonary and cardiac disruption visible by radiology (Fig. 3).
  - Eosinophilia.
- In the final stage, the dog may present with dependent oedema, ascites and cyanotic mucosae.

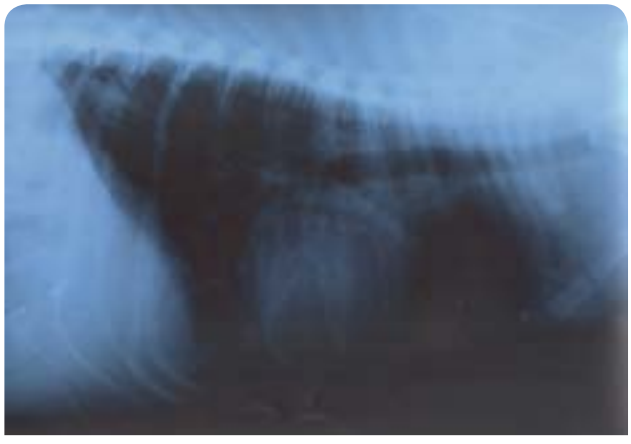


Figure 3. Radiographic examination: lateral view of the thorax of a dog with clinical signs of angiostrongylosis. Right cardiac hypertrophy, and bronchi significantly more visible than normal.

Pulmonary oedema can become acute, causing complications, and dyspnoeic crises can arise and lead to death of the dog.

An acute respiratory form has been described in young dogs and, in these cases, the coughing and dyspnoea is accompanied by fever.

Lesions

Pulmonary apparatus: the lungs are congested and present with purplish-white spots. They are covered in nodules a few millimetres in diameter, and this general appearance is described as "Roquefort cheese" and is comparable to the verminous pneumonia lesions seen in small ruminants. These nodules are actually granulomas centred on eggs and L1 larvae.

Histological lung sections show significant fibro-conjunctive infiltration of the parenchymal cells, surrounding alveoli containing *Angiostrongylus* larvae (Fig. 4). Emphysema can be seen in the pulmonary lobes, especially the anterior lobes.

Cardiovascular system: dilation of the right ventricle, presence of parasites in pulmonary valves and arteries (Fig. 5). Endarteritis, and occasionally, thrombus formation, can be seen.

Diagnosis

Clinical suspicion can be applied to hunting dogs which suddenly present with respiratory and cardiac signs: coughing, dyspnoea, tachycardia, fatigability, and even oedema and ascites.

This suspicion must be confirmed by identification of L1 larvae in the faeces (Figs. 6 and 7). These larvae measure 300–330 µm, and have a strongyloid oesophagus, a cephalic button on the front end and a subterminal spine before the S-shaped rear end. This allows them to be differentiated from the L1 larvae of other respiratory Strongylida in carnivores by faecal examination using the Baermann technique (see *Baermann test*, page 306). Since *Angiostrongylus* are fairly prolific, the sensitivity of coproscopy is adequate, but faeces should be collected for 3 consecutive days as excretion is intermittent.

A quick serological test is also available to detect specific *A. vasorum* antigens.

Prognosis

Prognosis is poor if untreated.

Control measures

Treatment

Symptomatic: cardiorespiratory analeptics.

Antiparasitic: some products containing either moxidectin or milbemycin oxime are licenced for the treatment of angiostrongylosis in dogs. Moxidectin is used at a dose of 2.5 mg/kg in a single topical application. A further veterinary examination is recommended 30 days after application, as some animals may require a second treatment. Milbemycin oxime, administered orally at a dose of 0.5 mg/kg, once a week for 4 weeks, can also be used to treat angiostrongylosis.

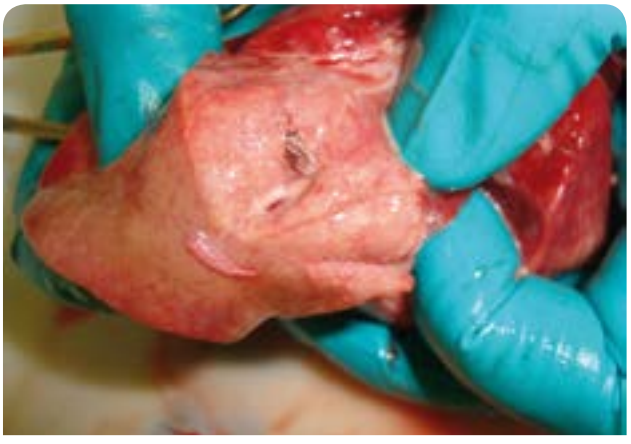


Figure 4. *In situ* localisation of adult *Angiostrongylus vasorum* in the lung of a dog.

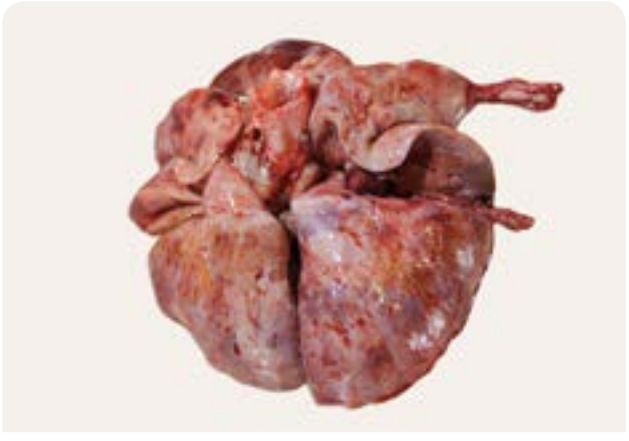


Figure 5. Necroscopic heart and lung sample from a dog severely infested by *Angiostrongylus vasorum*.

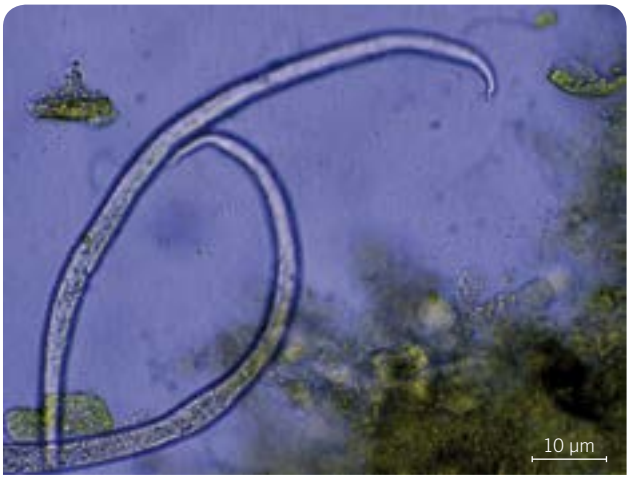


Figure 6. *Angiostrongylus vasorum* L1 larva. Microscopic coproscopy.

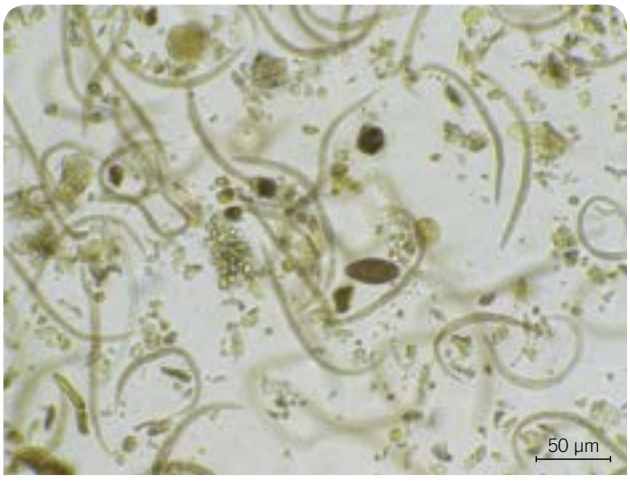


Figure 7. Numerous *Angiostrongylus vasorum* L1 larvae and one *Trichuris vulpis* egg. Microscopic coproscopy.

Fenbendazole has been used at a dose of 50 mg/kg for 5 days, and oxfendazole at 11.3 mg/kg every day for 7 days. Ivermectin, used off-label and at the discretion of the veterinary surgeon responsible, is active at a dose of 0.2 mg/kg, administered subcutaneously in 2 doses, a week apart.

Anthelmintic treatments work by breaking down the parasites, and the products of this lysis can cause allergic reactions (hypersensitivity) and thromboembolic disorders in the host.

Prevention

Some products containing either moxidectin or milbemycin oxime and administrated at monthly intervals are licenced for the prevention of angiostrongylosis in dogs, and hunting dogs, or dogs which regularly spend time in forests, must be regularly dewormed using effective formulations.

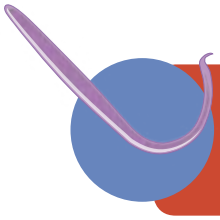
Controlling garden slugs can eliminate a source of infective L3 larvae, particularly in the countryside where foxes may sustain the cycle with these intermediate hosts.

Video 4.1

Binocular lens observation of the moving L1 larvae of lungworms (*Angiostrongylus vasorum*) collected from the faeces of an infested dog after Baermann sedimentation.

Video 4.2

Binocular lens observation of the moving adults of lungworms (*Angiostrongylus vasorum*) collected from the pulmonary circulatory system of an infested dog. Females have a typical "barber's pole" appearance.



Respiratory strongyloses in dogs

General comments

Respiratory helminthoses in carnivores are caused by the presence and development of Strongylida nematodes, Metastrongyloidea, in the respiratory system (trachea, bronchus, bronchioles and/or pulmonary alveoli).

Several species can be seen in dogs: ***Oslerus osleri***, ***Filaroides hirthi, Filaroides milksi, Crenosoma vulpis***, which are mainly parasites of wild carnivores, particularly foxes. Cats are infested by other respiratory Strongylida (see *Respiratory strongyloses in cats*, page 141).

The importance of these diseases is:

- Medical: they give the appearance of chronic tracheobronchitis which is resistant to treatment (in the case of oslerosis or crenosomosis) or bronchopneumonia (filaroidosis).
- Economic: caused by infestation of breeding animals. This applies to *O. osleri* (in Europe) and, to a lesser extent, *F. hirthi* and *F. milksi* (in the USA), because L1 emitted by adults of these parasites are directly infective.

Hosts

Respiratory Strongylida infest canids, mainly wild ones but sometimes dogs, except *A. abstrusus* and *T. brevior*, which infest cats. *C. vulpis* may also infest cats.

Geographical distribution

Respiratory strongyloses are found worldwide. Although the risk of infestation in dogs (especially hunting dogs) in rural and forest environments with large fox populations carrying *C. vulpis* is high, the epidemiology of oslerosis and filaroidosis is different: they are diseases more commonly found in kennels or canine breeding centres, where many dogs are kept together. Oslerosis mainly affects small breeds, probably because it goes clinically unnoticed in large breeds.

Taxonomy and morphology

Respiratory Strongylida are nematodes whose males have a copulatory bursa supported by the ribs. They belong to the superfamily Metastrongyloidea, which is characterised by a very small copulatory bursa and a rudimentary buccal capsule. They are also generally small in size. There are three distinct families: Angiostrongylidae (*Angiostrongylus* and *Aelurostrongylus* genera), Crenosomatidae (*Crenosoma* genus) and Filaroididae (*Oslerus* and *Filaroides* genera).

Male *C. vulpis* are 3–5 mm long and females, 12–15 mm. *Filaroides* and *O. osleri* are a similar size. The females are viviparous and lay L1 larvae which are 200 to 330 µm long and are expelled in the faeces. They can be seen by coproscopy to have a wavy (S-shaped) tail.

Aelurostrongylus are 4–10 mm long (females are larger than males) with a diameter of 50–80 µm.

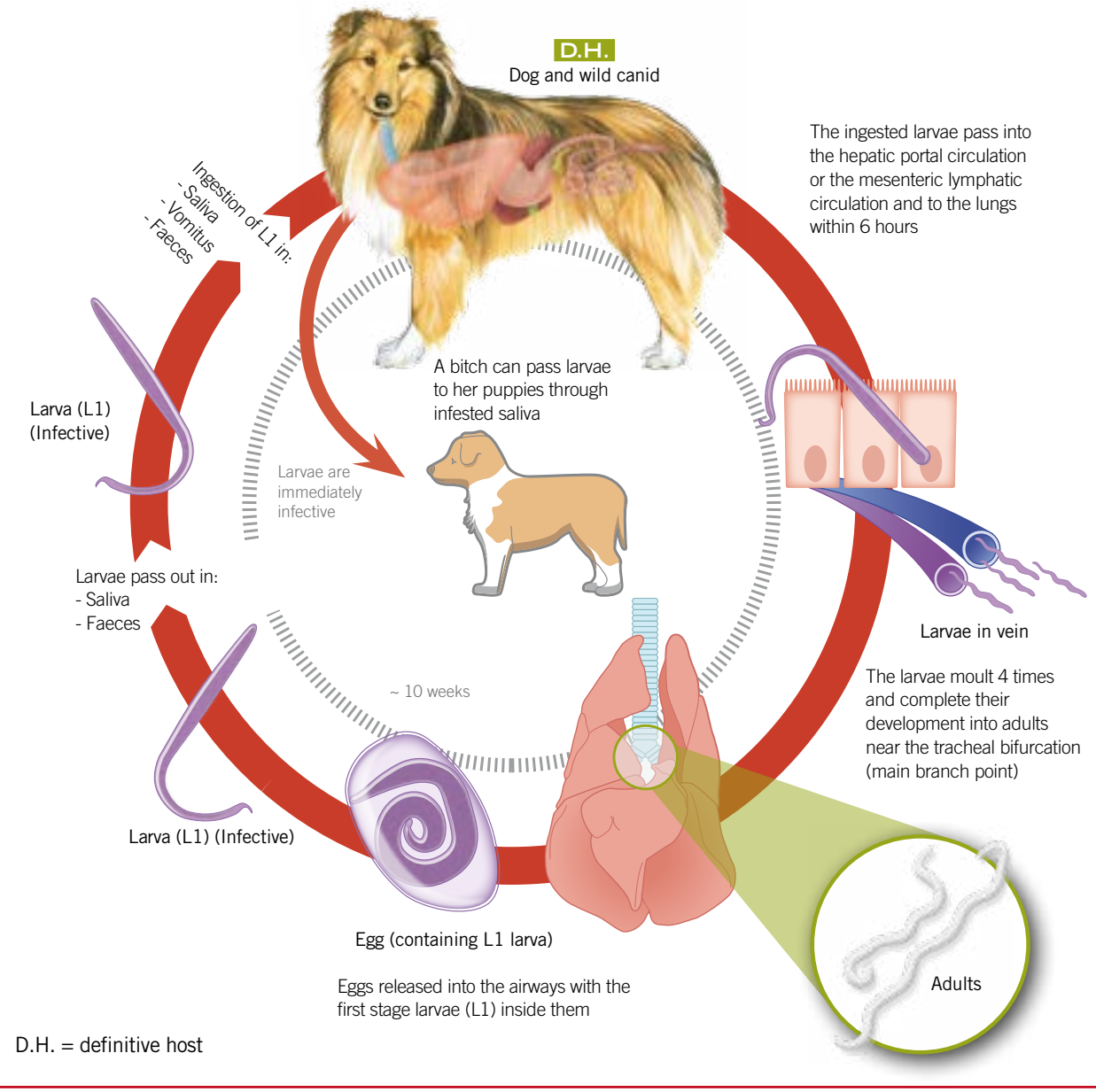
Biology

Localisation of respiratory Strongylida depends on the species:

- *C. vulpis* is localised to the lumen of the trachea and main bronchi.
- *F. milksi* and *F. hirthi* are parasites of the pulmonary parenchyma, so they are found in the bronchioles and alveoli.
- *O. osleri* is a parasite of the respiratory tract or tracheobronchial tree, but is localised to brownish fibrous nodules in the mucous membrane.

The pathogenicity of these nematodes is low and there are many healthy carnivore carriers. Oslerosis is more pathogenic in small breeds of dogs because the nodules in which the parasites live reduce the diameter of the respiratory tract as well as its mobility, making the animal more susceptible to coughing fits.

Life cycle of *Oslerus osleri* and *Filaroides hirthi*



Life cycle of *Oslerus osleri* and *Filaroides hirthi*

F. hirthi and *O. osleri* have a characteristic direct monoxenous cycle, since the L1 larvae excreted in the faeces are directly infective. Infestation occurs through ingestion of these L1 larvae, which cross the digestive wall, then migrate to the lungs.

In *Oslerus* infestation, larval eggs are expelled from the definitive host in nasal discharge, saliva or faeces. These eggs hatch immediately in the respiratory tract. In the case of *Filaroides*, embryonated eggs are laid in the pulmonary alveoli, where the adults are found.

The L1 survive only briefly in the soil and are ingested by another definitive host. Once ingested, they cross the intestinal wall, moult quickly into L2, L3 then L4 larvae in the mesenteric lymph nodes, before migrating through the lymphatic system towards the right heart, pulmonary arteries and arterioles. They enter the alveoli, where they develop into pre-adults then adults, which will either stay in the

alveoli (in the case of *Filaroides*), or go back up to the tracheobronchial bifurcation and lodge in the brownish cysts (nodules) (*O. osleri*). The prepatent period is approximately 3–4 months.

Life cycle of *Crenosoma vulpis*

C. vulpis has a dixenous cycle and it is transmitted via terrestrial gastropod molluscs, which are consumed by foxes.

In the definitive host, embryonated eggs are laid and rapidly hatch into L1 larvae in the bronchi and bronchioles. They are swallowed and eliminated with the faeces. The L1 survive for a short period in the external environment but then have to be ingested by terrestrial gastropods, mainly slugs (red slug, *Arion rufus*) where they develop into infective L3 larvae. When a definitive host ingest the infested mollusc (or possibly a paratenic host), L3 are freed in the digestive tract and pass through the intestinal wall. Moulting, into L4 then pre-adult, occurs in the mesenteric lymph nodes and the larvae migrate through the lymphatic system towards the right heart, then the arteries and pulmonary arterioles, until they enter the alveoli. The formation of adults occurs in the bronchi and bronchioles and the lifespan of the parasite is up to 2 years. The prepatent period is approximately 4 weeks.

Epidemiology

Respiratory strongyloses may be sporadic, and rural or sylvatic, especially when transmitted via the ingestion of a mollusc intermediate host, or a paratenic host, as is the case with crenosomosis. Strongyloses may be enzootic, or appear enzootic, like filaroidosis and oslerosis in canine communities.

Sources of parasites

- Reservoirs:
  - Wild canids (including foxes) for *F. hirthi*, *F. milksi* (with the involvement of mustelids) and *C. vulpis*. Crenosomosis is enzootic in foxes in Europe, with a prevalence of approximately 20–25 % according to various surveys (24 % of 100 foxes in a survey in Hungary in 2003).
  - Dogs for *O. osleri*.
- Direct sources:
  - Intermediate hosts (slugs) or paratenic hosts (rodents, birds, reptiles) for *C. vulpis*.
  - L1 larvae for *O. osleri*, *F. hirthi* and *F. milksi*.

Mechanisms of infestation

The only route of infestation is the ingestion of infective forms, either free forms (*O. osleri, F. hirthi* and *F. milksi*) or carried by intermediate hosts. Puppies cannot be infected through their mother's milk, and there is no risk of *in utero* infestation.

Predisposing factors

In the case of oslerosis and filaroidosis, life kennels or breeding centres facilitate contamination and the spread of parasites. Adults, particularly females, are sources of parasites for puppies. Oslerosis in Europe (especially in the UK) and filaroidosis in the USA are common helminth diseases in breeding centres.

Clinical signs and lesions

Clinical signs

Oslerosis

Oslerosis is characterised by chronic tracheobronchitis, caused by worms in voluminous nodules in the tracheal mucus. Dogs, mostly puppies, present with a bad coughing fits. They may also present with attacks of asphyxia, causing tracheal collapse. Hyperthermia is not usually seen. Antibiotic treatments have very often been administered without success but anti-inflammatories may induce temporary remission of clinical signs as they reduce tracheitis. Differential diagnosis must include tracheal collapse in small breeds (bichons, poodles, Yorkshire terriers, Westies, Scottish terriers, etc.) and in breeds where the disease is most common.

Filaroidosis

Filaroidosis is rarely symptomatic, although worms localised to the bronchioles and alveoli may sometimes cause dyspnoea.

Crenosomosis

Crenosomosis may manifest itself through dyspnoea and tracheobronchitis, with a pronounced cough in heavy infestations (adults are free in the bronchi, sometimes the upper respiratory tract).

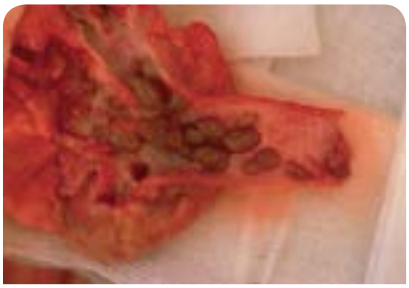


Figure 1. Necroscopic sample: respiratory tract of a dog infested by *Oslerus osleri*. Numerous brownish nodules containing adult worms.

Figure 2. Microscopic coproscopy: *Oslerus osleri* L1 larva. Size 250–350 µm, oesophagus is not visible, curved tail.

Figure 3. Microscopic coproscopy: *Crenosoma vulpis* L1 larva. Size 260–330 µm. Oesophagus is visible, tail is tapered.

Oslerosis lesions: presence of tracheobronchial nodules, 3–8 mm in diameter, brownish and transparent, so parasites can be vaguely seen. These nodules may develop into fibrosis and their large numbers may partially obstruct a bronchus or the trachea, causing significant breathing difficulties (Fig. 1).

Crenosomosis lesions are very subtle, except in heavy infestations when tracheitis and inflammatory bronchitis can be seen. The parasite's spinal cuticle can also irritate the mucosa significantly.

Lesions are similar to those of verminous bronchopneumonia, with granulomatous inflammatory foci centred on parasitised alveoli, giving the same appearance of many small greyish nodules on and in the parenchyma as in cases of filaroidosis.

Diagnosis

Clinical suspicion of oslerosis is based on observation of chronic tracheitis, resistant to standard treatment, in a young dog of a small breed. Observation of nodules on tracheal endoscopy confirms diagnosis. A biopsy of these nodules normally allows adult worms and numerous larvae to be recovered. L1 larvae may be seen by coproscopy (Fig. 2) but laying by adult females is irregular, and repeat examinations are necessary to increase the sensitivity of this technique.

In the case of other respiratory strongylosis, L1 larvae can be seen by coproscopy (Figs. 2 and 3). Diagnosis may be post-mortem, for example on foxes, by identifying lesions and parasites *in situ*.

Control measures

Treatment

Bioavailability of anthelmintic drugs is quite poor because of the alveolar or intranodular location of the parasites, and treatment is not always satisfactory. Lesions, especially oslerosis nodules, are sometimes irreversible and explain the persistence of clinical signs even after effective treatment.

Nevertheless, some anthelmintics seem to give good results:

- Oxfendazole and fenbendazole must be used at high doses for several days (e.g., oxfendazole at 50 mg/kg/day for 8 days).
- Avermectins/milbemycins are also effective, and some formulations are licenced for the treatment of *C. vulpis* infestation, such as moxidectin at 1.0 mg/kg and milbemycin oxime in a single dose of 0.5 mg/kg, which both reduce levels of infestation.

Prevention

Prevention of oslerosis in animal communities is only possible by treating adult carriers, and by regularly cleaning and disinfecting housing to eliminate infective L1 larvae.

Respiratory strongyloses in cats

General comments

Respiratory helminth infestations in carnivores are mainly caused by the presence and development of Strongylida nematodes (Metastrongyloidea) in the respiratory system (trachea, bronchus, bronchioles and/or pulmonary alveoli) of the animal. Cats are infested by respiratory Strongylida different from dogs. Two species are especially implicated: ***Aelurostrongylus abstrusus*** and, to a lesser extent, ***Troglostrongylus brevior***. ***Oslerus rostratus*** has also been described once, in a cat from Palestine.

Aelurostrongylosis is a helminthosis linked to the presence and development of a nematode, *A. abstrusus*, in the alveolar ducts and terminal respiratory bronchioles of wild and domestic felids. The genus *Troglostrongylus* (family: Crenosomatidae) includes four species of nematodes which infest the respiratory system of felids, through an indirect life cycle in intermediate and paratenic hosts. *T. brevior* (inhabiting the bronchi and bronchioles) and ***T. subcrenatus*** (inhabiting the trachea and the bronchi) have been found in domestic cats.

Geographical distribution

A. abstrusus seems to be distributed worldwide, as it has been reported from nearly all countries in Europe, in Australia and the Americas, and sometimes in Asia and Africa. This parasite was diagnosed for the first time in the former Yugoslavia. The geographical range of *A. abstrusus* appears to be expanding, although the reasons for this emergence are little known.

Distribution of the genus *Troglostrongylus* is less well-known and it has been mainly reported in Southern Europe (Italy, Spain) and in Africa (Malawi) to date.

Importance

Aelurostrongylosis and troglostrongylosis are rarely diagnosed and are not considered medically serious. The prevalence is therefore poorly understood and is probably underestimated. Even though *Troglostrongylus* spp. seem to be more pathogenic than *A. abstrusus* in domestic cats, very little information is available on the clinical impact of *Troglostrongylus* spp.

Morphology

A. abstrusus is a thin nematode measuring 4–10 mm long and 50–80 µm in diameter and, as with all respiratory Strongylida, the male's copulatory bursa is small. *Troglostrongylus* spp. are bigger worms with a body length which ranges from 5 to 24 mm.

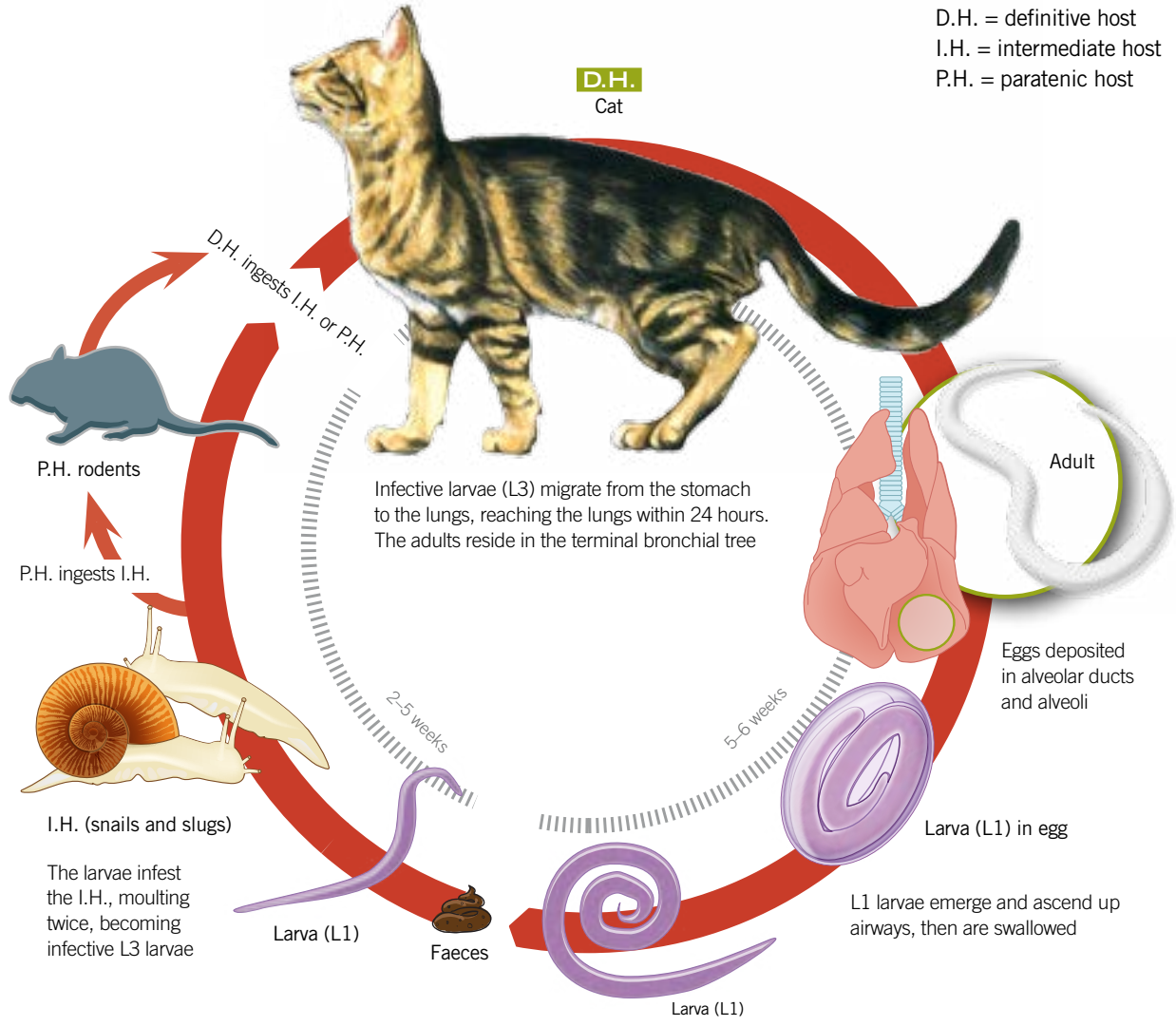
Biology

Adult worms are located in the alveolar ducts and terminal respiratory bronchioles (*A. abstrusus*) or in the bronchi and the bronchioles (*T. brevior*). Like *Angiostrongylus*, *Aelurostrongylus* are haematophagous. They are found in small nodules as a result of inflammatory infiltration. The lifespan of adults is several years and the female worms lay non-embryonated eggs which embolise and develop within the parenchyma, before hatching, releasing first stage larvae (L1) which pass up the respiratory tract to be swallowed via the pharynx, and are then excreted into environment in the host's faeces. L1 larvae actively penetrate their intermediate hosts (molluscs: snails or slugs), where they develop into the third, infective stage (L3) in approximately 3–5 weeks in *A. abstrusus* (Fig. 1). It has been demonstrated experimentally that *T. brevior* and *A. abstrusus* may develop simultaneously in the same mollusc species. Intermediate hosts are very varied and include:

- Slugs: *Arion ater, Arion lusitanicus, Arion rufus, Deroceras* spp., etc.
- Snails: *Helix aspersa, Helix pomatia, and the Cepaea, Euparypha, Sucuinea, Lymnacea, Physa, Planorbis species,* etc.

Rodents, frogs, lizards, snakes and birds may act as paratenic hosts for *A. abstrusus* and probably *T. brevior*. Cats become infested by eating snails, paratenic hosts or L3 larvae released by the death of the intermediate host and which survive for several days in the external environment. The L3 ingested into the intestine cross the intestinal wall and enter the right heart through the lymph ducts. The prepatent period lasts approximately 1–2 months and larva-shedding in the faeces continues for many months.

Life cycle of *Aelurostrongylus abstrusus*



Epidemiology

Aelurostrongylosis seems to be enzootic in populations of wild or stray cats, particularly in rural or forest areas. Cases are sporadic in domestic cats and they are infested by chance consumption of intermediate or paratenic hosts. There are two seasonal peaks, in spring and autumn, and these seem to be linked to the resurgence of intermediate hosts at these times.

Information on troglostrongylosis in domestic cats is mainly based on case reports, so there are no reliable epidemiological data on the distribution and incidence of *Troglostrongylus* spp. in cat populations. However, one epidemiological survey carried out on the island of Sardinia (Italy) reported a *T. brevior* prevalence of 6.5 % in domestic cats and catteries. Co-infestations by *T. brevior* and *A. abstrusus* have also been described in cats, suggesting that both species may exist in sympatry.

Source of parasites

Wild or stray felids expelling larvae in their excrement.

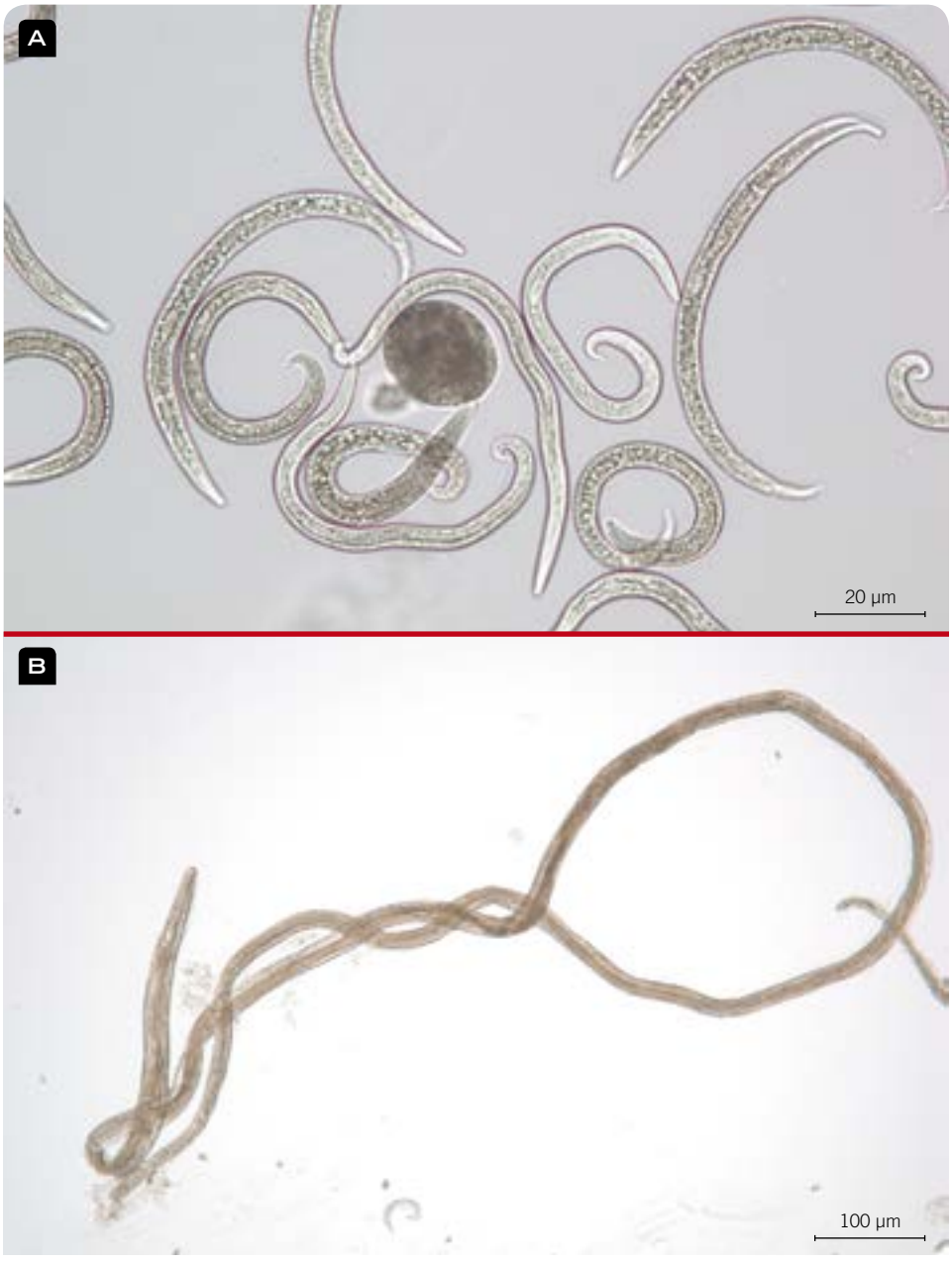


Figure 1. Different stages of *Aelurostrongylus abstrusus* collected after necroscopic examination and lung digestion of an infested cat. (A) L1 larvae; (B) adult worms.

Mechanisms of infestation

- Ingestion of intermediate and paratenic hosts (rodents, batrachians, reptiles and birds).
- A direct route of *T. brevior* transmission from an infested queen to her kittens has been suggested although it is still unclear whether transmission to kittens occurs via the placenta or lactation.
- Transmission of L3 larvae, from snail to snail in mucus balls has been demonstrated.

Predisposing factors

Rural and forest areas (contact with intermediate hosts). No influence of age or sex was demonstrated in aelurostrongylosis in the few cases studied, but some authors cite more cases in male cats, probably because they roam more. Kittens and young cats seem to be more susceptible to infestation, because of the possible vertical route of transmission.

Clinical signs and lesions

Aelurostrongylosis

Clinical signs are affected by worm burden, health status, age and the immune response of the infested animal. Indeed, *A. abstrusus* causes a wide spectrum of clinical pictures, ranging from asymptomatic, subclinical or mild disease up to severe, potentially fatal pneumonia, although this is rare. In the mild form of the disease, which is more common in adult cats and in cases of low worm burdens, the infestation may be self-limiting and respiratory signs gradually and spontaneously disappear within weeks.

- **Respiratory signs:** aelurostrongylosis is characterised by mild to intense chronic coughing, sneezing, wheezing, mucopurulent nasal discharge, dyspnoea, tachypnoea, tachycardia, and open-mouthed abdominal breathing. Severe respiratory signs and death are more common in young, debilitated or immunosuppressed cats. Clinical cases may be complicated by pyothorax and pneumothorax when migrating larvae carry intestinal bacteria with them. Infestation with *A. abstrusus* has been implicated in anesthetic-associated deaths.
- **General signs:** development is chronic. Hyperthermia is inconsistent and, when it does occur, it can be linked to secondary bacterial infections (such as pneumonia). General signs, like lethargy, depression and weight loss may occur and blood counts frequently indicate hypereosinophilia. Diarrhoea has also been reported in some cats. Development is slow and the animal usually recovers spontaneously after a few months; more rarely, its condition deteriorates, with cachexia and bacterial pneumonia.
- **Lesions of aelurostrongylosis**

  The lungs are congested and the parenchyma is covered in many small, greyish granulomatous nodules measuring 1–10 mm in diameter There are many of these nodules on the surface of the pulmonary lobes and greyish and fibrous plates may also appear on the lobes (Fig. 2).

  The general appearance is similar to the lesions caused by verminous pneumonia in small ruminants.

  The nodules are granulomas centred on eggs and L1 larvae and, when cut, a fluid rich in parasitic elements may flow out. Adult worms are still very difficult to observe.

  Histological lung sections show significant fibro-conjunctive infiltration of the parenchyma cells, surrounding alveoli containing *Aelurostrongylus* eggs and first stage larvae (Fig. 3). Functional alveoli disappear from lysed areas and the arterial system is also lysed: endarteritis and possible thrombosis can obliterate blood vessels.

  The pathogenic effect of the parasites is linked to the immuno-inflammatory response of the pulmonary parenchyma and endarterium of the infested capillaries (tissue infiltration by polynuclears, monocytes then fibro-conjunctive reaction) on one hand, and the formation of emboli consisting of eggs and first stage larvae in pulmonary capillaries, resulting in type III and IV hypersensitivities and thrombosis, on the other.

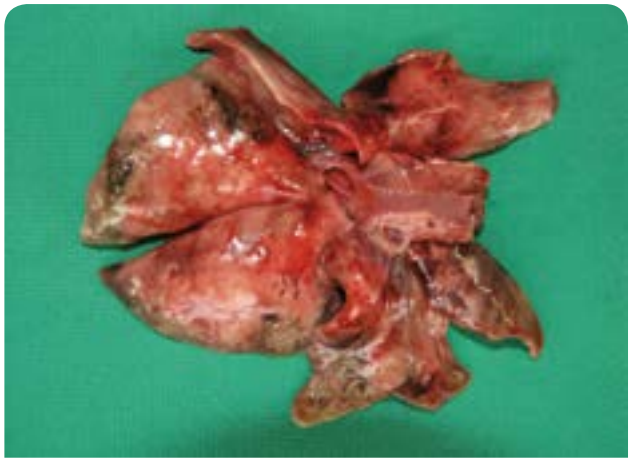


Figure 2. Necroscopic sample: subpleural, greyish-white, coalescing parasitic granulomatous nodules involving the parenchyma of both lungs of a cat presenting respiratory failure due to severe aelurostrongylosis.

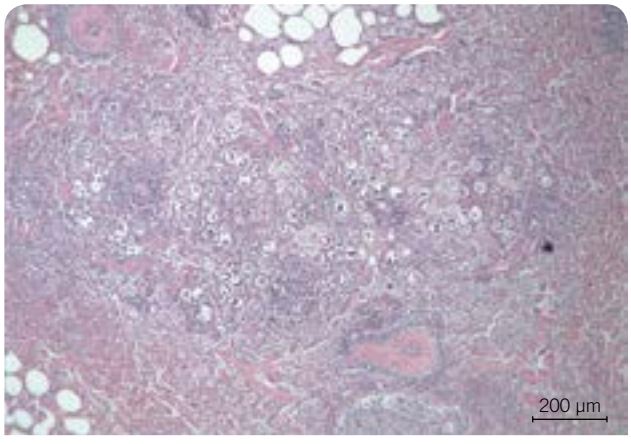


Figure 3. Section of a cat lung stained with haematoxylin and eosin. Alveolar lumina filled with several *Aelurostrongylus abstrusus* adults and larvae. The surrounding parenchyma shows a mixed inflammatory infiltrate.

Troglostrongylosis

Little information is available on the clinical impact of *Troglostrongylus* spp. in domestic cats. The larger size of adult *T. brevior* and *T. subcrenatus* and their anatomical localisation in the upper airways (i.e., trachea and bronchi) suggest a high pathogenic potential. Respiratory signs are mainly dyspnoea, cough and nasal discharge. Fatal infestations by either *T. brevior* or *T. subcrenatus* have been reported in kittens (Figs. 4 and 5) and the age of infested animals seems to play a key role in the clinical outcome of the disease.

Necropsy of dead animals has revealed pulmonary oedema, enlargement and congestion, lungs with multi-focal haemorrhages, diffused hepatisation and lobular inflammation, or areas of consolidation, and catarrhal exudate in the trachea (*T. subcrenatus*).

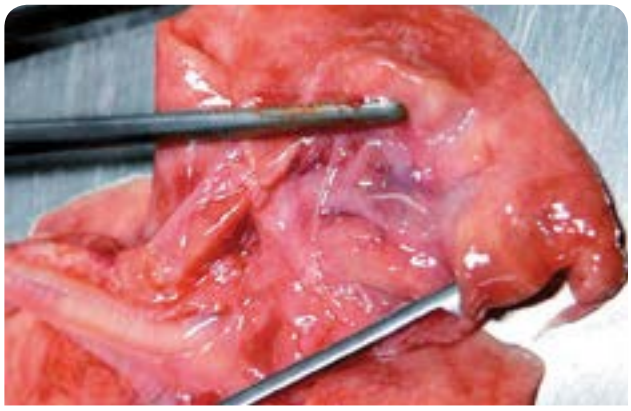


Figure 4. Adult of *Troglostrongylus brevior* in the lumen of the caudal bronchus of a shorthair kitten which died from respiratory failure. Courtesy of Donato Traversa, Angela Di Cesare and Emanuele Brianti.

Diagnosis

Epidemiology

Respiratory strongylosis in cats are not easily interpreted in current veterinary practice, because other overlapping conditions should be considered in any differential diagnoses, e.g., mycoses, viral and bacterial infections, nasopharyngeal polyps, allergic bronchitis, foreign bodies and respiratory neoplasms.

Cats which go outside often and present suddenly with a cough, dyspnoea, tachypnoea, weight loss and fatigability may be clinically suspected. No sign is pathognomonic, so epidemiological conditions will point to complementary testing.

Vets usually misdiagnose aelurostrongylosis and treat the condition as an allergic respiratory disease or cat bronchial disease/asthma. As treatment is symptomatic, the infested cat may show clinical improvement after administration of corticosteroids and bronchodilators, so clinicians have no reason to suspect that they have made a misdiagnosis.

Figure 5. First stage larva of *Troglostrongylus brevior.* (A) View of the entire larva; (B) magnification of the anterior end of the larva, showing a pointed extremity and a sub-terminal oral opening; (C) magnification of the tail of the larva, showing a deep dorsal incisure and a shallower ventral one. Courtesy of Donato Traversa, Angela Di Cesare and Emanuele Brianti.

Medical imaging

Radiography does not allow a definitive diagnosis, but confirms the pulmonary disorder: pulmonary densification, nodular images (fibrous tissue), and dilation of the pulmonary arteries, and the images can also indicate tumours, pneumonia, etc.

Parasitological examination

The technique of choice is microscopic coproscopy and the identification of L1 larvae (Figs. 5 and 6). *Aelurostrongylus* is fairly prolific, so the sensitivity of coproscopy is good, although it can be useful to carry out several tests (3 at 48 hour intervals).

The Baermann migration method is the gold standard to diagnose cat aelurostrongylosis with a sensitivity of ≈90 %, although it requires 24–36 hours before larvae can be found, and specific skill in detecting L1.

A differential diagnosis must be made from other larvae which can be found in the faeces: L1 of *Crenosoma vulpis* (rarer), L3 of *Ollulanus tricuspis* (rarer and L3 present in vomit), hookworm larvae which may be present in samples that have been allowed to incubate, and larvae from free nematodes if the faeces are not collected fresh and have sat in the soil, even for a few hours.

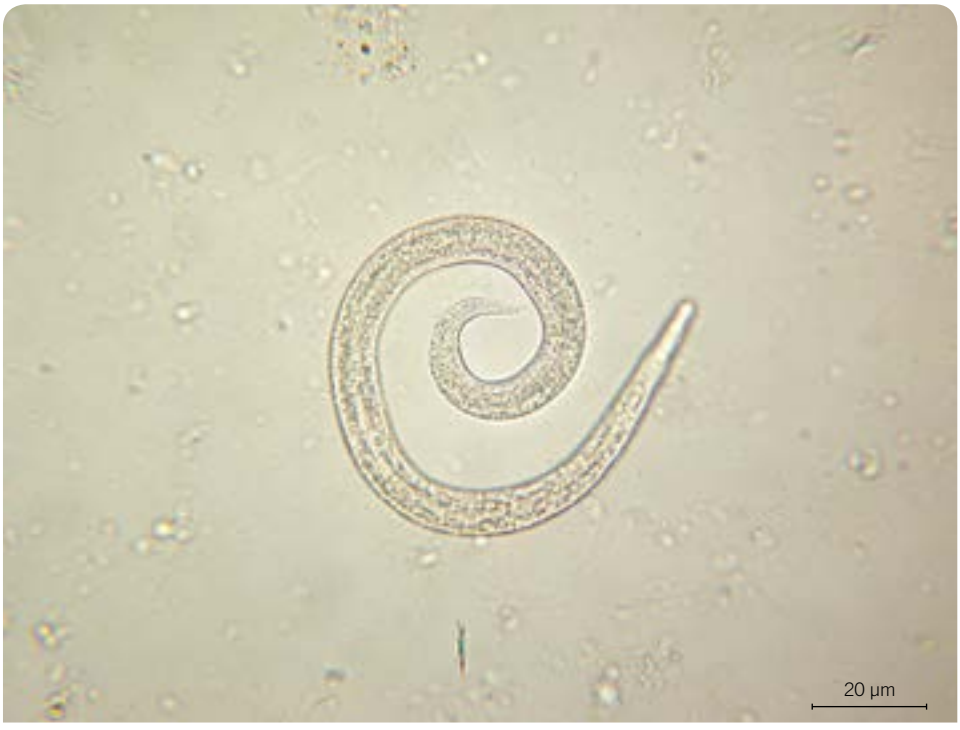


Figure 6. First stage *Aelurostrongylus abstrusus* larva. Microscopic coproscopy (Baermann technique).

Table 1. Morphological characteristics of *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* L1 larvae.

	Aelurostrongylus abstrusus	*Troglostrongylus brevior*
Length	360–400 µm	300–357 µm
Diameter	15 µm	18–19 µm
Oesophagus	Strongyloid oesophagus	Strongyloid oesophagus
Tail appearance	• Undulating tail, with a convex sub-terminal kink (S-shaped) • Distinct knob-like or small finger-like projections at the tip of cuticular spines	Tail gradually tapered to the extremity and bears a deep dorsal incision and a shallower ventral one, near its tip
Anterior extremity	Rounded with terminal oral opening	Pointed anterior extremity

Molecular analyses

Molecular methods have been developed to distinguish and identify L1 larvae of *A. abstrusus* and *T. brevior*.

Post mortem examination

In the event of a post mortem examination, the size and the localisation of worms in the lungs is useful to discriminate between *Troglostrongylus* spp., which localise in the upper airways and *A. abstrusus*, which inhabit the lung parenchyma. Given that other species of lungworms, e.g., *Oslerus rostratus* and *Capillaria aerophila*, present similar localisation and sizes to *Troglostrongylus* spp., careful morphological and morphometric identification of recovered worms is always advisable.

Control measures

Symptomatic treatment

Anti-cough and antibiotic treatment may be administered to prevent secondary bacterial infection.

Specific treatment

Information on anthelmintic treatment of cat aelurostrongylosis has been meagre for a long time, and the majority of the information is from anecdotal and empirically-derived protocols, mostly used in single clinical cases or small case series. Those studies have shown that benzimidazoles are effective, as are avermectins/milbemycins and emodepside.

In Europe, fenbendazole at a dose of 50 mg/kg *per os* for 3–5 consecutive days is licenced for the treatment of aelurostrongylosis in cats, although this treatment scheme does not clear the infection in all cats. Oxfendazole at a dose of 11.3 mg/kg every day for 7 days has also been described as effective.

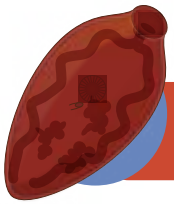
Ivermectin, used off-label in cats, at the discretion of the veterinarian responsible, is active at a dose of 0.4 mg/kg, administered subcutaneously once a week for 2 weeks.

Selamectin (6 mg/kg), moxidectin (1 mg/kg) and milbemycin oxime (0.5 mg/kg) have also been demonstrated to be effective. They are applied as a spot-on with one or two applications at monthly intervals.

A spot-on formulation combining eprinomectin (0.5 mg/kg), praziquantel, fipronil and (S)-methoprene has been licenced for the treatment of infestations with L3 and L4 larvae, immature adults and adults in cats. A single administration of the combination is usually effective, but treatment may have to be repeated monthly to clear the parasite from the cat. A coproscopic examination performed 28 days after the first administration enables the requirement for a repeat treatment to be assessed. A field study demonstrated that this formulation was effective against *A. abstrusus* (90.5 %) and *T. brevior* (100 %) infestation in cats living under natural conditions. *T. brevior* seems to be more susceptible to eprinomectin than *A. abstrusus*.

Prevention

Regular deworming of cats which frequently spend time in forests could prevent these diseases and controlling slugs in gardens may eliminate a source of infective L3 larvae, especially in rural areas where wild or stray cats may be present and sustain the cycle with intermediate hosts/paratenic hosts.



Paragonimoses

General comments

Paragonimoses are found in many mammals, particularly carnivores and humans, and are caused by the presence and development of Troglotrematidae trematodes of the genus ***Paragonimus*** in the pulmonary parenchyma. The diseases are characterised by afebrile respiratory signs linked to parasitic pneumonia: dyspnoea, breathlessness, and coughing, but the parasites may be carried by many healthy animals.

There are more than 50 species in the genus *Paragonimus*, but many species are not officially fully recognised as such. Three species are important in domestic carnivores and humans: ***P. kellicotti*** (Fig. 1), found in North America and Central Asia; ***P. westermani***, found in Asia; and ***P. africanus*** in tropical Africa.

No autochthonous case has been described in Europe, but imported cases are possible as a result of animal movements. A case of sudden death in an autochthonous dog was described in Israel in 1997, suggesting that the parasite exists in this region of the Mediterranean area.

The main species affected are wild carnivores, which are likely to consume second intermediate hosts (crayfish and fresh water crabs) and otters, racoons, foxes, mink, black-footed ferrets, wild cats, dogs and domestic cats.

Morphology

Paragonimus, or lung flukes, are trematodes with two distinct suckers. The genital pore is situated behind the ventral sucker, which is located nearby, and the two testicles are posterior and situated next to each other.

The adults are fleshy parasites measuring approximately 10–15 by 5–7 by 5 mm.

Each egg contains a miracidium larva and is ovoid, capped, brownish-orange, and measures approximately 90 × 60 µm.

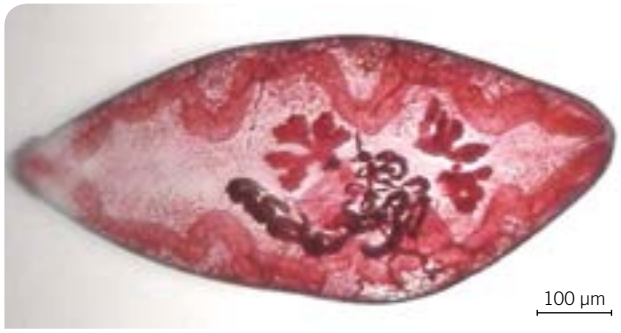


Figure 1. Adult *Paragonimus kellicotti*, red carmine staining. Trematode approximately 10 mm long, with a fleshy body with a flattened side and domed side, making it looks like a fresh coffee grain.

Biology

Trematodes of the genus *Paragonimus* have a trixenous life cycle. The adults live in intrapulmonary cysts in the respiratory tract, bronchi and pulmonary bronchioles in the definitive host and are usually found in pairs in these cysts. The adults release eggs into the lungs, where they are coughed up the pharynx and swallowed, then expelled either in expectoration or with the faeces.

If the eggs fall into an aquatic environment (fresh water), they mature in 2–3 weeks and hatch into miracidia. This survives very briefly and must actively penetrate a freshwater amphibious gastropod, the first intermediate host (*Pomatiopsis, Melania, Ampullaria* and many other genera).

The miracidia develop into sporocysts, rediae and then into cercariae, which are formed in 75 to 100 days, before being released.

Swimming cercariae will actively infest the second intermediate hosts: fresh water crustaceans, especially crabs (*Eriocher* spp., *Patomon* spp., *Sesarma* spp., *Pseudotelphus* spp., etc.) and crayfishes (*Astacus* spp., *Cambarus* spp., etc.). Cercariae develop into encysted metacercariae in these hosts.

The definitive hosts are infested by ingesting freshwater crustaceans. Immature adults (also called adolescariae) are released in the stomach where they cross the digestive mucosa and migrate directly, for approximately 14 days, to the peritoneal cavity via the diaphragm, then to the pulmonary parenchyma where they encyst, usually in pairs. Some parasites may occasionally migrate to, and encyst in, erratic locations: hepatic parenchyma, kidneys, myocardium, diaphragm.

The prepatent period is around 1 month (30–36 days).

Epidemiology

Domestic carnivores and humans are infested following consumption of raw or insufficiently cooked crustacean intermediate hosts.

Rats can serve as paratenic hosts, and vertical transmission has been suggested in cats.

Clinical signs and diagnosis

Infestation is often asymptomatic but it can result in weakness and weight loss in some animals. Migrating immature *P. kellicotti* and pulmonary cysts containing adult flukes cause a mild intermittent cough, breathlessness, occasional haemoptysis, dyspnoea and, occasionally, secondary bacterial pneumonia, pneumothorax, paroxysmal cough and dyspnoea. Epistaxis is possible and rupture of the pleural space can cause sudden death.

Blood exhibits hypereosinophilia on examination.

Pulmonary radiography reveals interstitial nodular densities, containing small air cavities, and pneumatocysts with irregular, sharply defined margins, especially in older infestations, which resemble parenchymatous pneumonia.

Lesions are typical of granulomatous pneumonia; the parenchyma is infiltrated by inflammatory cells, including eosinophils. Conjunctival cysts containing suckers may be seen (Fig. 2).

Differential diagnosis must include all causes of pneumonia.

Definitive diagnosis is made by the identification of the eggs, which are typical (80–120 × 50–60 µm, with a thick brown shell, distinct operculum and, ocassionally, a knob on the opercular end) using sedimentation of multiple faecal samples or tracheal wash fluid (Fig. 3).

Control measures

Treatment is by administration of high doses of praziquantel: 23–25 mg/kg 3 times a day for 3 days.

Fenbendazole at a dose of 50 mg/kg per day for 14 days also seems to be active.

Prophylaxis relies on monitoring domestic carnivores' food and preventing access to host crustaceans.

Figure 2. Tracheal lesion with cystic nodule containing two *Paragonimus* sp. trematodes.

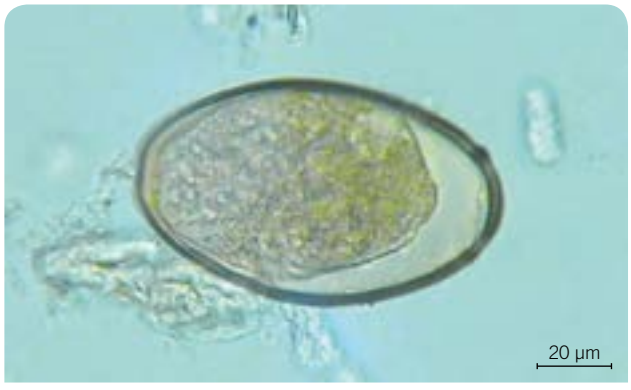


Figure 3. *Paragonimus* egg, microscopic coproscopy. Size approximately 80–100 × 50–60 µm.

Respiratory capillarioses

General comments

Carnivore respiratory systems may be infested by capillary nematodes (Capillaridae).

Two species can be seen: ***Capillaria aerophila*** and ***Capillaria boehmi***. The adult filamentous worms live beneath the epithelium of bronchi and trachea. *C. aerophila* (syn. *Eucoleus aerophilus*) is the agent of respiratory capillariosis in the dog, fox and cat, caused by nematodes in the tracheal and bronchial lumina. *C. boehmi* (syn. *Eucoleus boehmi)* is a parasite of the nasal cavities of the fox, which causes epistaxis, sneezing and nasal pruritus.

Respiratory capillariosis mainly affects wild carnivores, especially foxes and mustelids but they can occasionally be found in dogs and cats. They are found in cold temperate regions. Cats are less receptive to these nematodes than dogs and *C. aerophila* may also infest humans.

Morphology

These nematodes are characterised by their very small diameter, hence the name "capillary". They are whitish, thread-like, and 1–3 cm long.

Biology

Females lay eggs that are coughed up, swallowed and then released in the faeces and nasal discharge into the environment. These eggs are elongated, with a thick, smooth shell and have a shallow polar prominence at each end. They are smaller than whipworm eggs, measuring approximately 65 × 30 × 40 µm. They mature, develop into embryonated eggs, and become infective in about 40–60 days. The cat acquires the infestation by ingesting environmental embryonated eggs which hatch in the intestine. Within 1 week, the larvae migrate in the bloodstream or the lymphatic system to the lungs, where they invade the mucosa and reach adulthood in about 6 weeks. It has been suggested that earthworms could be involved as intermediate or paratenic hosts, but their actual role has not yet been elucidated. The prepatent period is approximately 40 days.

The cycle of *C. boehmi* is identical but the adults are localised in the nasal cavities of foxes.

Epidemiology

Capillarioses are commonly found in wildlife and affected species are the wild canids, especially foxes and mustelids although dogs and cats are sometimes infested accidentally. This often applies to dogs that hunt or frequently spend time in rural or forest areas.

It is enzootic in Western and Central Europe, and a survey conducted in Hungary reported *C. aerophila* in 66 % of foxes and *C. boehmi* in 8 %. In the past decade, clinical reports and epidemiological surveys have revealed the nematode in dogs and cats from Europe, North America and Australia. Infestation has also been described in humans from Ukraine, Russia, Morocco, Iran, France and Serbia.

The role of foxes as a reservoir for domestic carnivores is increasing as a result of their population growth and increasingly suburban or even urban distribution.

Clinical signs and lesions

Clinical signs

Animals harbouring *C. aerophila* may either be asymptomatic or present respiratory distress, ranging from subclinical or mild disease to severe and potentially fatal pneumonia.

Respiratory capillariosis due to *C. aerophila* is characterised by the development of chronic tracheobronchitis, causing a persistent cough which is resistant to standard treatment. When accompanied by secondary bacterial infections, *C. boehmi* causes nasal pruritus and serous to purulent discharge and, occasionally, epistaxis.

Adult parasites damage the lung parenchyma and cause bronchovesicular breath sounds, sneezing, wheezing, and chronic dry or moist and productive cough, especially when bacterial complications occur.

When the parasite burden is heavy, the disease may lead to mortality due to complicated bronchopneumonia and respiratory failure.

Lesions

Clinical capillariosis seems more severe in young, debilitated or immunosuppressed animals.

Necropsy of infested animals shows a serous exudate embedding worms in the trachea and bronchi, along with small calcified local granulomas throughout the lung parenchyma.

Histological examination reveals erosion of the tracheal mucosa, with chronic submucosal inflammation, and cellular infiltration immediately beneath the parasite.

Human capillariosis can mimic clinical and X-Ray findings of a lung neoplasia, with a cough, mucoid sputum, haemoptysis, fever, dyspnoea, and eosinophilia.

Diagnosis

Clinical diagnosis is difficult. Different antibiotic and anti-inflammatory treatments have often been used without any success, and a parasitic cause has only been considered as a last resort.

Definitive diagnosis is made by identification of the typical trichurid eggs in faecal samples examined using conventional coproscopy. *C. aerophila* are barrel-shaped, and present asymmetry of the bipolar plugs with no ring thickening, and a net-like outer shell with depressions and irregular anastomosing ridges and bridges. The long and short axes of the eggs are 65 and 30–40 µm, respectively (Fig. 1). Some morphometric and morphological features of these eggs overlap with other trichurid ova which may be shed in dog faeces, i.e., eggs of whipworms, or of other capillarids infesting cats and dogs.

Control measures

Knowledge of anthelmintic treatments for animals infested by *C. aerophila* is poor, although different doses and repeated applications of injectable or oral levamisole have been effective to some extent. Various publications indicate that ivermectin (off-label) administered subcutaneously to infested dogs at a dose of 200 µg/kg is effective. Other avermectins/milbemycins are also active. A spot-on formulation containing moxidectin 1 % has been used successfully to treat pulmonary capillariosis in cats, and another spot-on formulation, containing eprinomectin (0.5 mg/kg) combined with praziquantel/fipronil/(S)-methoprene recently proved effective in the treatment of infested cats.

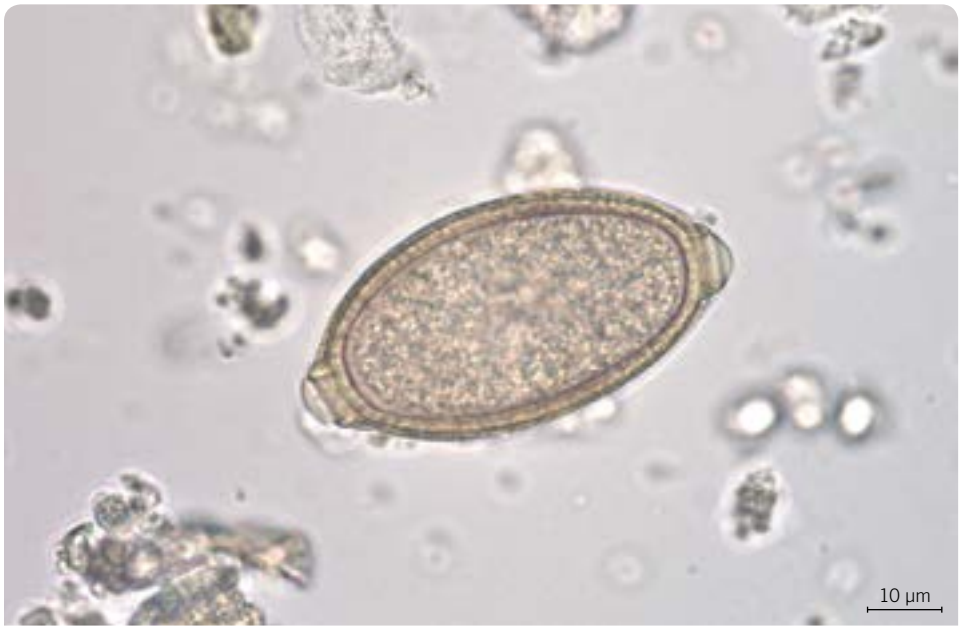
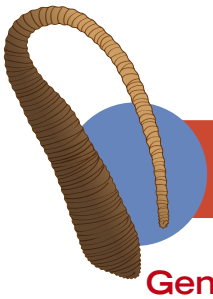


Figure 1. *Capillaria aerophila* egg. Coproscopy (×40): the egg is barrel-shaped, and presents asymmetry of bipolar plugs, no ring thickening, and a net-like outer shell with depressions and irregular anastomosing ridges and bridges. The long and short axes of these eggs are 65 × 30–40 µm, respectively.



Linguatulosis

General comments

Linguatulosis is a parasitic rhinosinusitis caused by the presence and development of a crustacean parasite, ***Linguatula serrata*** (syn. *Linguatula rhinaria*) in the nasal cavity and sinuses of carnivores.

Linguatula organisms belong to a homogenous group of parasites previously known as pararthropods, in the class Pentastomida (Heymons, 1926). Some recent molecular studies have compared them to parasitic crustaceans, the Copepod subclass in particular so Pentastomida is now an order of this subclass.

In their adult state, many species are parasites of reptiles (Porocephalidae family Heymans, 1922). *L. serrata* (Fröhlich, 1978) is the only representative of the Linguatulidae (Shipley, 1898).

This parasite is distributed worldwide but it seems to be more common in tropical countries (North Africa, Central Asia, and the Middle East). It has only been reported sporadically in Europe.

As the life cycle requires ingestion of the raw viscera of mammalian intermediate hosts (ruminants, rabbits or rodents), it mainly involves wild carnivores, particularly canids: foxes, wolves, although dogs and, occasionally, cats may also ingest the parasitised viscera and become infested.

Rare human cases have also been described.

Morphology

Adult pentastoma are whitish crustaceans with elongated, worm-shaped bodies which are sometimes striated (pseudo-segmentation) and more or less flat. Females are generally larger than males. They have a complete digestive tract.

At the anterior end, the mouth is surrounded by two pairs of small rudimentary legs, with articulated hooks (made up of two segments) and localised to the fossa. The name pentastoma comes from these four appendices, plus the mouth.

Adult male *L. serrata* measure 18–20 × 3–4 mm and females, 18–130 × 8–10 mm. They are elongated, flat and tongue-shaped, with a tapered posterior end (Figs. 1 and 2).

The brownish eggs measure 90 × 70 µm and they have a thick, smooth shell and contain an embryo with two pairs of hooks.

The first egg-derived larval stage measures 500 µm. This stage is not striated and has no buccal orifice, and it develops in nine successive moults to the nymph stage, which is 4–6 mm long. The nymph is morphologically similar to the adult and is only differentiated by its small size and absence of reproductive organs.

Biology

The parasitic cycle is a dixenous cycle. The adult *Linguatula* live in the nasal cavities or, more rarely, in the sinuses or pharynx of the definitive hosts, which are carnivores, held there by their hooks.

Figure 1. Macroscopic view of an adult *Linguatula serrata* (approximately 7 cm long) extracted with tweezers from a nasal cavity. Courtesy of Parasitology Unit, Alfort Veterinary School.

Figure 2. Adult *Linguatula serrata* (approximately 7 cm long) extracted with tweezers from a nasal cavity. Observation after lactophenol clarification. The striated appearance of the whole body and presence of four hooks at the anterior extremity can be seen. Courtesy of Parasitology Unit, Alfort Veterinary School.

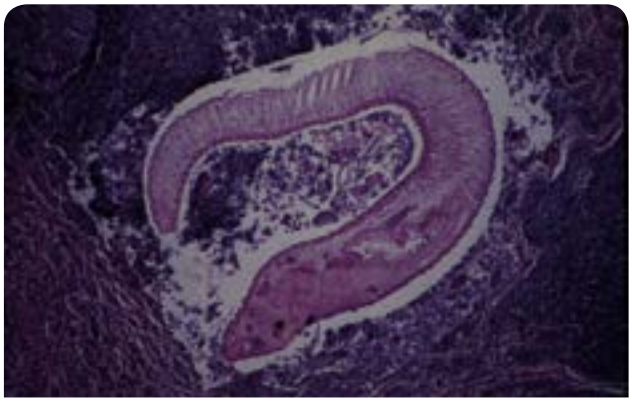


Figure 3. Histological section of the rumen mucosa of a cow, showing a larva of a *Linguatula* sp. curved around itself. Obj. ×10. Courtesy of Parasitology Unit, Alfort Veterinary School.

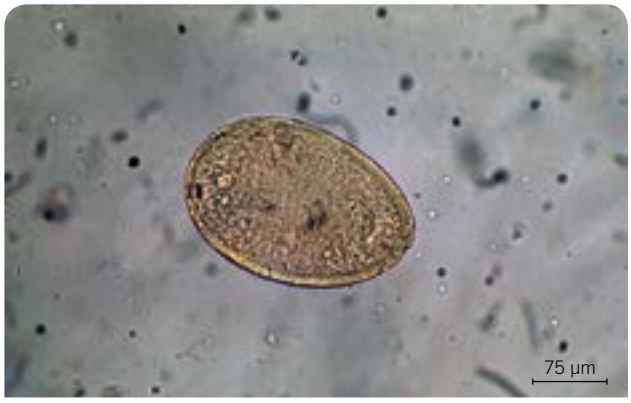


Figure 4. *Linguatula* sp. egg, excreted with nasal mucus. Microscopic examination.

The males die quickly after fertilisation (4 months after infestation), whereas the female lifespan is approximately 15 months. The females lay eggs which are either expectorated with nasal discharge or, more rarely, expelled with the faeces.

Eggs are directly infective and can survive for a few days in the soil. If they are ingested by herbivores (ruminants, horses, rabbits, rodents), they release stage 1 larvae (L1) which resemble small mites. The larvae encyst in the intestinal mucosa or migrate in the lymphatic vessels towards the mesenteric lymph nodes, liver or other organs. They may spread through the whole organism. After developing for several months (going through successive moults), the nymph which resembles an adult is formed. It resembles an adult, and is usually localised within a small cystic gall bladder. After developing for 7 months, the nymph comes out of its cyst and enters the thoracic or abdominal cavity.

If a carnivore consumes raw viscera from an infested herbivore, the cycle continues and the nymph travels up from the stomach towards the buccal cavity, then enters the pharynx and nasal cavity, where it remains and becomes an adult. The period of development is approximately 6 months in dogs.

Epidemiology

Linguatulosis is sporadic in domestic carnivores, particularly dogs, in Western Europe. The natural cycle takes place in the wild, and involves carnivores, herbivores, lagomorphs and rodents.

Linguatulosis can be enzootic in other areas, such as Central Asia and Africa.

Prevalence may be particularly high in stray dogs and a survey in Iran in 2003 reported that 62.2 % of dogs were infested and carrying 1–29 *Linguatula*. Dogs over 5 years old were significantly more infested than dogs up to 4 years old.

Clinical signs and diagnosis

Linguatula's size and mechanism of fixation are responsible for sinus and nasal inflammation which causes clinical signs.

Infestation may be asymptomatic or manifests itself through only mild clinical signs: animals sniff and sneeze, and epistaxis is common. Dogs present with abundant nasal discharge, serous where there areno complications, but often haemorrhagic. Expulsion of parasites during sneezing attacks is possible.

Differential diagnosis must be made between all causes of rhinitis in dogs and cats: the clinical signs of linguatulosis are resistant to antibiotic treatment, but may improve with anti-inflammatory treatment.

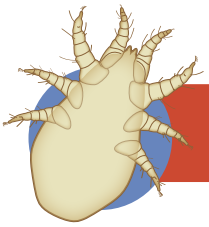
Definitive diagnosis depends on visualisation of adult *Linguatula* (by rhinoscopy) or eggs in discharge or faeces (Figs. 3 and 4).

Control measures

It is sometimes possible to remove *Linguatula* from a tranquilised dog with forceps.

Active antiparasitic drugs are effective against haematophagous parasites, notably nitroxinil, administered subcutaneously at a dose of 10 mg/kg. Closantel could be tested. Avermectins/milbemycins can be used at an insecticidal/acaridicidal dose.

Prophylaxis relies on preventing carnivores from accessing ruminant or lagomorph offal.



Pneumonyssoidosis

General comments

Pneumonyssoidosis is a respiratory acariosis in dogs caused by a mesostigmate mite, ***Pneumonyssoides caninum*** (Chandler and Ruhe, 1940), in nasal cavities and frontal sinuses.

This mite was first observed in the United States in 1904, but has since been found in most continents and many countries: North America, Australia, Japan, South Africa and Europe.

Pneumonyssoidosis has been diagnosed sporadically in Germany, Spain and France and it is enzootic in Scandinavian countries, sometimes with very high prevalence (up to 24 % of dogs found to be infested in surveys of autopsies in Sweden).

This parasite is host-specific and no infestations have been reported in cats or humans.

Infestations have been described in foxes but they do not seem to be a habitual host.

Morphology

P. caninum is a mesostigmatan mite belonging to the super-family *Dermanyssoidea* and to a family close to Dermanyssidae, Halarachnidae. The members of this family are obligate parasites of the respiratory system.

Dermanyssoidea are large mites, 300 µm to 2.5 mm long, characterised by a pair of respiratory stigmata next to coxa III and surrounded by a stigmatic plate or elongated perimeter. They have one or two dorsal chitinous plates and several ventral plates, and the legs are long and in an anterior position. The buccal apparatus is elongated, chelicerae are very long and styliform, and the maxillary palps are also long.

P. caninum is a mite with a yellowish, oval body. The females are 1–1.5 mm long; males, 0.8–1 mm; and hexapod larvae, 0.6–0.7 mm long. The adults have a small and irregular chitinous plate, the sternal ventral plate is small and square and the anal plate is small and round (Figs. 1 and 2).

Biology

The life cycle of the parasite is little understood; male and female adult mites and larvae are known and observed as parasites, but nymphs have never been described.

Reproduction has also never been observed *in vivo* but females containing eggs have been extracted from the nasal fossae of dogs, suggesting that reproduction occurs in the host. The eggs contain fully-formed larvae and they have never been observed in lesions, suggesting that the females are ovoviviparous. The nymph stage could be ephemeral.

Adults can survive in the environment for more than 19 days under experimental conditions, which suggests the possibility of an external phase, but it seems likely that contamination is direct and that the cycle takes place entirely in the nasal cavities and sinuses of dogs. Infestation does not tend to disappear in isolated dogs, which supports the idea that it is self-sustaining in the host.

When dogs are anaesthetised or at rest, it is possible to see many parasites in the nose or coming out of the nose and this is probably the mechanism of transmission (Figs. 3 and 4).

Epidemiology

Various studies cite infested dogs as the main source of parasites.

This parasitic disease is chronic and animals remain carriers of an often significant number of mites (several hundred) when left untreated.

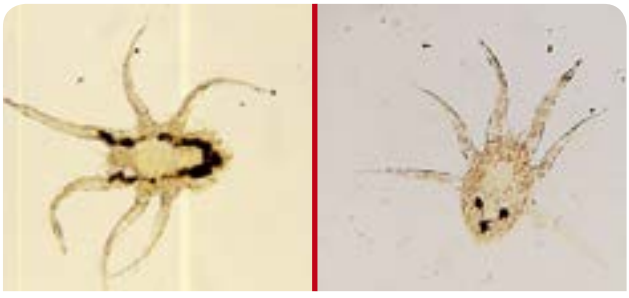


Figure 1. Adults *Pneumonyssoides caninum* observed between slide and coverslip in Amann's lactophenol. Size approximately 700 µm. Courtesy of Parasitology Unit, Oniris.

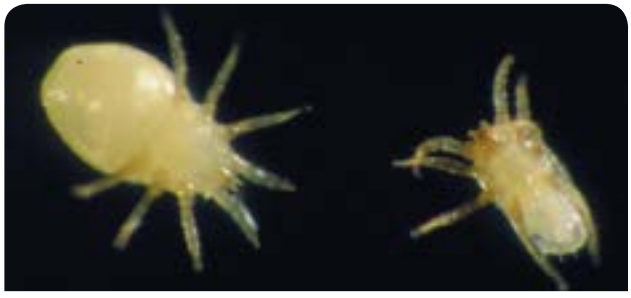


Figure 2. Adults *Pneumonyssoides caninum* observed fresh under a binocular magnifier. Size approximately 700 µm. Courtesy of Patrick Bourdeau, Parasitology Unit, Oniris.

The cycle seems to take place entirely in the dog. The closest relative to this species, *Pneumonyssus simicola*, a very common parasite of the pulmonary parenchyma in rhesus macaques (*Macaca mulatta*), with a prevalence of around 100 % in macaque colonies, completes its cycle entirely in the lungs. Transmission occurs directly between monkeys through discharge and sneezing.

Primary transmission seems to be direct, through contact between dogs or through the environment where an infested dog lives.

Clinical signs and diagnosis

Most infested dogs do not present with any clinical sign though other animals, which are more sensitive or have a higher parasitic burden, may present with certain fairly characteristic clinical signs:

- Nasal or facial pruritus (rubbing the muzzle on the ground and furniture).
- Rhinitis with serous discharge (secondary bacterial infections are possible but rare).
- Sneezing.
- Reverse sneezing.
- Epistaxis and dyspnoeic attacks are less common.

These clinical signs are linked to inflammation of the nasal mucosa and infiltration by eosinophils and mastocytes is usually seen.

Differential diagnosis must be made between all causes of rhinitis or reverse sneezing. The latter, in the form of snoring, is very common in brachycephalic breeds where it is caused by flaccidity of the soft palate.

Clinical signs do not recede on administration of antibiotics and does so only partially with anti-inflammatories.

Definitive diagnosis relies on visualisation of the mites and can be made by examination of nasal discharge after the instillation of physiological fluid, or by rhinoscopy (Figs. 3 and 4).

Some laboratories in Scandinavia have established serological techniques but they mainly used for epidemiological surveys.

Prognosis is good, clinical signs are often absent or limited, and treatment is simple.

Control measures

Avermectins/milbemycins are the antiparasitic treatment of choice.

Ivermectin and doramectin have long been used off-label, administered subcutaneously at a dose of 200–400 µg/kg but they have now been replaced by:

- Milbemycin oxime, taken orally at a dose of 0.5 mg/kg. Two or three administrations repeated at an interval of a week, or by
- Selamectin spot-on at a dose of 6 mg/kg, administered three times. Moxidectin spot-on probably has similar activity.
- New insecticidal/acaricidal molecules belonging to the isoxazoline family, which should be tested (afoxolaner, fluralaner, sarolaner).

There are no preventative measures for this disease, which is sporadic except in Northern Europe.

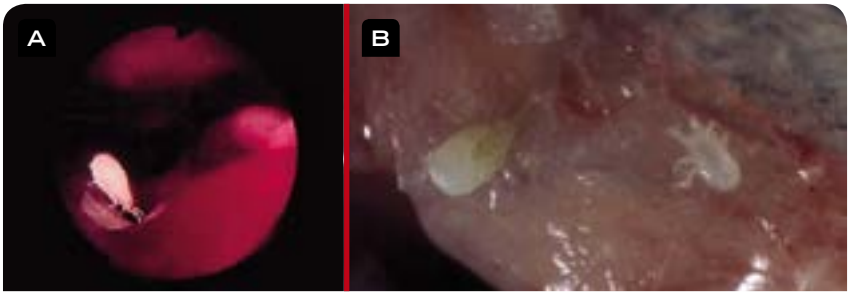


Figure 3. *Pneumonyssoides caninum in situ* by (A) intra-nasal endoscopy and (B) necroscopy. Courtesy of Patrick Bourdeau, Parasitology Unit, Oniris. From the thesis of Lotta Gunarsson.

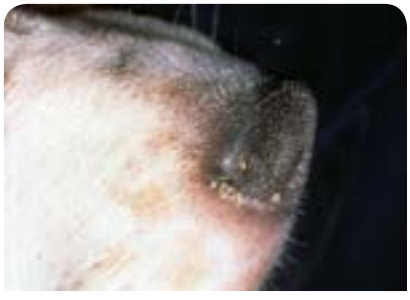


Figure 4. Presence of adults *Pneumonyssoides* on the nose of a dog. Courtesy of Patrick Bourdeau, Parasitology Unit, Oniris. From the thesis of Lotta Gunarsson.

Urinary parasitoses

Bladder capillariosis

General comments

The bladder of dogs and cats can be infested by nematodes belonging to the Capillariidae family. Two species are observed: ***Capillaria*** (syn. *Pearsomena)* ***plica*** in both dogs and cats, and ***Capillaria*** (syn. *Pearsonema)* ***feliscati*** which infests mainly cats, but sometimes also dogs. These nematodes are characterised by their small diameter, hence their name "capillaries". They are whitish and look like thread, 1–2 cm long (Figs. 1 and 2) and mainly affect wild carnivores, particularly foxes, but they are sometimes also observed in dogs or cats. They are present in cold temperate regions and are described in Europe and in North America.

Biology

The life cycle is thought to be dixenous, although the involvement of earthworms as actual intermediate hosts or only paratenic hosts is sometimes discussed.

Adults are present in the wall of the bladder and females lay eggs in the bladder lumen. These are then expelled in the urine. Eggs are elongated, with a smooth, thick shell and a shallow polar prominence at each end (Fig. 3). They are smaller and shorter than *Trichuris* eggs, measuring 65 × 25 µm, and contain a single cell when they are emitted; they develop into embryonated eggs containing L1 larvae in the soil in 10–30 days. When ingested by the intermediate host (earthworms of the genera *Lumbricus* or *Dendrobaena* the L1 larvae hatch in the earthworm's intestine and then burrow through the intestinal wall and become embedded

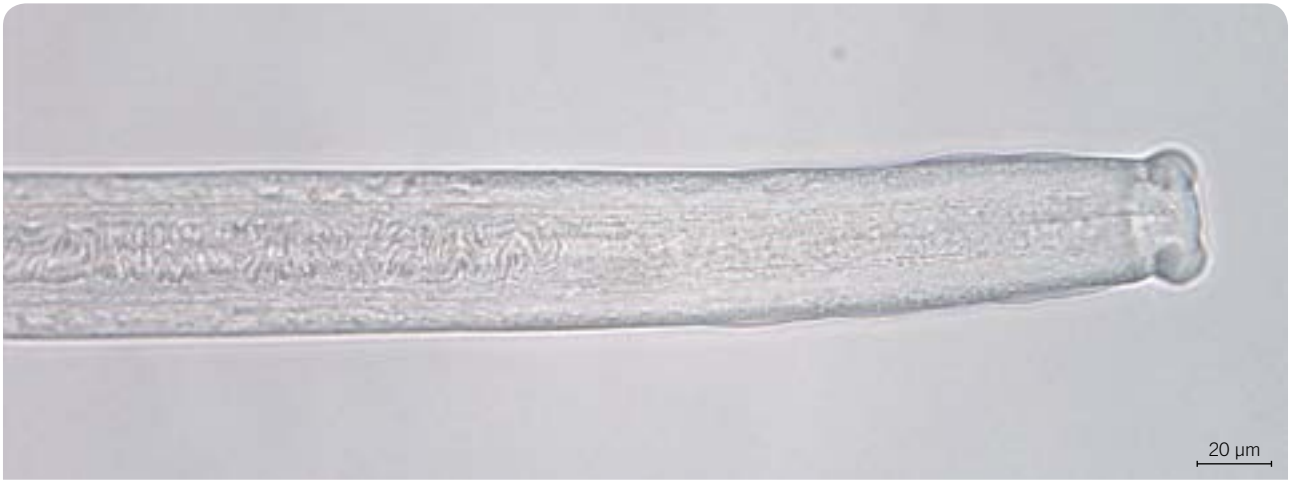


Figure 1. Anterior end of a male *Capillaria plica.*

Figure 2. Body of a female *Capillaria plica* showing vulva and eggs.

in connective tissue throughout the body of the worm. If the earthworm is eaten by a suitable mammalian host, the larvae moult into second stage larvae (L2), burrow through the intestinal wall, and moult again into third stage larvae (L3). The L3 are carried through the circulatory system to the glomeruli of the kidneys and, from there, they travel down the ureter to the urinary bladder. By 33 days post infestation, L3 and L4 larvae are found in the urinary bladder, where they mature into adults and reproduce sexually, shedding eggs into the host's urine within about 60 days of infestation (prepatent period).

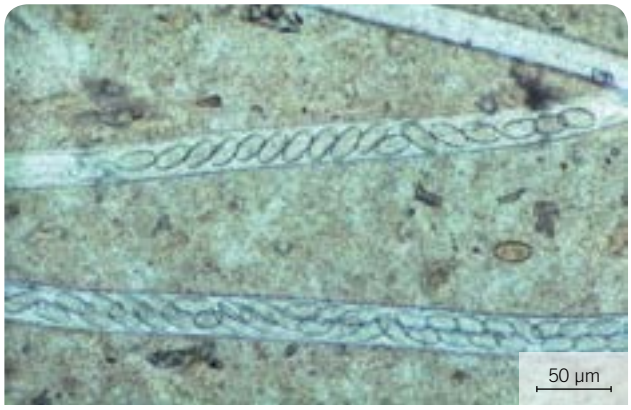
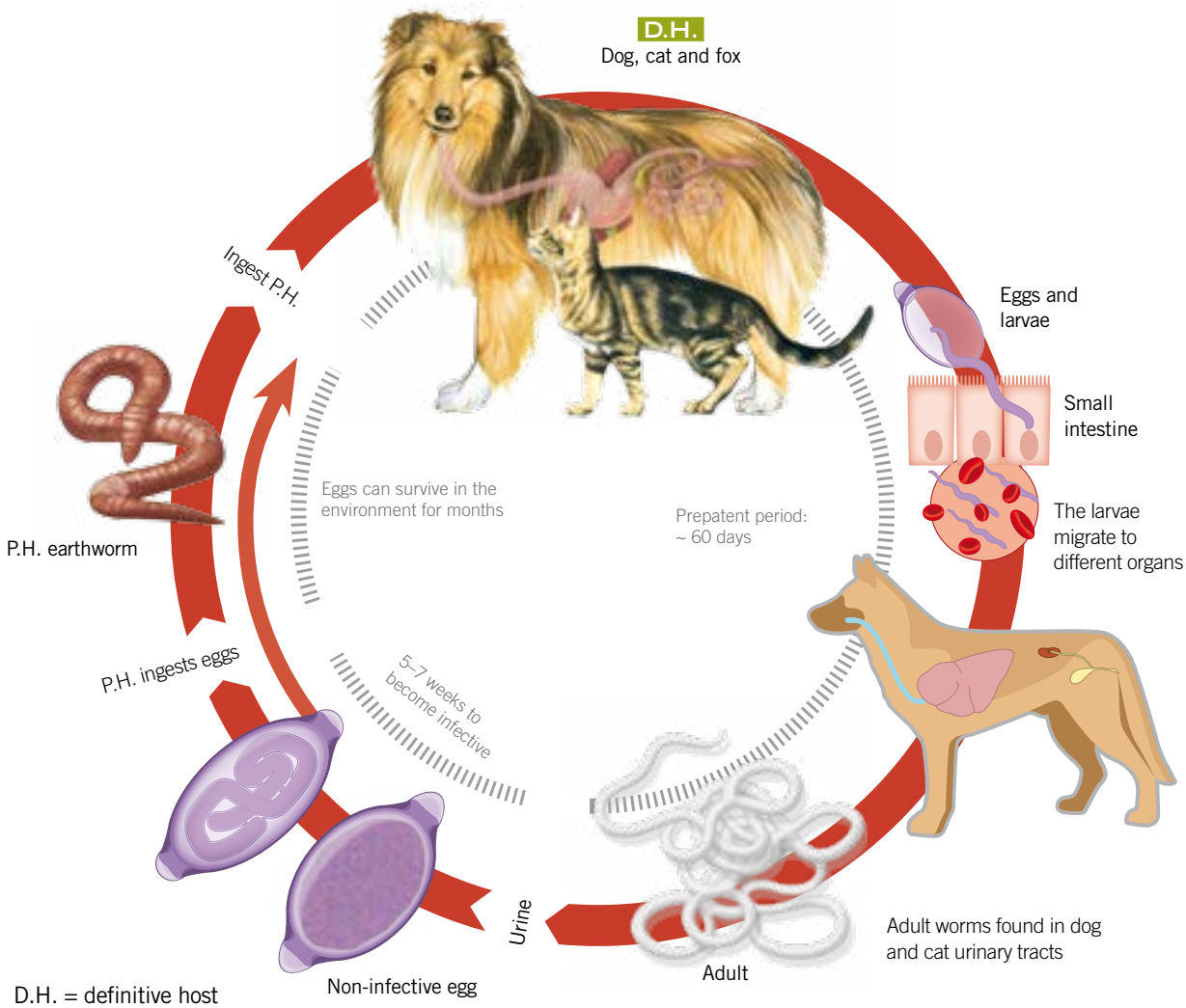


Figure 3. Body of a female *Capillaria* sp. showing eggs with plug-like structures at each end.

PDF

Life cycle of *Capillaria plica*



Epidemiology

As with respiratory capillariosis, bladder capillariosis is observed in wild fauna above all. Affected species are wild canids and mustelids, foxes and minks in particular. Dogs and cats are affected sporadically and accidentally, and this often applies to pets which hunt, or spend time in rural or forest areas. Bladder capillariosis is enzootic in European foxes and a survey carried out in 2003 in Hungary identified capillary worms in the bladder of 52 % of the 100 foxes captured.

As with respiratory capillaries, the role of the fox as a reservoir for domestic carnivores can only increase as a result of their population growth and their increasingly suburban or even urban distribution.

Clinical signs

Clinical signs of bladder capillariosis are those of cystitis, with urinary frequency, dysuria and haematuria. This cystitis is resistant to antibiotic treatment.

Diagnosis

Clinical diagnosis is difficult and different antibiotic and anti-inflammatory treatments have often been employed without success, and a parasitic cause has only been considered as a last resort. Confirmation of parasitic aetiology is only possible experimentally, through identification of eggs typical of capillary worms in urinary sediment (Figs. 4 and 5).

Figure 4. *Capillaria* sp. egg in the urine sediment of a domestic carnivore.

Control measures

A spot-on formulation which combines eprinomectin (0.5 mg/kg) with praziquantel, fipronil and (S)-methoprene, has been licenced for the treatment of infestations in cats. The off-label use of ivermectin, at a dose of 200 µg/kg administered subcutaneously, has been demonstrated to be effective. Other avermectins/milbemycins drugs (selamectin, moxidectin) should also be active.

Figure 5. *Capillaria plica* egg. Microscopic examination.

Dioctophymosis

General comments

Dioctophymosis is a helminthosis of the kidney caused by parasitism by ***Dioctophyme renale*** (or ***Dioctophyma renale***).

This parasite is a nematode belonging to the Dioctophymatoidea order, which does not contain any other nematode of medical or veterinary importance and it is commonly known as the "giant kidney worm". It is the largest parasitic nematode that can infest pets.

D. renale is distributed worldwide, but is less common in Africa and Oceania. It affects fish-eating mammals, especially otters and mink, and dogs to a lesser extent. Human infestation is rare, but results in destruction of the kidneys. It may also accidentally infest pigs and horses.

Morphology

D. renale is large roundworm, 20–80 cm long (Fig. 1). Both sexes are bright red in colour and taper at both the anterior and posterior ends. The posterior end is bell-shaped. Male *D. renale* worms have a bursa, which is used for attachment during mating, but it is not supported by chitinous ribs as are the copulatory bursa of Strongylida males (Fig. 2).

Biology

The adults are localised to the renal pelvis, mostly in the right kidney, and sometimes even in the ureters, and they cause atrophy of the renal parenchyma and an associated reduction in renal filtration, with the onset of lower back pain and haematuria.

The life cycle is aquatic and dixenous. The females lay eggs which are expelled in the urine, and develop in an aquatic environment into embryonated eggs containing L1 larvae in 1–3 months. If these eggs are ingested by the intermediate host, an oligochaete annelid (such as *Lumbriculus* sp., *Cambarincola* sp.), they develop into L3 larvae (after 100 days) but may re-encyst in numerous paratenic hosts: batrachian or freshwater fishes, including catfish (*Ichtalurus melas*).

The intermediate host or paratenic host is then eaten by a definitive host, where the L3 larvae penetrate the intestinal lining and migrate to the liver. After maturing for approximately 50 days, they migrate to the kidneys and moult into adults. The prepatent period is of 4–5 months. Upon maturation, *D. renale* can survive for approximately 5 years in its host.

Figure 1. Adult *Dioctophyme renale*, isolated after extraction.

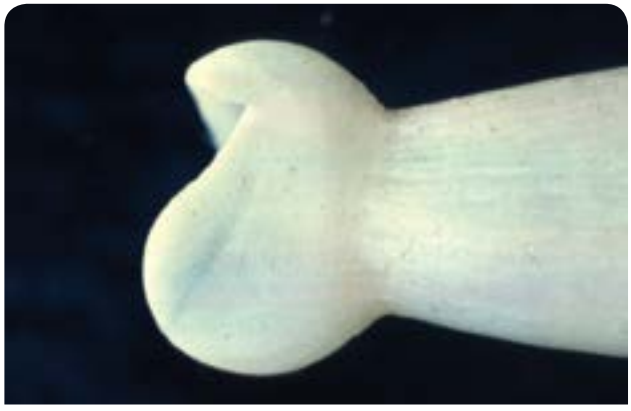


Figure 2. Observation of a male's bell-shaped caudal bursa.

Epidemiology

D. renale is found in all cold temperate regions of the Northern hemisphere where freshwater is available, as its cycle requires an aquatic environment.

The adults, measuring 20–80 cm in length, mainly parasitise piscivorous mammals, in particular mustelids (otters, minks) and sometimes canids, or humans. Dioctophymosis is principally a parasitosis of wild fauna and is found sporadically in domestic carnivores.

The consumption of contaminated fish, which act as paratenic hosts, is the main source of infestation in mammals.

Clinical signs and lesions

Clinical signs

Dioctophymosis is characterised by slow development of renal insufficiency and the animal presents with acute lower back pain on palpation of the kidneys (particularly the right kidney, which is infested in more than 90 % of cases).

A change in general condition is also seen. Nervous signs are possible: paresis, paraplegia, and rabies-like clinical signs.

Blood count shows high levels of urea and creatinine and constant or intermittent haematuria may be seen.

Lesions

The infested kidney is reduced to its capsule and its volume is significantly increased. Lesions in the kidney parenchyma consist of connective tissue proliferation in the interstitial tissue, tubular atrophy and fibrosis, and periglomerular fibrosis. The renal pelvis is stretched while the parenchyma is totally atrophied and non-functional (Fig. 3). 1–5 worms are usually seen in this stretched bladder, which contains a haemorrhagic fluid. Some nematodes can attach in erratic locations, such as in the ureters or the bladder and sometimes they are found in the abdominal cavity.

Figure 3. Lesion of the renal pelvis (right kidney) of an infested dog containing an adult *Dioctophyme renale*. Observation after nephrectomy.

Diagnosis

Diagnosis is based on epidemiological and clinical criteria.

Imaging techniques have recently proven to be useful diagnostic tools and the most commonly used of these methods are radiological sonography and renal echography.

Diagnosis is confirmed by observation of eggs in urinary sediment. Eggs are characteristic: elongated, barrel-shaped, and measure 75–80 × 50 µm. They have a thick shell which is undulating and punctuated, except at the poles, and they contain an embryo (Fig. 4).

Post-mortem diagnosis of *D. renale* parasitism is also very common.

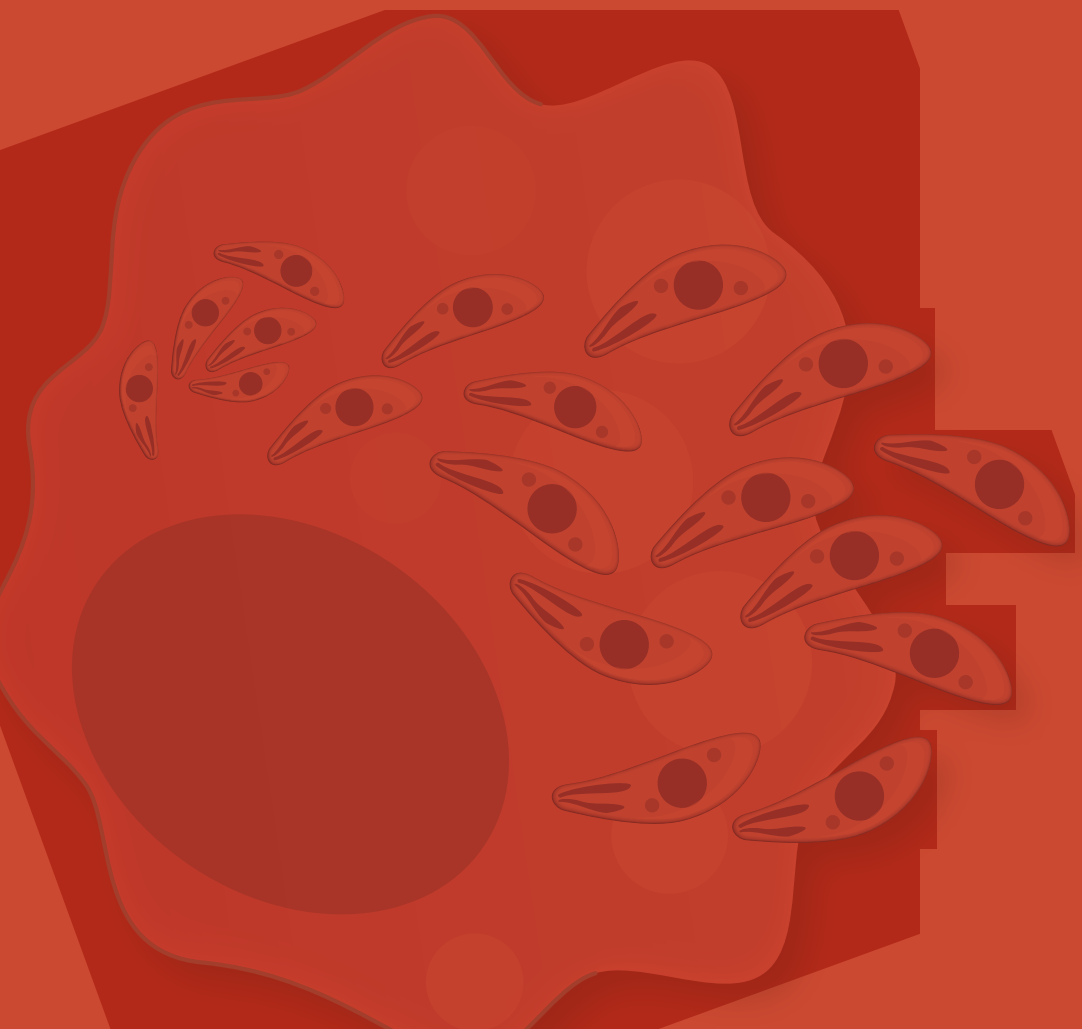
Figure 4. *Dioctophyme renale* egg recovered from the urinary sediment of an infested animal. The egg is elongated, barrel-shaped, and measures 75–80 × 50 µm. It has a thick shell which is undulating and punctuated, except at the poles, and it contains an embryo.

Control measures

Knowledge of anthelmintic treatments effective in animals infested by *D. renale* is poor but it seems that ivermectin (off-label) is active in a single subcutaneous administration of 200 µg/kg. Other avermectins/milbemycins should also be effective, although surgical excision is often the treatment of choice. Some authors recommend surgical nephrectomy, when just one kidney is affected, and nephrotomy if both are affected (Fig. 5).

Prophylaxis consists of avoiding consumption of raw freshwater fish.

Figure 5. Extraction of an adult *Dioctophyme renale* after nephrectomy (right kidney).



Disseminated parasitoses

Leishmaniosis

General comments

General canine leishmaniosis is an infectious protozoan disease, transmitted by phlebotomine sandfly bites (Fig. 1). It is caused by the presence and multiplication of flagellate protozoa belonging to the ***Leishmania (donovani) infantum*** species in cells from the mononuclear phagocyte line. It is characterised by visceral and mucocutaneous damage, hence the name "general leishmaniosis", and by damage to all organs and tissues containing macrophage cells.

Leishmania infantum is a zoonotic parasite, the agent for human visceral leishmaniosis, otherwise known as Mediterranean kala-azar (as opposed to Indian kala-azar, which is caused by *L. donovani donovani*).

Leishmania infantum was previously called *L. canis* as it infects domestic and wild canids (foxes in France), but lagomorphs and rodents, including black rats, mice and hamsters, can also be infected with some strains.

Some cases of leishmaniosis have been reported in cats and horses but they are still rare.

In humans, this infection has historically affected mainly children, hence the name *infantum*. Currently, the most cases are seen in immunosuppressed individuals, especially those infected by HIV.

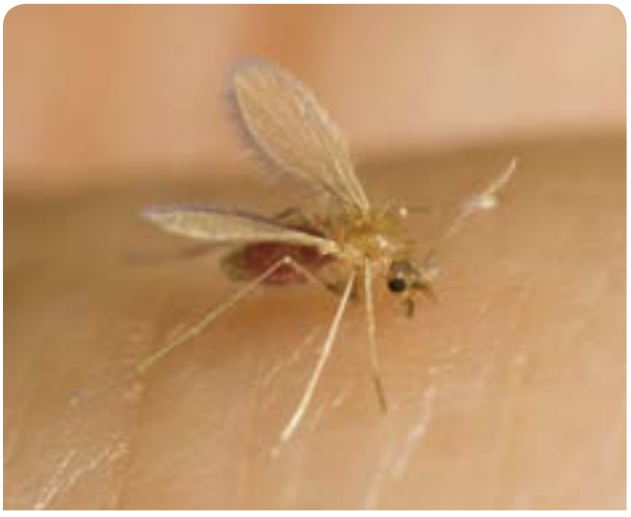


Figure 1. Female sandfly (*Phlebotomus*), vector of leishmaniosis in the Old World, including canine leishmaniosis due to *Leishmania infantum*.

Importance

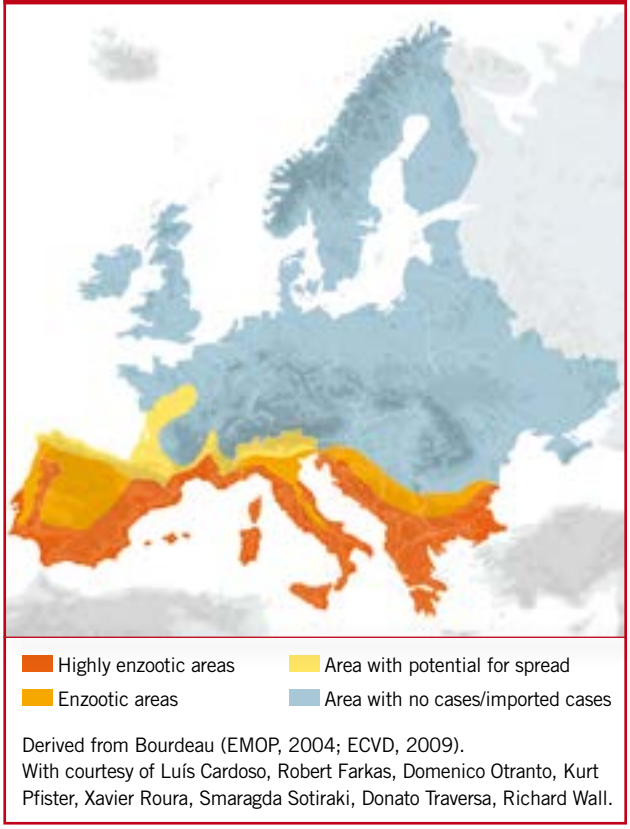
Veterinary importance for the dog is linked to the severity of the disease. It usually advances gradually until the animal dies and treatment provides only temporary clinical recovery. It does not eliminate the parasites and relapse is common. There is a threat to public health because dogs act as a parasite reservoir for humans but it is very rarely contagious from dogs to humans.

Geographical distribution

The distribution of *Leishmania* reflects that of its vectors. *L. infantum* is found in the Mediterranean Basin, the Near and Middle East, Central Asia and China, as well as Sub-Saharan West Africa. It was imported to South and Central America by European colonists and there, the parasite is known as *L. chagasi*, but it is the same as *L. infantum*.

In Europe, leishmaniosis is enzootic in Italy (except in alpine areas), Sardinia, Sicily, Spain, Portugal, the southern third of France, Corsica and Greece.

Figure 2. Distribution of canine leishmaniosis due to *Phlebotomus* spp. in Europe.

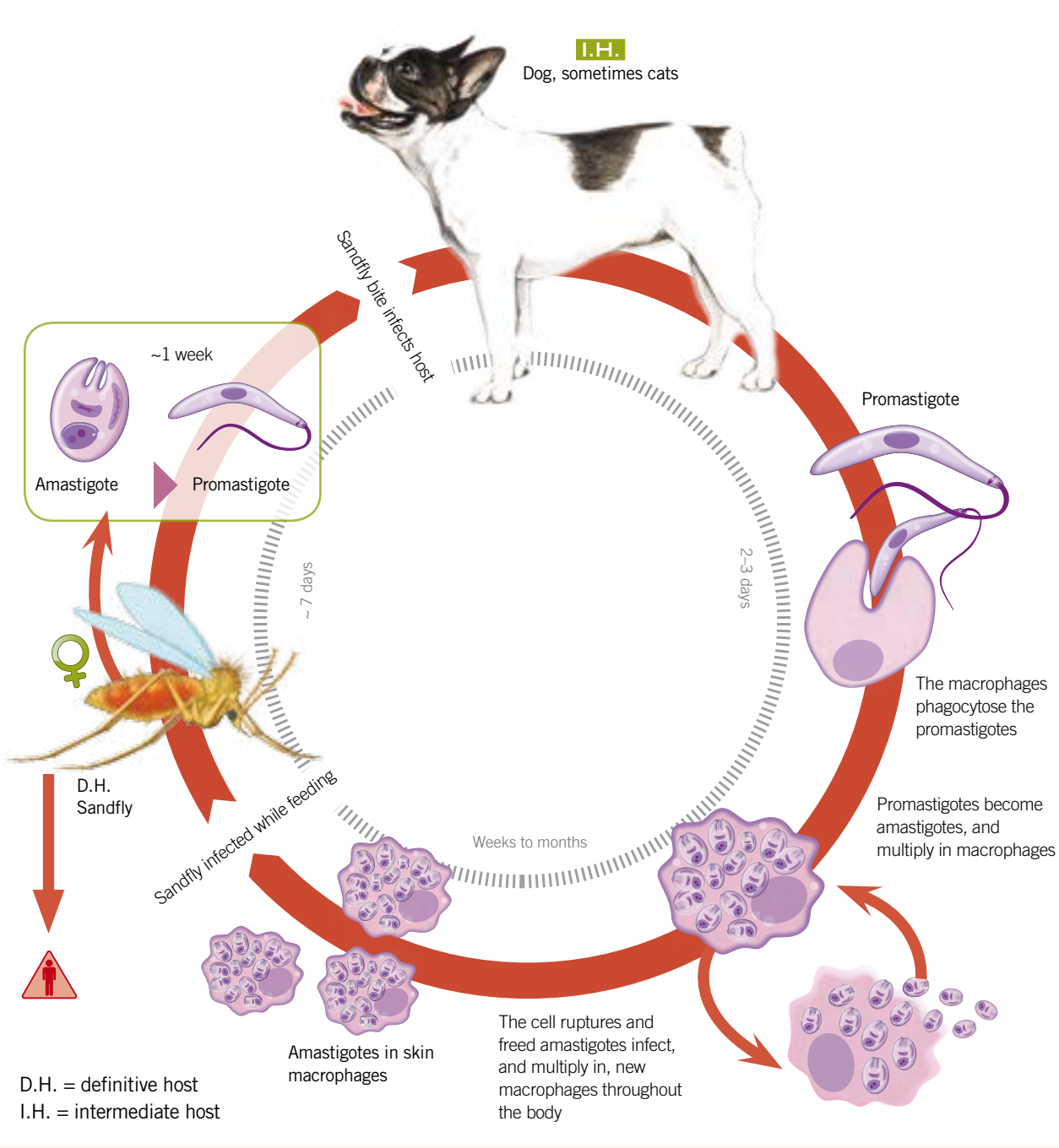


Derived from Bourdeau (EMOP, 2004; ECVD, 2009).
With courtesy of Luís Cardoso, Robert Farkas, Domenico Otranto, Kurt Pfister, Xavier Roura, Smaragda Sotiraki, Donato Traversa, Richard Wall.

Cases have also been reported in the North of France, in Belgium, the Netherlands, the UK and Germany, but these are imported rather than autochthonous cases due to the transport of infected dogs (Fig. 2). *L. infantum* is reported in North America. In this case, the parasite is mainly transmitted by vertical infection.

PDF

Life cycle of *Leishmania infantum*



Morphology and biology

Leishmania are flagellate protozoa (phylum Sarcomastigiphora, order Kinetoplastida, family Trypanosomatidae) and exist in two morphologically distinct forms in vertebrate and invertebrate vectors.

True extracellular flagellate forms called promastigotes are seen in sandflies or in culture, while dogs only harbour intracellular and non-flagellate parasites called amastigotes.

Amastigotes are found inside the parasitophorous vacuole in parasitised macrophages (Fig. 3). They are oval and measure 3–4 × 2 µm and contain a large nucleus and a stick-shaped element, the kinetoplast. Observation of infected macrophages, after MGG staining, is a classic diagnostic technique. Amastigotes survive phagocytosis, then oxidative stress in the macrophage. They multiply by longitudinal binary fission.

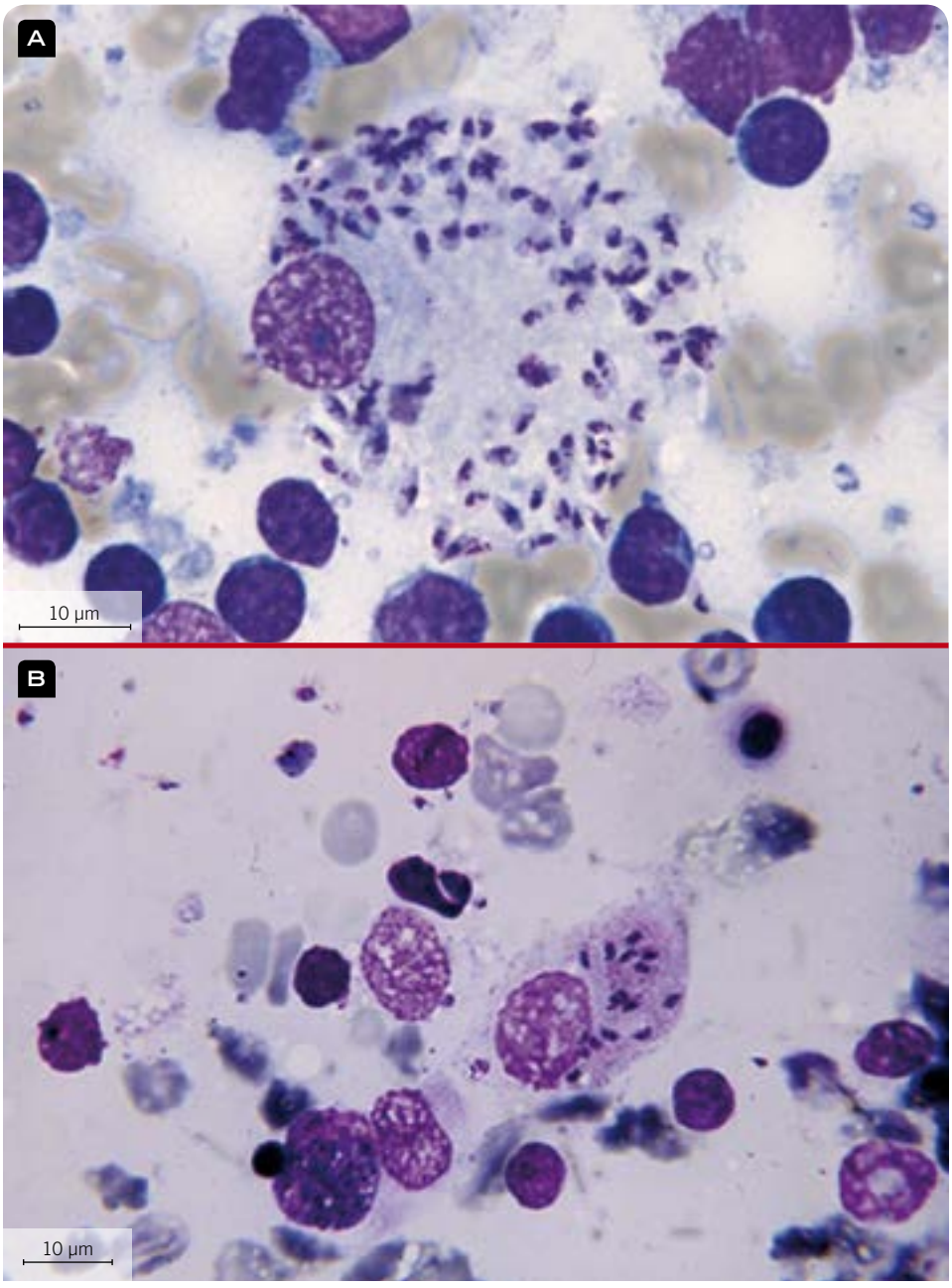


Figure 3. *Leishmania infantum* amastigotes inside macrophages. MGG-stained lymph node puncture. (A) Courtesy of the Parasitology Unit, Alfort Veterinary School.

Leishmania may be found in an extracellular position when macrophages are lysed or altered by sampling techniques or staining. Infected macrophages are enlarged and have the appearance of a blackberry.

The life cycle involves sandflies and dogs. Haematophagous female sandflies ingest amastigotes during a blood meal so the amastigotes are then found in the insect's midgut, where they start to develop. After 15 to 20 days, depending on the temperature, the promastigotes that infect vertebrates can be found in the insect's salivary glands.

The sandfly's meal is traumatic telmophagous ie creating a "lake of blood and lymph", which allows the promastigotes to come into contact with macrophagic cells and to be phagocytosed. They then start to multiply, and are transmitted from one macrophage to another by phagocytosis until they are distributed throughout the whole organism.

Epidemiology

Descriptive epidemiology: leishmaniosis is enzootic in areas with a high vector density, e.g., the Mediterranean area. The prevalence of infection exceeds 10 % in some areas of the South of France, Spain and Italy, reaching up to 60 % in Greece. Infections are seasonal, occurring between spring and autumn, but clinical expression is distributed throughout the year due to the extremely variable incubation period. Rural and peri-urban epidemiological cycles have been reported. The highest prevalence is observed in hinterland villages, but cases in dogs living in city suburbs are becoming more and more numerous.

The domestic parasite reservoir is the canine population. Dogs clinically affected with mucocutaneous lesions, i.e., approximately 50 % of the infected population, are the main source. 10 % of dogs appear to have a spontaneously regressive infection and are not sources. The final 40 % are dogs with the disease in incubation or clinically healthy dogs. These latest 40 % harbour parasites in the dermis and must be considered a source of *Leishmania*, even if their role is less important than that of clinically affected dogs.

In some areas, the sylvatic reservoir represented by foxes is significant.

When humans are infected, they develop visceral leishmaniosis and the cycle cannot be continued, except with some rare dermotropic strains. Cats are only occasionally affected and play no epidemiological role.

Sandflies are the only direct source of parasites. Two main vector species are known in Europe: *Phlebotomus ariasi* and *Phlebotomus perniciosus*.

Phlebotomus ariasi is a sandfly that is active in the summer and found mainly in the West (northern Spain, South-West France). It is found outside houses and on small hills, which explains the rural nature of this enzootic insect in these regions.

Phlebotomus perniciosus is ubiquitous and found in the whole of the Mediterranean region. It lives near human habitation and its activity is crepuscular. Its population peaks in the spring and autumn. It dislikes the wind and so is not found on the coast, but inland. This gives a rural and suburban character to the endemic.

In Serbia and Croatia, the main vector is *Phlebotomus perfiliewi*, and in Greece it is mainly *Phlebotomus major.*

Sandflies inoculate *Leishmania* by biting glabrous areas of the dog, such as the nose and pinna. However, the dog's coat does not offer protection, even if it is long.

Direct contagion between dogs, or from dog to human, is extremely rare if event existent because it would require contact between a wound with exudate rich in *Leishmania* and another injured area.

In utero transmission is possible.

Dog sensitivity does not depend on breed or sex or age, although the risk of infection does increase with age, as older animals have had a longer period of potential exposure to the parasite. Dogs in a poor condition are at risk of developing more pronounced clinical forms or having relapses soon after treatment.

Dog lifestyle has a large effect on the risk of infection, as dogs which live outside (guard dogs, shepherd dogs) are more likely to be bitten by sandflies. The suburban development of housing estates with gardens may explain the spread of leishmaniosis, since these areas create numerous environments favourable to vector proliferation.

Pathogenesis

The symptomatology of leishmaniosis is linked to infiltration of all tissues and organs by macrophagic cell lines, which cause functional disorders and destroy tissues. The synthesis of cytokines responsible for many effects, such as IFN, IL-1, and TNF, helps to explain many of these disorders. Leishmaniosis is a predominantly immunological disease.

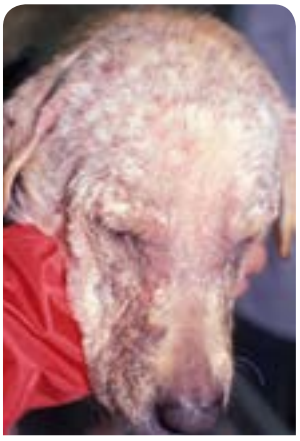


Figure 4. Dog with leishmaniosis: view of the head. Wasting of the masseter and frontal muscles, making the dog look old. Diffuse alopecia, significant squamosis. Courtesy of Blaise Hubert.

Figure 5. Amyotrophy in a dog with leishmaniosis (right). Courtesy of Blaise Hubert.

Leishmania survive inside macrophages by inhibiting their activity and adjusting the host's immune response so that the macrophage phagocyte system is not activated: stimulation of a type Th2 (humoral) response to the detriment of a type Th1 (cellular) response.

L. infantum is dermotropic and viscerotropic in the dog. Pathogenicity is linked to the infection of cells which are part of the immune system, causing an immunopathological disorder.

Leishmania, like all parasites, have a complex antigenic structure, including surface antigens (lipophosphoglycan, surface glycoprotein and somatic antigens). The antigenic coating differs between infective promastigotes and amastigotes, which is a mechanism for escaping immune recognition.

An immune response follows infection of the dog and it is cellular and humoral. The non-protective humoral response is early and intense and manifests itself by the appearance of antibodies, mainly of the IgG type. These can facilitate phagocytosis by macrophages, so they seem not to play any protective role; quite the opposite. Their abundance and the formation of complexes with antigens are responsible for immunopathological signs: glomerulonephritis, arthritis. Specific serum IgGs are found by various techniques: ELISA, agglutination or indirect immunofluorescence, which is still the reference.

The response to cellular mediation in the dog is generally insufficient to eradicate the parasite. It is based on the phenomena of cytotoxicity mediated by killer lymphocytes (CD8+ and NK) and intense oxidative reaction by macrophages, induced by various cytokines (IL-1, TNF-alpha, IFN-gamma). *Leishmania* promote a mainly humoral immune response by stimulation of the TCD4+ Th2-type lymphocytes at the expense of a cytotoxic response (Th1 type).

Clinical signs

These appear after a very variable incubation period, however this is usually between 3 months and a year after infection, so *Leishmania* can be seen in dogs which returned from enzootic areas several months, and sometimes several years, earlier. As the incubation period is long, serology is often already positive when signs start to appear.

Leishmaniosis is clinically very polymorphic, causing a variety of clinical signs, both general and cutaneous. The presence of a single sign must arouse suspicion of the disease, especially in enzootic areas.

Clinical signs may be more or less pronounced and vary in the length of time they take to develop.

General clinical signs

- Character change: a relatively consistent sign and one often reported by owners. The dog becomes apathetic, less playful, depressed. This state can progress as far as torpor. Appetite is also reduced.
- Amyotrophy: dogs show signs of muscle wasting, which affects the head first, especially the temporal and jaw muscles. The temporal fossae deepen, giving the animal a rather typical "old dog's head" (Fig. 4). Later, even the limbs get thinner, as well as the hips, which become prominent (Fig. 5).
- Weight loss: this accompanies the muscle wasting. The dog starts to look like a sad, old dog.
- Inconstant hyperthermia: this is particularly seen in young dogs, less than 2 years old.
- Blood and biochemical changes: anaemia, leukopaenia and thrombocytopaenia are usually noted. Leukopaenia is accompanied by monocytosis and hyperproteinaemia is soon seen. Globulins increase, resulting in a reversal of the albumin/globulin ratio from 1 to 0.3–0.1.

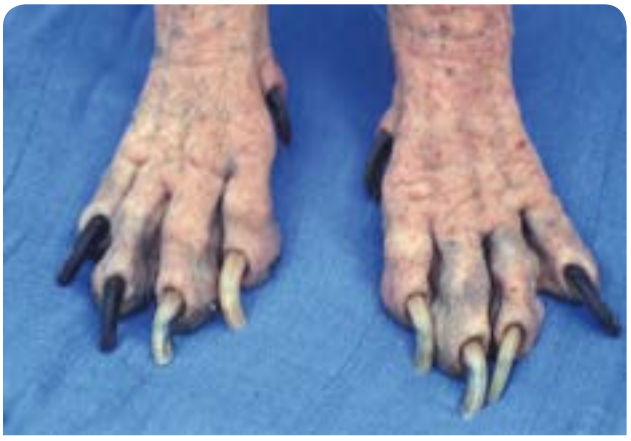


Figure 6. Onychogryphosis (accelerated claw growth). A clinical sign quite characteristic of leishmaniosis, linked to infected macrophages in the inguinal matrix and chronic inflammation which stimulates claw growth. Courtesy of Blaise Hubert.

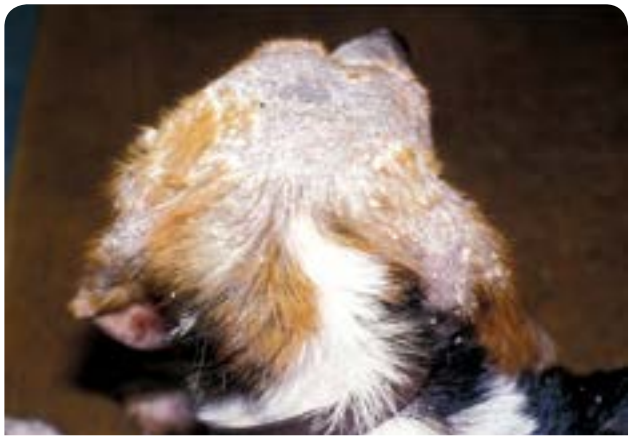


Figure 7. Dog presenting with significant squamosis on the face and the pinna, with thick dandruff which sometimes causes bright reflections. Courtesy of Blaise Hubert.

Mucocutaneous signs: dry dermatitis with squamosis

- Hair loss: diffuse alopecia, thinning of the coat without clearly localised baldness, never nummular. Hair loss is more pronounced on the limbs and head, including the area around the eyes, ears and tail.
- Onychogryphosis: multiplication of *Leishmania* in the claw matrix sometimes causes the characteristic sign that is constantly and rapidly growing claws (Fig. 6), linked to the presence of infected macrophages in the inguinal matrix and to chronic inflammation which stimulates horn growth.
- Keratogenic disorders: significant squamosis, with lots of large, bright scales (Fig. 7). This dandruff reforms very quickly when the dog is groomed. Hyperkeratosis may be associated with this problem; the epidermis thickens and becomes pigmented (melanosis), making the skin look pleated and greying, generally on the nose and ears, then spreading out.
- Ulcers: mucosal damage is shown by the appearance of ulcers, in cupules, which tend to spread, resulting in a flow of serous fluid rich in *Leishmania* (liquid form). Ulcers may sometimes heal temporarily (dry form). The most common locations for ulcers are the inner ear (corresponding to the main chancre of inoculation by the sandfly) (Fig. 8) and the nose (Fig. 9), paw pads (causing intense pain and reflex lameness [Fig. 10]), pituitary mucosa (causing nosebleeds and epistaxis, signs suggestive of leishmaniosis in endemic areas), oral and digestive mucosae, etc.
- Subcutaneous nodules: proliferation of macrophagic cell lines in the dermis may form nodules several centimetres in diameter. These nodules are palpable and painless, and this type of nodule seems to affect some breeds, such as Boxers, more than others.

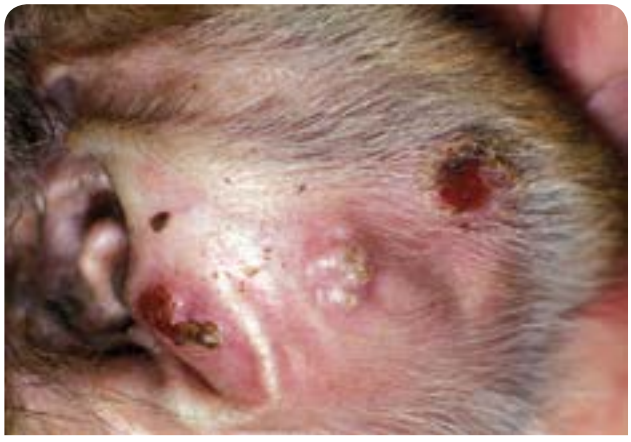


Figure 8. Internal face of the pinna: ulcers which can correspond to primary chancres of *Leishmania* inoculation. Ulcers in this case are located around a sandfly bite. Courtesy of Blaise Hubert.

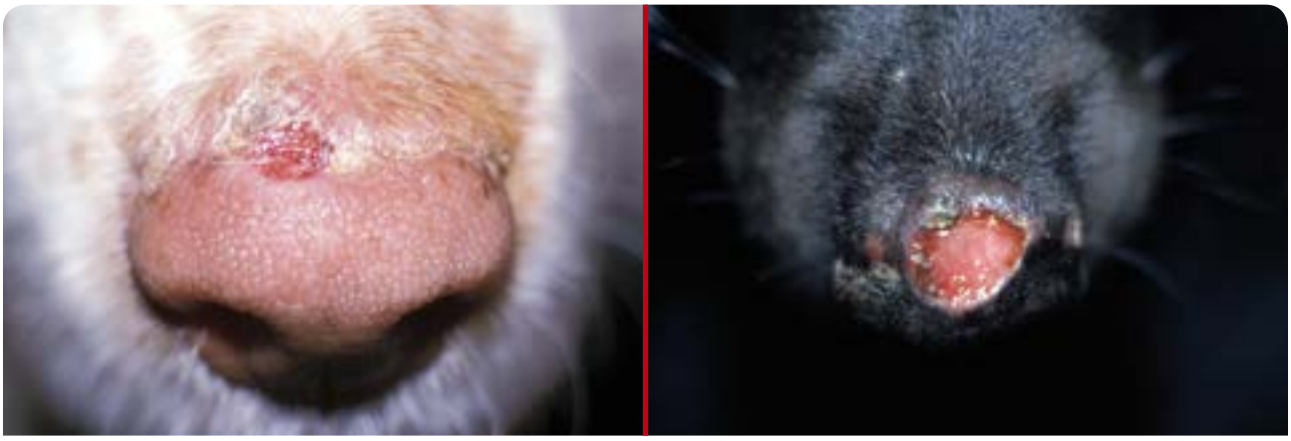


Figure 9. Ulcers on the nose of a dog. Courtesy of Blaise Hubert.

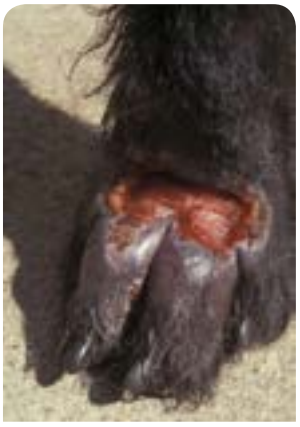


Figure 10. Ulcer on the foot of a dog with leishmaniosis. Courtesy of Blaise Hubert.

Clinical signs linked to damage to the mononuclear phagocyte system (MPS)

All organs which contain monocytes/macrophages are infected, including the spleen, liver and lymph nodes.

- Polyadenopathy: lymph nodes are hypertrophied and superficial nodes are easily palpable. They are not painful and puncturing them is useful for diagnosis.
- Splenomegaly: consistent in humans but inconsistent in dogs. Onset occurs in the later stages and is accompanied by pain.

Other clinical signs

Other clinical signs can be seen, with varying frequency. This is the case with **ocular signs:** blue keratitis, conjunctivitis, anterior uveitis, chorioretinitis. Motor and sensory nervous disorders have also been described.

The abundance of immune complexes explains most clinical disorders, including progressive development of **chronic renal failure** due to glomerulonephritis, and **polyarthritis**.

Leishmaniosis is a chronic disease and the animal can maintain a satisfactory condition for several months, but it usually progresses to cachexia, then death. Only 10 % of dogs remain "healthy carriers" or eliminate this parasite. Treatment does not neutralise the parasite or clear it from the host, so relapses are possible. Disease progression can be accelerated by the development of polyarthritis or immune-mediated glomerulonephritis.

Lesions

Histologically, cutaneous lesions are characterised by the formation of inflammatory granulomas centred on parasitised histiocytes (lympho-monocytic granulomas).

The general effects are also linked to tissue infiltration by MPS cells, which causes lympho-monocytic granulomas and histiocytic perivascular sleeves. Some immune complex deposits are found in joints and renal glomeruli.

Diagnosis

Clinical diagnosis

The diagnosis of leishmaniosis is differential and is based primarily on epidemiological and clinical considerations. Many diseases are involved in the differential diagnosis:

- Dermatoses: demodicosis, pyodermatitis, tinea, and especially other autoimmune dermatoses which may be clinically very similar to leishmaniosis. These conditions are sometimes associated with leishmaniosis, especially in dogs living in kennels or in large groups.
- General diseases: cancers, pyodermatitis, erhlichiosis (depression and epistaxis), and systemic lupus erythematosus (with an overall clinical presentation almost identical to leishmaniosis).

Laboratory diagnosis

- Suspicion: blood and biochemical changes (measured by protein electrophoresis and analysis of plasma profile): monocytosis (4 to 40 %), hyperproteinaemia (55 g/l to 85 g/l or even higher) with gammaglobulinaemia (block of β and γ globulins).

  The formol-leuco gel test is simple and useful: it consists of adding 2 drops of formaldehyde to 1 ml of serum. If a gel is formed and the mixture turns opaque within minutes, this indicates hyperglobulinaemia.
- Definitive diagnosis: this is achieved in 2 ways: either by serological screening of the infection (indirect diagnosis) or by detecting *Leishmania* amastigotes (direct diagnosis).
- Direct diagnosis: *Leishmania* live inside macrophages: dermal, splenic, hepatic, or in the bone marrow. However, they are rarely found in blood monocytes, which makes blood a very bad diagnostic sample in dogs.
- *Leishmania* can be revealed by MGG staining and microscopic observation with oil immersion and microscopic observation (1000x) from various samples: a layer of skin from a wet ulcerated lesion, skin shavings, popliteal peripheral lymph node puncture, or bone marrow aspirate. Lymph node puncture is fairly painless, easy to perform and safe for the animal. Simply extract a drop of lymph and cells using a syringe and a needle of a suitable diameter (1.2 mm).

  Leishmania generally occur in sufficient quantities in dogs with pronounced clinical signs, but it sometimes takes a long time to see them.
- Indirect diagnosis: infected dogs develop a premature antibody response (approximately 3 weeks after infection). These immunoglobulins can be detected by different techniques: ELISA, indirect immunofluorescence (IIF). Immunodiffusion tests are directly available to veterinary practitioners. IIF can be performed in specialised laboratories.

  Serology may be used to evaluate prognosis: the higher the antibody levels, the worse the prognosis. Treatment must reduce antibody levels by at least two dilutions, preferably below 1:320. Serology is performed 1 month after the end of treatment, then every 6 months, to monitor the disease in the patient.

Control measures

The course of treatment is lengthy and expensive so the owner must be fully committed and the dog's condition good enough before embarking on it. Clinical observation of a seropositive but healthy dog with a fairly low antibody level (1:160; 1:320) can be increased, rather than initiating treatment. Due to the zoonotic nature of *Leishmania* and the role played by dogs as a reservoir, euthanasia may be recommended for animals with many external lesions and in generally poor health.

Non-specific treatment

Given the sometimes pronounced renal failure, it may be necessary to delay the specific treatment and to adopt the following approach: drip, and immediate administration of corticosteroids to limit the formation of immune complexes and induced lesions (1 mg/kg *per os* of prednisone for 4–5 days).

Specific treatment

Meglumine antimoniate in a daily dose of 100 mg/kg, administered subcutaneously for 30 days. The maximum dosage for a large dog is 5 g. A new wave of clinical signs can be seen in the first week, related to the release of *Leishmania* antigens into the body. As this may affect the liver and kidneys, especially in weakened dogs or those with chronic renal insufficiency, supportive therapy with liver protectors and diuretics may be required.

Allopurinol (licenced for the treatment of gout in humans) can be used off-label as a leishmaniostatic in dogs. The dose for dogs is 15 mg/kg, twice a day for 30 days.

In terms of clinical efficacy, which can be confirmed by biological criteria, the meglumine antimoniate-allopurinol combination has proven its worth compared to traditional treatment based on meglumine antimoniate alone, including by reducing the risk of therapeutic failure. Allopurinol can also be administered immediately after diagnosis is confirmed, even if the animal's health necessitates renal support therapy which would prevent the immediate administration of meglumine antimoniate.

This treatment is followed by the administration of allopurinol for life, at a dose of 15 mg/kg/day. If treatment is discontinued, most dogs suffer relapses in the months after drug therapy ceases (an average of one relapse every 6 months).

Continued administration of allopurinol seems to limit the recovery of parasite numbers, explaining the absence of relapses. However, allopurinol does not sterilise the parasites and *Leishmania* can still be identified in culture or by PCR of samples of bone marrow, peripheral blood or lymph node puncture in dogs which have regained a satisfactory clinical state.

Miltefosine can be used in dogs alone or in combination with allopurinol. It is administered orally at 2 mg/kg/day for 30 days. It is recommended for the treatment of dogs with side effects due to meglumine antimoniate. Other molecules are mentioned in the literature: marbofloxacin, domperidone, metronidazole with no established efficacy. Amphotericin B is reserved for the treatment of human visceral leishmaniosis.

Combined treatments

The need for a diuretic has already been mentioned, but clinical improvement can be made in some cases, especially involving polyarthritis or ocular disorders, by using corticotherapy at immunosuppressive doses. Where clinical signs are linked to immune complex involvement, administration 1 mg/kg/day of prednisolone for 15 to 20 days reduces antibody synthesis and allows lesions to clear. This corticotherapy may be considered when the blood urea is greater than 1.5 g/L.

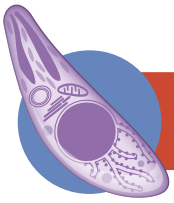
Prevention

Prophylaxis is limited and difficult because the vector is difficult to destroy. It is impossible to control the external environment chemically, so sandfly bites must be avoided. Simple measures, such as keeping dogs inside at dusk, are useful. Some molecules have a repellent effect on sandflies, as is the case with permethrin and deltamethrin. Their activity depends on the initial concentration, the method of application (collars or spot-on formulations), the individual animal and its way of life, and climatic conditions (wind, temperature, humidity).

Vaccines containing *L. infantum* excreted/secreted proteins (ESP) or recombinant proteins are commercialised in Europe and South America. They are intended for the active immunisation of *Leishmania*-negative dogs from 6 months of age, to reduce the risk of developing an active infection and clinical disease after contact with *L. infantum*. The recommended vaccination schedule is 3 injections as primary vaccinations and annual boosters.

Feline leishmaniosis

Rare cases of feline leishmaniosis have been described in Southern Europe. The clinical presentation is ambiguous so diagnosis is often established after a skin, lymph node or bone marrow biopsy. Treatments to date have often been disappointing.



Toxoplasmosis

General comments

Toxoplasmosis is an infectious disease caused by the multiplication and pathogenic activity of the protozoan species ***Toxoplasma gondii***, which infects cells in the mononuclear phagocyte system (MPS). However, the parasite's location and the pathogenic stage lead to the definition of two different entities, according to clinical and epidemiological criteria and public health criteria:

- Toxoplasmic coccidiosis, only affecting cats and other felids, which are the parasite's definitive hosts. Similar to other intestinal coccidioses, caused by the sexual multiplication and pathogenic activity of the parasite in the enterocytes, leading to the formation then excretion of unsporulated oocysts in the faeces.
- Toxoplasmosis *sensu stricto* due to the multiplication and pathogenic activity of the same parasite, this time in the form of tachyzoites and then bradyzoites, in exenteral locations (inside all types of cells, except red blood cells) in intermediate hosts, which are warm-blooded vertebrate mammals (including cats, dogs and humans) and birds.

This disease is important for various reasons:

- Because it is responsible for abortions in livestock, particularly sheep, and therefore for economic losses.
- Because it sometimes causes serious medical conditions in animals. These are quite rare in dogs and cats but common in some other mammals, especially marsupials (kangaroos, wallabies), otters, etc.
- Above all, because toxoplasmosis is zoonotic. In Europe, the serological prevalence in humans varies according to the country, from 10 % to more than 50 %. Human congenital toxoplasmosis is the most frequent congenital disease after Down's syndrome. Cerebral toxoplasmosis of immunosuppressed individuals affects approximately 40 % of people with AIDS.

Toxoplasmosis is a major zoonosis in which the veterinarian plays an important role to safeguard public health.

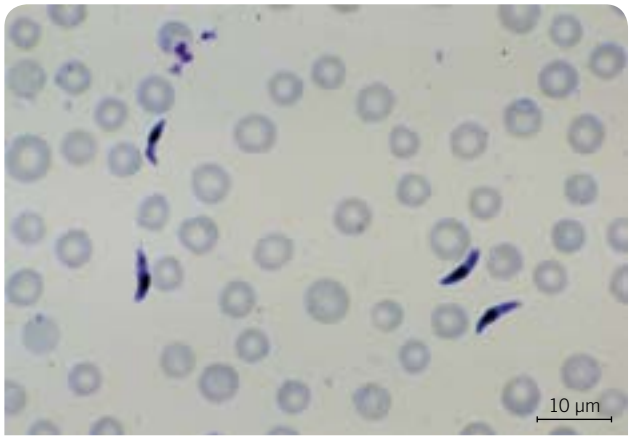


Figure 1. *Toxoplasma gondii* tachyzoites. MGG staining. Sporozoites collected in ascites of an infected mouse.

Taxonomy, morphology and biology

T. gondii is an Apicomplexa protozoan belonging to the order Coccidiorida, suborder Eimeriorina which contains coccidia of veterinary and medical importance.

The Isosporidae include coccidia whose sporulated oocysts contain two sporocysts with four sporozoites each (as opposed to four sporocysts with two sporozoites in the Eimeriidae) (Fig. 1). *T. gondii* belongs to the Toxoplasmatinae subfamily, along with the genera *Hammondia*, *Besnoitia* and *Neospora*. The other subfamilies are: Isosporinae, which includes the genus *Isospora*, and Sarcocystinae, with the genus *Sarcocystis*.

Toxoplasma is a protozoan which seems to be quite recent. It is estimated to have existed as a species for only 10,000 years, whereas *Neospora caninum* seems to be 12 million years old; *Hammondia*, 52 million and the genera *Sarcocystis* and *Isospora*, between 50 and 100 million years.

Morphology

Only the stages of asexual multiplication are seen on the intermediate host:

- Bradyzoites in tissue cysts (Fig. 2).
- Tachyzoites in pseudocysts (Fig. 3).

These two stages are not morphologically distinguishable; they differ in the speed of multiplication (bradyzoites having a slow form of multiplication, and tachyzoites a rapid form) and in the morphological modifications to the cell which harbours them.

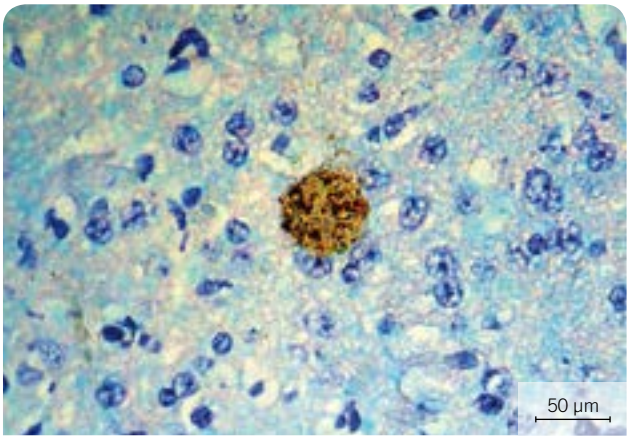


Figure 2. Cyst with bradyzoites. H&E staining. Brain histology.

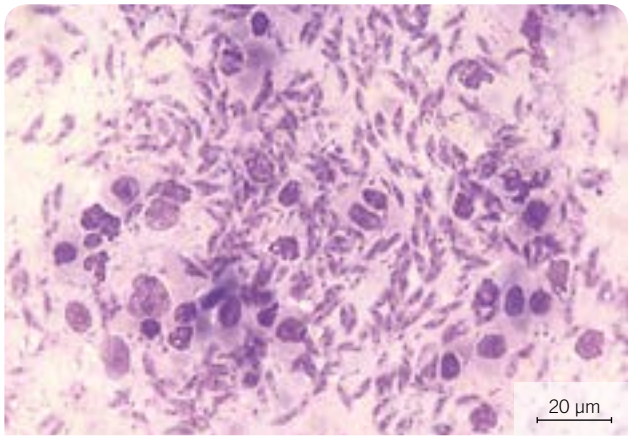


Figure 3. *Toxoplasma gondii* pseudocysts. Intense tissular multiplication. Acute toxoplasmosis in a kangaroo imported into the Forest Park in Noumea, New Caledonia. Tissue smear. MGG staining.

These stages are grouped together, along with sporozoites (seen in the sporulated oocyst), in the category of infectious germs. They consist of crescent-shaped elements (toxo = bow), measuring 5–6 µm × 2–3 µm, and presenting two different ends: one pointed, containing the apical complex, the other more rounded, considered to be the rear, with a nucleus which occupies a third of the cell.

The **tachyzoite** is the rapid form of parasite multiplication, responsible for the clinical expression of toxoplasmosis. It can be seen in a parasitophorous vacuole in the host cell. The cell is not deformed; it presents a distinct nucleus and constitutes the pseudocyst (15–30 µm in diameter).

The **bradyzoite** is the element which follows the first, quiescent form, and is found in a deformed host cell (the parasitophorous vacuole has disappeared), whose nucleus is flattened and pinned against the cell wall, and which constitutes the cyst (60–100 µm in diameter). This cyst is the element that will resist and persist in the organism with time.

Habitat

Unlike the sexually reproductive forms which can be clearly seen in enterocytes, bradyzoites and tachyzoites are not cell-specific. They can be found in monocytes, histiocytes, macrophages, but also in epithelial cells, fibroblasts, hepatocytes, neurons, etc. They never, however, infect red blood cells (in mammals).

Reproduction

The life cycle of *T. gondii* is very complex. This parasite is fundamentally dixenous, but it can also be strictly monoxenous in cats. Transmission between intermediate hosts is also possible and this is considered the predominant route of infection.

Infection occurs in three ways:

- By ingesting sporulated oocysts, in contaminated food or water.
- By ingesting the meat from an intermediate host which carries cysts, so by carnivorism: this is the most likely occurrence in cats and dogs, which consume infected prey (such as rodents) or undercooked/raw meat.
- *In utero*, when tachyzoites contaminate a non-immune pregnant female, the absence of immunity enables the passage of tachyzoites to the foetus, resulting in congenital toxoplasmosis.

Monoxenous cycle (only in cats)

This is similar to the cycle of the *Isospora* genus coccidia. It has two phases: exogenous and endogenous.

- Endogenous phase: schizogony, then gametogony, takes place in the enterocytes and can cause non-characteristic and benign diarrhoeic enteritis. It can also lead to the excretion in the faeces of simple oocysts, which are

non-infectious and morphologically indistinguishable from other coccidian genera. After ingesting sporulated oocysts, a cat will re-excrete some oocysts within 15–20 days and for approximately 2 weeks. Epidemiological surveys indicate that 1–2 % of cats under 1 year old excrete *Toxoplasma* oocysts.

- Exogenous phase: in the external environment, oocysts undergo sporogony for between 48 hours and 5 days and become infectious. They are very resistant and can infect other cats or intermediate hosts. They are, however, sensitive to heat and desiccation (20 min at 60 °C), as well as to putrefaction and anaerobic conditions. They are resistant to numerous chemical agents (oxidants which actually encourage their development) but are destroyed by formol and ammonia.

Dixenous cycle

Transmission occurs between a definitive host and an intermediate host, or between two intermediate hosts. The intermediate host, the cyst carrier, is a potential source of parasites for the definitive host (the cat) or for another intermediate host, of the same species or another. The dog is an epidemiological dead-end, as it is not consumed by another carnivore.

When a cat ingests *Toxoplasma* cysts, the enteritic phase is short, and does not require schizogony, unlike the ingestion of oocysts. In this case, oocyst excretion takes places 4 to 6 days after the ingestion of the cyst.

If the cat is the single definitive host, it is also the intermediate host, as it can present with toxoplasmic coccidiosis and toxoplasmosis at the same time.

If quiescent tissue cysts are "reactivated", the cat may present once more with an enteric cycle, resulting in renewed oocyst excretion. This recurrence is usually induced by another coccidiosis, another disease or immunosuppression.

Epidemiology

Intermediate hosts are infected in two ways, either by ingesting sporulated oocysts or by ingesting cysts from the tissues of other intermediate hosts: ingestion of raw meat, consumption of small mammals (mice, etc.).

Cats are usually infected at a young age, under a year old, including through a cat-mouse cycle.

The cat is a recurrent source of *Toxoplasma* oocysts and it spreads them in the external environment. Oocysts sporulate in 24–48 hours. Excretion by cats takes place during the primary *Toxoplasma* infection, but also during transient immunosuppression or infection by other species of coccidia (*Isospora rivolta* or *Isospora felis*).

The consumption of (raw or undercooked) meat of herbivorous mammals (especially mutton), omnivores or birds, through the intermediary of the cyst, is the main source of infection. Eating commercial diets or well-cooked meat can prevent such a contamination.

Human contamination occurs in a different way:

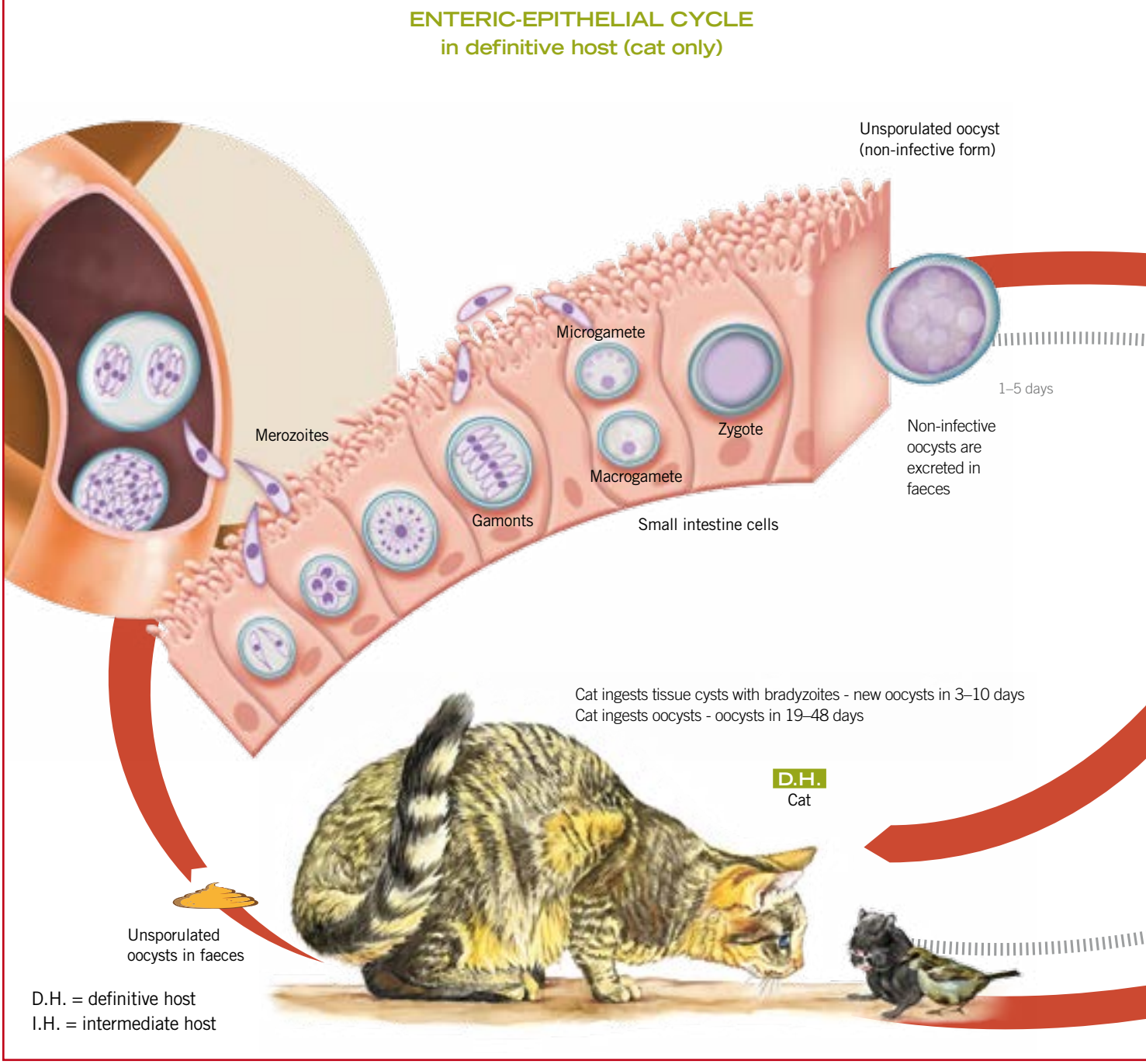
- Through the consumption of raw or insufficiently cooked meat containing *Toxoplasma* cysts. Pork is the most significant risk in the USA, and lamb in Europe. The role of beef is considered to be less important. Rabbit and poultry may be infectious.
- Ingestion of sporulated oocysts present in the environment: contamination of vegetation, possibly of cat litter, and only occasionally from the cat's coat, which is too dry to allow the oocysts to sporulate.
- *In utero* in congenital toxoplasmosis.

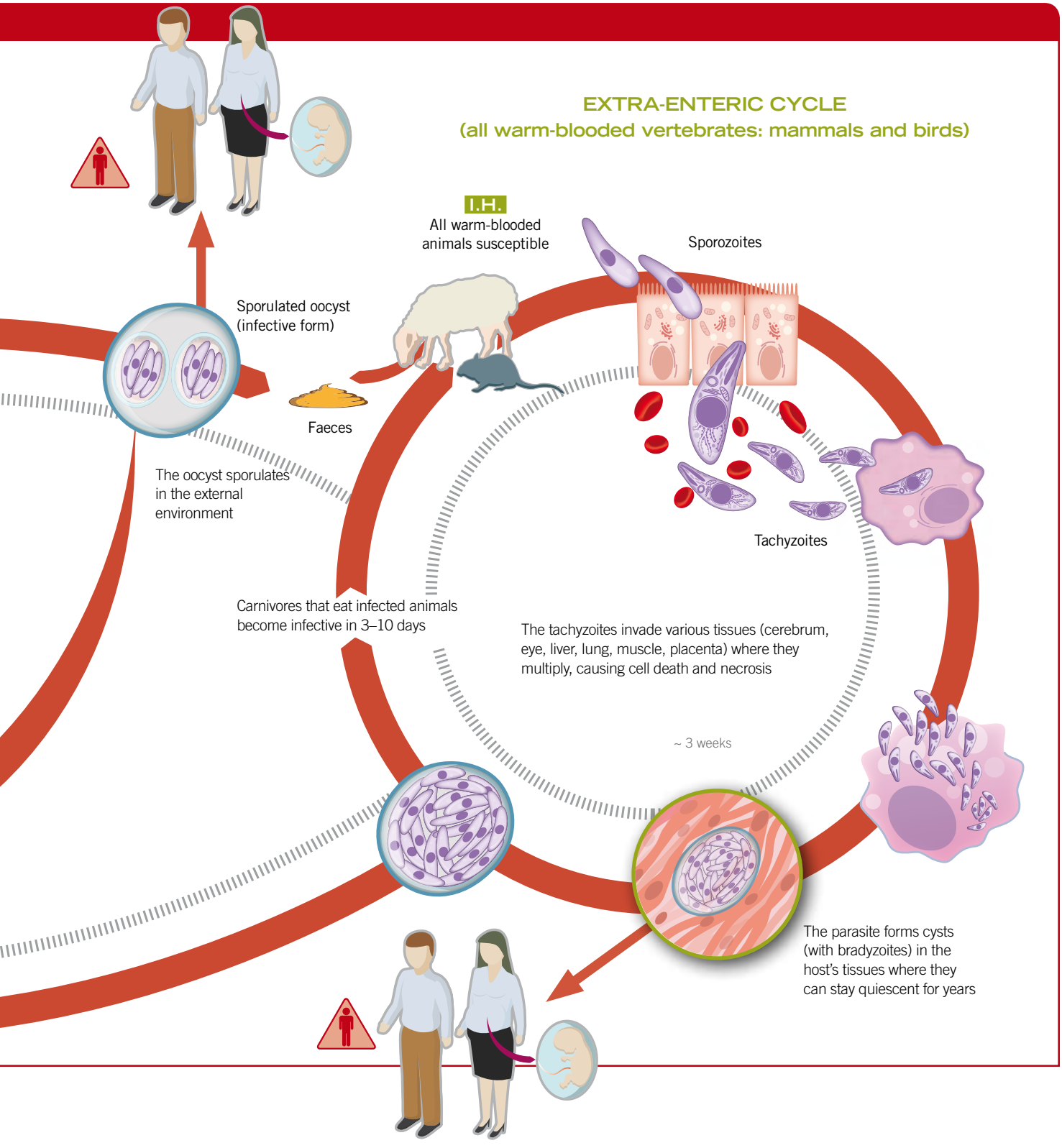
The prevalence of toxoplasmosis in humans is quite variable: 30 to 60 % of adults are immune in France, Germany and the Benelux countries, as opposed to under 30 % in Scandinavian countries and the British Isles and 20 to 50 % in Southern Europe. The prevalence is lower in Asia and the Americas (<15 %) and between 20 and 50 % in humid regions of Africa.

In France, the rate of seroconversion during pregnancy is approximately 0.5–1.5 per 1,000 births. More than 61 % of children born after *in utero* infection are born healthy.

Prenatal infection results in miscarriages and embryo resorption. During the first trimester of pregnancy, the infection may cause toxoplasmic meningoencephalitis (macrocephaly, hydocephaly, and ocular and nervous problems). For the remaining two trimesters, infection causes fatal visceral forms or delayed formswhich are only visible after birth (chorioretinitis, developmental delay, etc.). If infected at the end of gestation, the baby is usually born with no pathology.

Life cycle of *Toxoplasma*




EXTRA-ENTERIC CYCLE
(all warm-blooded vertebrates: mammals and birds)
I.H.
All warm-blooded animals susceptible
Sporozoites
Sporulated oocyst (infective form)
Faeces
The oocyst sporulates in the external environment
Tachyzoites
Carnivores that eat infected animals become infective in 3–10 days
The tachyzoites invade various tissues (cerebrum, eye, liver, lung, muscle, placenta) where they multiply, causing cell death and necrosis
~ 3 weeks
The parasite forms cysts (with bradyzoites) in the host's tissues where they can stay quiescent for years

Clinical signs

Infection in cats nearly always manifests itself through asymptomatic toxoplasmic coccidiosis.

In the majority of cases in all types of intermediate host-*Toxoplasma* infection does not manifest itself through any clinical signs, or expresses itself through a mild form of adenomegaly.

If the infection occurs in a pregnant dog or cat which is not immune, the tachyzoites can cross the placenta and contaminate the foetus, leading to foetal death in early pregnancy, abortion, the birth of deformed foetuses or death of the young from septicaemia before the age of 2 months.

In non-immune adult cats and dogs, the clinical presentation is very varied and the following can be seen:

- Respiratory forms: bronchopneumonia associated with irregular fever, cough and dyspnoea; lesions consist of scattered miliary necrotic foci in the form of small white spots with irregular contours.
- Nervous forms similar to distemper (it should be noted that this can be associated with toxoplasmosis; the virus causing the disease encourages parasite multiplication): central (encephalitis) and peripheral (polyradiculoneuritis) disorders causing seizure, epilepsy, paresis, paralysis and myoclonus. However, it is very difficult to attribute to *Toxoplasma* the only (or predominant) role in the apparition and evolution of these clinical cases, since the presentation is very similar to that of distemper, and because some cases attributed to *Toxoplasma* are in fact caused by *N. caninum*.
- Some atypical forms (sometimes associated with pulmonary and nervous forms): digestive (gastric, hepatic and/or fatal acute necrotising pancreatitis); ocular, which is rare in dogs (unlike in cats): chorioretinitis with pigmentary modification.

Diagnosis

- Diagnosis is never clinical, and must therefore be based on laboratory confirmation.
- Various methods can be used to confirm a suspicion of toxoplasmosis in mammals: direct methods to reveal the parasite or indirect methods based on serology.

Direct methods

- Since the parasite is likely to multiply in the various tissues and systems of the body, it is possible, in cases of clinically-expressed progressive toxoplasmosis, to find tachyzoites in lymph aspirate, bone marrow, bronchoalveolar lavage fluid, cerebrospinal fluid, muscular tissue, etc. after centrifugation and staining.
- The sample can also be inoculated by intraperitoneal injection into a sensitive animal (mouse) in which examination of ascitic fluid 3–5 days later can reveal many tachyzoites.

Serological methods

These aim to detect antibodies (total Ig, IgM or IgG) with the help of figured or soluble antigens, or to detect circulating antigens.

Many methods are available, ELISA and haemagglutination, or latex particle agglutination. The latter have the advantage of functioning with serum from any animal species, and not requiring specific conjugates, as ELISA reactions do.

Despite specificity and sensitivity which are often satisfactory, these serological reactions must be viewed and interpreted in the clinical context.

These techniques only allow antibodies to be detected. The presence of antibodies signifies that the animal has been in contact with the parasite and has established a humoral immunological reaction against it. This is not synonymous with the disease toxoplasmosis, or rather, *Toxoplasma* infection.

Kinetics and a double serological reaction (first on Ig totals then just IgG) allow progressive toxoplasmosis to be detected:

- If the serological results from samples taken at intervals of at least 15 days reveal a significant difference in the titre, progressive toxoplasmosis can be suspected.
- If the suspect animals' serum titres, the first untreated, then treated with 2-mercaptoethanol (which "destroys" IgM), are significantly different, this difference can be attributed to a high IgM level and therefore to progressive and/or recent toxoplasmosis.

No immunoglobulin isotypes are detectable before 15 days after contamination, so the following approximate kinetics can be seen:

- IgM from W2 to W6 in a bell curve: no detection before day 15, smaller quantities at the detection limit after day 90; their detection in significant numbers indicates acute and/or recent infection.
- IgG increasing progressively and slowly from day 15 to reach a slightly later peak from that of IgM, followed by a regular and very weak decrease, such that these Igs are detectable for several years and indicate an old infection.

PCR

It is possible to detect specific fragments of *Toxoplasma* DNA from different samples. This direct methodology is sensitive and may be more practical than serology.

Control measures

Treatment

Specific treatment for toxoplasmosis uses clindamycin, a molecule likely to stop tachyzoites multiplication and to diffuse throughout the whole organism after it is absorbed in the intestine. Different clinical forms (respiratory, nervous, etc.) require complementary symptomatic treatment.

Clindamycin: 10– 40 mg/kg/day, in 2–4 doses, orally for 4–6 weeks.

Prevention

Various prophylactic measures directly address the sources of contamination, as defined by the life cycle.

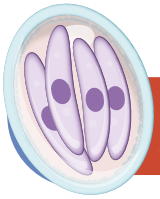
The cat, a recurrent source of *Toxoplasma* oocysts spread in the external environment (consumption of food or water containing sporulated oocysts): it is practically impossible to reduce significantly this excretion for several reasons:

- Toxoplasmic coccidiosis in cats is most often asymptomatic, very rarely associated with diarrhoeic enteritis.
- The excretion of oocysts is unpredictable. It occurs later than the potential symptomatology. It is massive and possibly recurrent: at the end of the patent period, excretion of oocysts can restart, in particular when the immune system is supressed or when there is an associated infectious or parasitic disease (for example, another, non-toxoplasmic coccidiosis).
- Anti-coccidia (sulfamides) are active only against the pathogenic forms before oocysts appear (schizonts and gamonts) and not on oocysts themselves. These medicines also cannot be administered continuously.

Measures to prevent human contamination mainly target food (consuming correctly washed/boiled vegetables; meat cooked to the centre) and the usual principles of hygiene (wearing gloves to clean out cat litter). The cat itself is a source but oocysts do not sporulate on the cat's coat (insufficient moisture). It is not necessary to get rid of cats where there is a non-immune pregnant woman, since cats are not the habitual source of contamination. As a precaution, the cat can be serologically tested and fed a commercial diet.

Research conducted in cats does not provide a definitive answer:

- When coproscopy is positive: the cat contaminates its environment and can re-excrete because there is no sterilising immunity, and recurrence is possible.
- When coproscopy is negative: serology is recommended:
  - If it is negative, the cat may be infected, particularly if it hunts or goes outdoors. Only by confinement and a commercial diet can infection be avoided completely.
  - If it is seropositive, the animal has been in contact with the parasite, it is partly immune but may potentially re-excrete if subsequently immunosuppressed, although this is rare in adult cats. Nonetheless, the risk is present.



Neosporosis

General comments

Canine neosporosis is an infectious disease caused by the development, multiplication and pathogenic activity of an Apicomplexa protozoan similar to *Hammondia* and *Toxoplasma*: ***Neospora caninum***.

The original description of neosporosis goes back to 1984, when Bjerkas et al., Norwegian authors, cited a parasite similar to *Toxoplasma* and responsible for paresis in three litters of Boxer puppies. The serological survey did not reveal any response corresponding to *Toxoplasma*. Independently, in 1988, Dubey et al. described a protozoan responsible for a series of clinical cases observed in dogs and identical to those described as being caused by toxoplasmosis, due to a new agent which they gave the binomial *Neospora caninum*. It was later demonstrated that the parasite shown in Norway was *N. caninum*, and that some preserved samples which had been taken from corpses of dogs thought to have died of toxoplasmosis were in fact infected by *Neospora*.

Taxonomy

N. caninum is an Apicomplexa protozoan (i.e., having at one pole of a group of organelles used to penetrate the host cell). It belongs to the subclass Coccidiasina, order Eimeriorida, family Isosporidae, subfamily Toxoplasmatinae. They are dixenous coccidia characterised by the excretion of oocysts containing two sporocysts with four sporozoites.

In order to understand this parasite, it is important to differentiate between the two life phases of *Neospora*:

- The parasite undergoes sexual reproduction in the digestive epithelium of canids, like classic coccidian, so the dog is the definitive host. We can therefore talk about coccidiosis due to *Neospora* as we talk about coccidiosis due to *Toxoplasma* in the cat. This digestive infection is, according to current understanding, non-symptomatic.
- The parasite is capable of asexual multiplication in various cells, especially neurons, in many intermediate hosts. Infection in intermediate hosts is referred to as neosporosis. If the clinical signs are mostly neurological, a disseminated form of the disease is involved, affecting the central and peripheral nervous systems, and also muscular tissue, the digestive system, skin, etc. The dog may behave as an intermediate host.

N. caninum therefore seems to be the equivalent to *T. gondii*, but with dogs and other canids as the definitive host. Cats are only intermediate hosts to *N. caninum*. This protozoan does not infect humans, unlike *T. gondii*.

Recent phylogenetic studies indicate that *N. caninum* is a relatively ancient protozoan, estimated to be 12 million years old. It seems to have separated from the genus *Hammondia* (thought to have originated more than 50 million years ago). *T. gondii* is much more recent, estimated at just 10,000 years old, and is probably a genetic derivative of *Neospora*.

Species affected

N. caninum are reported in dogs, cattle (where it is considered to be responsible for a large number of abortions), sheep, goats, cervids and horses.

Subcutaneous, intramuscular, intraperitoneal and intravenous experimental infections have been carried out on rodents (rats, mice, gerbils), rabbits, cats and rhesus macaque monkeys. No spontaneous cases have been reported in humans, including in immunosuppressed individuals. Epidemiological surveys carried out by various teams indicate an absence of serological trace in humans.

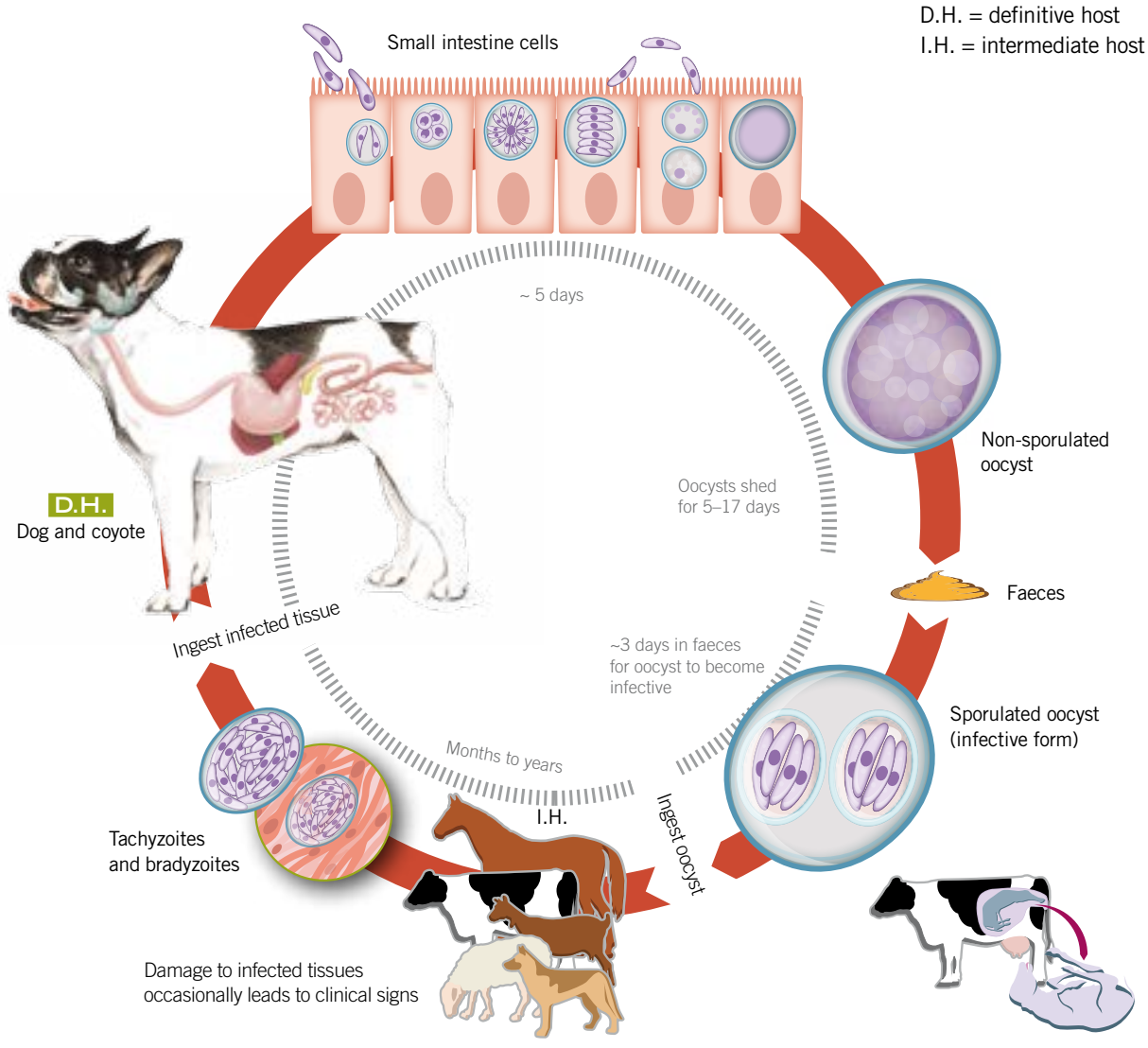
Biology

Morphology and habitat

Three elements are likely to be seen in dogs:

- The unsporulated oocyst: found in the faeces after experimental infection or a natural cycle. This oocyst is the result of sexual reproduction in the intestinal mucosa, the process responsible for the coccidiosis caused by *Neospora*. This oocyst measures approximately 10 µm in diameter, i.e., approximately half of an *Isospora ohioensis* oocyst or a third of an *Isospora canis* oocyst. It is morphologically identical to *Toxoplasma* and *Hammondia* oocysts.
- Tachyzoites: elements that replicate rapidly, are oval or globular (measuring 3–7 × 1–5 µm), and localised in the tissue and cells. Tachyzoites can be found in many types of cells: neurons, macrophages, fibroblasts, vascular endothelial cells, monocytes in many muscle groups, renal tubular epithelial cells, hepatocytes, spleen cells and lymph nodes. They are present in the cell cytoplasm, usually in a parasitophorous vacuole (up to 100 per cell).

Life cycle of *Neospora caninum*



- Bradyzoites: stages characterised by slow replication, measuring 6–8 × 1–2 µm, and present in cysts from infected cells. It is possible to differentiate between *Neospora* cysts and *Toxoplasma* cysts, since the former have a thick wall: 3–4 µm versus 1 µm in the latter. It is also possible to observe differences in the organelles which make up the apical complex by electron microscopy. Bradyzoites are found only in the central nervous system (brain, spinal cord), peripheral nervous system (nerves) and the eye, inside thick-walled (3–4 µm thick) cysts whose diameter can reach 100 µm (Fig. 1).

Life cycle

The life cycle resembles the cycle of dixenous coccidia, circulating between the definitive carnivorous host and the intermediate hosts consumed by the carnivorous hosts. In this cycle, the dog, like the cat for toxoplasmosis, seems to suffer simultaneously from "neosporic coccidiosis" (as the definitive host) and neosporosis (as an intermediate host). The asexual cycle (multiplication of the parasite in the tachyzoite form) precedes the sexual cycle (gametogony leading to the formation of oocysts in the intestinal lumen).

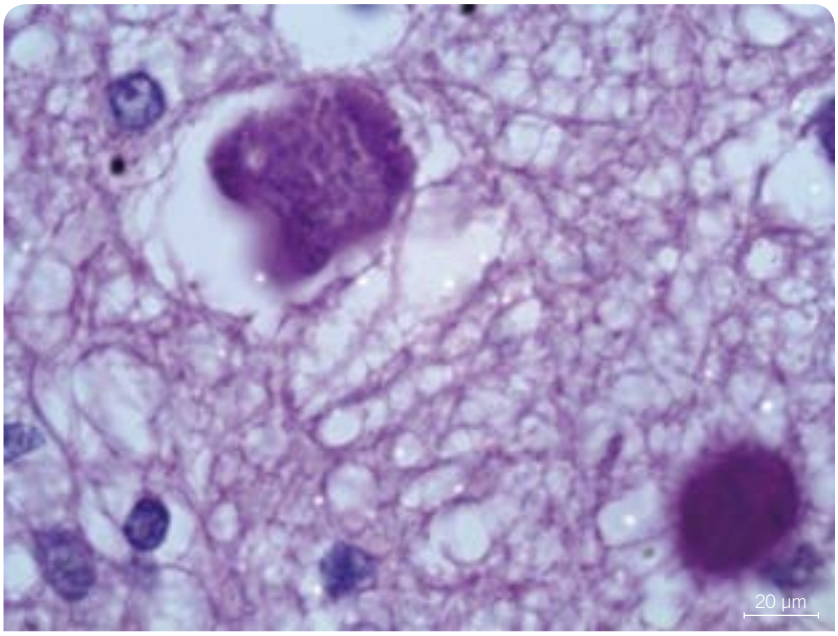


Figure 1. *Neospora* cyst containing bradyzoites in the brain tissue of a puppy with clinical signs of neosporosis. Courtesy of *Laboratoire Territorial de Diagnostic Vétérinaire de Nouvelle-Calédonie*.

The role of dogs as definitive hosts has been demonstrated experimentally by examining the faecal excretion of oocysts after ingestion of tissue from infected mice.

Epidemiology

Source of parasites

- The **definitive host** contaminating the environment of the intermediate hosts (cattle or other mammals), notably grasslands, with infective oocysts. Dogs are definitive hosts, but shed few oocysts expelled in the experimental infections carried out. Other carnivores are certainly involved: foxes (and perhaps mustelids).

  Epidemiological surveys do not always confirm the dog as having a major role in cases of neosporosis in cattle. Some authors consider that they do not have a greater presence in cases where seroprevalence and abortions due to *Neospora* are frequent or pose problems.
- **Intermediate hosts**, which are consumed by carnivores, therefore continuing the dixenous cycle.
- **Pregnant females**, whose bradyzoite cysts are likely to be activated during gestation, releasing tachyzoites, which can vertically infect the foetus.

Mechanisms of infection

Neosporosis is transmitted horizontally, by ingestion of sporulated oocysts, and vertically, from the mother to the foetus, in the majority of species, especially cattle. This congenital transmission can occur during any gestation and not only at primary infection, as occurs in toxoplasmosis. This mode of transmission seems to be the main explanation for the survival of *Neospora* in cattle herds for several generations.

Predisposing factors

Many animals are healthy carriers, as with *Toxoplasma*, as *Neospora* can be transmitted without causing neosporosis. It seems that the immune status of the host plays an important role in explaining the presence or absence of 1) vertical transmission, 2) abortion, and 3) post-natal congenital neosporosis.

Geographical distribution and prevalence

Distribution is thought to be worldwide. The serological and/or parasitological prevalence in cattle is significant in the USA, Canada, New Zealand, and Australia, but also in Europe (France, Germany, UK), where this parasite is responsible for up to 20 % of abortions (the second most common cause of abortion in cattle).

During the last decade, tests have been carried out on dogs all over the world which have demonstrated that the parasite circulates through the entire canine population. Seroprevalence rates of between 5 % and 67 % have been observed, with the highest rates observed in farm animals. Seroprevalence is usually 20 % in clinically healthy dogs. Purebred dogs appear to be more prevalent in case reports of clinical neosporosis, with Boxers, German Shorthaired Pointers, Labradors, Golden Retrievers, Basset Hounds, and Greyhounds being the most commonly affected breeds.

Neosporosis predominantly affects young dogs, a few weeks old (the majority are less than 3 months old). Cases resulting from congenital transmission are the most severe.

This transmission by the mother is possible during several successive gestations.

Not all puppies in a litter are affected and present with the disease (some are clinically unaffected), and diseased puppies will be affected to a variable extent (moderate to fatal forms).

Prognosis for puppies will be worse if the mother was infected in early gestation. Experimental infection of a dog at day 35 of pregnancy resulted in a stillbirth (but the apparently normal development of the other puppies. However, administering corticoids in immunosuppressive doses to these puppies and their mother can lead to generalised fatal neosporosis.

The forms observed in adult dogs are clinically different, less characteristic, often disseminated. It is impossible to define the origin of the disease in adults: the classic, new infection of a previously healthy individual, or reactivation from quiescent cysts as a result of immunosuppression.

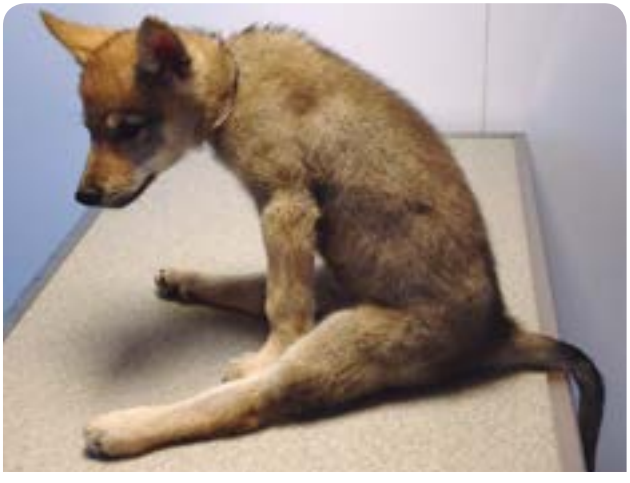


Figure 2. The typical "seal" position in a young dog with neosporosis. Courtesy of Magali Charve.

Clinical signs

Several clinical forms have been described, affecting very young individuals, adults or even old dogs, mainly characterised by nervous manifestations.

Classic form

This is the most common, seen in puppies which are a few weeks old (less than 4 months in most cases), following congenital infection.

Within the litter, some individuals present with progressive paresis of posterior limbs (far less often, the forelimbs), which are often hyperextended and rigid (forced flexion is impossible), making the animal adopt the so-called "seal" position, where it moves solely or primarily on its front legs (Fig. 2). This paresis is caused by damage to motor neurons and muscles, leading to contraction (combination of polyradiculoneuropathy and myositis). This is the most common presentation (more than 80 % of cases observed in dogs under a year old). This paraparesis is combined with a reduction in spontaneous tail movements, reduced perineal sensitivity and the development of bowel and bladder incontinence.

The disease progresses with increasing paralysis of the thoracic limbs, apparent cervical weakness or, conversely, cervical rigidity, and signs of encephalitis: dysphagia, abnormal food prehension, musculoskeletal disorders, circular movements, nystagmus and seizures, resulting in death in the majority of cases. The clinical presentation may develop over several months.

Some dogs (approximately 10 %) present with signs of septicaemia (pneumonia, encephalitis, myocarditis, adenomegaly). The parasite is found in many tissues and organs: lymphatic system, liver, spleen, pancreas, and central and peripheral nervous system, causing a high rate of stillbirth.

More or less atypical presentations, not associated with nervous or general signs, have been described:

- Ocular presentation: nystagmus, aniscoria, absent pupillary reflexes.
- Myocarditis causing sudden death, following other indicative clinical signs (diagnosis confirmed by autopsy, demonstrating severe disorders of the myocardium and numerous other organs).

Cutaneous form

This seems to be found solely in adult, even old dogs, in which it is not possible to define the precise origin of infection with the current state of understanding. Clinically, this dermatosis is expressed in two forms:

- Either by the presence of a single or a few ulcerated nodule(s), several centimetres in diameter and without any associated neurological signs, blood disorder or general deterioration in health, in various parts of the body (limbs, torso, etc.), painless and non-adherent.
- Or in the form of extensive nodular dermatosis, moderately pruritic, affecting large parts of the body, associated with satellite adenomegaly and to a drastically altered general state of the animal: anorexia, tiredness, dyspnoea, changes in the blood composition (leucocytosis, thrombocytopaenia), and cardiac arrest.
- These forms correspond to disseminated neosporosis.

Other clinical signs seen in adult dogs

- Various neurological and muscular signs: difficulties with food prehension and swallowing, paralysis of the jaw, flaccidity of any muscle.
- Signs of cardiac insufficiency due to myocarditis, which may cause sudden death.

Lesions

- Multifocal failure of the central nervous system: necrosis, discoloration of white and grey matter, gliosis, small foci (1–3 mm diameter) affecting many segments of the spinal cord (lumbar, thoracic, etc.) consisting of an abundant lymphoplasmacytic infiltrate, and characteristic cysts.
- Diffuse myositis, sometimes affecting many muscles (skeletal and smooth muscles, myocardium), cellular necrosis and lymphoplasmacytic infiltrate.
- Polyvisceral disease: affecting the liver, spleen, lungs, and lymph nodes.
- Diffuse pyogranulomatous necrotising dermatitis, sometimes severe and deep, surrounded by a large conjunctive reaction and associated with ulcers and vasculitis: accumulation of numerous macrophages and polynuclear neutrophils, lymphocytes and plasmocytes in the dermis and the underlying adipose tissue, destruction of connective fibres and related tissues. Macrophages, polynuclear neutrophils and epithelial cells may contain many tachyzoites.

Diagnosis

Neosporosis can only be suspected when the dog is under observation for some of the following:

- Neurological disorder characterised by paresis, possibly paralysis of hind limbs, which are hyperextended.
- An uncharacteristic stillbirth within a litter.
- Presentation of septicaemia or disseminated disease with polyvisceral disorder (hepatic, muscular including cardiac, nervous, etc.).
- Inconstant and indicative biological anomalies: leukocytosis, anaemia, and increased muscle-derived and hepatic enzymes.

Diagnosis must be confirmed by the identification of the parasite in bronchoalveolar lavage fluid, cerebrospinal fluid or in any biopsy sample: either there will be barely detectable tachyzoitic forms, or cysts with bradyzoites with a thick wall (4 µm) indicating *Neospora*.

The suspicion of *Neospora*, based on histological examination, can be confirmed by specific immunohistochemical testing using monoclonal anti-*Neospora* antibodies or polyclonal serum obtained from a laboratory animal, or by serology based on two methods:

- Indirect immunofluorescence.
- Indirect agglutination.

Coproscopy will be negative when the dog has been infected as an intermediate host.

Prognosis

Prognosis is always bad, either because neosporosis may progress in a disseminated manner, or because functional recovery of paralysed limbs is nearly always impossible, causing the animal to be immobile or to have extreme difficulty moving; the definitive handicap can be incompatible with normal life.

Control measures

There is no approved or curative treatment for canine neosporosis. Clinical disease is best arrested when treatment is initiated before contracture or paralysis occurs. Dogs typically die without treatment, and some dogs die even with treatment.

The following treatment regimens are used to control clinical neosporosis:

- Clindamycin (7.5–25 mg/kg PO or IM every 12 hours for 4 weeks), even if there is no remission of clinical signs.
- Trimethoprim-sulfadiazine (15–20 mg/kg PO every 12 hours for 4 weeks) in combination with pyrimethamine (1 mg/kg PO every 24 hours for 4 weeks).

The trimethoprim-sulfadiazine combinations seem to be the least active.

If clinical improvement is seen to be slow, treatment should be extended beyond the recommended 4 weeks until 2 weeks after clinical signs have plateaued.

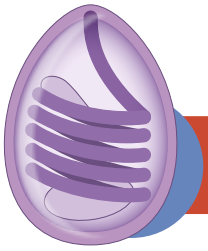
All littermates of affected puppies should be treated, regardless of clinical signs.

Passive range of motion exercises and massage may be beneficial in some cases.

It has been demonstrated that transmission in dogs, as in cattle, is vertical, therefore it is advisable that bitches that have given birth to infected litters should be removed from reproduction.

In the case of a bitch which has given birth to infected puppies, it is possible to administer preventative treatment during subsequent gestations: clindamycin at 7 mg/kg/day, twice a day, from the 15th to the 25th day of gestation (optimal period for transmission of the parasite across the placenta) to avoid foetal contamination. The treatment of a pregnant bitch based on trimethoprim-sulfonamide is ineffective.

Video 5
A young dog with congenital neosporosis. Courtesy of Magali Charve.



Encephalitozoonosis

General comments

Encephalitozoonosis is an infectious disease caused by the presence and multiplication of a Microsporidia, ***Encephalitozoon cuniculi***, in various tissues and cells, such as vascular endothelial cells or uriniferous epithelial cells.

Taxonomy

Encephalitozoon cuniculi belongs to the phylum Microspora, which includes obligate intracellular parasites. Microsporidia have historically been considered to be "primitive" protozoa, however, recent molecular phylogenetic analysis has revealed that these organisms are a group of highly adapted unicellular fungi not related to protozoa. These organisms are best known for their very simple cellular and genomic features, an adaptation to their obligate intracellular parasitism. Genome sequence data from these pathogens has revealed how obligate intracellular parasitism can result in radical changes in the composition and structure of nuclear genomes, and how these changes can affect cellular and evolutionary mechanisms that are otherwise well-conserved among eukaryotes.

Despite being recognised as a fungal disease, encephalitozoonosis will be addressed in this book of parasitology because it is still often described as a protozoan infection.

Hosts

This disease primarily affects lagomorphs, in which the parasite was discovered in 1924. The infection occurs, and is chronic, in many rabbit populations, such as the European rabbit population. Dogs are receptive to infection and some surveys have indicated high prevalence rates: 18 % in South Africa, 13 % in stray dogs in England. Foxes are also affected, as are cats. Zoonotic infections have also been reported in immunosuppressed humans.

Biology

Encephalitozoon cuniculi is characterised by its infective form, which is a complex resistant spore. Each spore is ovoid, measuring 1.5 × 2.5 µm and contains a sporoplasm with a nucleus, posterior vacuole and tubular filament.

During cell infection, the spore extrudes its polar tubular filament near a host cell and injects the infective sporoplasm into the cell via the filament. Inside the cell, the sporoplasm is located in a parasitophorous vacuole, where it undergoes extensive nuclear multiplication. After several successive schizogonies, sporonts are formed; they divide once to make two sporoblasts, which quickly turn into spores. Once the spores increase in number and completely fill the cytoplasm of the host's cell, the cell membrane is disrupted and releases the spores to the surroundings. These free mature spores can infect new cells, continuing the cycle. Spore formation takes just 48 hours. Many cells may be infected: uriniferous tubes, intra-tissular macrophages, hepatocytes, and vascular endothelia, including those located in the brain.

Spores are excreted in the urine of infected animals.

Epidemiology

Spores are present in the environment, where they can survive for a long time. They are found on substrates contaminated by the urine of infected animals, or by the lysis of infected corpses.

Infection takes place through licking or sniffing of contaminated substances. Ingestion of infected prey by a carnivore is also a means of infection, and transplacental and sexual transmission is suspected. The majority of rabbits seem to be infected with *E. cuniculi* by their mothers. Human-to-human transmission is also possible, via transplantation of solid organs from an infected donor.

Clinical signs

In the dog, infection is often asymptomatic at first but can progress in a few weeks, especially in young dogs, to reveal clinical signs:

- Nervous disorders: motor incoordination, ataxia, blindness and, sometimes, behavioural problems (aggression, biting, barking).
- Renal disorders: severe nephritis.
- Hepatitis with significantly raised enzyme (transaminase) levels.

If clinical signs appear, death often follows within weeks or months.

In the cat, infection is mostly asymptomatic. Cases of chronic infection, predominantly nervous disorders, have been reported. Cats presented with core signs of depression and progressive paralysis leading to death. Ocular disorders (keratitis) have also been reported.

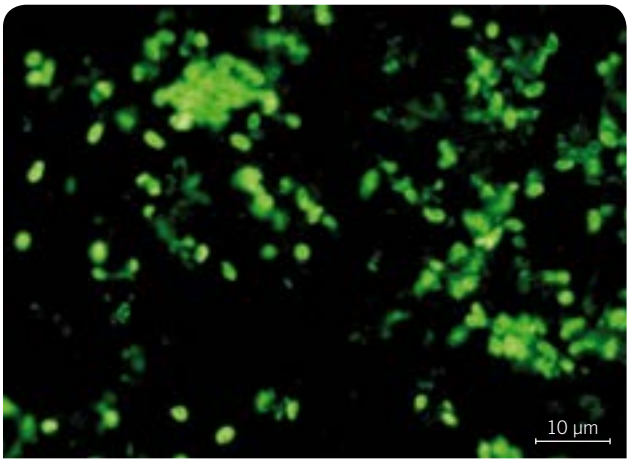


Figure 1. Section of liver from a patient with AIDS and infected by *Encephalitozoon hellem*. *Encephalitozoon* spores stained by immunofluorescence. Anti-*E. cuniculi* polyclonal antibody serum. Fixed by conjugated FITC goat anti-rabbit IgG (Antonella Tosoni et al. 2002. Modern Pathology. 15(5): 577–583).

In rabbits, carriers are often asymptomatic and the few animals presenting with clinical signs have a nervous disorder dominated by fairly characteristic torticollis and signs of ataxia.

Lesions

E. cuniculi is the cause of encephalitis and nephritis in all species, and in immunosuppressed patients.

Lesions indicate septicaemia with multivisceral necrotic foci. Interstitial nephritis can be seen, as well as vascularity, notably encephalic.

The spores, whether isolated or within pseudocysts which resemble toxoplasmic cysts, can be found in various tissues, including the brain.

Diagnosis

Clinical diagnosis is impossible, since there are no characteristic clinical signs in carnivores (not the case in rabbits with torticollis).

Diagnosis may be based on serology (ELISA or IIF), on histology from autopsy, or by identification of spores in urinary sediment (Fig. 1).

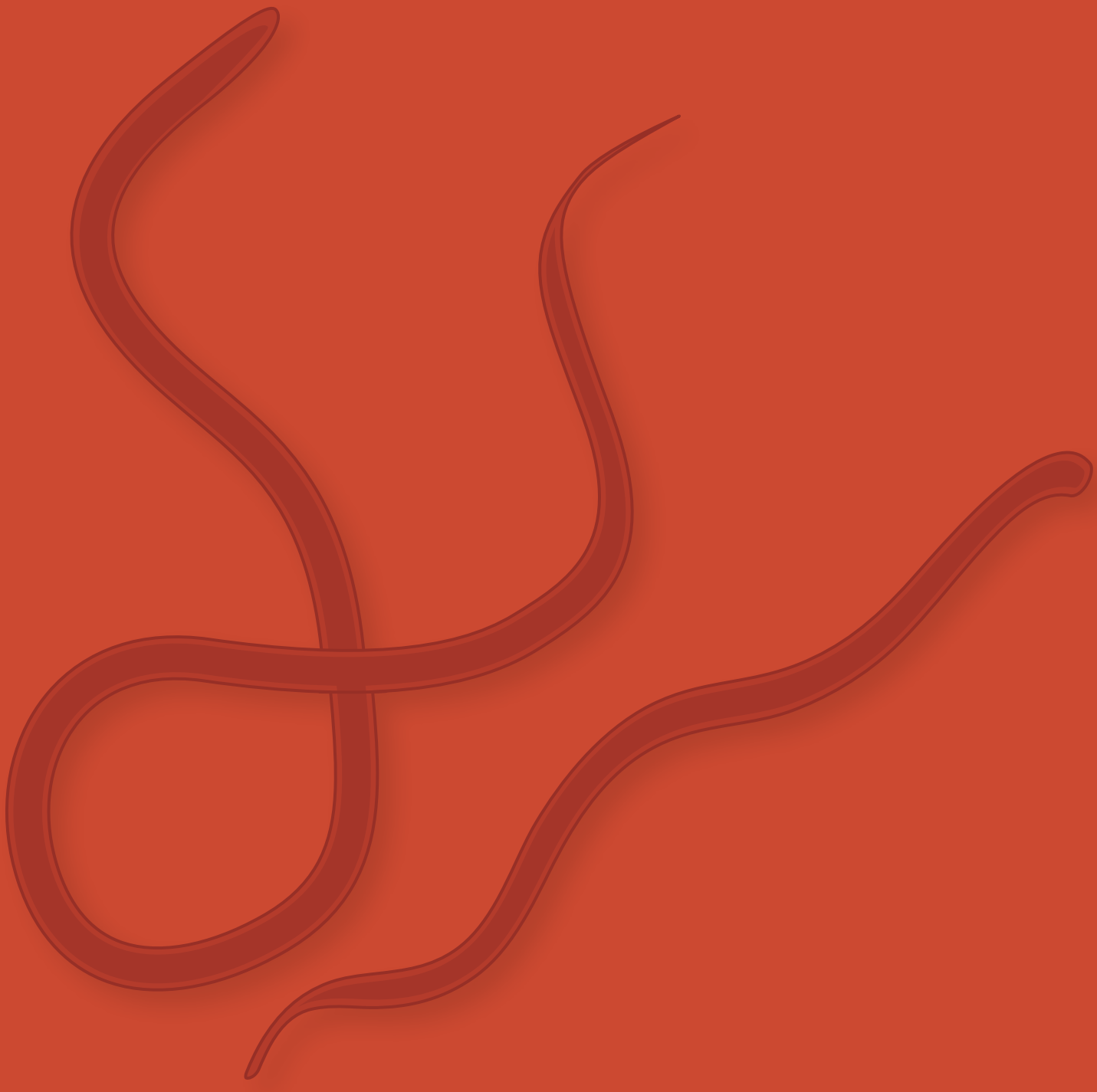
It is also possible to detect parasitic DNA by PCR from biopsies or in the urine.

Control measures

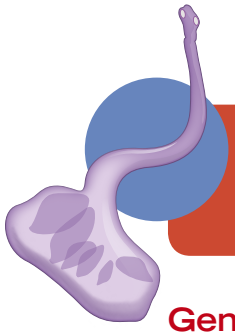
There is no specific treatment. Trials using fenbendazole have produced very good results in rabbits, with a daily administration of 20 mg/kg for 21 days. Albendazole is used with good result in humans, although it is relatively toxic for dogs, for whom fenbendazole is preferred.

For ocular disorders, the antibiotic fumagillin has been applied topically each day until keratitis regressed.

There are no particular preventative measures, since spores are resistant in the external environment. Rabbits and rabbit farming are factors contributing to the presence and concentration of spores.



Miscellaneous parasitoses



Subcutaneous cysticercosis

General comments

Metacestodoses are caused by the larval stages of cestodes from the family Taeniidae. Dogs and cats usually act as definitive hosts and harbour adult cestodes in their gastrointestinal tracts. They may also become intermediate hosts and develop metacestodosis, but this is very rare. It mainly occurs with ***Taenia crassiceps*** and, more exceptionally, with other Taeniidae such as *Taenia* (*Multiceps*) *serialis*, *Taenia* (*Multiceps*) *multiceps* or *Echinococcus multilocularis*.

Cases of subcutaneous cysticersosis due to *T. crassiceps* have been reported in dogs, as well as in humans.

Morphology and biology

Adult *T. crassiceps* are found in the intestines of canids, especially foxes. Cysticercal larvae develop in the subcutaneous tissues, and sometimes in the peritoneum, in wild rodents, notably red-backed voles. This cestode is also used as a laboratory model for cysticercosis because the strain can be maintained through successive rodent infestations (Fig. 1). *T. crassiceps* has been used in an extensive series of experiments as a source of antigens and as a model for immunological studies on cysticercosis immunity. It may also be used to evaluate cestodicidal molecules.

T. crassiceps has a dixenous life cycle, as do all cestodes. The definitive host is a canid which hosts an adult tapeworm, approximately 20 cm long, in its small intestine, and expels egg segments in its faeces.

These eggs are ingested by wild rodents, mainly voles. Embryos (oncospheres) are released, cross the wall of the intermediate host's intestine and develop into cysticercal larvae subcutaneously or intraperitoneally. These larvae are commonly known as cysticerci. These cysticerci measure 0.5–2 × 3–5 mm and can multiply asexually by internal and external budding (Figs. 2–4). This multiplication may be seen under natural conditions, but is more marked in some laboratory rodents and in immunocompromised hosts.

Epidemiology

T. crassiceps is one of the most common parasites in foxes, with a prevalence of 5–30 %, and sometimes even more.

The epidemiological cycle is sylvatic, taking place between the definitive host (wild canid) and intermediate host (vole).

Dogs are infested by hunting wild rodents (carrying *Taenia* spp. larvae) or by ingesting eggs directly. In the latter case, dogs act as intermediate hosts and may develop subcutaneous cysticercosis.

Dogs living in rural environments seem to be predisposed and most are more than 7 years old. Subcutaneous cysticercosis in dogs and humans appears to be linked to predisposing factors: dysendocrinism, age and immunosuppression (e.g., HIV infection). The parasite can modify the type of immune response from the host.

Figure 1. Parasitic peritonitis due to *Taenia crassiceps* in an experimental mouse model showing invasion of the peritoneal cavity by thousands of cysticerci (semolina-like appearance). Courtesy of Parasitology Unit, Alfort Veterinary School.

Figure 2. Cysticercus presenting the characteristic budding process of *Taenia crassiceps*. Courtesy of Parasitology Unit, Alfort Veterinary School.

Clinical signs

In all cases reported to date, the onset of subcutaneous tumour-like swellings caused dog owners to consult a veterinary surgeon. These swellings, 5–15 cm in diameter, are very often soft and localised to the flank, elbow or knee (Fig. 5). Surgical exeresis does not prevent a relapse in the following months, nor propagation to new areas, and the animal's general health deteriorates. Swelling is caused by the development of tissue cysts containing several cysticerci in a serohaemorrhagic fluid (Fig. 1).

The zoonotic potential of this new pathogen must be monitored because of its significant prevalence in fox populations. Fox populations are also increasing and they are becoming more suburban, bringing them into more frequent contact with humans and domestic carnivores.

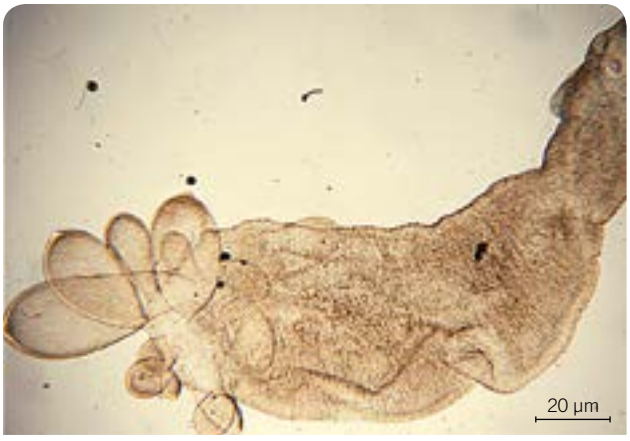


Figure 3. Cysticercus presenting the characteristic budding process of *Taenia crassiceps*. The start of invagination and strobila formation can also be seen. Courtesy of Parasitology Unit, Alfort Veterinary School.

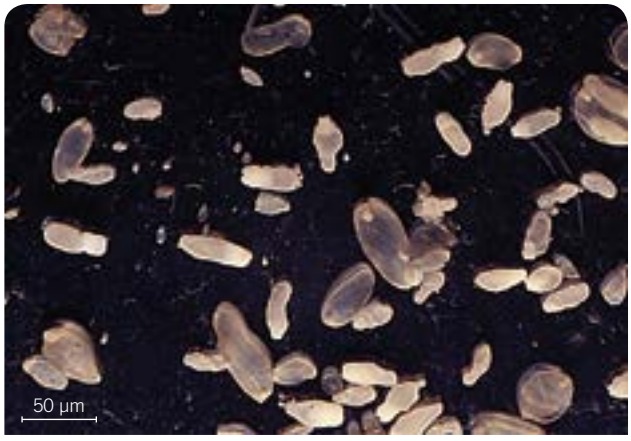
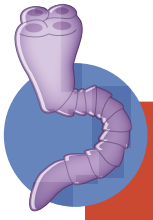


Figure 4. Numerous cysticerci obtained by puncturing the deformed subcutaneous mass in the dog in Figure 5. Courtesy of Parasitology Unit, Alfort Veterinary School.

Figure 5. Swelling of around 10 cm in diameter on the left flank of a dog. Courtesy of Parasitology Unit, Alfort Veterinary School.



Peritonitis due to *Mesocestoides* larvae

General comments

Parasitic peritonitis refers to the proliferation of cestode larvae in the peritoneal cavity of domestic or wild carnivores. The main parasites responsible are *Mesocestoides* spp. tapeworms, especially ***Mesocestoides lineatus***. Rare cases of peritoneal infestation by *Taenia crassiceps* larvae have also been described but this species more usually causes subcutaneous cysticercosis (see *Subcutaneous cysticercosis*, page 192).

Morphology and biology

Mesocestoides spp. tapeworms are approximately 50 cm long, sometimes even longer, and they live in the small intestines of wild and domestic carnivores

The first larval stage develops in oribatid mites present in the soil. The second larval stage is called a tetrathyridium larva and it usually develops in rodents or reptiles (such as lizards). The tetrathyridium larva is whitish, non-vesicular, and up to 30 mm long. It has a wide anterior end due to its invagination and it may be pseudo-segmented.

Tetrathyridium larvae are localised to the peritoneum, where they may be free or attached.

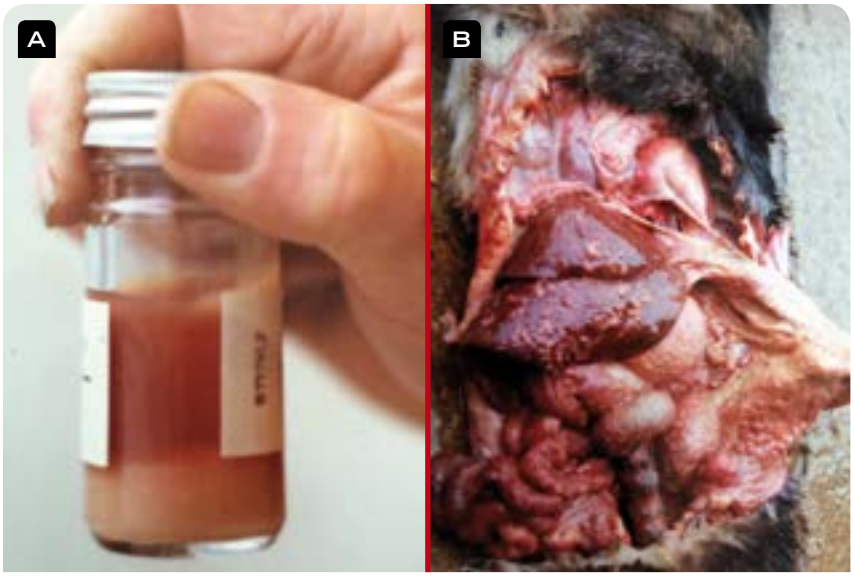


Figure 1. Ascitic fluid caused by parasites and *Mesocestoides* larvae in the abdomen of a dog. Courtesty of Jean-Paul Lemonnier.

Epidemiology

Mesocestoides spp. infestation is a mainly sylvatic cestodosis which affects foxes and wildcats.

Domestic carnivores may become involved in the cycle by consuming intermediate hosts and, in the majority of cases, they host adult tapeworms. Larval stages sometimes pass into the body cavity, where they proliferate.

Clinical signs

Infested animals present with a bloated abdomen, linked to fluid accumulation ("parasitic ascites") (Fig. 1). The condition is not always painful and it develops gradually, with possible diarrhoea and weight loss.

Tetrathyridium larvae are sometimes detected during abdominal surgery when puncturing the abdominal cavity releases a serohaemorrhagic fluid containing numerous opaque, whitish vesicles which are 10–30 mm in length and 1–3 mm in diameter and look just like grains of tapioca (Fig. 1).

Diagnosis

Parasitic peritonitis may be suspected in dogs with apyretic ascites and the appearance of abdominal puncture fluid is characteristic.

The ascitic fluid is an exudate rich in proteins (>25 g/L) and it contains many cells (>1,500/mL), usually including eosinophils (up to 10 %).

Control measures

Treatment is surgical, completely removing the ascitic fluid and cleaning out the peritoneal cavity with physiological solutions. A suspension of mebendazole at 40 mg/kg can be administered intraperitoneally, but daily oral administration of high doses of cestodicides gives disappointing results.

Peritoneal and subcutaneous filarioses *

General comments

Dogs and cats may be infested by filarial species other than *Dirofilaria immitis*. These are less pathogenic but must be included in any differential diagnosis when blood microfilariae are observed.

The main species of Filarioidea (order Spirurida) which parasitise dogs and cats in Europe belong to the family Onchocercidae and these are:

- *Dirofilaria immitis*.
- ***Dirofilaria repens***, located in subcutaneous tissue and muscle fasciae (Figs. 1–3).
- ***Cercopithifilaria*** (formerly *Dipetalonema*) ***grassii***, located in perirenal, peritoneal and subcutaneous tissues.
- ***Acanthocheilonema*** (formerly *Dipetalonema*) ***dracunculoides***, located in the peritoneal cavity.
- ***Acanthocheilonema*** (formerly *Dipetalonema*) ***reconditum***, located in the peritoneal cavity and subcutaneous tissue.

Vectors and intermediate hosts are:

- Only female Culicidae mosquitoes for *D. repens*.
- Ticks and fleas for the genus *Acanthocheilonema*.
- *Rhipicephalus sanguineus* ticks for the genus *Cercopithifilaria*.

Species affected: canids, notably dogs, but also foxes, jackals and fennec foxes. Filarial species can also infest cats in highly enzootic areas.

Geographical distribution

The subcutaneous filarial species *D. repens* is found worldwide, but seems to be absent from North America. The enzootic areas of *D. repens* and *D. immitis* overlap in many regions of Europe. Transmission frequency and the spread of *D. repens* depend on environmental factors, such as temperature, the density of vector populations and the presence of microfilaraemic dogs and wild canids, which are the main reservoirs of infestation.

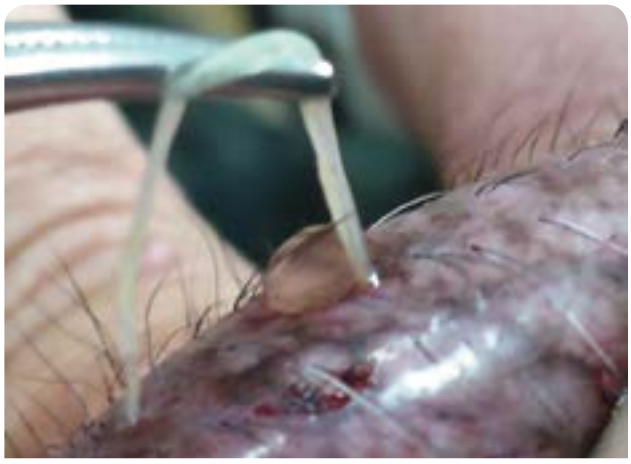


Figure 1. The adult *Dirofilaria repens* worm is easily removed in a minimally invasive procedure. Courtesy of Laura Rinaldi.

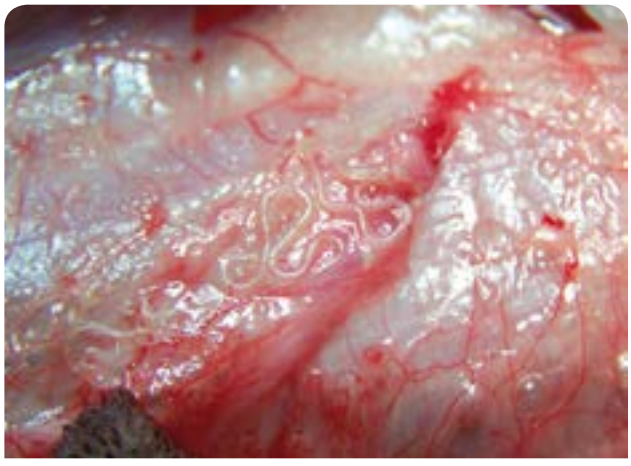


Figure 2. Adult *Dirofilaria repens* in the subcutaneous tissues of dogs sin an incidental finding during surgery to remove a skin tumour. Courtesy of Laura Rinaldi.

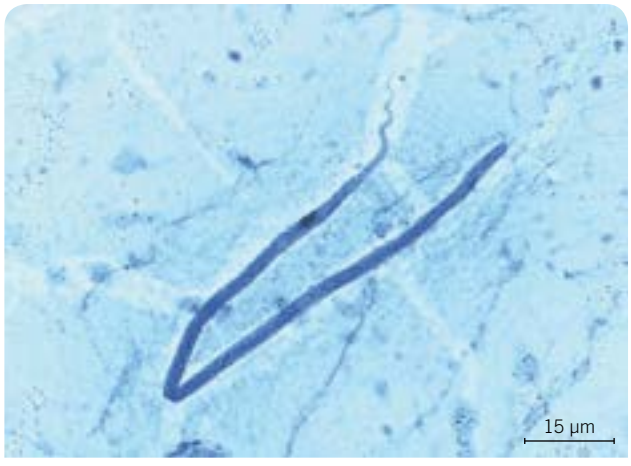


Figure 3. *Dirofilaria repens* microfilaria stained with acid phosphatase. There is only one red spot (2 spots for *Dirofilaria immitis*).

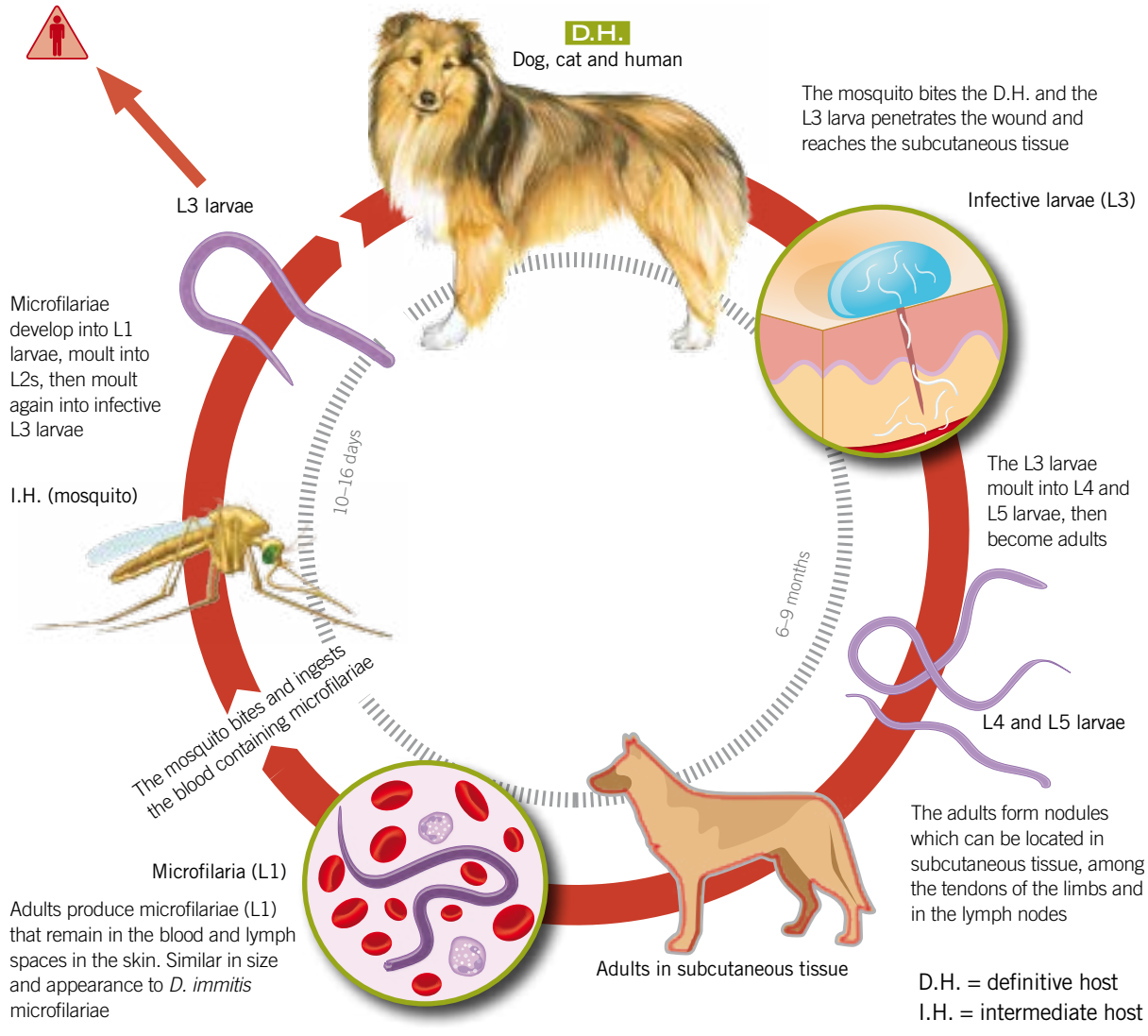
Acanthocheilonema and *Cercopithifilaria* spp. are found worldwide and *A. dracunculoides* infestation is up to 14 % in hunting dogs and dogs living outdoors in some European areas, such as Spain and Southern Italy. *A. reconditum* is quite common in Sardinia, Italy.

Importance

These filariae are of little medical importance because infestation with filarial species (other than *D. immitis*) is mostly asymptomatic.

D. repens is zoonotic and infested dogs, cats and wild canids are reservoirs of microfilariae for mosquitoes, which transmit the parasite to humans.

Life cycle of *Dirofilaria repens*



Morphology

Table 1 lists the morphological features of adult filariae and blood microfilariae of the species which infest dogs and cats in Europe.

Biology

After inoculation by haematophagous arthropods (mosquitoes, ticks or fleas), L3 larvae migrate towards their definitive site: subcutaneous or muscle tissue for *D. repens*, perirenal or peritoneal fat for *Acanthocheilonema* species. The adults develop in 2–3 months and the females often survive for several years. They release embryos or microfilariae into the blood, in the case of *D. repens*, *A. reconditum* and *A. dracunculoides*, and dermotropically in *C. grassii*.

Arthropods become infested during their blood meal and, in the case of ticks, *Acanthocheilonema* larvae are transmitted between developmental stages.

These filarioses are sporadic and are usually detected accidentally, when a blood sample is analysed. Their transmission is seasonal in temperate countries and depends on the biology of the corresponding vector (mosquitoes, ticks or fleas).

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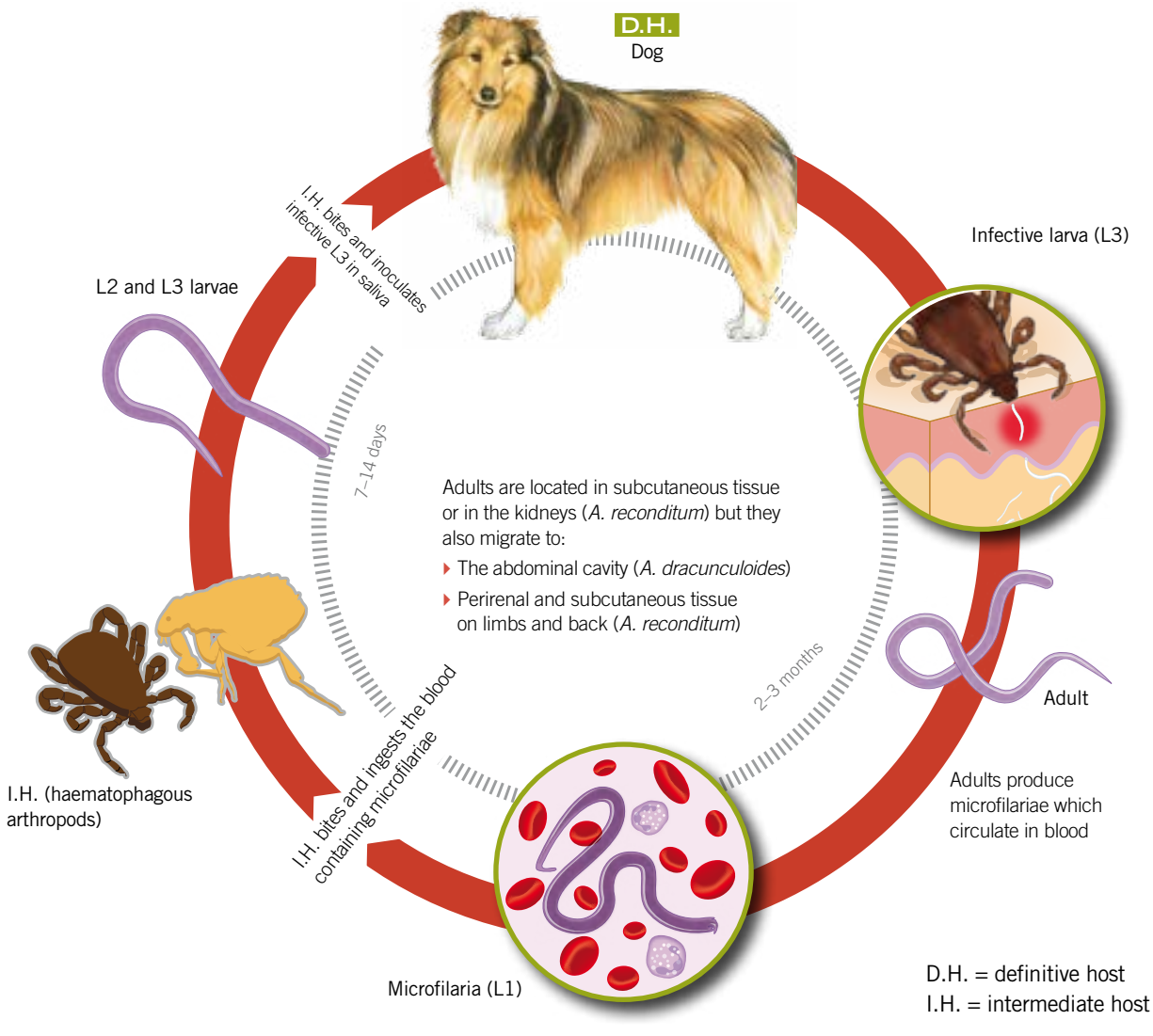
Life cycle of *Acanthocheilonema reconditum* and *Acanthocheilonema dracunculoides*



Clinical signs

Infestation with filarial species other than *D. immitis* is mostly asymptomatic.

Adult *D. repens* filariae can be found in subcutaneous nodules which are 3-6 cm in diameter, may be ambulatory and usually soft, with a serohaemorrhagic contents. These nodules are usually considered to be tumours or pseudo-tumours and they are usually surgically removed.

Free filariae in a peritoneal or perirenal location do not cause any clinical signs but microfilariae, and the embolisms they cause, circulating through blood capillaries may be responsible for immunoinflammatory vascular lesions. The main clinical signs include: onset of renal insufficiency, necrosis of the extremities (especially the ear pinnae and the tail) and pruritus.

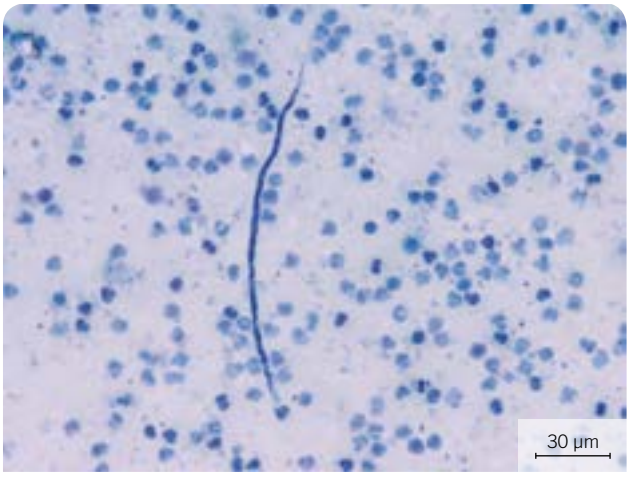


Figure 4. Microfilaria of *Achantocheilonema reconditum* detected by Knott technique.

Diagnosis

Infestation by *D. repens* and *Acanthocheilonema* species can be detected by blood tests, which detect circulating microfilariae (Fig. 4). Morphological differentiation of the microfilariae by their length is often difficult due to the overlapping sizes of most species (Table 1). In the case of *C. grassii*, lymph or dermotropic microfilariae can be detected by cutaneous biopsy. Adult filariae may more rarely be found in subcutaneous nodules.

Control measures

Moxidectin is labelled for the prevention of *D. repens* infestation in dogs, and the treatment of circulating microfilariae.

A microfilaricidal treatment based on ivermectin (50 µg/kg) can be administered when clinical signs suggest microfilariae (off-label use).

There is no known effective adulticide for *D. repens* and there are no prophylactic measures other than vector control.

Table 1. Morphological features of filarial species in dogs and cats (adapted from ESCCAP Guideline 5).

Species	Adults	Microfilariae (in the blood)		
		Length	Width	Features
Dirofilaria immitis	M: 12–18 cm F: 25–30 cm	290–330 µm	5-7 µm	No sheath, cephalic end pointed, tail straight with the end pointed. APh-S: two activity spots located around the anal and excretory pores
Dirofilaria repens	M: 5–7 cm F: 10-17 cm	300–370 µm	6-8 µm	No sheath, cephalic end obtuse, tail sharp and filiform often ending like an umbrella handle. APh-S: one spot around the anal pore
Acanthocheilonema reconditum	M: 9–17 mm F: 21–25 mm	260–283 µm	4 µm	No sheath, cephalic end obtuse with a prominent cephalic hook, tail button hooked and curved. APh-S: activity throughout the body
Acanthocheilonema dracunculoides	M: 15–31 mm F: 33–55 mm	190–247 µm	4-6.5 µm	Sheath, cephalic end obtuse, caudal end sharp and extended. APh-S: three spots which include an additional spot in the medium body

M: male; F: female; APh-S: acid phosphatase stain.

Thelaziosis

General comments

Thelaziosis is caused by the presence and development of nematodes of the genus *Thelazia*. These nematodes, called eyeworms, are responsible for epiphora, conjunctivitis, keratitis and even corneal ulcers.

Eyeworms are frequently reported in cattle (*Thelazia rhodesii*, *T. skrjabini* and *T. gulosa*) and horses (*T. lacrymalis*) but they can also be found in carnivores, especially dogs and foxes. The cat is not a common host but several cases have been described in enzootic areas of Europe. The species responsible for canine thelaziosis are ***T. callipaeda*** (Railliet and Henry, 1910) in Europe and Asia, and ***T. californiensis*** in America. These two species can infest humans.

T. callipaeda infection has proved to be widespread in the past two decades among dogs from the northern Aosta valley and southern Basilicata regions of Italy. It is also increasingly reported in western France (Dordogne area), Switzerland, Spain, and Portugal. In 2014, the first autochthonous cases of thelaziosis were described in red foxes, dogs and a cat living in Bosnia and Herzegovina and Croatia. Cases are also reported in Romania, Greece and Serbia.

Morphology and biology

Thelazia species are characterised by a serrated cuticle and a crown-shaped buccal vestibule. Adult females are characterised by the position of the vulva, located anteriorly to the oesophageal-intestinal junction.

Thelazia callipaeda is a whitish filiform nematode, 7–17 mm in length by 0.2–0.3 mm in diameter (Fig. 1).

The female releases L1 larvae, which are found in dog tears. *Thelazia* species are transmitted by the various species of flies which feed on the definitive host's lacrimal secretions. *T. callipaeda* L1 larvae undergo three moults in the vector (taking about 14–21 days) and the infective L3 may be transmitted to a new host, developing into the adult stage in the ocular cavities within 1 month. The competence of the drosophilid fly *Phortica variegata* (Fig. 2) as a *T. callipaeda* vector has been demonstrated. Interestingly, only male *P. variegata* were found to be infested with *T. callipaeda* under natural conditions in Italy.

Adults *T. callipaeda* may survive several months in the dog's conjunctival sacs.

Figure 1. Adult *Thelazia callipaeda*. Direct examination after extraction.

Figure 2. *Phortica variegata*. Courtesy of Domenico Otranto.

Epidemiology

Thelaziosis is a seasonal nematodosis linked to the presence and density of drosophilid vectors and there is a peridomestic cycle between wild or stray dogs and vectors.

Dogs and cats are infested in the summer by male drosophilid flies, which deposit L3 larvae on the conjunctiva or around the eyes. Clinical signs usually occur in winter.

Clinical signs and diagnosis

Thelaziosis may be asymptomatic but noticeable clinical signs are sometimes reported as a result of the irritant nature of the eyeworm cuticle. These clinical signs include:

- Blepharospasm.
- Epiphora.
- Keratitis.
- Conjunctivitis.
- Intense lachrymation.

The condition may be uni- or bilateral.

Secondary bacterial infections are possible and keratoconjunctivitis may rapidly become severe and purulent.

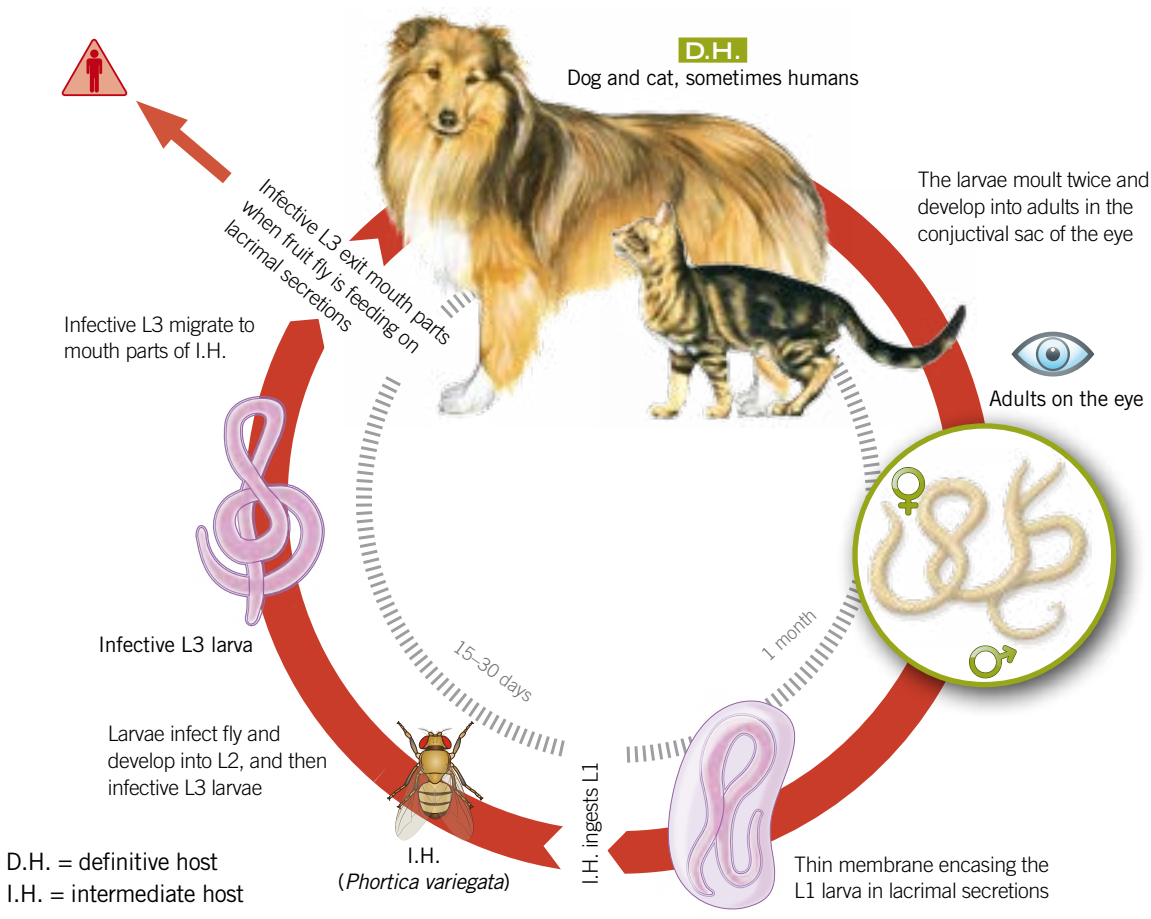
Differential diagnosis of keratoconjunctivitis must include: post-traumatic bacterial infections, immunological keratitis, and keratitis linked to other diseases (especially leishmaniosis in enzootic areas).

Definitive diagnosis relies on the visualisation of whitish nematodes on the conjunctiva and conjunctival sacs (Fig. 3) and the conjunctival fornix can be tested using a sterile cotton swab. L1 larvae (approximately 200 µm long) can be seen by direct examination of the tears.

Figure 3. Clinical infestation with *Thelazia callipaeda* in a cat (A) and a dog (B). Courtesy of Domenico Otranto.

PDF

Life cycle of *Thelazia callipaeda*



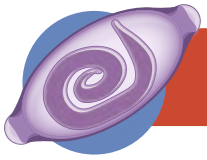
Control measures

Treatment is based on the direct removal of nematodes the eyes of affected animals (after local or general anaesthesia) and the use of an antiparasitic drug. Moxidectin applied in a spot-on, oral milbemycin oxime and subcutaneous ivermectin have all proven to be effective treatments for thelaziosis in dogs. Antibiotic ointments or ocular drops s are recommended in cases of bacterial infection.

Prophylactic measures include the use of macrocyclic lactones in a spot-on form administered monthly or in a slow-release form during the vector's active period.

Video 6

Adult *Thelazia callipaeda* on the surface of the nictitating membrane of a dog. Courtesy of Olivier Pennant.



Trichinellosis

General comments

Trichinellosis is a non-contagious zoonotic helminthosis common to numerous animals and humans. It is caused by the presence and development of *Trichinella* species nematodes, mostly ***T. spiralis***. The adults are localised to the intestine, and larvae to striated muscle tissue.

The various *Trichinella* species are distinguished according to biological, epidemiological, immunological and molecular characteristics. *T. spiralis*, ***T. nelsoni***, ***T. britovi***, ***T. nativa***, and ***T. pseudospiralis*** are the most common among the different species and populations identified.

Hosts

All mammals (>150 species) may be affected, including humans and wild, domestic and peridomestic animals, and one species, *T. pseudospiralis*, also infects birds.

Geographical distribution

The disease is found worldwide, distributed according to the particular *Trichinella* species. Cases are either caused by consuming insufficiently cooked game (such as wild boar) or horse meat from America or Central Europe. In the latter case, the number of people affected is often very significant as one infested carcass may be distributed between several hundreds of consumers.

Other source of *Trichinella* in humans include bears in North America, seals in the Arctic, warthogs in Africa, and dogs and pigs in Asia.

The epidemiology of infestation in domestic carnivores is the same as for humans. Trichinellosis is common in dogs in Asian countries, and it can also be seen in other regions, notably in hunting dogs which consume raw game. Dogs and cats which hunt rats are susceptible to infestation, trichinellosis being endemic in populations of these "peridomestic" rodents.

Importance

- Medical: limited in veterinary medicine.
- Economic: linked to the loss of parasitised meats, cost of screening and limits placed on meat distribution.
- Veterinary public health: humans are receptive and sensitive. Trichinellosis is a zoonosis which can be medically serious or even fatal in humans.

Biology

Trichinella are intracellular nematode parasites. Adult *Trichinella* are tapered, and the males are 1–1.5 mm long and females 2.5–3.5 mm long. The newborn larvae are 100–160 µm long and 9 µm in diameter and the infective larvae are 1 mm long and 30 µm in diameter (Fig. 1). They are quite similar to the adults but their ends are rounded. The general arrangement of the body is spiral and the reproductive system is as yet undifferentiated, although it is possible to distinguish between males and females.

Figure 1. Infective *Trichinella spiralis* L1 in muscle. Length 900 µm. Pepsin digestion of muscle fragments from a wild pig killed in New Caledonia.

Two days after mating, which takes place in the lumen of the small intestine, females start to release L1 larvae and they release approximately 1,500 of them during their 6-week lifetimes. These L1 (known as newborn L1) enter the bloodstream and are distributed throughout the organism. They then penetrate striated muscle fibres and cause muscle fibres to transform into "nurse cells": myofibrils disappear, the nucleus divides and peripheral neovascularisation occurs. The nurse cell is surrounded by a protective wall and becomes a lemon-shaped cyst (called a trichinian cyst) measuring approximately 400 × 200 µm. It takes approximately 3 months to form completely (Fig. 2) and the larva can be seen coiled in the cyst, hence the name "spiralis". Several larvae may be found in the same cyst and the cyst is infective for any mammal that consumes it. It survives for several years in its host and resists putrefaction and the cold, and some can even survive freezing (*T. nativa*, found in the Arctic) for several weeks.

When a cyst is ingested, the L1 larva is freed from the muscle and it transforms into an L2, L3 and L4, then into a phase 5 and adult within 48 hours. The cycle then restarts: any mammal is therefore both a definitive and intermediate host and the cycle is described as auto-heteroxenous.

T. pseudospiralis and *T. papuae* are different because their larvae remain free in the muscle fibres and do not develop inside cysts.

Their pathogenicity is significant, especially in humans, for whom the ingestion of 50 larvae is enough to produce clinical signs. Allergic reactions are frequent in humans but, although wild reservoirs are receptive, they are not very sensitive. Pathogenicity depends on the species and strain of *Trichinella*.

Epidemiology

Trichinellosis develops enzootically in animal populations in different parts of the world and wild epidemiological cycles can be distinguished from domestic cycles. Transmission is linked to meat consumption, including predation and scavenging. Carnivorous birds such as ravens and magpies, may excrete infective cysts in their droppings. Scavenging beetles may also carry viable cysts. Predisposing factors include the presence of rats near livestock, tail and ear biting, and cannibalism linked to overpopulation in swine.

Humans become infested by consuming wild game or farmed meat. Ingestion of raw, or insufficiently cooked or cured meat is usually the cause. The infective dose is low, so licking a knife which has been used to prepare the meat or ingesting bread on which it has been wiped is sufficient.

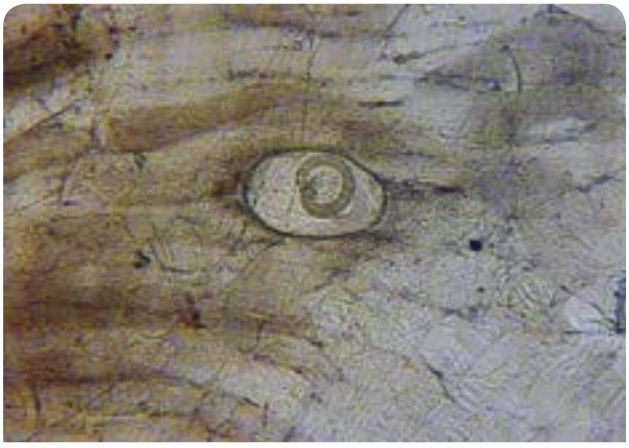


Figure 2. *Trichinella* cyst (approximately 400 µm in diameter) observed on crushing a muscle fragment between two glass plates (trichinoscopy).

Dogs and cats are infested accidentally by sharing the same food as their owners. In some parts of the world, trichinellosis in carnivores is more common, as is the case in Southeast Asia and China and carnivores, which may sometimes be consumed by humans, can also become a source of infestation.

Clinical signs and lesions

Clinical signs

Usually absent in animals and present in humans, they are divided into two stages, one intestinal and one muscular, or general, stage.

The intestinal stage starts in the week following infestation, is accompanied by hyperthermia and lasts for approximately 1 month. It also causes colic.

The muscular or general stage occurs 3 weeks after infestation and combines myalgia, asthenia and allergic reactions, including facial oedema and an urticarial rash. Cardiac disruption is possible, although larvae are not localised to the heart, and abortion is not uncommon when trichinellosis affects pregnant women. Persistent muscular pain, as well as the general asthenia, is experienced by patients for several months or even years.

Digestive disorders, then muscular problems, have been reported in dogs, but allergic reactions seem to be quite specific to human infestation.

Lesions

Acute enteritis lesions can be seen, followed by eosinophilic myositis with the formation of *Trichinella* cysts. Overall pathogenicity is related to the combined pathogenicity of the intestinal adults and the larvae in the muscles, along with the host's immune and inflammatory response.

Diagnosis

Various methods can be employed to diagnose trichinellosis: serology (ELISA), which becomes positive approximately 3 weeks after infestation, PCR (from muscle biopsy), and detection of encysted larvae on muscle biopsy.

Control measures

Treatment

There is no treatment for livestock but high doses of benzimidazoles could be considered for use in domestic carnivores and humans.

Anthelmintic treatment is more effective when administered early, killing the intestinal females and larvae which have not yet encysted. No treatment is active after larvae have encysted. Corticotherapy limits allergic reactions in humans.

Prevention

- Animal infestation can be prevented by simple measures: rat control and disposal of carcasses, and prohibiting the use of raw abattoir waste in animal feed.
- Human infestation can be prevented in two ways:
  - Individual measures: cooking meat correctly before consumption (approximately 56 °C all the way through). Mandatory freezing of game for a month at -20 °C.
  - Collective, regulatory measures: screening of local and imported meat, in accordance with regulations in force.

EXTERNAL PARASITOSES

Entomoses

Flea infestation

Introduction

The cat flea, ***Ctenocephalides felis***, is the predominant flea species found on cats and dogs, with a prevalence of over 90 % cited in almost all publications (Fig. 1). However, other flea species can occasionally be seen on carnivores: ***Ctenocephalides canis*** (dog flea) (Fig. 2), ***Spilopsyllus cuniculi*** (rabbit flea), ***Ceratophyllus* spp.** (bird fleas), ***Xenopsylla cheopis*** (rat flea), ***Archeopsylla erinacei*** (hedgehog flea), ***Leptopsylla segnis*** (rodent flea) and ***Pulex irritans*** (human or fox flea). These fleas represent less than 1 % of the fleas found on cats, and are usually found on outdoor cats which hunt.

C. canis is the predominant flea species found on dogs in some parts of the world, such as Central Europe. *P. irritans*, the fox and human flea, is also common on dogs in some areas.

C. felis can be divided into four sub-species, three of which infest felids. *C. felis felis* (Bouché, 1935) is the predominant sub-species in Europe and North America, whereas *C. felis strongylus* is more common in Africa and the Middle East. *C. felis orientis* (Jordan, 1925) is found in Asia. These last two sub-species are morphologically very similar to *C. canis* (Curtis, 1826). *C. felis damarensis* (Jordan, 1936) infests small carnivores and is found in North America.

C. felis felis is not host-specific and can take its blood meal from various mammals (domestic and wild carnivores, opossums, rodents, rabbits, ruminants, humans, etc.) (Fig. 3). More than 50 hosts have been identified, even though this sub-species is called the cat flea. The owners of flea-infested cats and dogs are often bitten and frequently develop pruritic papules on their legs and ankles.

Figure 2. Adult *Ctenocephalides canis*.

Figure 1. Adults *Ctenocephalides felis*.

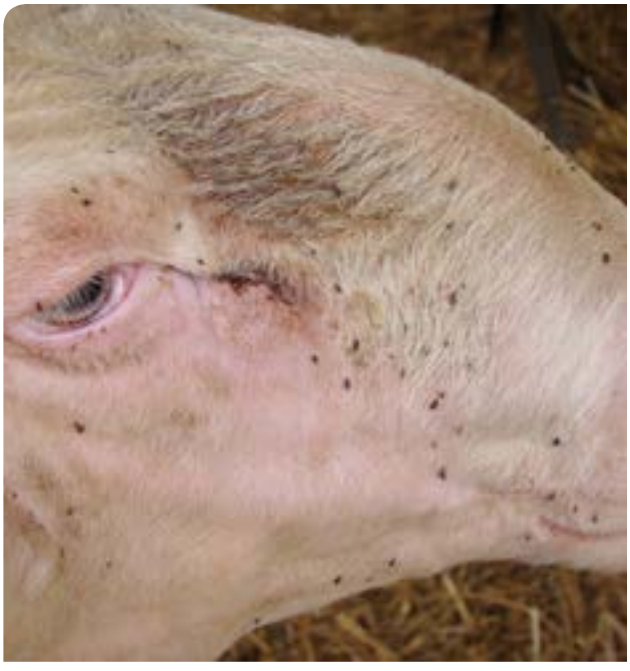


Figure 3. Sheep heavily infested by *Ctenocephalides felis*.

Fleas are the most common ectoparasites infesting pets in both rural and urban habitats. Cat fleas are adapted to their environment (outdoor and indoor) and persist throughout the seasons, even surviving through the winter in temperate countries. Nevertheless, infestation and clinical manifestation usually peaks between spring and autumn.

Cats usually tolerate fleas quite well but flea infestation can sometimes provoke intense pruritus and some animals will develop flea allergy dermatitis (FAD) with more pronounced skin lesions.

C. felis is usually recovered from 5 % to over 50 % of the cats studied in epidemiological surveys, and the variation is linked to methodology, country, season, treatment history, etc. *C. felis* probably originated in Africa and is better adapted to warm climates than cold. It is found in both rural and urban areas and on pets living indoors or outdoors.

Morphology

Fleas are wingless insects, 2–4 mm long and a yellowish-brown colour, belonging to the order Siphonaptera. There are approximately 2,500 flea species, divided into 15 families and 200 genera. Most fleas of medical and veterinary importance belong to the family Pulicidae.

Many species bear one or more "combs" or ctenidia, which are groups of sclerotinised spines. Flea classification is mainly based on the morphology of the head and the adult genitalia, and the number of ctenidia and their positions. *Ctenocephalides* fleas have two pronounced ctenidia: the genal ctenidia on the ventral margin of the head, and the pronotal ctenidia on the posterior margin of the head (Fig. 4).

Fleas usually have well-developed eyes, and antennae which are composed of three segments and located in antennal fossae on each side of the head. The mouthparts are well adapted to blood sucking: the two labial palps locate the feeding site, then the other mouthparts (the "stylets") are used to pierce the skin through to a capillary. They then form a feeding canal and a salivary canal.

C. felis felis belongs to the family Pulicidae. It is 2–4 mm long and orange to dark brown in colour. The front of its head is rounded and has two perpendicular combs lined with dark brown teeth. The body is laterally compressed to facilitate movement between hairs and the third pair of legs is very well developed and adapted to jumping. *C. canis* is smaller than *C. felis*, the head is shorter and the first spine of the genal ctenidia measures half of the second in *C. canis* and almost the same length in *C. felis*.

The third pair of legs is bigger than the others, and adapted to jumping to facilitate infestation of the host. The average jumping distance of *C. felis felis* is 20 cm (2–48 cm), and of *C. canis* is 30 cm (3–50 cm). Jump height is about 15 cm, with a maximum height of 25 cm attained by *C. felis*.

Flea eggs are small (0.2–0.5 mm), ovoid and white to yellow-white.

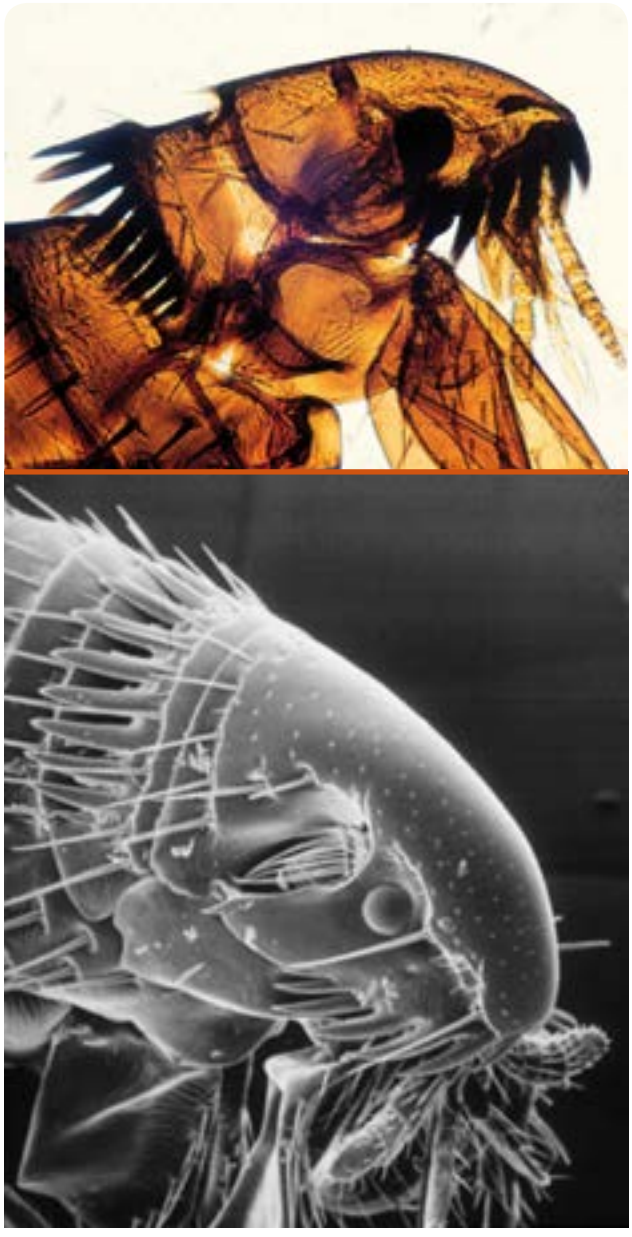


Figure 4. Head of *Ctenocephalides felis* showing the two ctenidiae.

There are three successive worm-like larval stages (L1-L3): eyeless, legless, with a head and 12 posterior segments. They measure from 1.5 mm (L1) to 8 mm long (L3).

Flea pupae are formed in sticky cocoons, often surrounded by debris which helps provide camouflage.

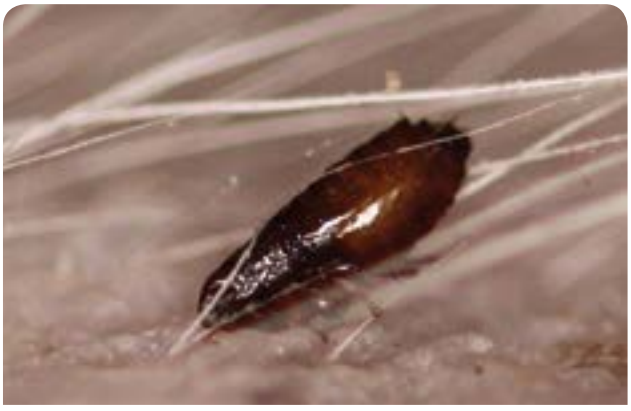


Figure 5. Flea taking a blood meal on a dog.

Figure 6. Fleas mating.

Figure 7. Female flea laying an egg on its host.

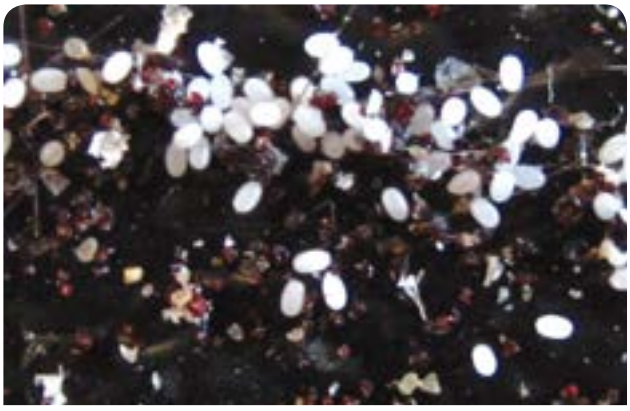


Figure 8. Flea eggs on the ground.

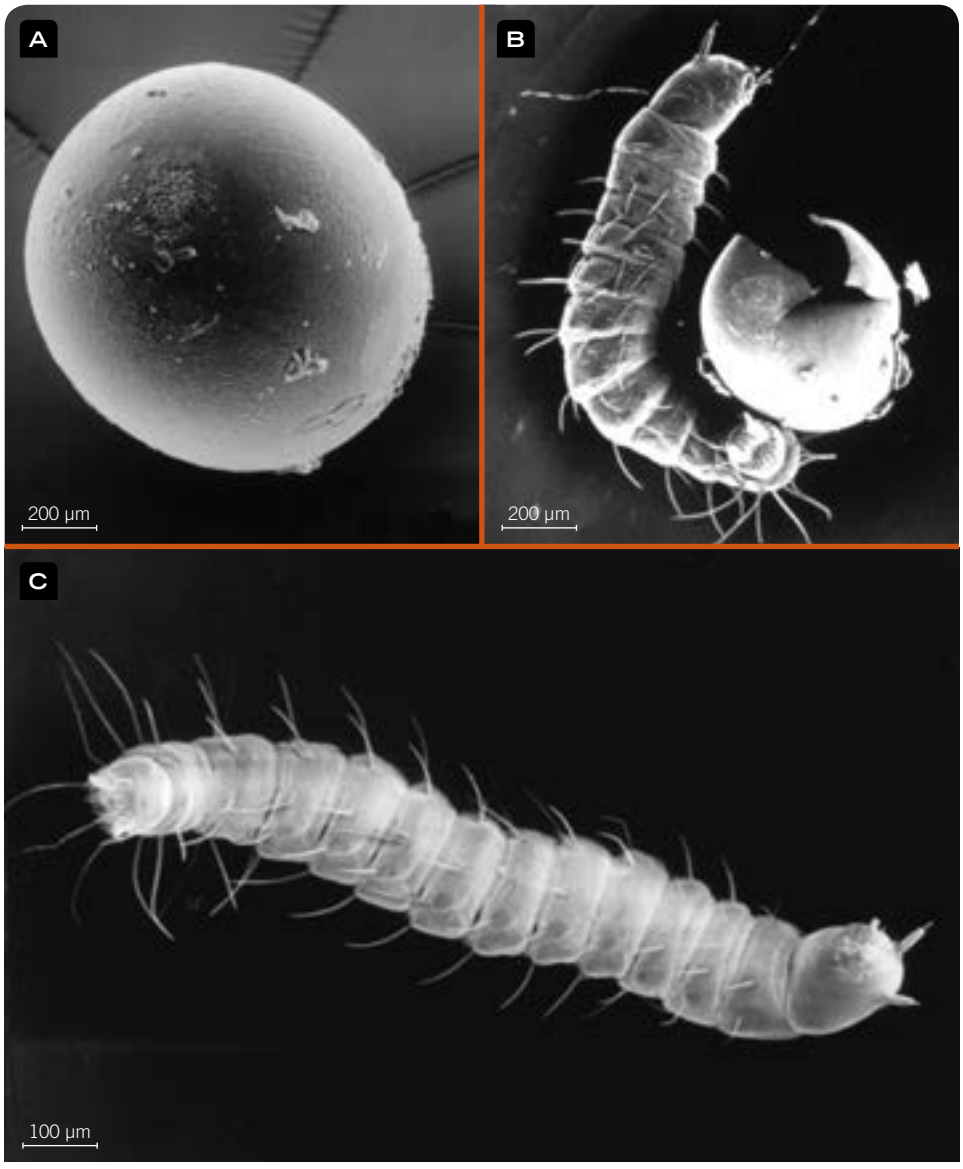


Figure 9. Electronic microscopy showing a flea egg (A and B) and a recently hatched stage 1 larva of *C. felis*.

Figure 10. Stage 3 *Ctenocephalides felis* larva.

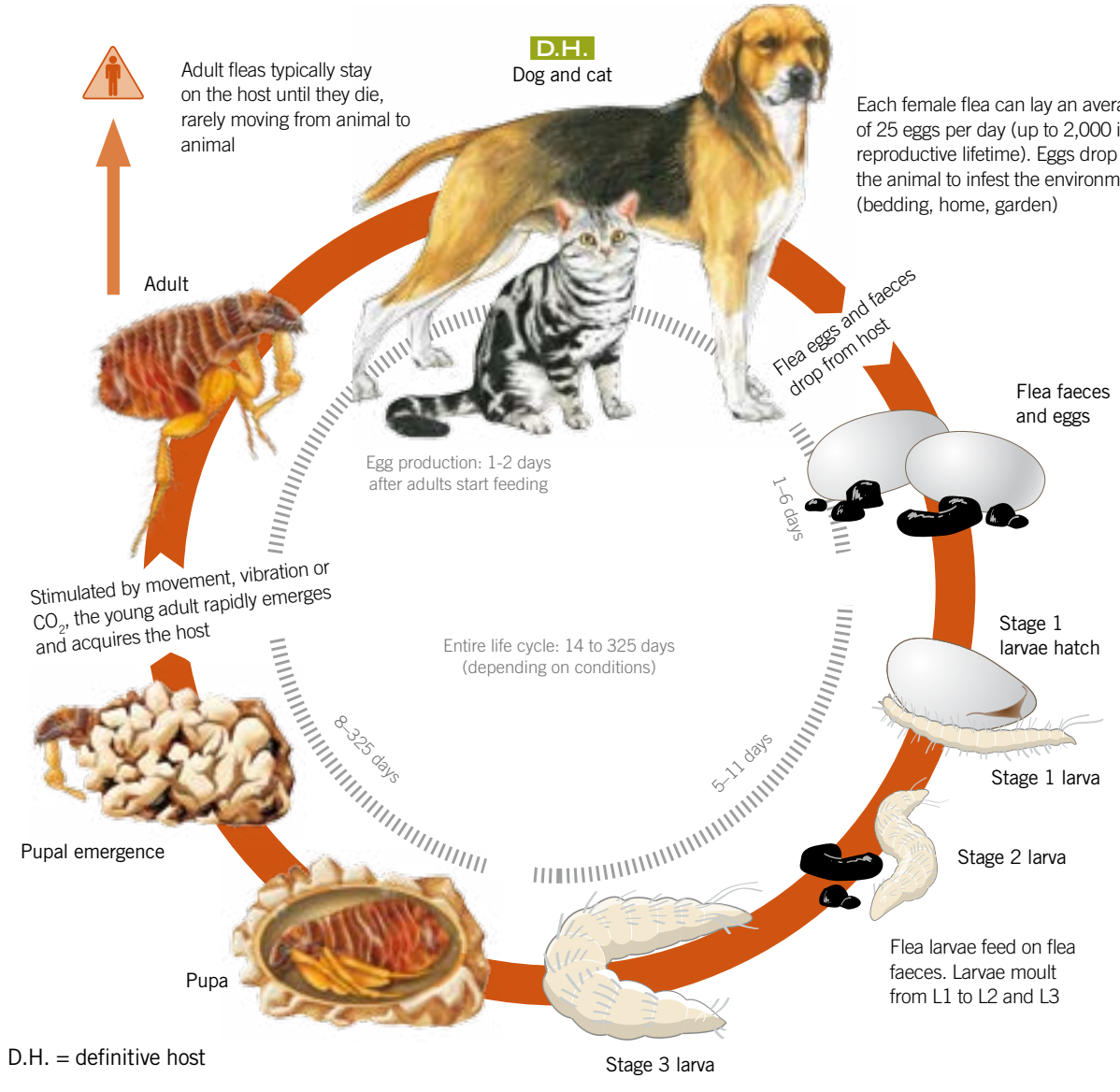
Figure 11. Flea cocoons and stage 3 larvae on a sofa.

Biology of *Ctenocephalides* fleas

Over the last 20 years, studies on the biology of fleas on pets have improved our understanding of the flea life cycle, providing us with essential information to construct an effective flea control programme. Some ideas about cat fleas have been found to be wrong, for example, the adult flea is no longer considered a transitory parasite, found on the cat or dog only when feeding, but a permanent parasite which tends to stay on the same animal. It only survives for 3 to 5 days in the external environment if it falls off. Adult fleas on pet animals exceptionally change host and infest other animals. However, the epidemiological role of these few mature fleas which transfer from one host to another can be significant due to the prolificacy of each female.

Adult fleas take their first blood meal 30 minutes to 1 hour after arrival on the animal and then breed within the following 48 hours (Figs. 5 and 6). Each female can lay up to 50 eggs per day at her peak, starting to lay a few eggs 36 hours after infesting the host and averaging 20–30 eggs per day (Fig. 7). Females lay eggs throughout their whole life, which is usually short (15 to 30 days). They probably take 4 to 10 blood meals per day, each lasting several minutes.

Life cycle of *Ctenocephalides* spp.



The eggs are not fixed to the host and fall to the ground as the animal moves around (Figs. 8 and 9). It has been estimated that the eggs remain on the animal's skin for around 2 hours before falling off. This enables contact with any insecticides or insect growth regulators (IGRs) that may be present on the animal's skin. When temperature and humidity are optimal, eggs hatch in 3–7 days on the ground. The maximum number of eggs will usually be found where pets are resting.

The larvae are a few millimetres in length and are non-parasitic, feeding on organic debris, mainly skin debris and adult flea faeces (desiccated blood). They prefer dark and humid conditions and can move horizontally for about 20 cm in secluded places (for example, under sofas, carpets and rugs, or in pet bedding) L1 and L2 are very sensitive to UV light and desiccation.

Having passed through three larval stages over a period of a week to a month, each L3 spins a cocoon in which it metamorphoses into a pre-emerged flea within about 10 days. The cocoon is sticky and is surrounded by debris which protects it (Figs. 10 and 11).

Adult fleas quickly emerge from the pupae if hosts are nearby but, if no hosts are available, the non-emerged adult fleas can survive for 6 to 12 months, protected by their cocoons. Pre-emerged fleas are an important reservoir of new fleas which are relatively resistant to insecticides while protected by their cocoon They are easily transported from one place to another by animals and humans as they are sticky and attach to shoes, socks, trousers, etc.

Newly emerged fleas actively seek out a host (preferably a cat or dog) and can survive for about a week without a blood meal.

Environmental conditions affect the development and timing of flea life cycles. Each stage in the life cycle is susceptible to desiccation and relative humidity of 85 % is optimal. Temperature can accelerate or slow down development. The minimum temperature for *C. felis* seems to be 22 °C, with the optimum being 25–26 °C. Temperatures above 30 °C reduce adult lifespans. In winter, an outside temperature below 0 °C is fatal to larvae and pupae. The life cycle slows down considerably at 17–19 °C, but pre-emerged adults survive, waiting for more favourable conditions. This means that fleas can survive all year round, with a sudden population explosion in spring.

When humidity is favourable, the life cycle of *C. felis* takes 14 days at 29 °C. On average, a complete life cycle can be considered to take 3 to 4 weeks.

The emergence of fleas from their cocoons is influenced by various factors: shadows, footsteps, vibrations (for example, from a vacuum cleaner) can all trigger emergence. Cats typically catch fleas by passing through an infested environment, either outdoors in the right season, or indoors (e.g., when visiting someone else's house), and they often bring fleas into their own house, where they then breed and become a source of infestation for other cats or dogs sharing the same environment.

Ecology

Most flea species infesting wild animals are nidicolous, so they live in nests or burrows and infest their hosts just to take their blood meal. Their reproductive cycle may run in parallel to their hosts', which is the case for the rabbit flea, *S. cuniculi*, whose population increases when their rabbit or hare hosts give birth to their progeny. These nidicolous fleas mainly infest small mammals (rodents, lagomorphs, bats) and birds.

The situation is different for fleas which infest carnivores as adult stages of these fleas are more permanent parasites, remaining in the host's fur. The environment is then contaminated by the immature stages (eggs, larvae, and pre-emerged fleas in their cocoons) which represent the source of infestation. It is this distribution between adults and pre-adults which makes controlling fleas on pets so difficult, as well as the low host specificity.

Most pet owners just wait until they see their pet scratching, then want to kill the fleas which are actually on the animal, but the most difficult aspect of flea control is that most pet owners do not realise the importance of the pre-existing environmental infestation or understand its relationship to flea biology.

This is why the concept of integrated flea control is so important. By the time a pet owner notices fleas on his/her pet, there is already a large biomass of flea life stages present in the pet's environment. Flea biology dictates that it will take approximately 1–2 months for these life stages to complete their development: for the eggs to develop into larvae, then into pupae and finally into pre-emerged adults ready to emerge from the pupal cocoon and jump onto a passing animal. It is therefore biologically impossible to eradicate a flea infestation overnight, regardless of which product is chosen to treat the animal.

It is important to understand that the length of time it will take to resolve any individual flea problem is governed by a number of factors that are unknown at the beginning of the treatment.

The most important ecological questions in flea control is:

- **Where are the fleas coming from:** indoors/outdoors or a combination of the two? The source of fleas can be neighbouring cats and dogs, but also stray cats and wild animals, such as opossums, raccoons, etc. The reservoir of pupae could be located outside the house, in the basement, in the garden or even outside the garden, in a place where dogs and cats often visit.
- **How many immature stages are already present in the environment?** The life cycle never stops in tropical and Mediterranean climates, but it stops outdoors, and slows down indoors, during cold periods (and also during hot and dry periods), and increases in spring, in temperate and continental climates.
- **How long will it take for the immature stages to complete their development?** This can vary according to fluctuations at a microclimate level.
- **Is there an ongoing source of new flea eggs in the environment?**

These unknowns explain why flea treatment results vary from household to household, from year to year, and even from season to season.

Remember, the flea life cycle does not only occur inside the house, there is a large reservoir of outdoor fleas due to the interaction between untreated pets/strays/feral and native animals and the flea. This outdoor life cycle is a constant source of reinfestation and the reason that fleas can still be seen on pets which are consistently treated with a long-lasting anti-flea product. Seeing a few fleas on a treated pet does not equate to product failure and expecting pets with access to the outdoors to be "flea free" in humid, tropical conditions is unrealistic.

Figure 12. Flea-infested dog scratching.

Clinical signs

Fleas are responsible for numerous clinical signs, and allergic animals should be differentiated from non-allergic animals. The vast majority of animals suffer from irritation and pruritus (Fig. 12): they continuously scratch, groom, lick or nibble at themselves, more or less vigorously, in an attempt to catch the fleas, swallowing them in the process. Cats are very successful, and this largely explains the infestation by *Dipylidium caninum* cestodes, with fleas as intermediate hosts. Tolerance of this infestation varies drastically from one animal to another: some cats are able to withstand infestation by hundreds of parasites and only express mild pruritus, whereas others present with allergic dermatitis with only a few fleas.

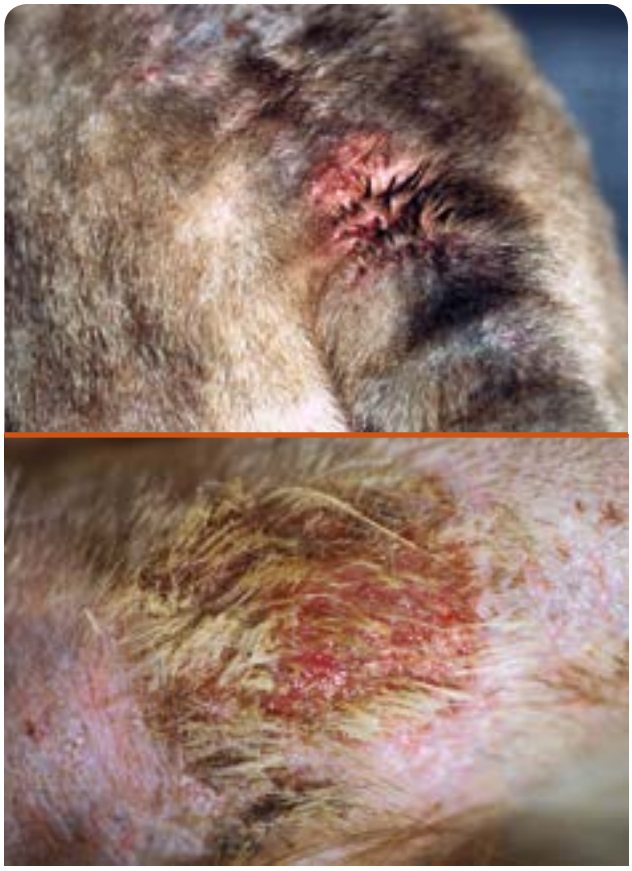


Figure 13. Acute exudative dermatitis in a dog infested by fleas. Pictures from Canine Dermatology Guide, E. Guaguère and P. Prélaud, Ed. Merial, 2008.

Flea allergy dermatitis (FAD) is triggered by individual factors and the antigens that provoke this immuno-inflammatory response come from flea saliva. Cats and dogs with FAD overreact and show some typical, and some less typical, clinical signs (Fig. 13–15). These clinical signs correspond to a mast cell-mediated cutaneous hypersensitivity reaction, with tissue infiltration by polynuclear cells (including basophils and eosinophils) and increased IgE production. Degranulation follows, and inflammatory mediators (especially histamine, serotonin and various leukotrienes) are released, provoking a skin hypersensitivity reaction.

Beside intense pruritus with scratching wounds, other lesions in cats and dogs include diffuse hair loss, primarily but not exclusively on the lumbosacral area. Chronic inflammation can alter the skin's appearance, which thickens (orthokeratotic hyperkeratosis) and acquires a greyish colouration (melanosis). Secondary infection by yeasts (*Malassezia pachydermatis*) or *Staphylococcus intermedius* is common.

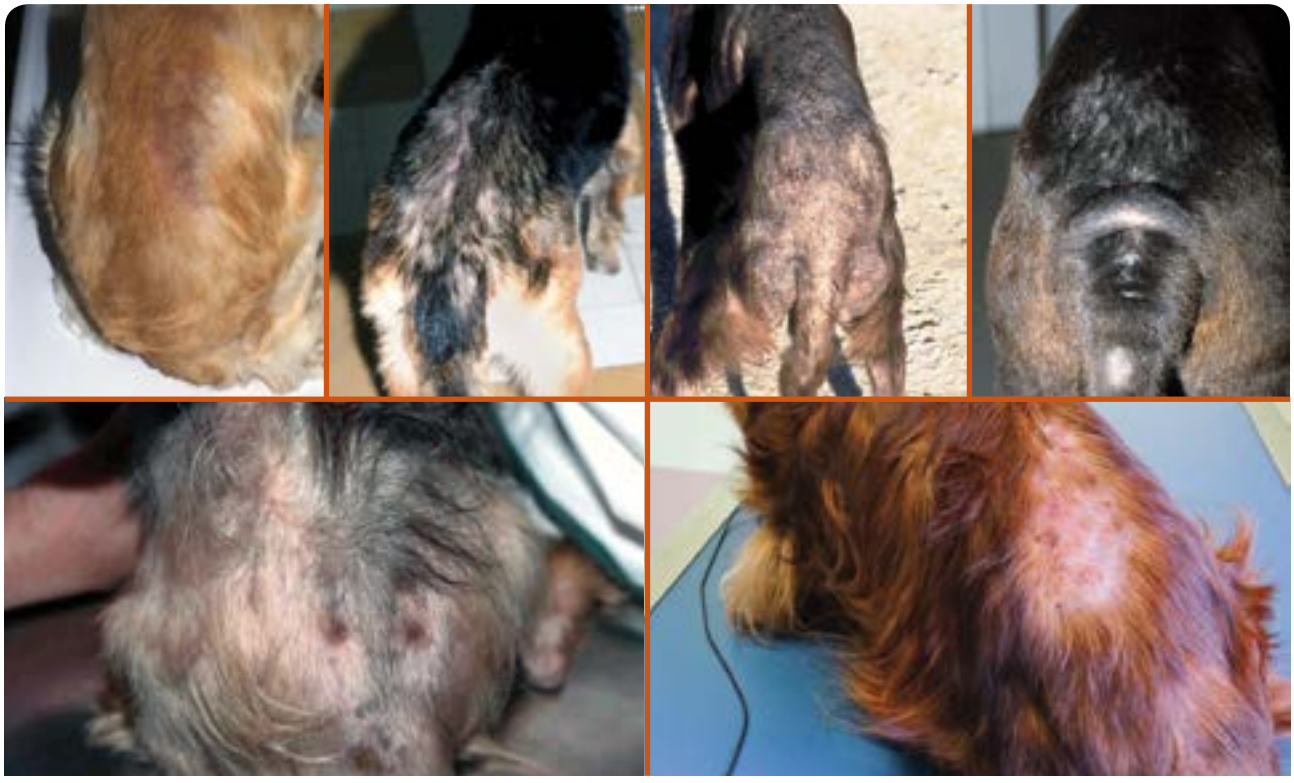


Figure 14. Typical appearance of flea allergy dermatitis in dogs with dorsal alopecia and squamosis. Pictures from Canine Dermatology Guide, E. Guaguère and P. Prélaud, Ed. Merial, 2008.

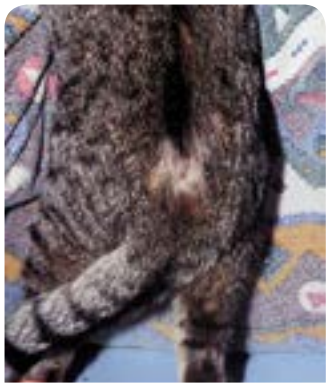


Figure 15. Flea allergy dermatitis in a cat, showing hair loss and hyperkeratosis at the base of its tail.

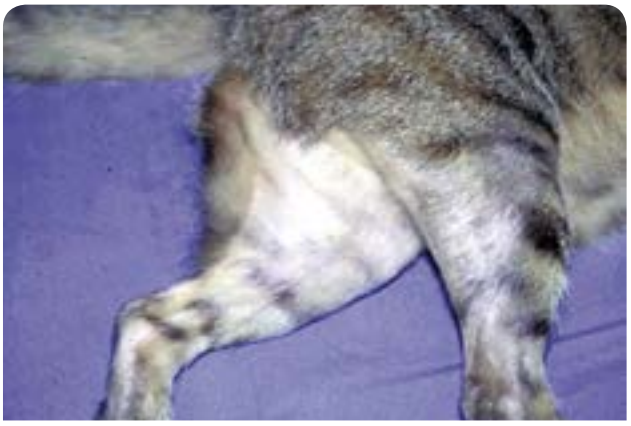


Figure 16. Skin of a cat with flea allergy dermatitis which has licked itself repeatedly.

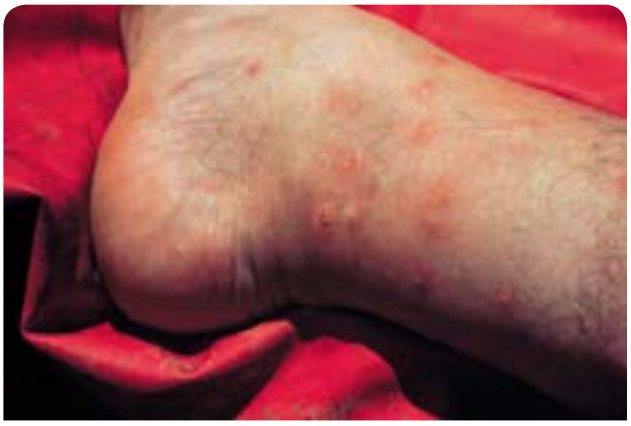


Figure 17. Hypersensitivity to flea bites in a human.

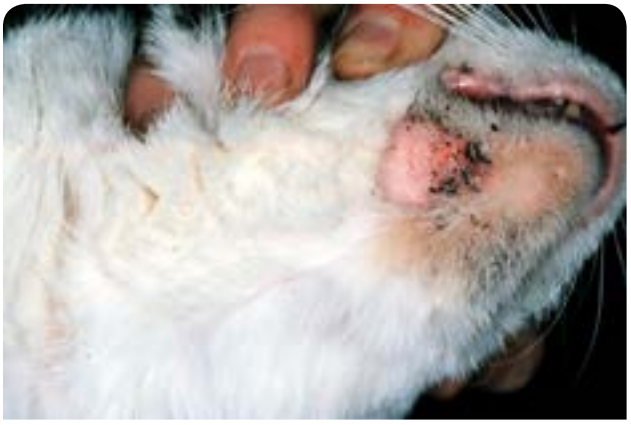


Figure 18. Flea faeces on a cat's chin, with scratching injury.

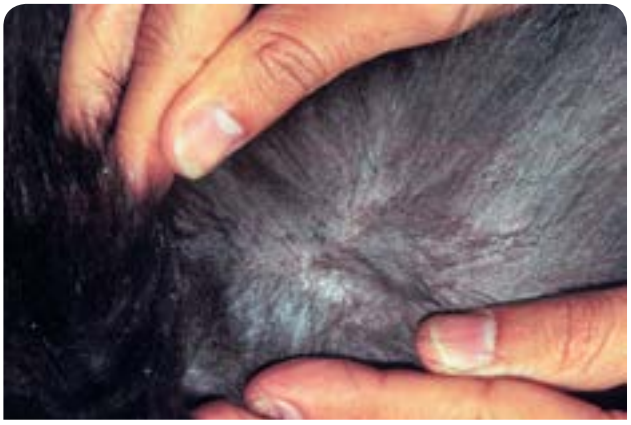


Figure 19. Flea faeces visible in the fur.

Other clinical signs are more specific to cats, such as miliary dermatitis, which is defined by numerous papules and scabs on the back and around the neck, which feels as if it is covered with sand. The animal scratches itself continuously and can even harm itself with its claws. Continuous licking and self-inflicted injury are also possible and hair loss can be seen on the abdominal area, legs, flanks or tail (Figs. 15 and 16) as a result. Flea saliva allergens may also result in feline eosinophilic complex with various clinical manifestations: granuloma or cutaneous eosinophilic plaques, labial ulceration, lymphadenopathy.

Besides this directly pathogenic involvement of fleas, they play an indirect role in pathogenesis by spreading *Dipylidium caninum*, *Taenia* and *Bartonella henselae*, the agent of "cat scratch disease" in humans (Fig. 17).

Diagnosis

The diagnosis of flea infestation relies on finding fleas in the coat, but it sometimes presents great difficulties due to the mobility of the fleas. The tail, ventral face and neck areas must be examined for fleas and finding the insects in the fur can be very difficult. It has been demonstrated that only 5–15 % of fleas carried on an animal are discovered. Finding just one flea, or flea droppings, therefore justifies treatment. The level of infestation is described as average (<5 fleas), high (5–10 fleas) or very high (>10).

Flea faeces are easier to find than the fleas themselves, appearing as a small "comma-shaped" grain of up to 1 mm in length when intact and, when dampened on white paper, the "flea dirt" appear as a reddish colouration due to the flea's blood diet (Figs. 18 and 19).

Role of fleas as vectors

Fleas are competent vectors for numerous pathogens of medical and veterinary importance. Plague and murine typhus have long been known, but cat and dog fleas can transmit other pathogens, such as bacteria responsible for cat scratch disease (a type of bartonellosis), feline anaemia (formerly "haemobartonellosis"), and flea-borne spotted fever (ricketssiosis).

Cat scratch disease due to *Bartonella henselae*

There is abundant literature concerning cat scratch disease and other *Bartonella* infections, and there is little indication that these bacterial infections are increasing or that their epidemiology is changing. However, the number of human cases of cat scratch disease is not decreasing, despite improved flea control, and it seems that bartonellosis is now affecting adults whereas it was formerly considered to be a childhood disease.

Feline infectious anaemia due to *Mycoplasma*

There is literature worldwide concerning the infection of cats by "Haemobartonella", now called *Mycoplasma haemominutum* or *Mycoplasma haemofelis*. The prevalence of infection is usually high (20 % to 40 %), but pathogenicity is still considered to be low, except where there is co-infection, for example, with FIV-FeLV in cats.

Flea-borne spotted fever due to *Rickettsia felis*

Rickettsia felis emerged recently as a new pathogen in humans, responsible for flea-borne spotted fever, also called cat-flea typhus.

This bacterium was first detected in the cat flea, *C. felis*, in 1990, using molecular biology. DNA fragments of this organism were then detected in blood samples taken from the first human patient, in Texas. It is thought to be distributed worldwide, like its main vector, *C. felis.*

The prevalence of *R. felis* can be very high, and it varies with environment and season. Although *C. felis* is the main biological vector for *R. felis*, this bacterium has also been detected in *C. canis*, *P. irritans* (the human flea), and *A. erinacei*. *R. felis* has also recently been found in *X. cheopis*, the Oriental rat flea.

To date, only a few clinical cases have been reported worldwide, although the disease is probably ubiquitous. It may also be misdiagnosed as a tick-borne rickettsiosis. Classic symptoms are fever, maculopapular rash and eschar.

Very few clinical cases due to *R. felis* in animals have been reported in the literature. Interestingly, *R. felis* DNA was found in the serum of a dog living in a house in Spain where two people were suffering from flea-borne spotted fever, evidenced by PCR. The dog did not present with fever, but fatigue, vomiting and diarrhoea were reported. In a similar situation in Germany, a dog from a family in which two people suffered from flea-borne spotted fever was found to be infected by *R. felis* but showed no clinical signs.

Infestation by *Dipylidium caninum*

Although this is not a true vector-borne disease, fleas are the cestode's natural intermediate hosts. Taeniosis due to *D. caninum* should be included in the list of diseases related to the presence of fleas. This cestode can be zoonotic in the event that a human accidentally ingests a flea.

Putative transmission of other pathogens

Some authors have suggested the possible role of fleas as vectors for many pathogens. FIV and FeLV viruses have been studied, but no proof of a vector role has yet been published. It is important to remember that finding pathogen DNA in fleas collected from cats, dogs or other animals through PCR only means that the flea has ingested the pathogen (dead or alive, whole or fragmented) in the blood of its host, and nothing more. There has recently been some controversy regarding the role of fleas in the transmission of *Leishmania infantum* to dogs, but the distribution of canine leishmaniosis would likely be very different if fleas could act as vectors. Experimental designs using natural transmission are necessary to demonstrate a vector role: from host to vector, and from vector to host. In the case of fleas, transovarial transmission should also be demonstrated, bearing in mind that adult fleas do not usually move from one host to another.

Control measures

Fleas both on the animal and in its environment must be considered to prevent infestation. Preventive agents must have two properties: an immediate effect and a long-lasting effect (sustained action) (Fig. 20). To interrupt the insect cycle, fleas must be eliminated before they can reproduce and lay eggs, therefore before the end of the first 36 hours, as indicated on most insecticides. Some products have a rapid effect (less than 24 hours) combined with sustained efficacy and these interrupt the cycle completely. The combination of IGR and insecticides to treat dogs and cats has been proven to be useful to accelerate flea eradication in the environment, especially when owners do not treat for fleas regularly.

Controlling fleas in the environment requires all of the potentially infested areas (sleeping areas, carpets, cars, furniture) to be defined, which can be difficult. The environment should be considered in its larger context, not only the habitat, or places where the animal travels to, but also other animals (other cats, dogs) which may come into contact with the animal. Where possible, all animals encountered should be included in the prevention programme and regularly treated for flea infestation. Environmental (household) formulations usually contain both an insecticide and a growth regulator (juvenile hormone analogue) which interfere with the normal development of immature stages. Diffusers are a volumetric treatment which enables widespread distribution of insecticide molecules. Sprays are particularly useful for inaccessible zones but, as these products cannot reach all environmental life stages, efficacy remains partial.

Figure 20. Anti-flea treatment applied topically (A) or given orally (B) to a dog.

Video 7.1
The flea risk.

Video 7.2
Adult fleas in a dog's fur.

Lice infestation

General comments

Lice infestation in dogs is most commonly caused by the chewing (or biting) louse ***Trichodectes canis*** (family Trichodectidae; Fig. 1) than by the sucking louse ***Linognathus setosus*** (family Haematopinidae; Fig. 2), characterised by an elongated head, like other sucking lice, such as the human louse *Pediculus humanus*. Mixed infestations can also occur.

The only cat louse is the chewing louse ***Felicola subrostratus*** (Fig. 3).

Lice infestation is a rare ectoparasitosis in domestic pets but a common one in both stray cats and dogs. It can be found on pets which lead an outdoor life, bringing them into direct contact with other animals. Louse species are very host-specific, and there is no risk of transmission between pet species, or to humans.

Lice are small, grey-brown insects, dorsoventrally flattened and wingless. All louse species spend their entire life on the host and are very host-specific. Adult female lice lay individual eggs and attach them to individual hairs. Immature lice (nymphs), resembling small adults, hatch from the egg after approximately 1 to 2 weeks and then develop into mature adults over the next 3 weeks.

Figure 1. Adult *Trichodectes canis*.

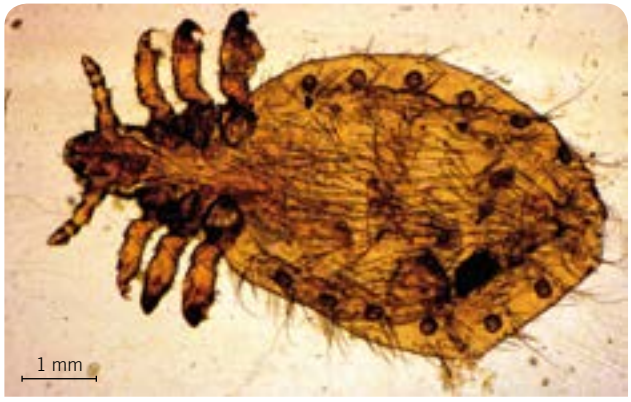


Figure 2. Adult *Linognathus*.

Figure 3. Adult *Felicola subrostratus*.

Figure 4. Adult *Heterodoxus spiniger*.

Morphology and biology

Lice belong to the order Phtiraptera, and suborder Anoplura (sucking lice) or Mallophaga (chewing lice).

Louse infestation in cats is only caused by the chewing louse *F. subrostratus* (Fig. 3). Chewing lice have a head which is broader than their thorax, unlike the sucking lice, which have a narrow head. *F. subrostratus* is taxonomically close to *T. canis*, the chewing louse infesting dogs and they both have visible antennae (which defines the superfamily Ischnocera), composed of three segments (family Trichodectidae) with one claw at the end of each leg.

Unlike cats, dogs can be infested by *T. canis* (Fig. 1) but also by the sucking louse, *L. setosus* (Fig. 2). In tropical countries, dogs can also be affected by *Heterodoxus spiniger*, another chewing louse (family Boopidae) which originated in marsupials and has adapted to canids (Fig. 4).

Felicola and *Trichodectes* lice are small (1–2 mm long), yellowish, dorsoventrally flattened, and wingless.

Life cycle of chewing lice (Mallophaga)

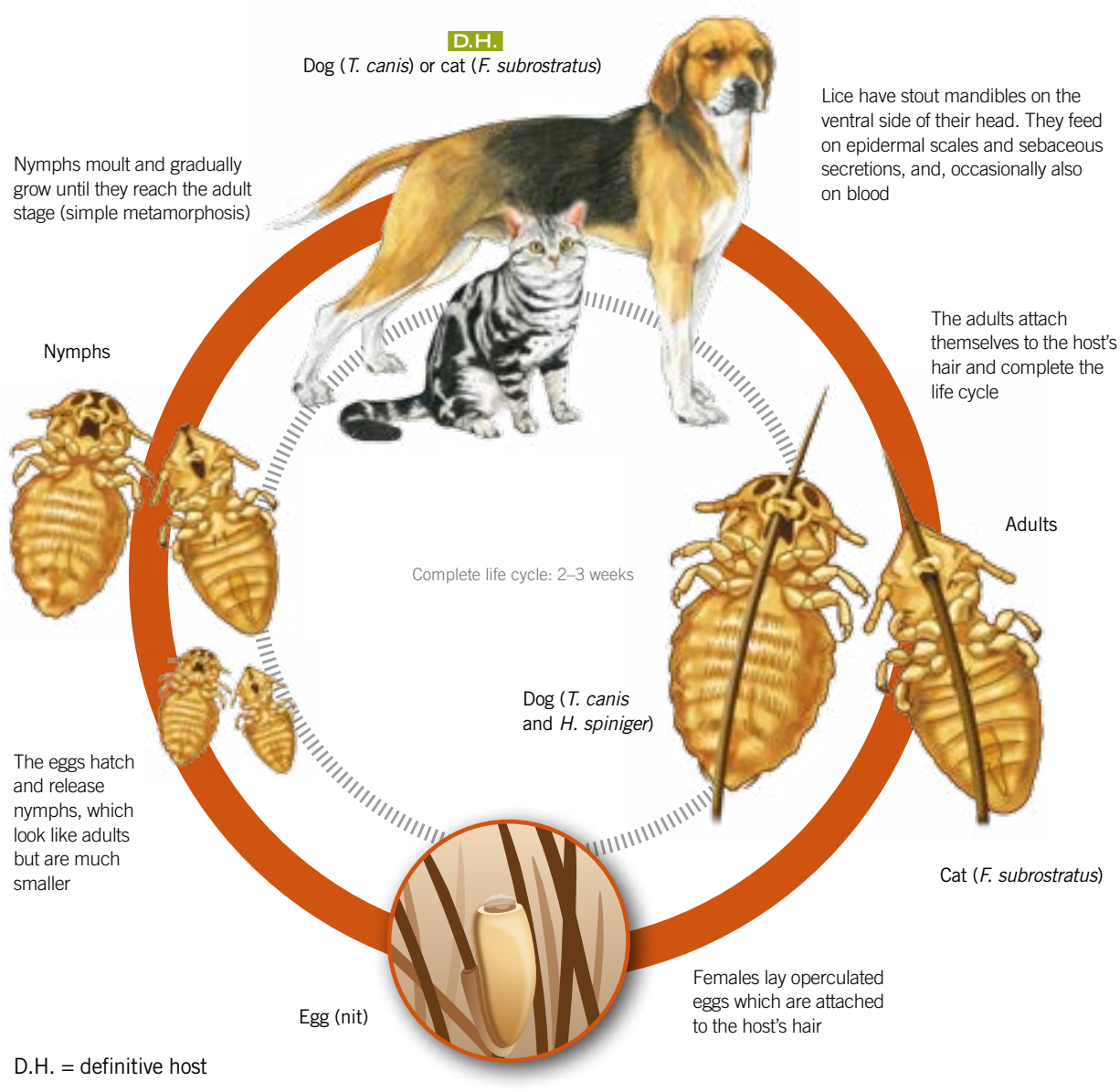
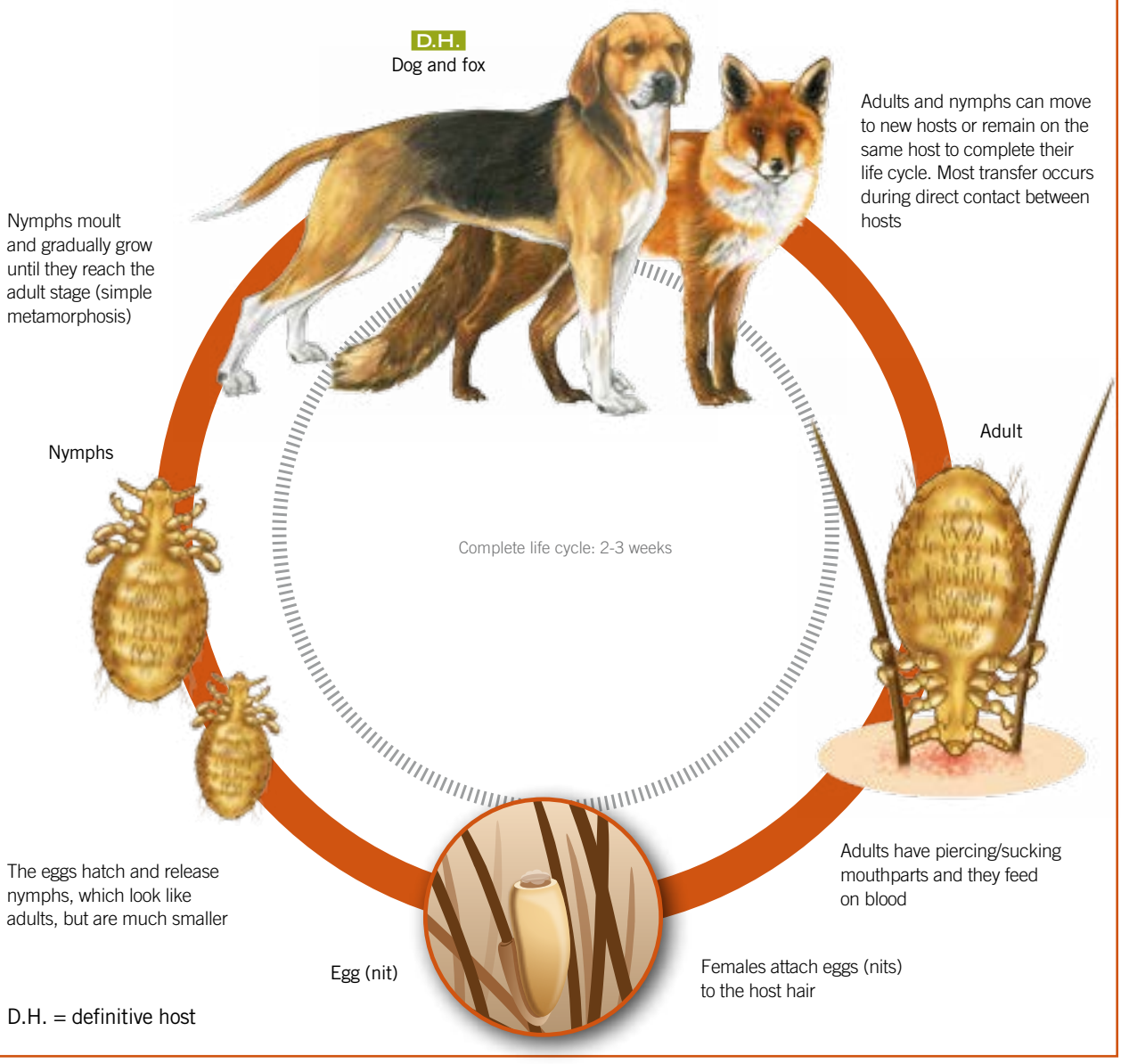


Figure 5. Louse egg (nit).

Life cycle of sucking lice (Anoplura)



The head of *Felicola* is triangular with the point directed forwards (Fig. 3), whereas the head of *Trichodectes* is rectangular (Fig. 1). The head has a median longitudinal groove which fits around the host's hair and helps the louse attach during egg laying (Figs. 5 and 6).

Adult lice have a claw at the end of each tarsus which allows them to remain attached to the coat and they are found mostly around the hair base. Lice are permanent parasites that feed on skin scales, cutaneous debris, hair, and inflammatory exudate where there is dermatitis. They remain on the surface of the epidermis and can move quite quickly. It can be hard to see the adults.

The females lay their whitish eggs (called nits) at the base of the hairs. These nits are operculated, 1 mm long and attached to the hair by cement so, as the hairs grow, nits are found along their whole length (Fig. 7). They hatch out in about 6 days, giving rise to nymphs (sometimes also called larvae), which look like smaller and less sclerotinised adult lice (immature stages of these paurometabolic insects are morphologically similar to adults, apart from their size). Nymphs moult three times before becoming adults and the life cycle usually takes about 3–4 weeks. Each female lays about 300 eggs and lives between 6 and 8 weeks.

Transfer of lice between hosts is by close contact or fomites, such as grooming equipment. Lice do not like cold or hot conditions and need to feed continuously, so they cannot survive off the host for more than a few days (3–4 days at the most). Kittens and puppies often catch lice from their infested mothers and louse numbers tend to be highest on young, elderly and debilitated animals. The latter is due to an inability to self-groom effectively, which normally helps keep louse numbers low. Clinical signs are therefore seen in these animals, and some healthy animals carry a low burden without obvious signs.

Lice are less common on domestic pets nowadays and regular flea treatments are assumed to play a role in eliminating them.

Figure 6. *Felicola subrostratus* egg.

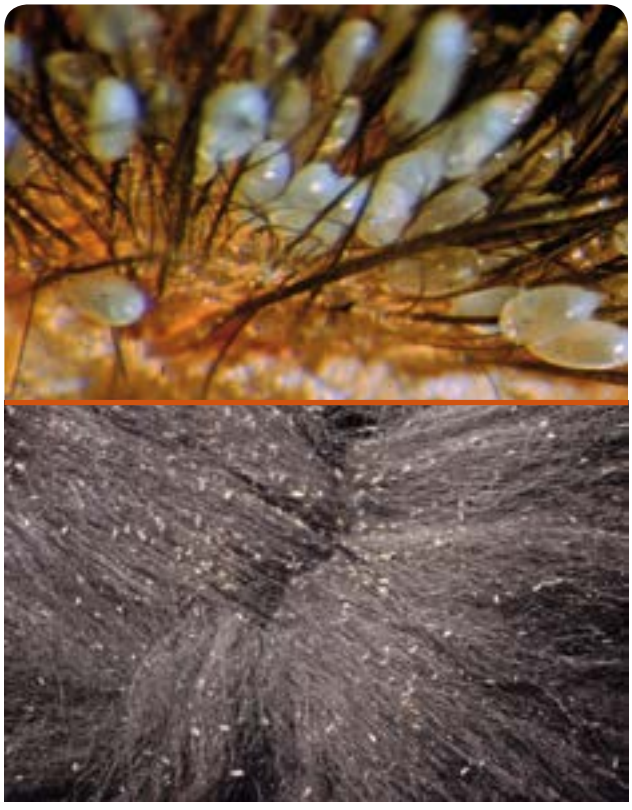


Figure 7. Louse eggs.

Chewing lice (*T. canis, F. subrostratus* and *H. spiniger*) have abrasive mouthparts that bite the skin, and then the disrupted surface layer is ingested. This activity causes pruritus that increases as louse numbers increase, resulting in secondary trauma, caused by rubbing or scratching, that may appear as areas of alopecia.

L. setosus may be tolerated relatively well as it causes less pruritus but it can result in substantial blood loss and anaemia when present in large numbers.

Clinical signs

Small numbers of lice may go unnoticed but heavy infestations, particularly of chewing/biting lice, may cause alopecia and dermatitis. Depending on coat colour, louse eggs cemented to hairs may be easily visible (particularly against dark hairs) or the lice themselves may be visible against pale skin. A heavy *L. setosus* infestation may cause anaemia.

Diagnosis

Lice are visible to the naked eye and are characteristically flattened dorsoventrally. Eggs can be seen as pale structures attached to hairs (Fig. 8).

Figure 8. Lice infestation in a dog.

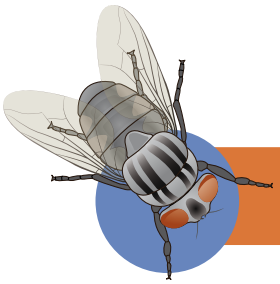
Control measures

A range of insecticides are recommended for treatment of chewing lice on cats and dogs, and all animals of the same species in the household should also be checked and/or treated at the same time as the animal with the louse infestation.

No treatments are indicated for sucking lice in dogs, but treatments indicated for chewing lice should be effective.

A single treatment may be all that is required if the product's residual activity ensures that immature lice hatching from eggs after application will also be killed (egg shells are not very permeable to insecticides). However, it is worth checking that there are no remaining signs of lice after approximately a month, particularly where infestation has been heavy, to evaluate whether further treatment is necessary.

Although it has not been studied, the insect growth regulators (IGRs) used to control fleas (i.e., pyriproxyfen, (S)-methoprene, lufenuron) may have an effect on louse reproduction and development of the nymphal stages.



Myiasis

General comments

Myiasis is a seasonal condition caused by dipteran insect larvae (maggots). These facultative or obligate parasites contaminate wounds and/or healthy skin. Carnivores are occasional hosts for species which are mainly found in sheep, but also in cattle, horses and pigs. Dogs are more commonly infested than cats.

Biology

There are only four genera of myiasis-causing flies in Europe: ***Wohlfahrtia*** (***W. magnifica***), ***Lucilia*** (***L. sericata***) (Fig. 1), ***Calliphora*** and ***Musca*** (Figs. 2 and 3). Other flies are found in other regions: ***Cochliomyia hominivorax*** (South America), ***Dermatobia hominis*** (South America), ***Curetebra*** **spp.** (North America) and ***Cordylobia anthropophaga*** (tropical Africa) (Fig. 4).

Dogs can sometimes be infested with flies that normally feed on carrion, such as the flesh fly *Sarcophaga* (Fig. 5). These flies cause opportunistic myiasis.

Only the larvae are parasitic, obligate or facultative depending on the species and they feed on tissues. The three larval stages seen in healthy skin or wounds use their buccal sclerites (hooks) to meander through skin and subcutaneous connective tissue.

Free-living adults are active in the summer and they survive the winter in the external environment as pupae. The life cycle of all species is quite rapid in the active season, with some important differences relating to external temperature.

Wohlfahrtia magnifica are attracted by warm-blooded animals and lay approximately 150 larvae on healthy or damaged skin.

Figure 3. *Musca* houseflies.

Figure 1. *Lucilia sericata.*

Figure 4. *Cordylobia* sp. stage 3 larva.

Figure 2. *Musca* sp. fly.

Figure 5. *Sarcophaga sp.* fly.

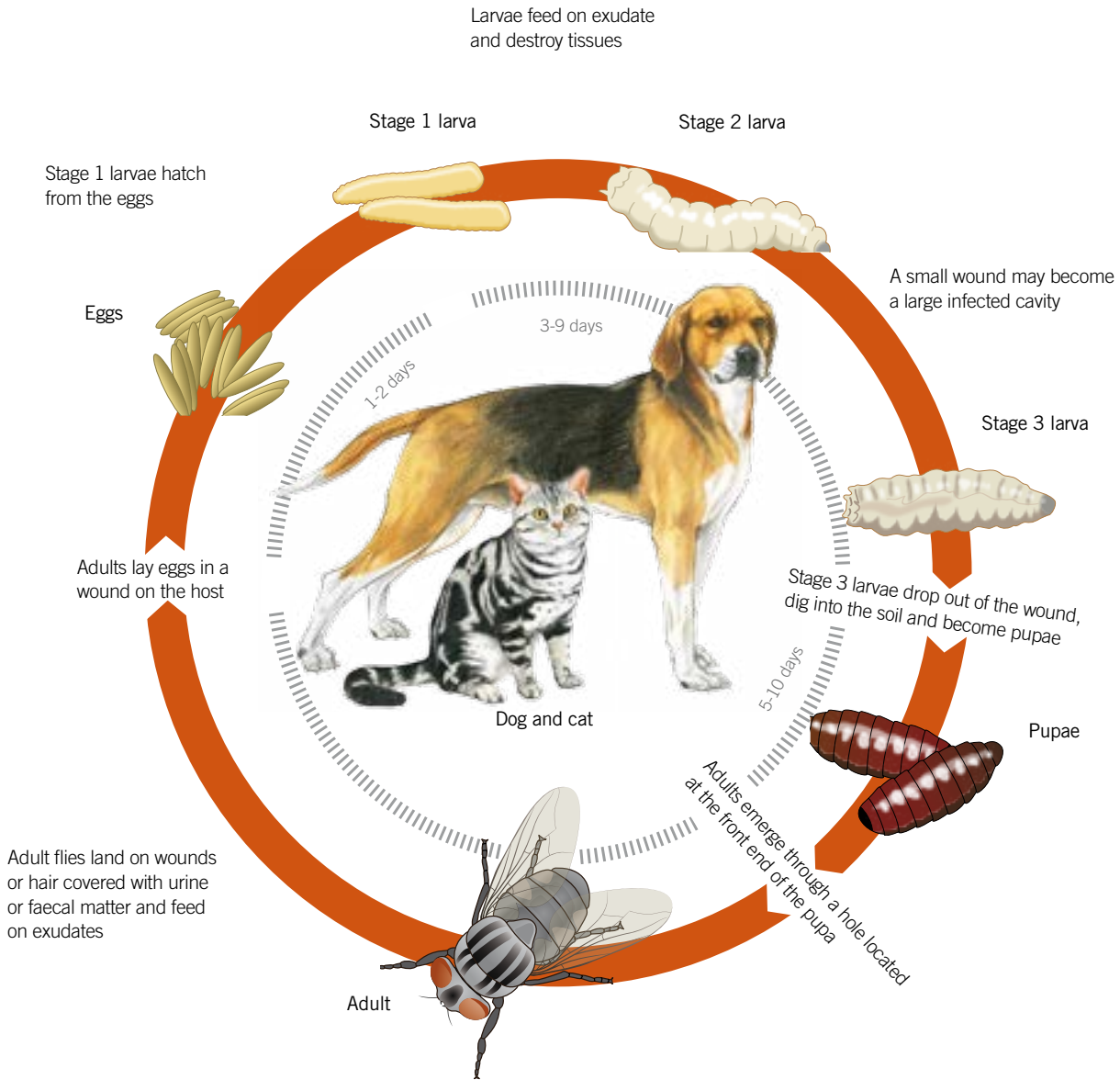
Lucilia sericata are attracted by moist areas of the body, skin lesions, urine and diarrhoea, where they lay several thousand eggs in clumps of 50–100.

Cutaneous myiasis mostly occurs in the summer. Predisposing factors include unprotected wounds, constant moisture on the body (caused by urine and faecal staining in recumbent, debilitated dogs, for example) and close contact with sheep (especially *W. magnifica* and *L. sericata*).

Powerful hooks and proteolytic enzyme secretion give larvae a great capacity for tissue destruction which then attracts more flies.

D. hominis and *C. anthropophaga* larvae may also parasitise humans.

Life cycle of flesh flies



Clinical signs

Dermatological signs: painful and often foul-smelling areas of ulceration, draining tracts and necrosis. A channel can sometimes be seen at the point of larval penetration and maggots are often visible (Fig. 6). Pressure points (legs, elbows), lips, vulva, prepuce and tail are most affected (Figs. 7 and 8). Systemic signs are variable and include fever, lethargy, anorexia and pain, shock and signs relating to the underlying condition.

Diagnosis

Diagnosis is based on medical history, clinical signs and identification of larvae.

Microscopic examination of the larvae, especially their stigmatic plates, can be used to identify species.

Prognosis is often very guarded, particularly if the animal is systemically ill.

Control measures

Treatment involves clipping the affected area, thorough cleaning of infested wounds and irrigating the site with an appropriate antiseptic solution. Larvae must be removed by hand and necrotic areas surgically resected. Wound dressings should be applied to protect against further fly strike. Systemic antibiotics, anti-inflammatories and analgesics are also indicated.

It can sometimes be difficult to remove all the larvae, and some form of insecticide may be needed. This can be topical or systemic (macrocyclic lactones, isoxazolines), and should be applied on several occasions.

Prevention consists of eliminating predisposing factors: good hygiene for recumbent, debilitated dogs and insecticides for susceptible sheep are recommended.

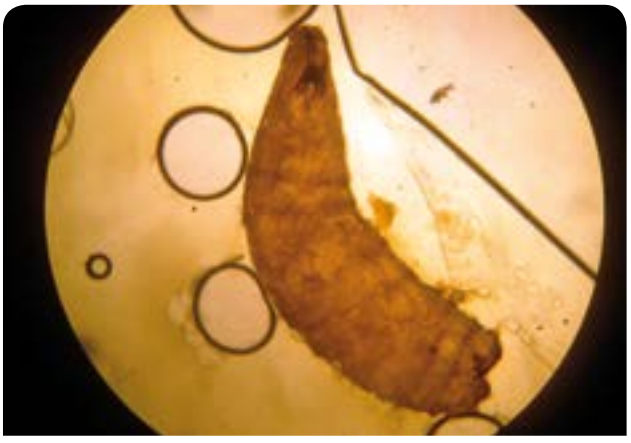


Figure 6. Maggot.

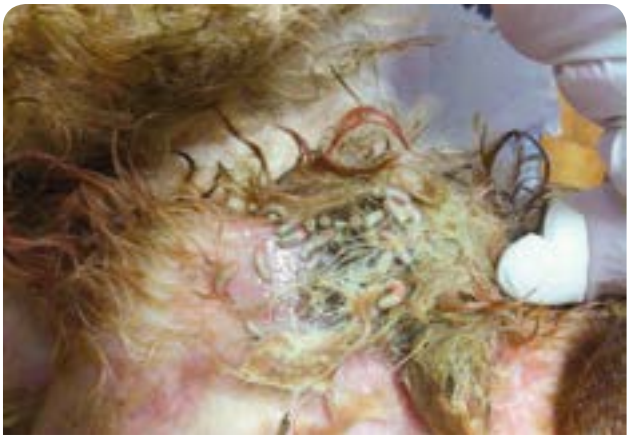


Figure 7. Myiasis in a dog.

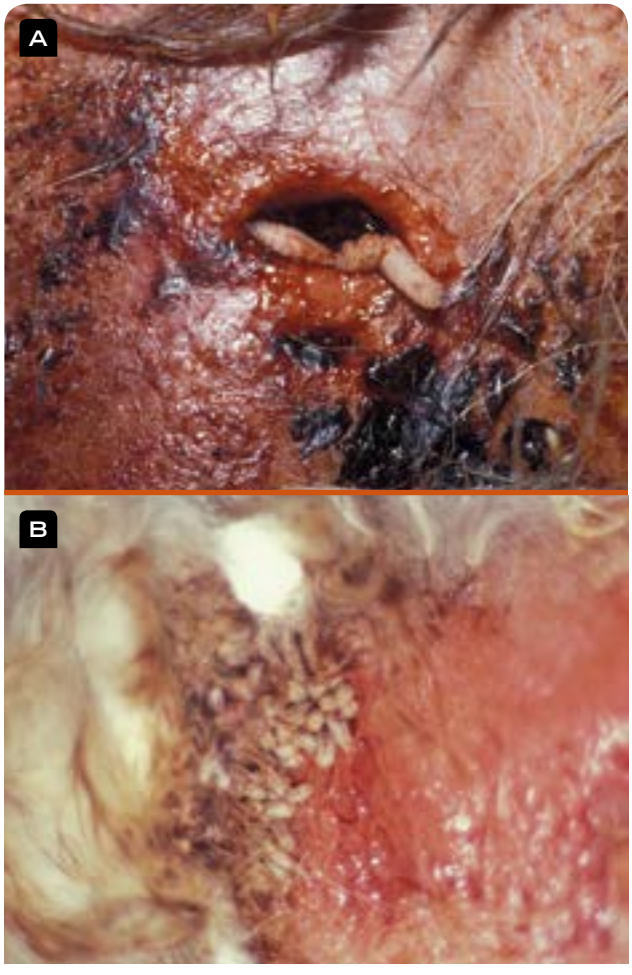


Figure 8. Myiasis lesions in (A) a dog and (B) a cat. Courtesy of Blaise Hubert.

Table 1. Major species responsible for myiasis in the dog.

Myiasis-causing flies	Classification	Morphology
Musca spp. (worldwide)	Diptera, Brachycera, Cyclorrhapha, Muscidae, Muscinae	• Adult: black and whitefly with mouth parts (proboscis) designed for licking. *Musca domestica* (common house fly) 6–9 mm. • Larva: white to yellowish maggot, blackish buccal sclerites clearly visible. 1–6 mm according to stage (L1 to L3). Anterior end thin, final segments larger. Posterior stigmatic plates in L3 composed of three very sinuous clefts.
Lucilia sericata (worldwide, common in Europe)	Diptera, Brachycera, Cyclorrhapha, Calliphoridae	• Adult: 5–10 mm. Metallic green, sometimes copper-coloured. Dorsal thorax hairy. • Larva: white to yellowish maggot, blackish buccal sclerites clearly visible. 1–6 mm according to stage (L1 to L3). Anterior end thin, final segments larger. Posterior stigmatic plates in L3 composed of three very straight clefts.
Cochliomyia hominivorax (tropical America)	Diptera, Brachycera, Cyclorrhapha, Calliphoridae	• Adult: 8–10 mm with blue-green metallic sheen. Three black bands on thorax. • Larva: white to yellowish maggot, blackish buccal sclerites clearly visible. 1–6 mm according to stage (L1 to L3). Anterior end thin, final segments larger. Posterior stigmatic plates in L3 composed of three very straight clefts.
Cordylobia anthropophaga (tropical Africa)	Diptera, Brachycera, Cyclorrhapha, Calliphoridae	• Adult: Cayor fly, tumbu fly. 6–12 mm. Yellowish-brown. • Larva: white to yellowish maggot, blackish buccal sclerites clearly visible. 1–6 mm according to stage (L1 to L3). Anterior end thin, final segments larger. Posterior stigmatic plates in L3 composed of three very straight clefts converging on the button.
Calliphora spp.	Diptera, Brachycera, Cyclorrhapha, Calliphoridae	• Adult: flesh fly. Large with metallic sheen. 12 mm. Thorax and abdomen steely blue, reddish eyes. • Larva: whitish maggot, blackish buccal sclerites clearly visible. 1–6 mm according to stage (L1 to L3). Anterior end thin, final segments larger. Stigmatic plates, very pronounced in L3, composed of three very straight clefts converging on the terminal button.
Dermatobia hominis (South America)	Diptera, Brachycera, Cyclorrhapha, Cuterebridae (related to Oestridae)	• Adult: large (12 mm). Dark blue mouth with orange eyes. • Larva: first stage (3 mm) subcylindrical. Spines on each segment. Very characteristic second stage (5–6 mm) divided into anterior globular part (11 segments) and posterior part extending to the tail. Last stage (10 mm) cylindrical, with small spines on intermediate segments and two distinct clumps of spines on the anterior surface.
Wohlfahrtia magnifica	Diptera, Brachycera, Cyclorrhapha, Sarcophagidae	• Adult: hairy, greyish fly with no metallic sheen. 8–14 mm. Round spots on abdomen. • Larva: yellowish maggot, blackish buccal sclerites clearly visible. 1–6 mm according to stage (L1 to L3). Anterior end thin, final segments larger. Posterior stigmatic plates very pronounced in final segment of L3, and composed of three very straight clefts.
Sarcophaga spp.	Diptera, Brachycera, Cyclorrhapha, Sarcophagidae	• Adult: hairy, greyish fly with glossy sheen. 8–12 mm. Black lines on abdomen. • Larva: yellowish maggot, blackish buccal sclerites clearly visible. 1–6 mm according to stage (L1 to L3). Anterior end thin, final segments larger. Posterior stigmatic plates very pronounced in final segment of L3, and composed of three very straight clefts.

Precise identification of dipteran larvae is very difficult and can only be carried out by a specialist. Identifying adults is easier although there are many dipteran species.

General description of dipteran larvae: usually three stages, L1, L2 and L3.

Larvae (sometimes called maggots, especially those of the fly family Muscidae) usually conical, pointed in front and truncated behind.

Larvae have 13 segments but the first two are fused (so only 12 are visible). Acephalic, eyeless, no antennae. Mouth with cephalopharyngeal chitinous exoskeleton; two anterior hooks (labial sclerites) and two posterior, pharyngeal sclerites used in classification.

Caudally, larvae have two respiratory stigmatic plates, each consisting of a chitinous, often circular, plate or peritreme, with a false orifice (button) and respiratory orifices, grouped together in three sinuous or straight stigmatic clefts.

Video 8
Cutaneous myiasis on a dog.

Flying insect bites

A number of flying insects can cause problems in animals, such as the Diptera and Hymenoptera.

Diptera, Brachycera

Biting flies include stable flies (***Stomoxys calcitrans*** [Fig. 1]), horn flies (***Haematobia irritans***), horse flies (***Tabanus* spp.**) and deer flies (***Chrysops***). They can induce severe pruritic lesions, such as crusts, erythema and oedema, often localised on the nose, face and ears (Fig. 2). Reactions are caused by hypersensitivity to the biting insects" antigenic saliva.

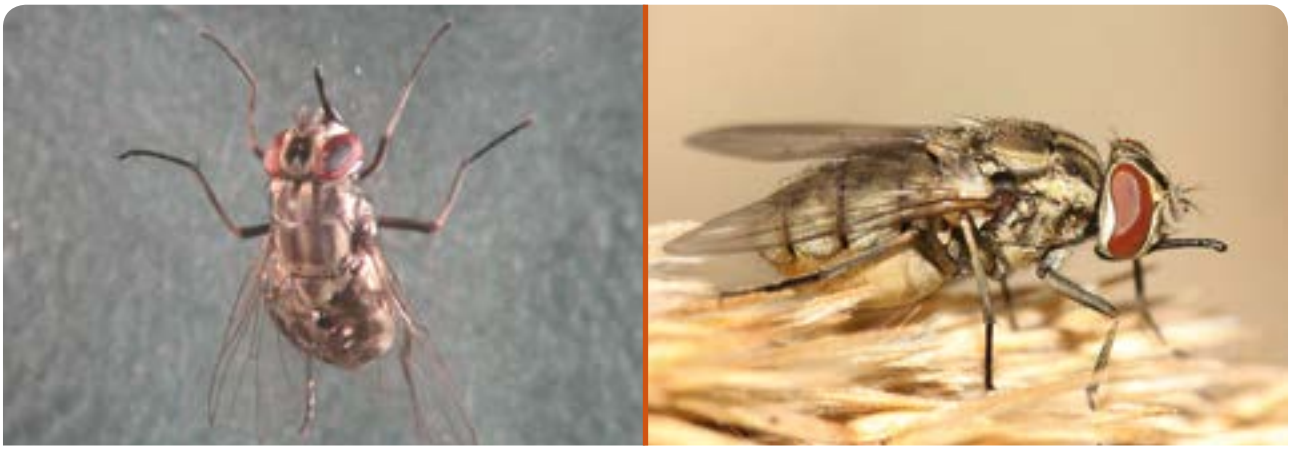


Figure 1. *Stomoxys calcitrans.*

Figure 2. Ear bite lesions due to *Stomoxys* in dogs.

Diptera, Nematocera

Mosquitoes (***Culex***, ***Aedes*** [Fig. 3], ***Anopheles***), sandflies (***Phlebotomus*** [Fig. 4], ***Lutzomyia*** [Fig. 5]), black flies (***Simulium***) and midges (***Culicoides*** [Fig. 6]) also bite both dogs and cats, and have been associated with hypersensitivity reactions.

Hymenoptera

Various hymenoptera (bees, wasps, yellow jackets, hornets, etc.) can sting dogs and cats. Their venom is irritating and can result in hypersensitivity reactions, including localised swelling, urticaria, anaphylaxis, and a condition known as facial eosinophilic folliculitis and furunculosis.

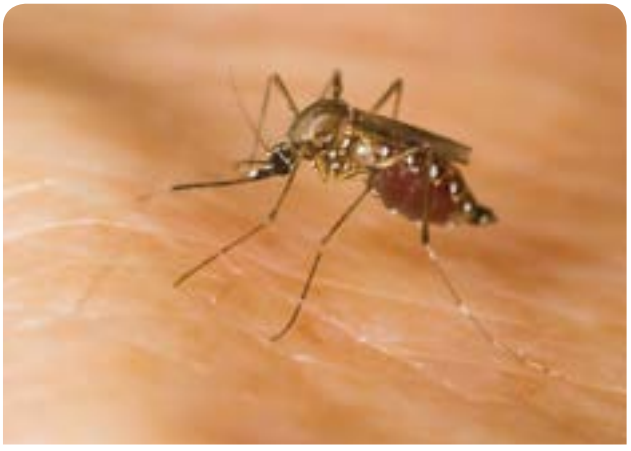


Figure 3. Mosquito feeding.

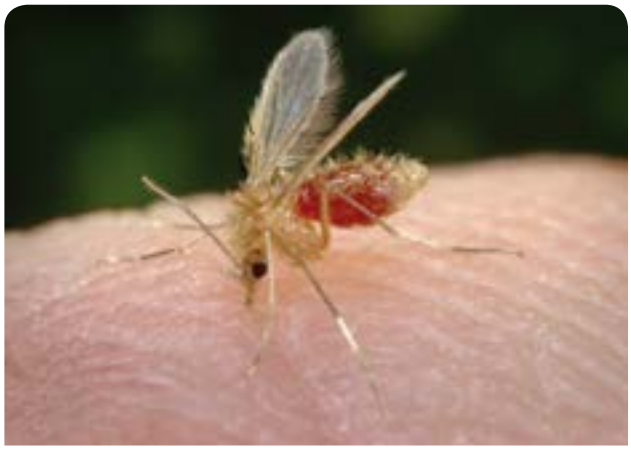
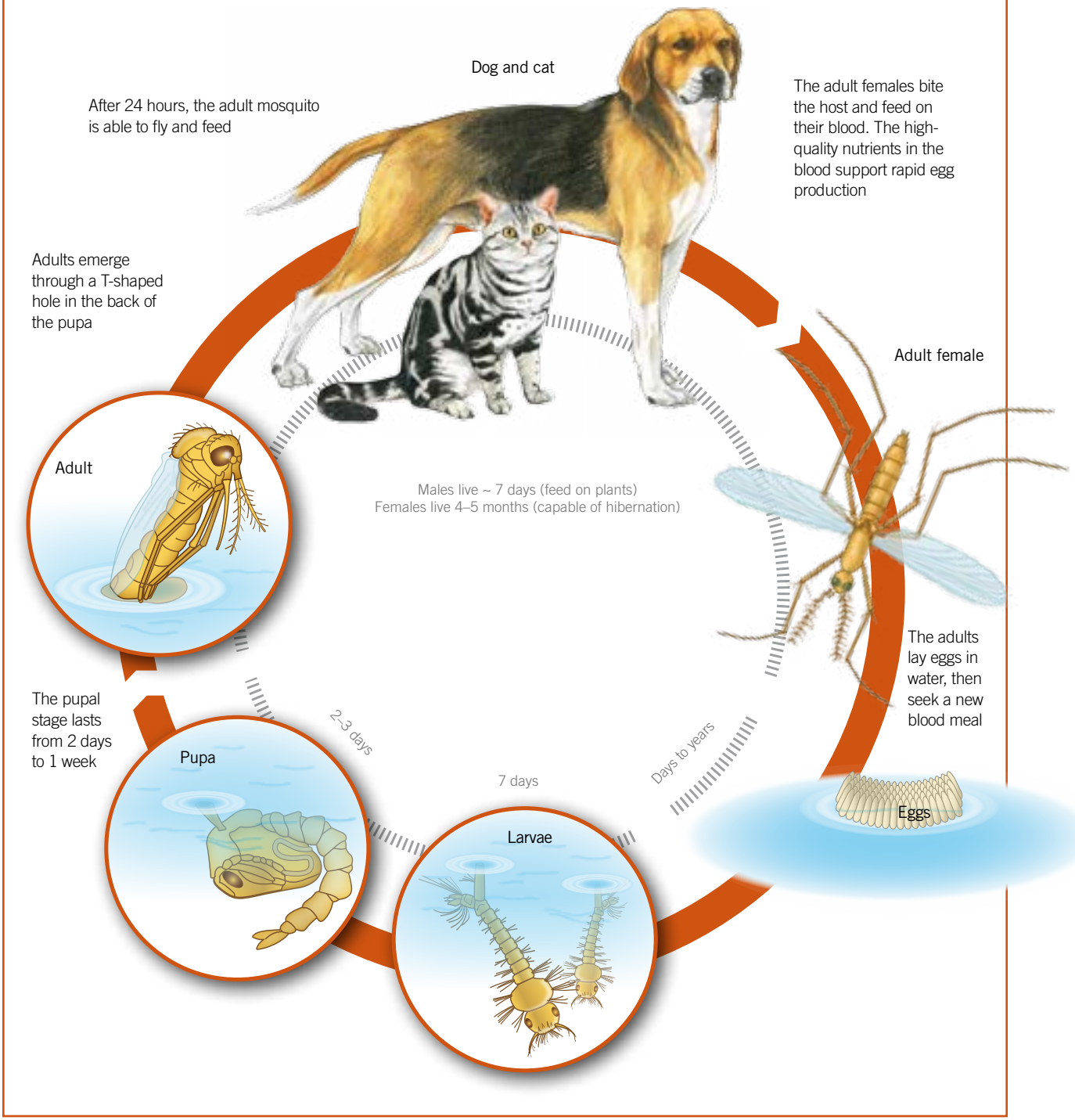


Figure 4. Sandfly feeding.

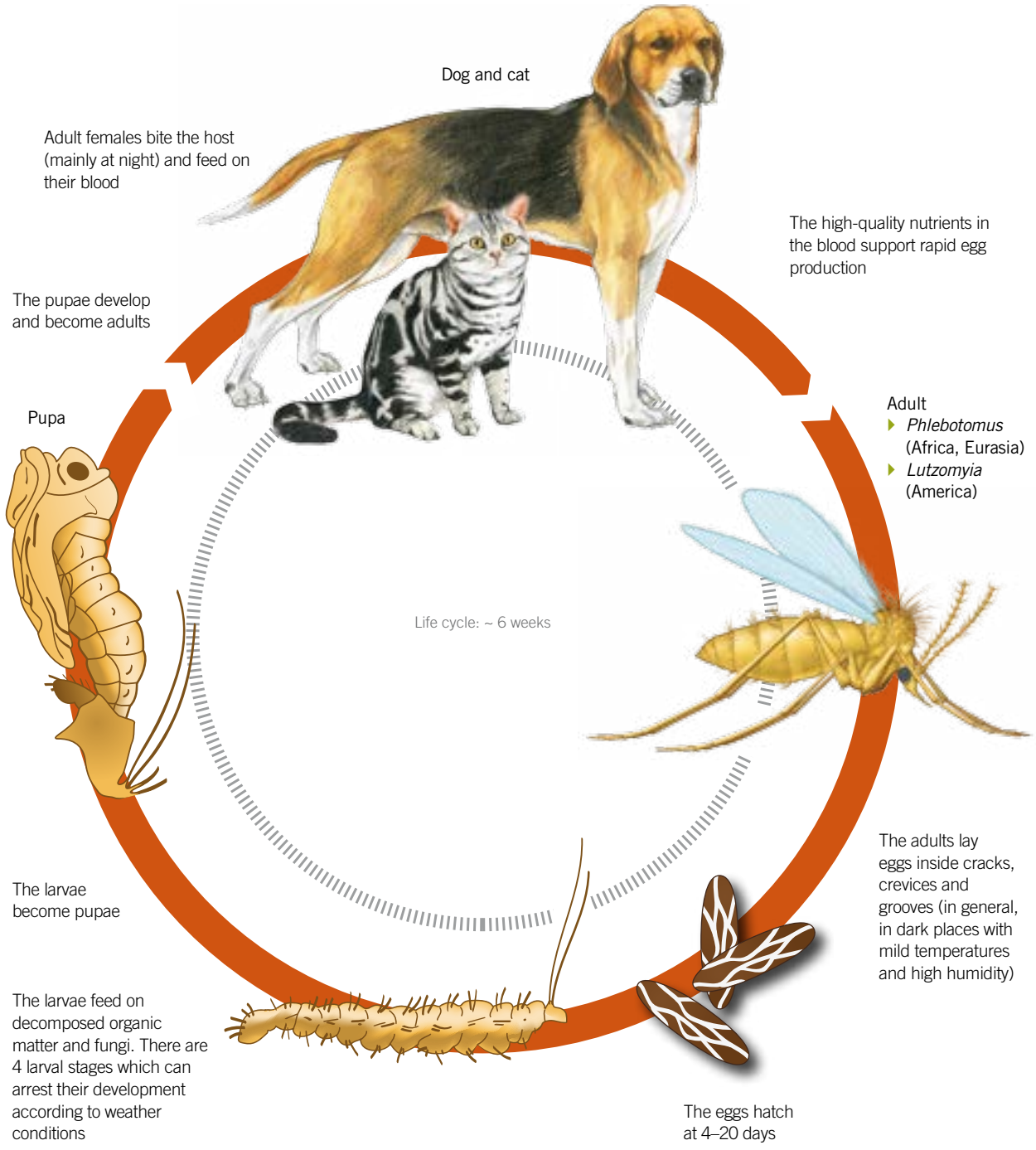
Figure 5. Sandfly after feeding.

Figure 6. Midge feeding.

Life cycle of mosquitoes



Life cycle of sandflies



Acarioses

Tick infestation

Introduction

Ixodidae, known as hard ticks, are giant mites. They have adapted to live in all habitats and to feed on any kind of host, from reptiles to mammals. In industrialised countries, they are also known for their major impact on animal health, including pets or sport animals (dogs, horses) and livestock (cattle). They are vectors for many pathogenic agents: viruses, bacteria, protozoa or helminths, some of which are common in humans and animals (Colwell et al., 2011). The most important tick genera infesting pets worldwide are ***Ixodes***, ***Rhipicephalus***, ***Dermacentor***, ***Amblyomma*** and ***Haemaphysalis*** (Fig. 1).

Ixodes sp. male.

Rhipicephalus sp. male.

Dermacentor sp. male.

Amblyomma americanum female.

Haemaphysalis longicornis male.

Figure 1. Major tick genera.

Tick infestation is very common in pets and can be seasonal in temperate zones, or year-round in warmer regions.

Distribution and density of the ticks which infest pets and humans is variable, as are the pathogens transmitted. The reasons for these changes are related to climate change (warmer winters in temperate zones), wildlife populations (increasing numbers of wild boars, foxes, deer, and rodents), human activity (creation of parks, riversides, walking trails in woodlands, forest management, spread of suburban areas with many gardens and green areas) (Fig. 2), pets travelling all around the world. It appears that the tick threat is now an increasing concern in many parts of the world.

Figure 2. Ticks infesting a human.

Main characteristics of ticks

Definition

Ixodoidea, a superfamily of the order Acari, comprises two main families: hard ticks (Ixodidae) and soft ticks (Argasidae). Ixodidae are characterised morphologically by a denticulate rostrum and a chitinous dorsal shield (scutum). They are characterised by three life stages: larva, nymph and adult, each requiring only one blood meal before developing into the next stage. The main genera are *Hyalomma* (27 species), *Amblyomma* (143 species), *Rhipicephalus* (79 species, including *Boophilus* species), *Dermacentor* (38 species), *Haemaphysalis* (166 species) and *Ixodes* (249 species).

Argasidae are mainly bird and reptile parasites, characterised morphologically by a downward-curving rostrum (on the underside of the head) and by the lack of a scutum, which together define their status as soft ticks. They are characterised by the succession of one larval stage, four to six nymphal stages, and one adult stage, and by having several blood meals in the nymphal and adult stages (only one in the larval stage). These ticks belong to three main genera: *Argas* (58 species), *Ornithodoros* (37 species), and *Otobius* (2 species). *Argas* ticks are often found under roofs where birds nest (pigeons in cities) and they can feed on mammals (carnivores, humans) when their usual host is unavailable. *Otobius megnini* is distributed in the Americas. It usually locates in the ears of mammals, including dogs and cattle (see life cycle, page 253).

The next paragraphs will only focus on hard ticks, given their medical and veterinary importance .

Geographical distribution and host preference

Hard ticks are distributed worldwide but each species is restricted to a particular biotope and climate (Fig. 3). Populations in each habitat may be subject to marked seasonal changes.

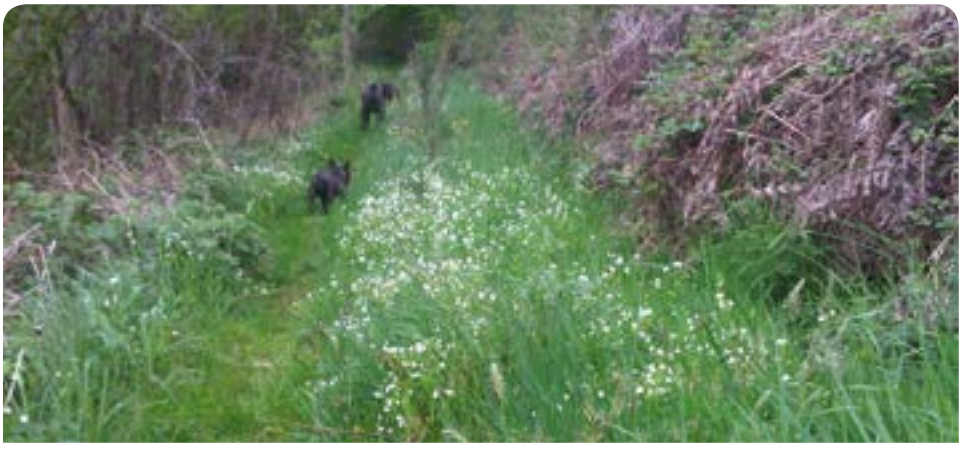


Figure 3. Typical biotope for the hard tick *Ixodes ricinus*.

Some ticks are adapted to desert conditions, especially the genus *Hyalomma*, such as *H. dromedarii*. Some are adapted to humid tropical climates, especially *Amblyomma* ticks like *A. variegatum* and *A. maculatum*. Others favour warmer climates, either tropical or Mediterranean, especially *Rhipicephalus* ticks (e.g., *R. sanguineus*). Some species are more adapted to cold temperatures (*D. reticulatus*, *I. ricinus*, *I. pacificus*) and/or cold continental climates (*I. ricinus*, *I. scapularis*).

Different biotopes may be suitable for the different free-living stages (larvae, nymphs and adults waiting for a host) of the same species. For example, *I. hexagonus* (hedgehog tick) and *D. reticulatus* (marsh tick) larvae and nymphs may be found in rodent or rabbit burrows, while adults are found in grass. All life stages of *I. scapularis* and *I. ricinus* (forest ticks) are found in grass, preferably under the forest canopy, but they do not have the same host preference.

Particular preferences correspond to particular parasitic life stages: larvae and nymphs may have a tropism for some hosts, such as micromammals (field mice, voles, hedgehogs) or birds, while adults look for herbivores (cattle, deer, horses) or canids, which is the case in *I. scapularis* and *I. ricinus*. This preference can be strict, or less so. For example, *Rhipicephalus (Boophilus) microplus*, the tropical cattle tick, is mainly restricted to cattle and almost never infests humans or pets; on the other hand, many other ticks have a non-restricted tropism, for example *Ixodes* ticks which can bite any mammal in the absence of their preferred hosts.

Figure 4 shows the distribution of ticks of medical and veterinary importance in Europe.

Figure 4. Distribution of *Dermacentor reticulatus* (A), *Ixodes ricinus* (B) and *Rhipicephalus sanguineus* (C) in Europe.

Abundant Present Sporadic reports

Courtesy of Luís Cardoso, Robert Farkas, Domenico Otranto, Kurt Pfister, Xavier Roura, Smaragda Sotiraki, Donato Traversa and Richard Wall.

* *Dermacentor reticulatus* is distributed in a highly focal pattern within its geographical range. This map represents the trend, but clustered foci of higher density are possible.

Morphology of hard ticks

Stages

- Larvae: 0.5–1 mm, hexapods (Fig. 5).
- Nymphs: 3–5 mm before blood meal, octopods (Fig. 6).
- Adults: 5–10 mm before blood meal, up to 30 mm for engorged females (Fig. 7), octopods, sexually dimorphic.

Figure 5. Tick larva - *Ixodes*.

Figure 6. Tick nymph - *Ixodes*.

Figure 7. Engorged female - *Rhipicephalus*.

Anatomy

- **Anterior extremity: mouthparts or gnathosoma**

  The gnathosoma is found in the capitulum comprising the rostrum or hypostome (single piece, toothed), two chelicerae and two pedipalps. Pedipalps are tactile organs that help the tick choose biting sites. Chelicerae, which end in harpoon-like structures, pierce the skin, anchor the tick to the skin after muscle contraction and help the hypostome penetrate the skin. Backward-pointing barbs on the hypostome secure the attachment. Ticks are either brevirostris (*Rhipicephalus*, *Haemaphysalis*, *Dermacentor*) or longirostris (*Ixodes*, *Amblyomma*, *Hyalomma*), according to the length of the rostrum. The tick always anchors its rostrum sideways because its gnathosoma cannot be bent (Fig. 8).

Figure 8. Tick rostrum.

- **Body or idiosoma**
  - **Dorsal surface:** the protective cuticular shield, or dorsal scutum, is more developed in males which cannot swell up, whereas females can increase in size substantially, given their smaller dorsal shield (surface area can increase 15-fold).
  - **Ventral surface:** the genital opening is located between the second pair of legs, the anal opening between the fourth pair of legs, and the two respiratory spiracles or peritremes are located directly behind the fourth pair of legs. The location of the anal groove, anterior or posterior to the anal opening, is a characteristic used to classify ticks into either Prostriata (*Ixodes*) or Metastriata (all other genera).
- **Legs**

  Each leg is composed of six segments: coxa, trochanter, femur, patella, tibia and tarsus. The tarsus of the first pair of legs bears Haller's organ, a complex sensory apparatus sensitive to vibrations and carbon dioxide, which is involved in host detection (Fig. 9). The pretarsus consists of paired claws and a sticky organ (adhesive pads) (Fig. 10).

It should be noted that ticks also bear other sensory organs:

- In female ticks, the dorsal surface and both sides of the capitulum are made up of open pores, which allow them to detect males and communicate with other ticks using pheromones.
- In both males and females, photosensitive organs ("eyes") can be present or absent, depending on the genus.

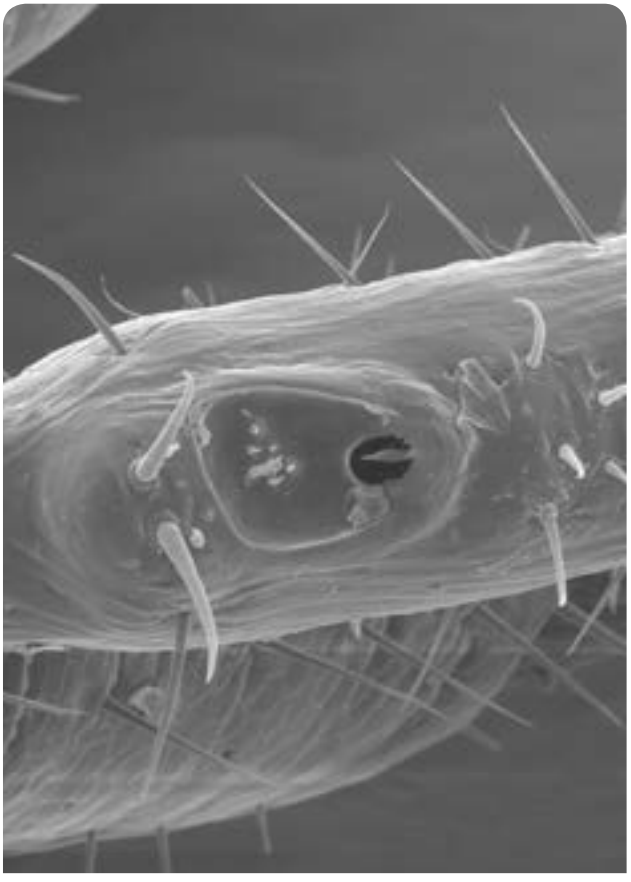


Figure 9. Haller's organ.

Figure 10. Tick claw and sticky pad to infest host.

Biology and ecology

Ticks are temporary ectoparasites and are not intermittent feeders like mosquitoes, or permanent parasites like lice. An obligate alternation occurs between free-living and parasitic stages.

Free-living stage

Variations in the living environment

Ticks which have endophilic stages must be distinguished from those with exophilic stages:

- **Endophilic domestic ticks**: *R. sanguineus* (all stages are found in the host dog's environment: on kennel floors, walls, etc.).
- **Exophilic ticks**: *I. ricinus*, *D. reticulatus*. All stages are found in forests and woods, along field margins, in vacant lots, public gardens and parks, riversides, etc. Some exophilic ticks are "troglodytes": found in the environment, preferably in burrows rather than on the surface, which is the case for *I. hexagonus* (the hedgehog tick).

Different stages of the same species may be either endophilic or exophilic.

Climate variations

In addition to the biotope, temperature and humidity play key roles in determining the presence of one species or another.

- **Hygrophilic ticks**: require humidity and do not tolerate excessive heat and desiccation.
  This is the case for many *Ixodes* such as *I. ricinus* in Europe, *I. scapularis* in the USA, *I. persulcatus* and *I. ovatus* in Asia and Japan, and *Dermacentor* ticks like *D. reticulatus* in Europe and *D. variabilis* in America.
- **Xerophilic ticks**: live in warm areas and may tolerate desiccation, but not frost.
  This is the case for *R. sanguineus*. Other may prefer warm, humid conditions and be susceptible to desiccation, like *H. longicornis* in Asia.

When conditions are unfavourable to activity, ticks undergo **diapause**. Ticks found in temperate and continental climates undergo winter and dry-season diapause. Resumption of activity often depends on the season e.g., spring and autumn peak in Western Europe) but activity can also resume during warm spells in winter, even temporarily. This can explain the occurrence of canine babesiosis cases throughout the year, when weather changes "wake up" *Dermacentor* ticks.

Parasitic stage

Choice of host

Ticks can be differentiated by their host preference. Euryxenous (polytropic) ticks are not host-specific and feed on a wide range of animal species. Stenoxenous (monotropic) ticks exhibit a narrow host preference and this affinity may also vary between life stages.

- ***I. ricinus* and *I. scapularis***: larvae and nymphs usually feed on micromammals whereas adults target ungulates (domestic and wild ruminants, wild boars). Larvae and nymphs can nevertheless bite birds or just about any mammal that they encounter (human, dog, cat, etc.), which is why Lyme disease can be transmitted to humans and dogs.
- ***I. holocylus*** (Australian paralysis tick): larvae, nymphs and adults usually feed on marsupials (small and large) but they can also bite any other mammal that they encounter (human, dog, cat, etc.), which explains cases of tick paralysis (involving the neurotoxin, holocyclotoxin, which is secreted with the tick's saliva) seen in dogs and children in northern and eastern Australia (mainly Queensland).
- ***D. reticulatus:*** larvae and nymphs usually feed on micromammals, whereas adults will await a passing dog or horse.
- ***H. longicornis***: larvae and nymphs usually feed on micromammals, but adults target ungulates (domestic and wild ruminants). All stages can also bite any mammal that they encounter (human, dog, cat, etc.), which is why this tick is the vector for canine babesiosis in Japan.
- ***R. sanguineus:*** all stages prefer to parasitise canids, but cats may also be infested.

Attachment to the host

Ticks find their hosts by detecting heat, vibration, shadows, breath (CO_2) and odour. They use the Haller's organs located on the tarsus of first leg, which help them locate a host and gauge their distance from it. In order to locate their host, ticks rely on features such as heat, smell, sight and touch.

Figure 11. Questing female *Dermacentor*.

Figure 12. Questing female *Ixodes*.

Ticks can be separated into two groups:

- **Questing ticks** (Figs. 11 and 12): these climb onto vegetation during the day, usually during the hours when the host is active (early hours of the day and dusk). They then remain immobile and wait for their host to approach (Fig. 13). They climb down to the ground and bury themselves in the at night or when conditions are unfavourable. **This category includes** *Ixodes*, *Dermacentor*, *Haemaphysalis*, *Boophilus*.
- **Hunting ticks:** these are highly mobile and will follow their hosts and they can "run" quite fast. This is the case for some *Amblyomma* and *Hyalomma*, and also *R. sanguineus* to a certain extent.

These two habits are quite similar to those seen in spiders, which belong to the order Araneae and the same Arachnida class as the Acari. Some spiders spin a web and wait for their prey to get caught while others hide and attack their prey.

Ticks infest theirs hosts rather quickly, in a matter of seconds. They usually do not jump down onto the host since they do not live in trees, but close to the ground and on stretches of grass. They grab onto the host with the sticky pad on their tarsus and then use their legs and claws to crawl through the fur and over the skin, to find a suitable place to attach and feed (Figs. 2 and 14).

Figure 13. Questing female *Ixodes* on grass. Courtesy of Phil Ward.

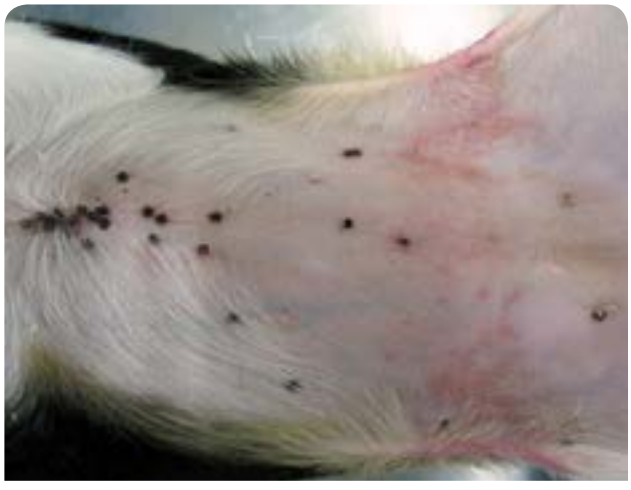


Figure 14. *Haemaphysalis longicornis* attached to a dog.

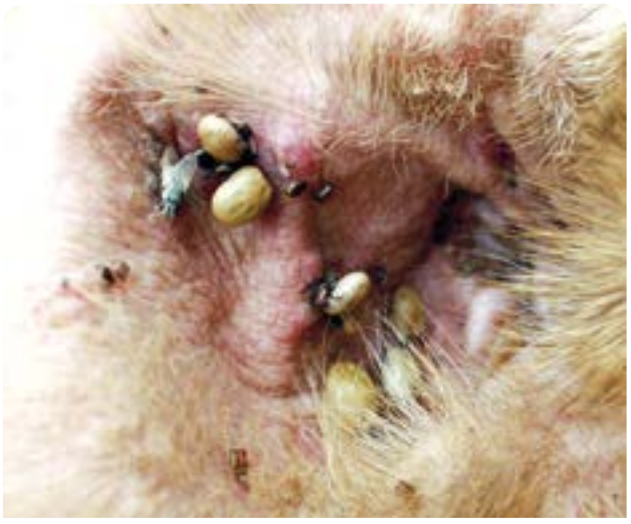


Figure 15. Attached ticks in a dog's ear.

Figure 16. Attached *Rhipicephalus sanguineus* ticks.

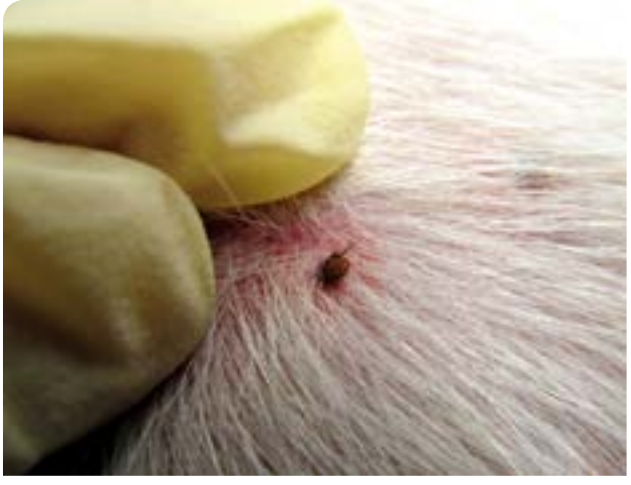


Figure 17. Attached *Ixodes scapularis*.

Choice of attachment site

Ticks usually favour areas of thin skin, such as the ears, underside of the limbs, scrotum, udder, etc.

Rhipicephalus ticks prefer to attach around or in the dog's ears (Figs. 15 and 16) while *Dermacentor* and *Ixodes* show less preference for this site (Fig. 17).

After infesting their host, it takes an average of 4 to 6 hours for the tick to find its attachment site.

Attachment

- The process of tick attachement involves many specific organs of the tick, such as the chelicerae and hypostome. First, the tick uses its tactile pedipalps to locate the attachment site, then it attaches with its chelicerae, which look like harpoons with two terminal hooks. These penetrate the skin and are retracted by the tick through muscular contraction which allows the skin to be penetrated obliquely by the hypostome.
- Upon attachment, the tick secretes cement for 10–30 minutes (primary and secondary cement produced by types II and III salivary gland acini). This cement is made up of glycoproteins that polymerise on exposure to air and skin.
- As soon as attachment occurs, some pathogens may be transmitted and the first pathogenic agents to be inoculated must be present in the saliva and immediately infective. This is the case for viruses, which may be inoculated within 15 minutes after the start of attachment.

Nutrition - blood meal

All stages feed: larvae, nymphs and adults (female and male). Males feed smaller volumes and can bite several times. It was thought that *Ixodes* males did not feed but it has now been proven that they may ingest some fluids, like in other genera.

The meal lasts 3 to 7 days on average. Larvae and nymphs feed for shorter periods (3 to 5 days) than females (5 to 7 days).

The meal is not strictly a blood meal, as it is for mosquitoes, as it contains not only blood but digested tissue and many leucocytes. Food intake occurs in two phases:

- The first, which can be called a preparatory phase, involves intense secretory activity during which the tick produces enzymes and peptides, inducing immunomodulatory, anticoagulant and proteolytic effects. This phase creates an area of haemorrhagic necrotising liquid through the digestion of subcutaneous tissue which attracts many leucocytes (monocytes, phagocytes, granulocytes). This preparatory phase lasts at least 3 days, during which the tick exchanges fluids with the host. The volume of the female tick does not increase much during this phase (from 2 to 30 mg).
- The second phase is the rapid ingestion phase: the tick ingests the fluids and cells and the volume of the female increases dramatically (from 30 to 250 mg) (Figs. 18 and 19). The female concentrates the ingested meal and excretes the excess fluid in order to prevent osmotic shock.
- Each phase involves the activity of different salivary gland acini. Besides enzymes, the saliva contains peptides which act as cytokines with an immunomodulatory role. This prevents a protective immune response by the host, which is uncommon in mammals except guinea pigs, which develop basophilic hypersensitivity to tick bites. This immunomodulation attracts many white blood cells , and these will form part of the meal and "help" the process of localised necrosis to occur. This also favours pathogen transmission, as occurs in other vectors, such as mosquitoes (*Plasmodium*) or sandflies (*Leishmania*).
- Pathogenic agents can be transmitted throughout the whole blood meal. The directly infective bacteria in the saliva are inoculated quite quickly, an average of 3 to 24 hours after attachment. This applies to *Ehrlichia*, *Anaplasma*, *Rickettsia*, etc. Other pathogens, such as *Borrelia burgdorferi sensu lato*, must undergo multiplication and antigenic variation to become infective, so transmission occurs later, usually after 36 to 48 hours. *Babesia* sporozoites have to become infective and migrate to the saliva so they are usually transmitted 48 to 96 hours after attachment.
- Females will actively detach at the end of the blood meal, and fall off the host onto the ground.

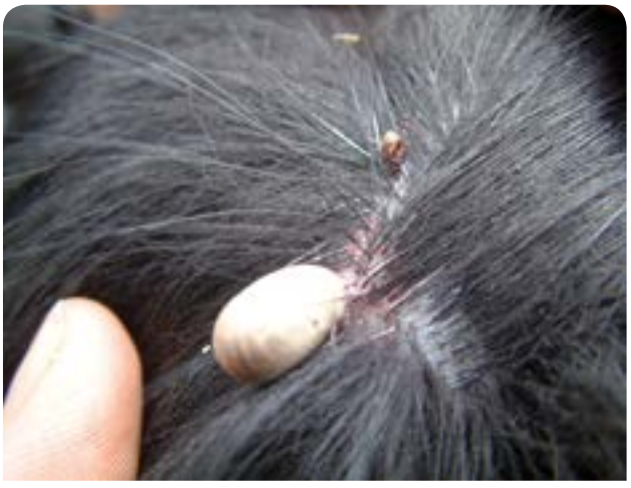


Figure 18. Engorged female tick inducing erythema.

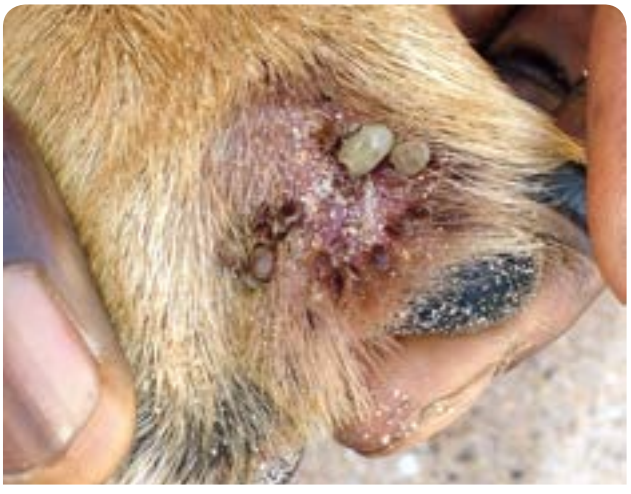


Figure 19. Engorging *Rhipicephalus* in kennelled dog.

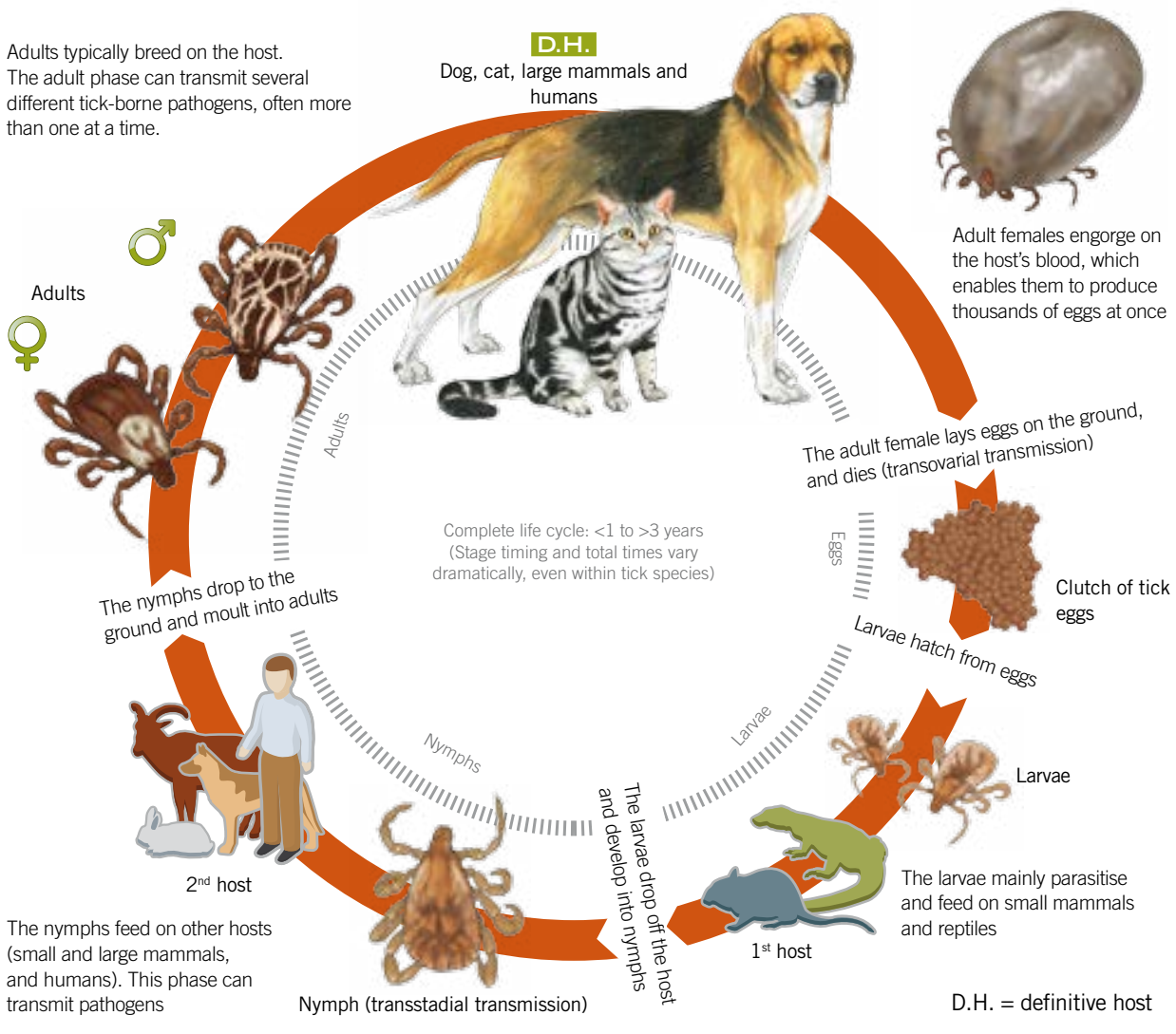
Figure 20. Engorged *Rhipicephalus* ready to lay eggs.

Figure 21. Ticks mating.

Figure 22. *Rhipicephalus* female laying eggs. Courtesy of Emanuele Brianti.

PDF

Life cycle of *Ixodes, Amblyomma, Dermacentor, Haemaphysalis* and *Hyalomma* ticks

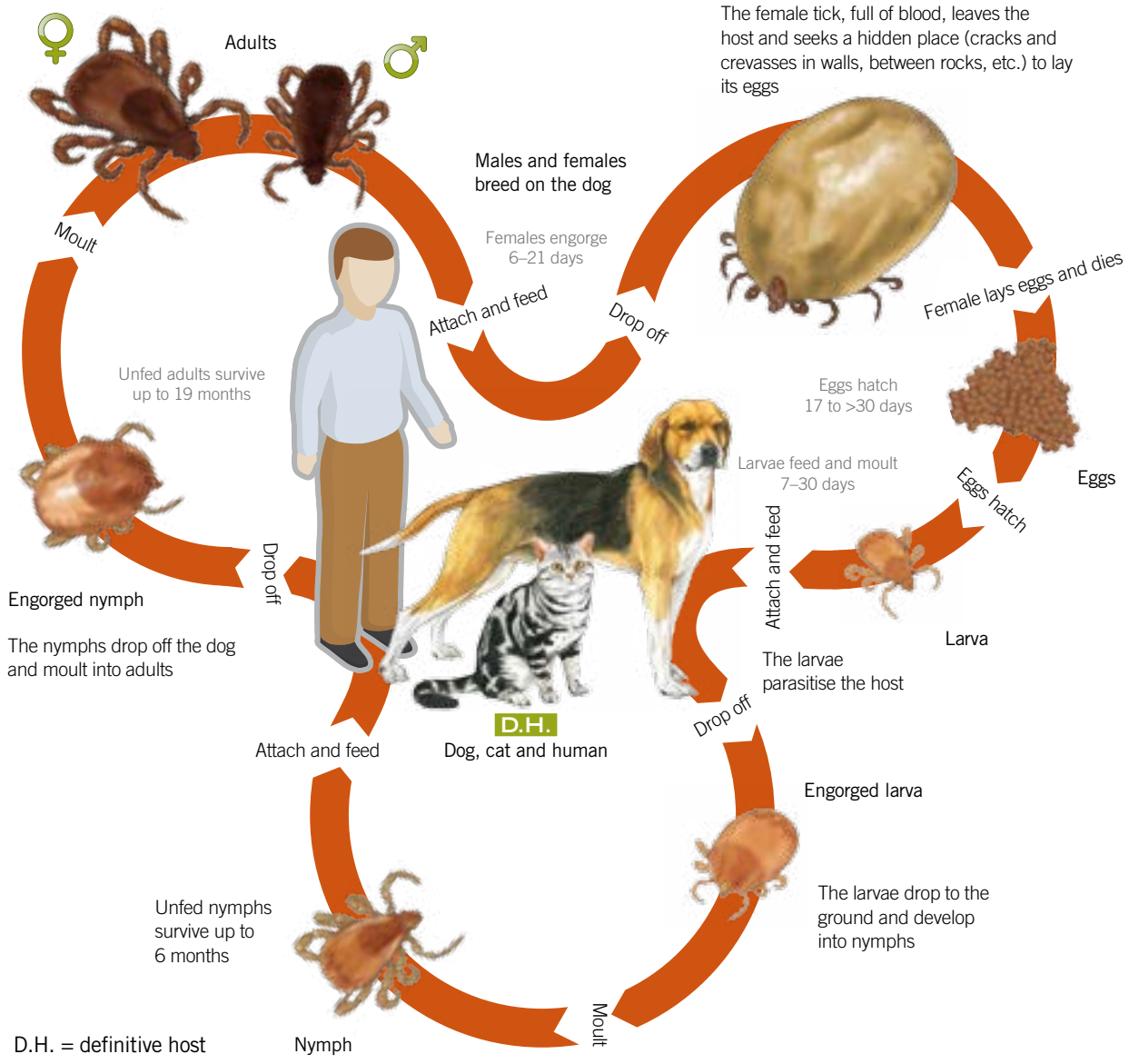


Reproduction and egg-laying

Females and males mate on the host and mating occurs before or during the female's meal (Fig. 20). Ticks of some genera secrete pheromones to attract ticks of the opposite sex. Pathogens can be exchanged between one tick and another during co-feeding at the same location.

In utero egg formation starts during the female's blood meal and, after the females fall off their host onto the ground, they will search for a crevice to hide in, and lay their eggs within 48 to 72 hours. The females bend their anterior extremity which splits dorsally on the capitulum (camerostomal fold). The egg-laying phase will last 48 to 72 hours and the female will die at the end. The eggs are protected and clustered together by a yellow lipid wax and an average of 3,000 to 10,000 eggs is laid at once (Figs. 21 and 22).

Life cycle of *Rhipicephalus sanguineus*



Life cycles

Originally, all ticks moulted in the environment, but some genera and species have evolved to moult on their host.

- **The triphasic life cycle:** each stage requires one meal, then the engorged stage drops off the host onto the ground and moults. The new stage, fasting, awaits a new host to complete the cycle. All important ticks encountered in Europe, North America, and Asia undergo this cycle, so every tick generation requires three hosts (one each for the larval, nymphal and adult stages).
- **Biphasic cycle:** the larva moults into a nymph on the first host, meaning that there are two hosts instead of three per tick generation. This is the case for some *Hyalomma* and *Rhipicephalus* ticks.
- **Monophasic cycle:** the two moults occur on the same host. This pattern can be seen in all *Boophilus* (infesting ruminants) and *Margaropus* (infesting giraffes, zebras and horses), and in some *Dermacentor* species.

Cycle duration varies widely and depends on both climatic conditions and host behaviour. The life cycle may be interrupted: egg diapause (exceptional); behavioural diapause in larvae, nymphs or adults, awaiting favourable conditions; diapause in fasting stages for up to a year, waiting for a host.

There may be just one stage per year in ticks like *I. ricinus* or *D. marginatus* i.e., a complete cycle and one generation in 3 years. There may be more generations per year if the climate is favourable and there are many hosts: 7 days per meal, moulting within 3–4 days in the environment, the same for egg-laying, waiting for new hosts, so a minimum of 2 months for one generation, at best.

If we link this to the vertical transmission of pathogenic agents from the female to the eggs, demonstrated over three or four tick generations in some pathogens like *Babesia canis*, the ticks in a "tick area" transform it into a "babesiosis area" for many years, without an infected dog having to enter that area. In this case, ticks are not only the vector, but also the reservoir of the disease. Ticks alone can maintain babesiosis levels and they do not need carnivores to survive, as they can feed on small mammals.

Direct pathogenic role

Spoliation

Female tick-induced blood loss of 2 to 4 cm^3 can be problematic in cases of continued parasitism in certain breeding areas (especially in equatorial areas) or in stray dogs living in warm areas harbouring many *Rhipicephalus* ticks.

Abscess-like wound complications are rare, but improper tick removal, leaving part of the rostrum behind, can often lead to the formation of inflammatory granulomas, which can persist for several weeks.

It is noticeable that tick attachment is usually painless, even during a meal; therefore hosts do not feel that their skin is infested. Ticks on humans are usually accidentally discovered by touching or seeing them, with absolutely no sensation.

Toxic action

Some ticks inoculate salivary glycoproteins that are true toxins and most of these target nerve receptors and cause ascending paralysis in their hosts: ascending tick paralysis in Australia (*I. holocyclus*), Africa (*I. gibbosus*), and America (*D. andersoni*). The Australian paralysis tick is the best-known, due to its potentially fatal effects and the thousands of cases diagnosed in dogs each year. This tick is a tropical *Ixodes* whose natural hosts are marsupials, but pet and human infestation is easy due to the presence of possums or other marsupials in backyards. An antitoxin is available in Australia due to this incidence of human cases, especially children.

Indirect pathogenic role

Ticks are the most important vectors in veterinary medicine because of the range of diseases transmitted, their economic importance in production animals and their zoonotic impact. This is not the case in human medicine, where mosquitoes are the predominant vectors.

Transstadial transmission is one of the conditions required for ticks to be vectors and the infected stage is never a vector.

The agents transmitted can be categorised in many groups:

- **Viruses** (>99 %): responsible for tick-borne encephalitis (classic tick-borne encephalitis virus, Powassan fever, Kyasanur forest disease, Omsk haemorrhagic fever and Langat virus, ovine encephalomyelitis or louping ill, Colorado tick fever, etc.).

- **Rickettsiae:** responsible for ehrlichiosis, anaplasmosis, coxiellosis (Q fever), cowdriosis, Rocky Mountain spotted fever, Mediterranean spotted fever, African spotted fever, Australian spotted fever, Queensland tick typhus, Siberian tick typhus, etc.
- **Other bacteria:** responsible for tularaemia, dermatophilosis, Lyme disease, etc.
- **Protozoa:**
  - ***Babesia:*** inevitable and exclusive biological vector, the tick is the definitive host.
  - ***Theileria:*** inevitable and exclusive biological vector, the tick is the definitive host.
  - ***Hepatozoon canis*** (transmission by ingestion of the tick).
  - **Helminths:** filarial parasites *(Acanthocheilonema* and *Cercopithifilaria).*

Diagnosis

Diagnosis is based on inspection of preferred attachment sites and observing ticks attached to the skin.

During tick seasons and in tick areas, owners must always be advised to search for ticks after walking their animal, even if their antiparasitic treatment is up-to-date.

Tick species, stages and level of engorgement should be identified to assess the risk of pathogen transmission.

Control measures (treatment of the animal during parasitic phases)

The tick control is still based on regular treatment of the animal with acaricides. These kill existing ticks and prevent new tick infestation. Protection can be short to long term, depending on the formulation and the molecule used.

When ticks are diagnosed, they must be immediately and carefully removed, then an anti-tick treatment should be applied to the animal.

Treatment should **limit infestation, providing "general repellency":**

- Disrupt tactile or olfactory chemoreception (direct repellency + irritant repellency);
- And/or disrupt attachment (repellency in general);
- And/or inhibit feeding (repellency in general);

Treatment should also kill quickly = acaricidal action specifically

The requirements for a good anti-tick product are:

- **To be curative and preventive:**
  - **Curative** = kills and detaches existing ticks;
  - Preventive = quickly kills any ticks infesting the animal, if possible before pathogen transmission;
  - Sustained: effect persisting for a certain period of time (from one month to several months).
- To be waterproof (swimming, rainy season, etc.) as dogs are usually infested during outdoor activities.
- To have a good distribution over the body (for products which act on contact).

The objective of anti-tick treatments is not only to kill ticks, but to reduce the risk of tick-borne pathogen transmission where possible. Prevention of *Babesia canis*, *Borrelia burgdorferi*, and *Ehrlichia canis* by several anti-tick products has been demonstrated and published.

Environmental control methods can be added in particular circumstances, especially against the kennel tick *Rhipicephalus sanguineus*:

- Clean up and reduce wild/feral animal habitats (destroy refuge areas for animals that serve as alternative hosts for ticks).
- Eliminate undergrowth (grass, weeds and brush), especially if they are close to buildings or animal housing.
- Prevent access to crawl spaces under homes, decks, or outbuildings.
- Pesticides can be used to treat the environment, especially kennel walls and cages, in cases of massive infestation. The risk of environmental pollution by spraying acaricides should be taken into account.
- Some zoological measures are available, such as the use of various tick-eating bird species ("tick birds" in tropical areas, e.g., *Buphagus* in Africa; Moluccan bluebird, *Acridotheres tristis* in Asia and the Pacific; and also chicken or Guinea fowl in gardens).
- The use of entomopathogenic fungi, including *Beauveria bassiana* and *Metarhizium anisopliae* conidiospores, has not been adopted in the field but should be considered as a promising strategy.

Risk to humans

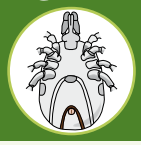
Ticks will infest humans directly from the environment but there is no risk of transfer from an animal to a human.

Diagnose of the main tick genera infesting dogs, cats and humans

- Phylum **Arthropoda**
- Absence of antenna, presence of chelicera: subphylum **Chelicerata**
- Aerial respiratory system: class **Arachnida**
- Body formed by the prosoma and the opisthosoma which are not clearly separated; 8 legs in the adult stage: order **Acari**
- Respiratory stigma behind the 4th leg; large acarines: **Ixodida** = ticks
- Terminal capitulum + dorsal scutum: **Ixodoidea** = hard ticks
  - Anal groove anterior to the anus: **Prostriata**
    - Long capitulum
    - Genus ***Ixodes***
      - **Ixodes ricinus** (Europe)
      - ***Ixodes scapularis*** (North America)
  - Anal groove posterior to the anus or absent: **Metastriata**
    - **Brevirostris** = short hypostome and palps
      - Rectangular basis capituli
        - Male has large coxa IV
          - ***Dermacentor***
          - ***Dermacentor reticulatus*** (Europe)
          - ***Dermacentor variabilis*** (North America)
        - Male has normal coxa IV
          - ***Haemaphysalis***
          - ***Haemaphysalis longicornis*** (Asia - Pacific)
      - Hexagonal basis capituli
        - Festoons present
          - ***Rhipicephalus***
          - ***Rhipicephalus sanguineus*** (worldwide)
    - **Longirostris** = long hypostome and palps
      - Dorsal scutum colorized
      - Male has no ventral plates
        - ***Amblyomma***
        - ***Amblyomma americanum*** (North America)

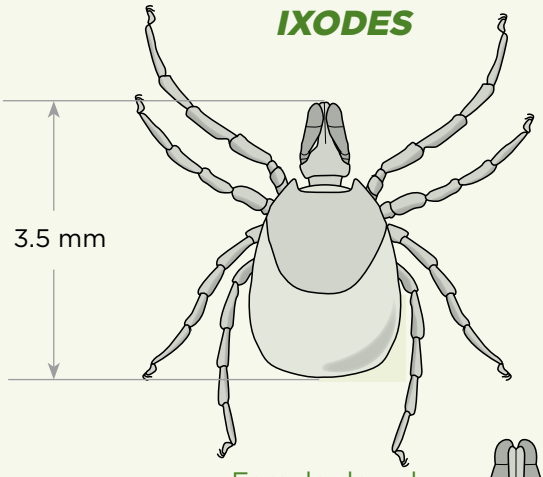
DIAGNOSIS of the main **tick** genera

ANAL GROOVE ANTERIOR TO THE ANUS: **PROSTRIATA**

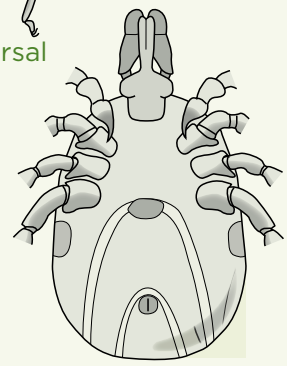


Long hypostome and palps

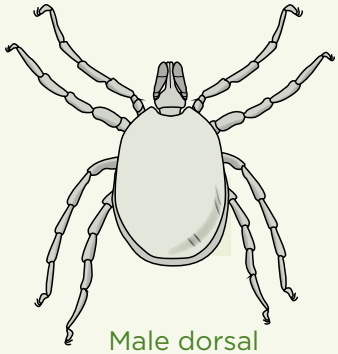
IXODES



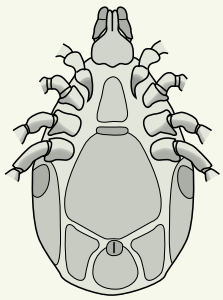
Female dorsal



Female ventral



Male dorsal

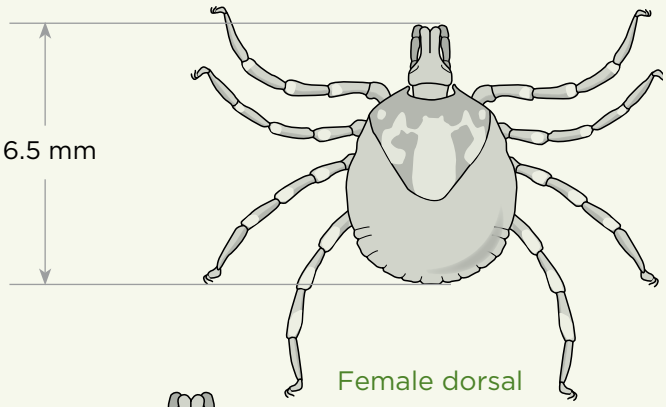


Male ventral

ANAL GROOVE POSTERIOR

Long hypostome and palps
Dorsal scutum well coloured
Male without ventral plates

AMBLYOMMA



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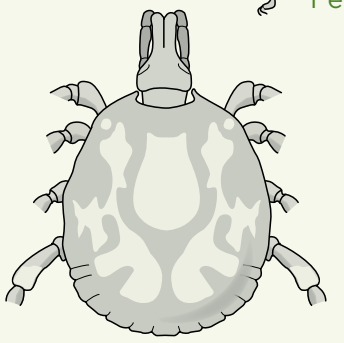
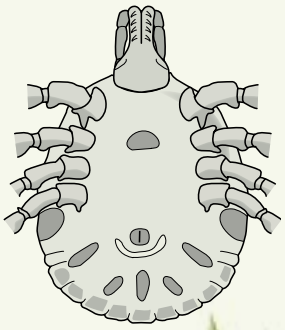


Male dorsal



Male ventral

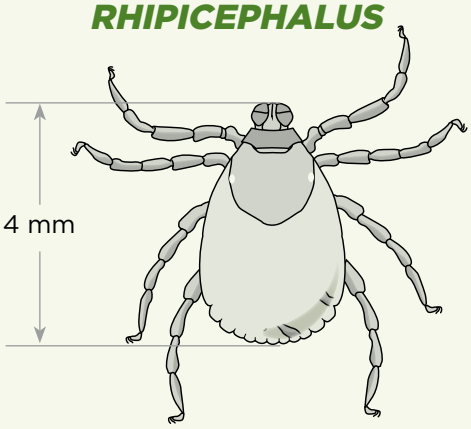
infesting dogs, cats and humans

TO THE ANUS OR ABSENT: **METASTRIATA**

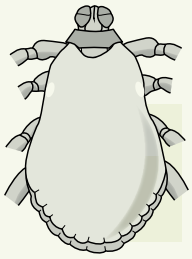
Short hypostome and palps

HEXAGONAL BASIS CAPITULUM

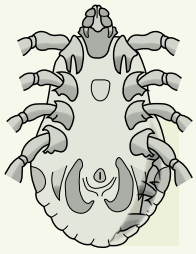
RHIPICEPHALUS



Female dorsal



Male dorsal

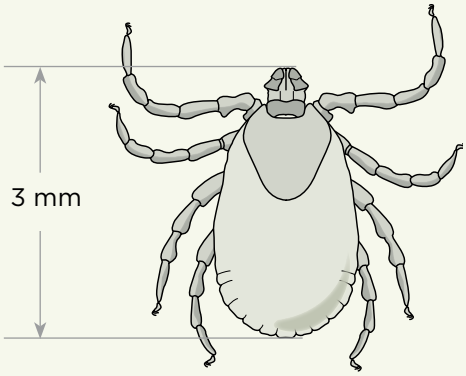


Male ventral

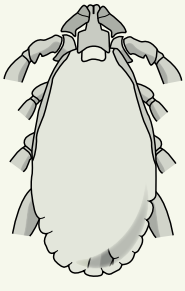
RECTANGULAR BASIS CAPITULUM

Male has normal Coxa IV

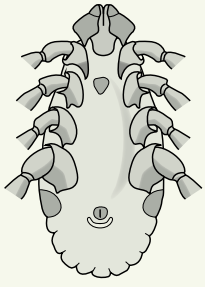
HAEMAPHYSALIS



Female dorsal



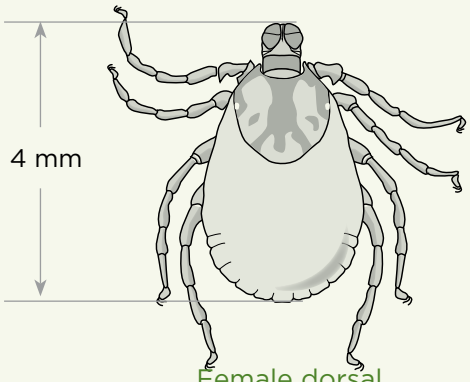
Male dorsal



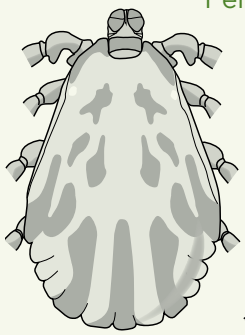
Male ventral

Male has large Coxa IV

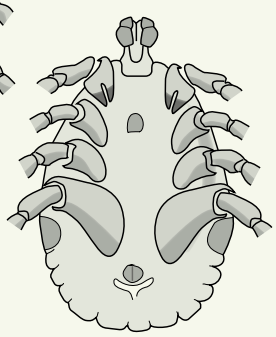
DERMACENTOR



Female dorsal



Male dorsal



Male ventral

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1. ***Ixodes***
Female dorsal

2. ***Ixodes***
Male dorsal

3. ***Rhipicephalus***
Female dorsal

4. ***Rhipicephalus***
Male dorsal

5. ***Dermacentor***
Female dorsal

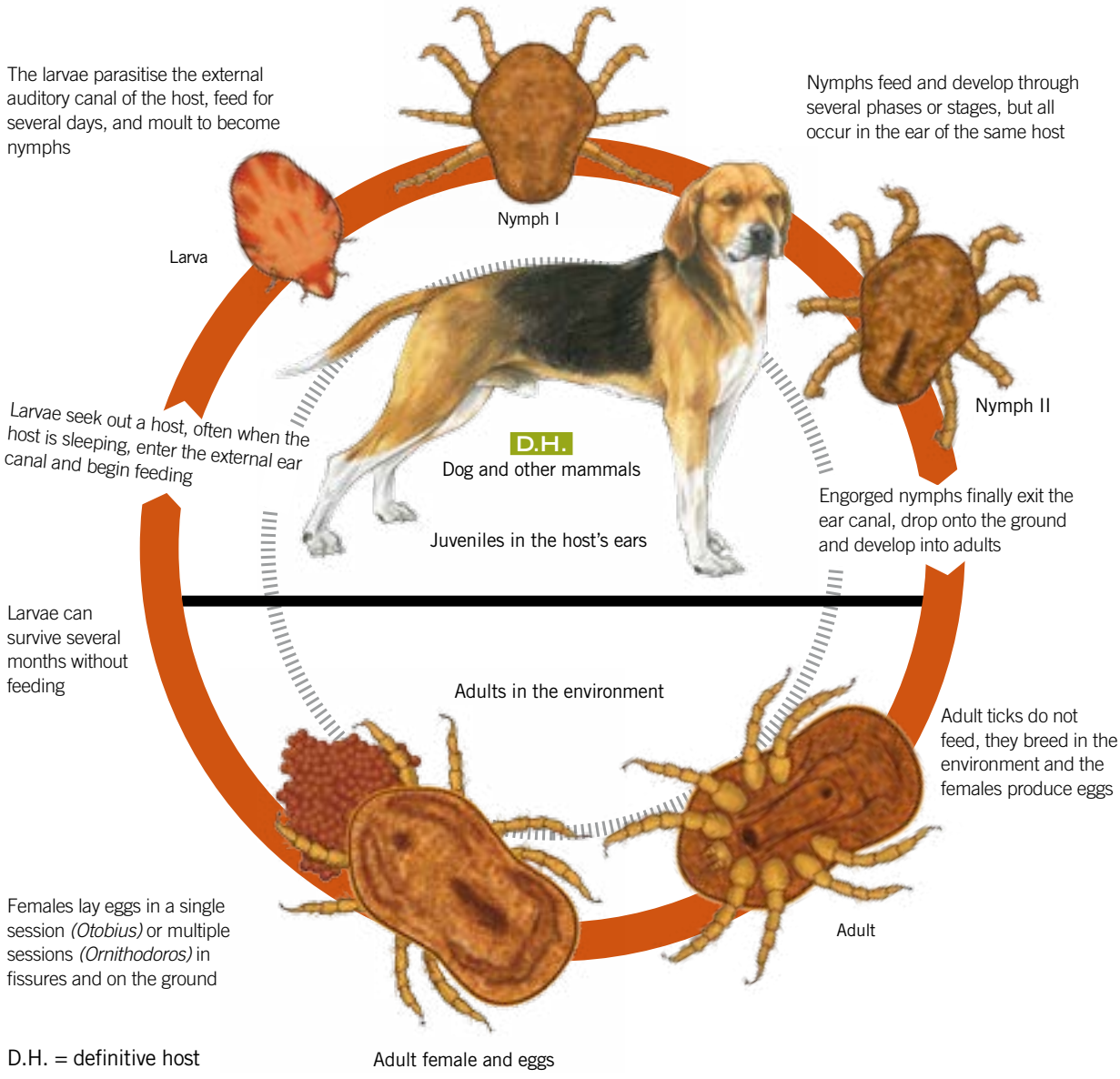
6. ***Dermacentor***
Male dorsal

7. ***Amblyomma***
Female dorsal

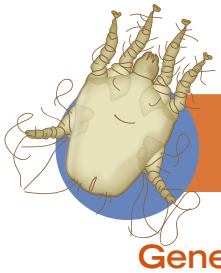
8. ***Amblyomma***
Male dorsal

9. ***Haemaphysalis***
Female dorsal

Life cycle of *Otobius megnini*, the ear soft tick



Video 9
The tick risk.



Otodectic mange

General comments

Description and morphology

Otodectes cynotis (family Psoroptidae) is an obligate parasite of dogs, cats and ferrets, which spends its entire life cycle on the host, mainly in the external ear canals. Comparison of ribosomal DNA gene sequence ITS2 from 16 *Otodectes* mites taken from different host species living in different continents revealed that the mites all belong to the same species.

Adult *O. cynotis* measures about 350–550 µm in diameter (Figs. 1 and 2). All stages (eggs, larvae, nymphs and adults) live in the external ear canal, where they also breed. Otodectic mites mainly feed on the inflammatory exudate they trigger when they bite the epidermis, but they also feed on cerumen (Fig. 3). Females lay their eggs in the external ear canal and the larvae develop into adults in 14–21 days. *Otodectes* can leave one ear canal to infest the other ear. They can also survive for some time in the coat and infest other parts of the body.

However, otodectic mites cannot survive for long (about 4 or 5 days) in the external environment. They are acquired by contact with infested cats and dogs or from a contaminated environment (e.g., crates, kennels and dog baskets).

Biology

Eggs hatch 3–4 days after being laid and hatching is followed by one larval and two nymphal stages, each 3–10 days long. The mites feed during this period, with a 1-day quiescent phase between each stage. On moulting into the adult stage, the males attach to deutonymphs, using adanal suckers on their posterior end, and remain in place until the nymphs moult. If a female adult emerges, copulation occurs immediately. This attachment and insemination is key to egg laying: females which moult and emerge without being mated are infertile (Fig. 4). Adult mites can survive on their host for approximately 2 months. Secondary bacterial and yeast infections are common, as they are in other mite infestations in dogs and cats, especially secondary otitis caused by *Malassezia pachydermatis*.

Epidemiology

Otodectes cynotis mites occur throughout the world and can infest pet carnivores of all ages and breeds, but cats are more commonly infested than dogs, and kittens and puppies more than adults. Infestation can occur year-round. Human infestation with *O. cynotis* is exceptional, and this mite should not be considered to be zoonotic.

Figure 1. *Otodectes cynotis* female.

Figure 2. *Otodectes cynotis* male.

Figure 3. *Otodectes cynotis* in cerumen.

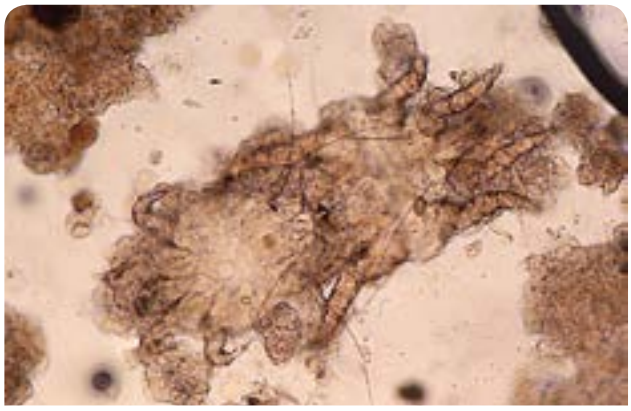
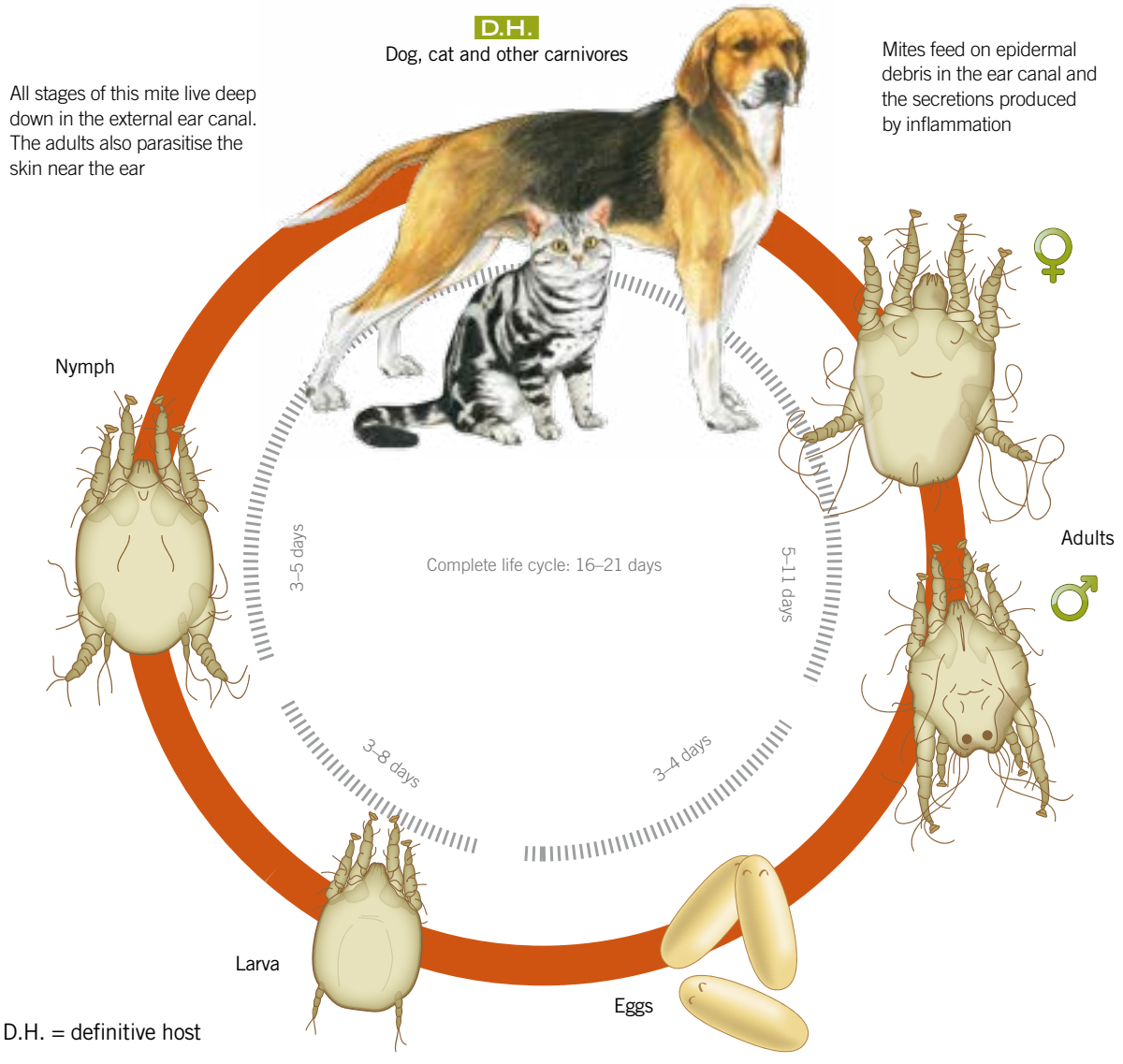


Figure 4. *Otodectes cynotis* mating.

PDF

Life cycle of *Otodectes cynotis*



D.H. = definitive host

Pathogenesis

O. cynotis causes irritation (mechanical and chemical) and type I and III hypersensitivity reactions in its host. The presence of immunoglobulins E and G, and circulating immune complexes has been demonstrated by passive cutaneous anaphylaxis tests. Antigenic cross-reactions shown by positive intradermal reactions to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are also extremely important, because they raise the possibility of false positive reactions to house dust mites in intradermal testing.

There are several primary causes of otitis externa, including *O. cynotis* and other parasites such as *Demodex* spp., atopy and foreign bodies. A number of predisposing factors are associated with otitis externa, such as moisture in the ears due to regular swimming or bathing, for example, and systemic disease, but none of these are pertinent to the establishment of *O. cynotis* infestation. Infestations are usually bilateral, affecting both ears and, in addition to being a source of discomfort and irritation in the ear, they can occasionally cause dermatitis elsewhere on the host. *O. cynotis* can induce hypersensitivity reactions, the host becoming exposed to mite antigens when the mite bites the host to ingest body fluids. Secondary bacterial or yeast (*M. pachydermatis*) infections, associated with the pruritus and consequent ear scratching and rubbing, are common, especially in dogs.

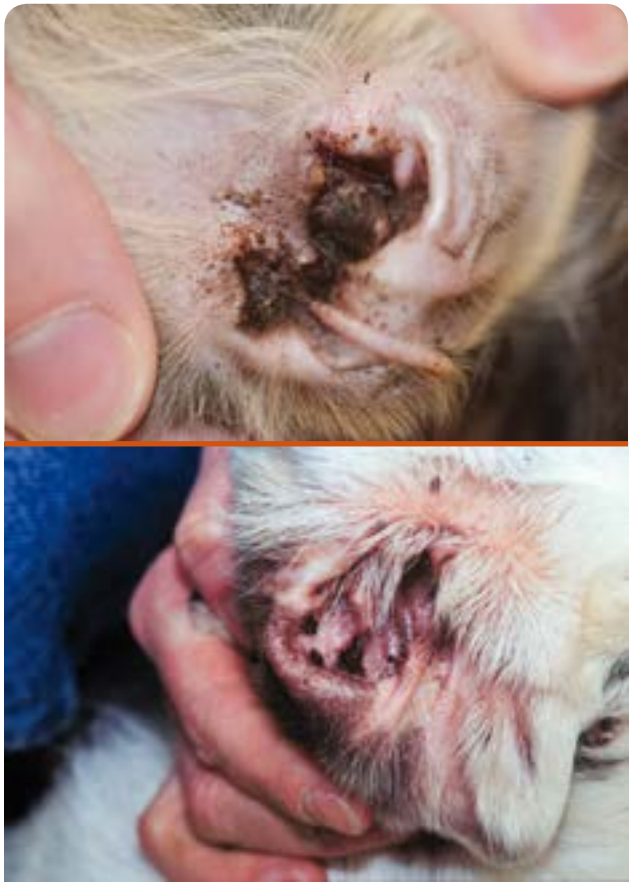


Figure 5. Ceruminous otitis.

Clinical signs

The incubation period lasts for about 2 to 3 weeks following infestation. Otitis, usually bilateral and erythemato-ceruminous, ensues with dry (flaky or powdery), brownish-black cerumen (Fig. 5). Aural pruritus is associated with a pinnal-pedal reflex, variable in intensity but apparently less intense in the dog than in the cat (Figs. 6 and 7). Self-induced erosive, crusting lesions are often seen behind the ears and secondary bacterial and fungal infections are common.

Skin involvement is rare but may occur when mites migrate from the ear canal to neighbouring areas of skin, such as the face (eyelids and interocular region in brachycephalic breeds), pinnae, neck and cranial carpi. Skin lesions involve hair loss, erosions, crusts and constant pruritus.

Systemic signs including aggression, fits and vestibular syndrome (following rupture of the tympanic membrane) may be seen.

Figure 6. Pruritic auricular reflex in an infested cat.

Figure 7. Audito pedal reflex in a cat infested by *Otodectes cynotis*.

Figure 8. *Otodectes cynotis* female and eggs.

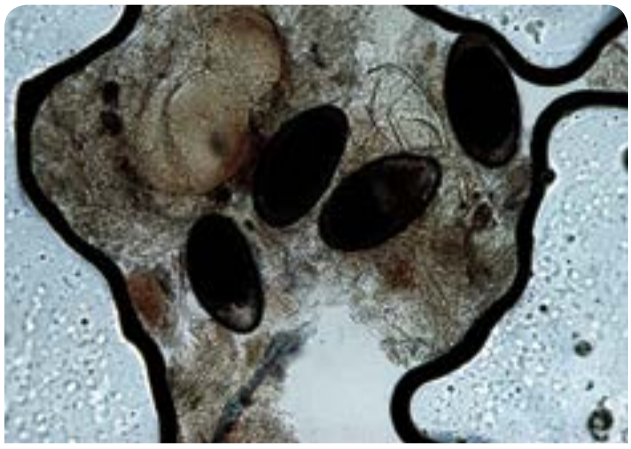


Figure 9. *Otodectes cynotis* eggs.

Diagnosis

Diagnosis of otoacariosis is based on medical history, distribution, clinical signs and detection of the parasite in any of its various stages.

Microscopic examination of a sample from the external ear canal, mounted in liquid paraffin, chloral lactophenol or 10 % potassium hydroxide may reveal otodectic mites; adults and immature stages (eggs, larvae and nymphs) (Figs. 8 and 9). However, detecting the parasite is not always straightforward.

Cytology of an ear canal swab often reveals cocci and yeasts (*M. pachydermatis*).

When the skin is involved, skin scrapings reveal far fewer *O. cynotis* (both adult and immature forms).

Differential diagnosis includes other causes of erythemato-ceruminous otitis, sarcoptic mange, cheyletiellosis, and trombiculosis.

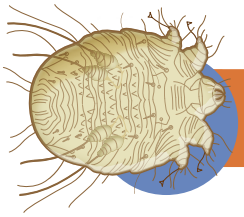
Prognosis

Prognosis is usually good, especially in young animals. It is more guarded in dogs which are continuously reinfested and also in older dogs with concurrent diseases, such as leishmaniosis, diabetes and lymphoma.

Control measures

Treatment has become much easier with the advent of topical spot-on ectoparasiticides with persistent efficacy, and clinical outcomes more certain due to improved compliance with the treatment by the owner. However, as most products do not have ovicidal effects unless they are effective for longer than 3–4 weeks, a second administration may still be required to break the parasite life cycle, even if this is not actually stated on the product label. Cleaning the ear to remove the ceruminous exudate is often omitted from product labels but is good practice and is thought to improve acaricidal efficacy. It is usually recommended that all dogs and cats in a household are treated simultaneously to prevent reinfestation from asymptomatic carriers.

Video 10
Moving ear mites in ear wax (*Otodectes cynotis*) observed under the microscope.
Courtesy of Stéphane Girodon.



Sarcoptic mange

General comments

Canine mange is a contagious parasitic skin condition caused by the mite ***Sarcoptes scabiei* var. *canis*** (family Sarcoptidae) which feeds on cutaneous debris and exudate. The primary domestic host is the dog. Sarcoptic mange does occur in cats and it has been reported in multi-pet households where cats cohabit with dogs, although it is rare for *S. scabiei* var. *canis* to cross-infest cats. *S. scabiei* mites are obligate parasites, completing their entire life cycle on a single host and living in the surface skin layers, with adults mating on the skin surface before the females burrow into the stratum corneum to lay their eggs.

The mite is small, the male measuring 250 µm in length and the female, 350–500 µm (Fig. 1). Morphologically, the variety infesting the dog is indistinguishable from the human variety.

Biology

The entire life cycle takes 14–21 days, with female adults starting to lay eggs within approximately 3 days of becoming adults. Egg-bearing females dig out tunnels or burrows in the horny layer of the epidermis, progressing about 2 mm per day and laying 2–3 eggs per day (Fig. 2). Tissue-feeding larvae hatch out after about 2 days and then either head for the skin surface, where they dig new moulting pockets, or stay in the tunnels where they hatched out. After 4–6 days, they moult into protonymphs, then into tritonymphs. Males mate with female tritonymphs. Males live for about 3 or 4 weeks, females up to 3 months.

Epidemiology

The *S. scabiei* mite is capable of infesting a large number of different mammalian species, although a number of different host-specific varieties have been described. The disease occurs in dogs worldwide. It is non-seasonal and highly contagious, with transmission normally occurring by direct host-to-host transfer of adult mites when animals are in close proximity. The extent of cross-infestation from foxes to dogs is not known, but is assumed to exist in countries with urban fox populations.

Scabies can affect dogs of all ages and breeds, and both sexes, although the incidence is higher in younger animals. Immunosuppressed animals are most at risk of severe disease. Infestation with *S. scabiei* var. *canis* can occur in other small domestic mammals as well as dogs and, occasionally, cats, and often causes transient dermatitis in pet owners. Sarcoptic mites do not survive for long (around 3 days) in the external environment and they are acquired either by direct contact with an infested dog or from a contaminated environment (e.g., crates, kennels and dog baskets).

Sarcoptic mange is common in dogs living in groups, dogs that roam and dogs belonging to homeless people. Sarcoptic mange is sometimes thought of as a disease of young dogs (<1 year old) but although young dogs are perhaps more likely to come into contact with infested animals and a contaminated environment (e.g., breeding kennels) and acquire the infestation, it is not unusual to see the condition in adult dogs, possibly in a milder clinical form.

Transmission to humans is often overlooked, and human involvement is seen in 25–30 % of cases, although this varies according to the owner-dog relationship. If the dog is often held in the owner's arms (as may be the case for a small dog)

Figure 1. Adult *Sarcoptes scabiei*.

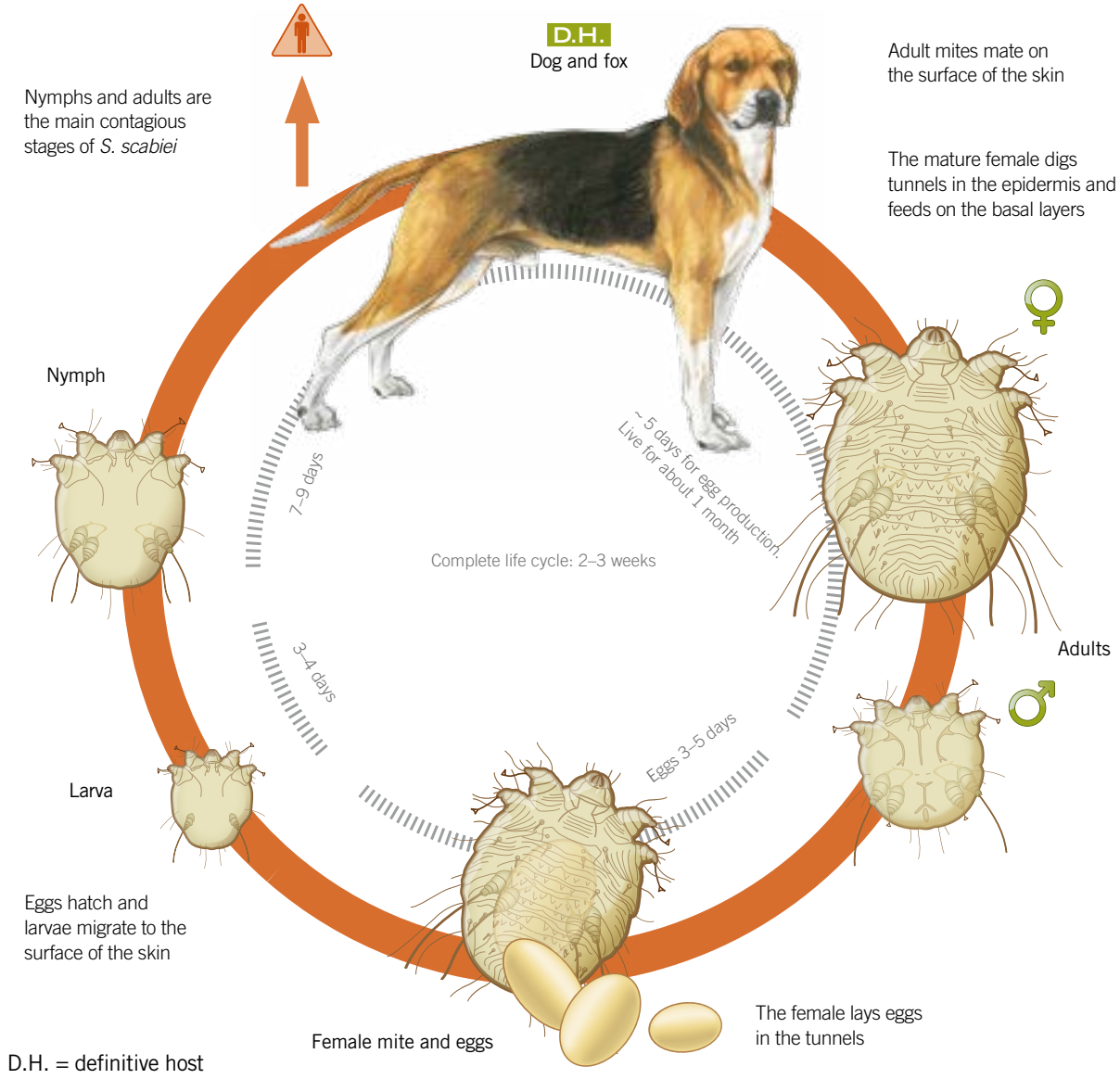
Figure 2. *Sarcoptes scabiei* nymph and eggs.

or shares the owner's bed, human transmission is more likely. Papules and small pruritic crusts are then seen on the forearms, legs and trunk, but these lesions recede rapidly once the affected dog has been treated. The human being is effectively a dead-end host, as canine sarcoptic mites cannot breed in human skin. Mites live only at the epidermal surface, for 2–3 weeks on average. They do not dig the tunnels and furrows seen in human mange caused by *S. scabiei* var. *hominis* which explains why the condition is under-diagnosed by human dermatologists.

Pathogenesis

The host's immunological response is well understood. Salivary antigens consisting of proteolytic enzymes and cuticular antigens resulting from the mite moulting have been identified and type I, III and IV hypersensitivity reactions have been described. Type III hypersensitivity is responsible for immune complex deposition in various organs, notably the kidneys, sometimes causing immune-mediated glomerulonephritis. Sarcoptic mange may therefore be considered a systemic illness.

Life cycle of *Sarcoptes scabiei* var. *canis*



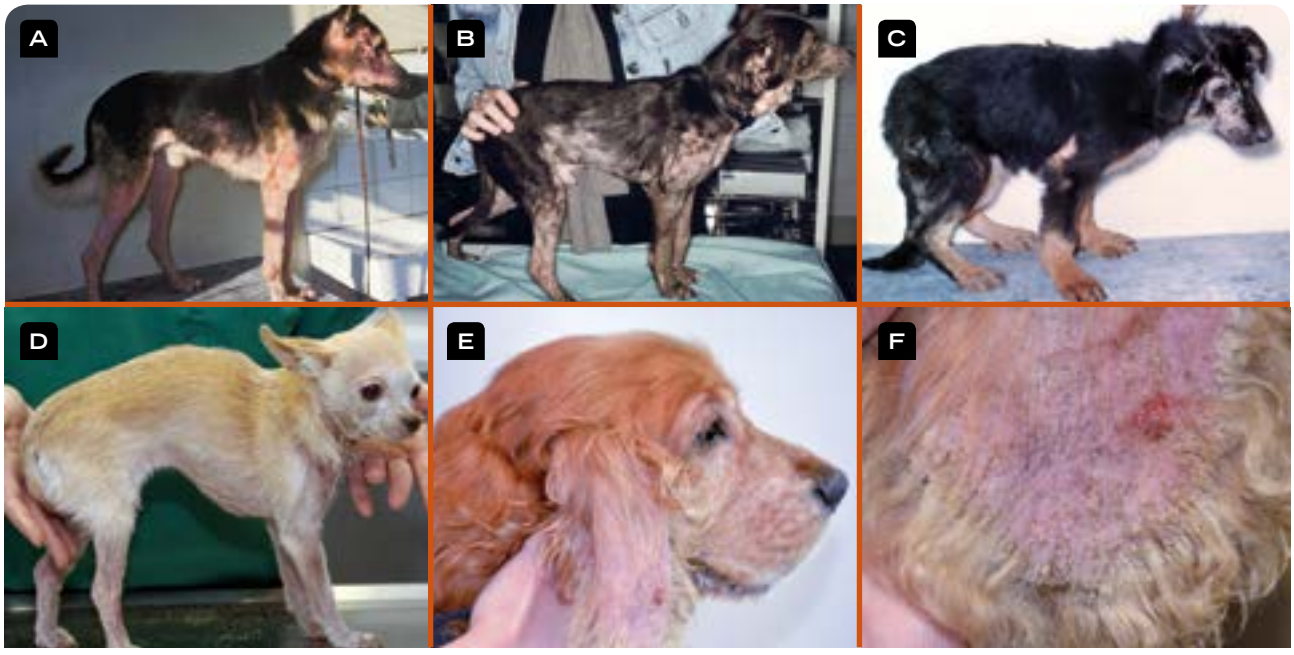


Figure 3. Clinical signs of sarcoptic mange. A, B, C and D (typical distribution of the early lesion of sarcoptic mange): diffuse alopecia of the legs, lower abdomen and face. E and F: diffuse erythema and crusted papules on the pinnal margins. Courtesy of Parasitology Unit, Alfort Veterinary School.

Experimental infestations in naive dogs are reported to have an incubation period of 10–21 days, but the same animals presented clinical signs within 3 days on subsequent re-exposure, indicating a hypersensitivity response to the mite. Disease lesions are first seen as urticaria, progressing to an intensely pruritic but initially localised dermatitis with papules, erythema, excoriation, crusting and alopecia (Fig. 3). If untreated, infestation may lead to generalised dermatitis, characterised by widespread alopecia, lichenification, hyperpigmentation and, in some cases, lymphadenopathy, reduced activity, weight loss and occasionally, death (Fig. 4). In some dogs, affected areas may recover spontaneously as immunity develops, with these animals becoming asymptomatic carriers.

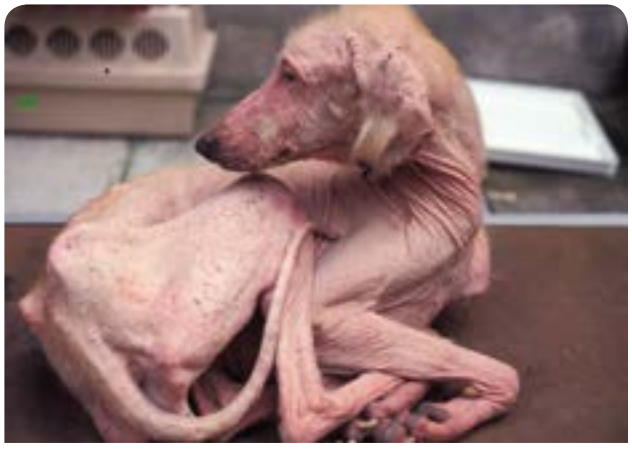


Figure 4. Extensive sarcoptic mange lesions in a greyhound. Courtesy of Blaise Hubert.

Clinical signs

Classic form

The incubation period lasts about 3 weeks after contact with contaminated material. The classical form is characterised by intense pruritus and a positive pinnal-pedal scratch reflex (note that this reflex is present in only 75–90 % of sarcoptic mange cases and can also be seen in other pruritic skin conditions). Initial distribution of lesions on the face (pinnal margins), lateral elbows and sternum is very indicative of sarcoptic mange. Primary skin lesions (diffuse erythema, papules, crusted papules where mites penetrate the epidermis, and patchy hair loss) are seen only at the onset. Urticarial lesions (papules and oedematous plaques) are seen in about 30 % of cases. Secondary lesions (excoriations, erosions, crusts, lichenification and hyperpigmentation) soon follow in the case of intense pruritus. Superficial pyoderma (folliculitis), bacterial proliferation syndrome and *Malassezia* dermatitis are commonly seen in cases of chronic mange.

Localised form

Localised, often chronic, forms are increasingly reported. They are characterised by erythematous, papular, crusting lesions, occasional excoriations, restricted to pinnal margins or lateral elbows for several months. Pruritus is variable and not necessarily severe and the pinnal-pedal reflex is often positive.

Juvenile form

This mild form is seen in young dogs, involving mild pruritus, truncal scaling and occasional pyotraumatic dermatitis.

Norwegian (or crusted) scabies

This is a rare form where thick crusts are seen on the face, lateral elbows and other parts of the body and pruritus is mild to moderate. This form is seen in debilitated animals with a concurrent disease, dogs that have received excessive glucocorticoid therapy and very old dogs.

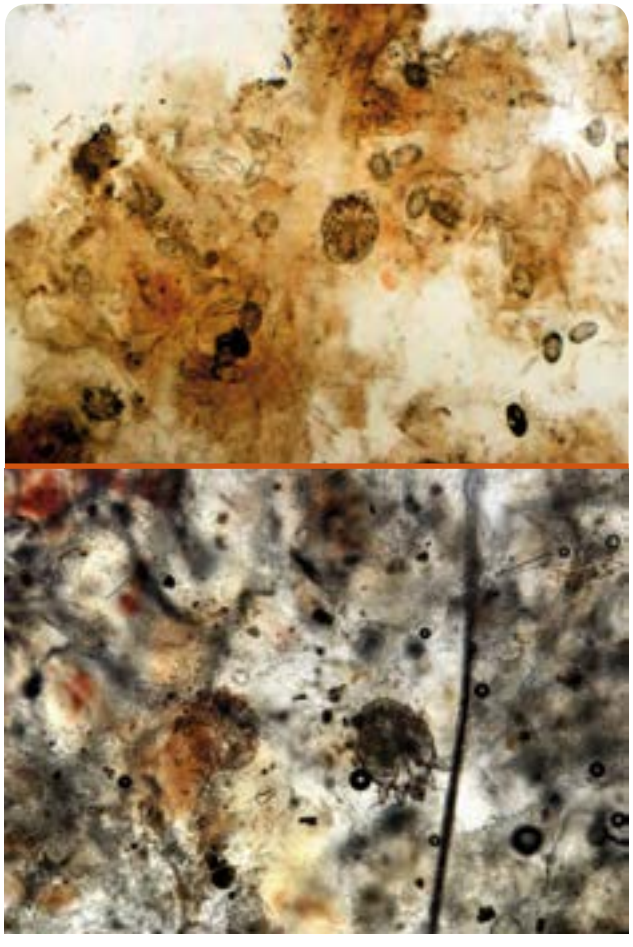


Figure 5. Skin scraping showing *Sarcoptes* mites and eggs.

Systemic signs

Although systemic signs are rarely suspected, they are sometimes present in chronic cases (of "several months" duration) and in old dogs. Anorexia, pyrexia, weight loss and polyuria-polydipsia (associated with immune-mediated glomerulonephritis) may be seen.

Diagnosis

Diagnosis is based on medical history, clinical signs and detection of the parasite in its various stages.

Skin scrapings

Multiple skin scrapings (5–10) should be carried out in areas likely to harbour sarcoptic mites (Fig. 5). Such areas include the pinnal margins (especially the small fold known as Henry's pocket), lateral elbows and every crusted papule (where present). Scrapings must be plentiful and examined in a clearing fluid e.g., chloral lactophenol or mineral oil (liquid paraffin) which has the advantage of not killing the parasites, making them easier to find on the slide. Microscopic examination is carried out at an initial magnification of ×40, then ×100 and ×250, under low to moderate lighting. Only a few parasites (1–2 adult sarcoptids) are usually found. Scrapings should also be examined for eggs and mite faeces. In Norwegian scabies, scrapings from thick crusts reveal huge numbers of mites in different stages of development (adult, larva and egg).

Serology

ELISA testing using a purified extract of *S. scabiei* is available and this test seems to be sensitive and specific, with no cross-reactivity in dogs with house dust mite sensitivity. It is particularly useful to distinguish sarcoptic mange from atopic dermatitis, however, dogs with sarcoptic mange show positive intradermal reactions and may have allergen-specific IgE and IgG to *Dermatophagoides farinae* and *D. pteronyssinus*.

Skin biopsies

Histopathology is of little use in diagnosis as sarcoptic mites are rarely detected, but it does alert the clinician to the presence of an inflammatory skin condition: superficial perivascular dermatitis mainly with eosinophils and mast cells, or an eosinophilic pustular dermatitis also seen in allergic dermatitis. It is not uncommon to see cases of sarcoptic mange treated for atopic dermatitis, purely on the basis of this type of histopathology report.

Other diagnostic tests

Impression smears (tape strips) are useful to identify bacterial and fungal proliferation (e.g., *Malassezia*), often associated with chronic mange.

Haematology and biochemistry profiles and urine analysis (looking for proteinuria) are recommended in old dogs and dogs with systemic signs.

The possibility of an underlying illness (hyperadrenocorticism, internal neoplasia) must always be investigated in cases of Norwegian scabies.

Differential diagnosis

This includes all pruritic skin conditions including other parasitic infestations (which may also be present) such as cheyletiellosis, trombiculosis and *Otodectes* skin infestations, atopic dermatitis, flea allergy dermatitis, *Malassezia* dermatitis and bacterial folliculitis. Pemphigus foliaceus should also be considered in adult animals and the pinnal-pedal scratch reflex is sometimes positive in this case.

Prognosis

Prognosis is usually good, although less so in chronic mange, Norwegian scabies or group infestations (in boarding or breeding kennels, for example). The condition can be hard to eradicate, with the on-going possibility of reinfestation from contact animals and the environment.

Control measures

Treatment (as per the instructions) with a licensed acaricide specifically for *S. scabiei* is essential. Trial therapy is recommended whenever the disease is suspected, even if scrapings are negative, because positive identification of the mites is so difficult. All dogs in a household should be treated simultaneously to avoid the risk of reinfestation, and a full course of treatment should be administered to all animals. Anti-inflammatories may be required to control pruritus, with antibiotics to treat secondary bacterial infections. Anti-seborrhoeic shampoos can be helpful in cleansing the skin, but care should be exercised in the timing of their use as they may reduce the persistence of topical acaricidal treatments.

Risk to humans

Sarcoptes scabiei is a zoonotic parasite, although lesions caused by the canine strain of the parasite (var. *canis*) in humans tend to be much more limited in extent and duration than those caused by the human strain of the mite (var. *hominis*). Human scabies induced by the canine mite variety usually resolves spontaneously on treatment of the canine primary host although, both canine and human immunosuppressed individuals may develop more severe clinical signs (Fig. 6).

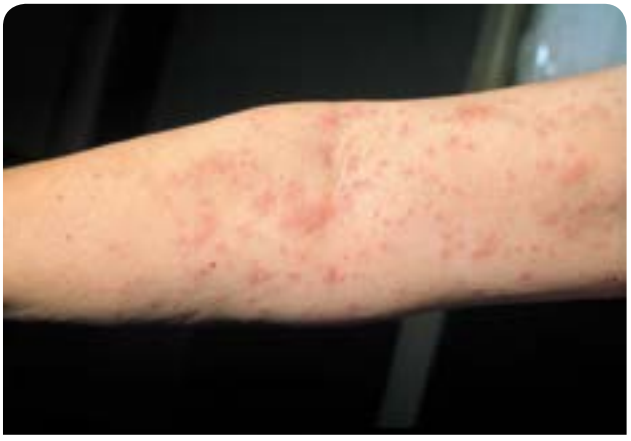
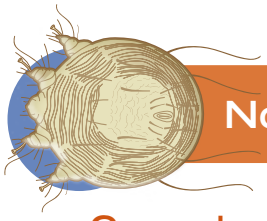


Figure 6. Cutaneous lesions due to the transmission of *Sarcoptes* mites from a dog to its owner.

Video 11
The pinnal-pedal reflex is usually positive in case of sarcoptic mange. Courtesy of Parasitology Unit, Alfort Veterinary School.



Notoedric mange

General comments

Notoedric mange is caused by the mange mite ***Notoedres cati***. The cat is the usual host for this mite, although infestations have been recorded in dogs. The aetiology of *N. cati* is very similar to that of *S. scabiei,* with the mites completing the whole of their life cycle in the surface layers of skin on a single host in approximately 21 days, and eggs hatching 3–10 days after being laid. Female mites lay their eggs in clusters in tunnels in the stratum corneum. Unlike *S. scabiei*, inter-host infestation with *Notoedres* is thought to occur through the transfer of larvae or nymphs, rather than by the movement of adults (Figs. 1 and 2). Secondary bacterial or fungal infections seem very common.

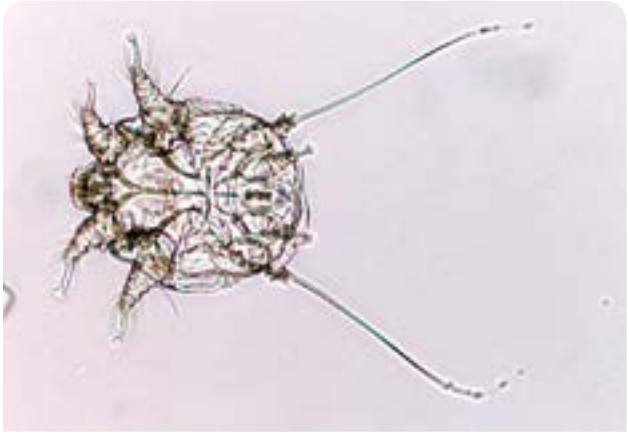


Figure 1. *Notoedres cati* male.

Figure 2. *Notoedres cati* female and larva.

Epidemiology

N. cati infests cats of all ages and both sexes. It can also infest foxes, rabbits and other small domestic mammals (pet rodents, especially rats). The mite is an obligate parasite which can survive for only a few days off the host. Notoedric mange is highly contagious, with localised outbreaks of infestation and it is spread by direct contact and by fomites. It is widespread, although incidence is low and it mainly occurs in stray cats (Fig. 3) rather than domestic cats.

The mites can temporarily infest humans, causing intensely pruritic dermatitis similar to that seen in the cat.

Pathogenesis

The incubation period for notoedric mange is similar to sarcoptic mange as the life cycles of the two mites are very similar. The exact pathogenesis is unclear, but it is thought that the acute pruritus associated with this disease may be due to the close proximity of mite burrows and egg deposits to nerve endings in the host's skin. Disease lesions usually begin as erythematous papules on the head, specifically around the leading edge of the ears, with rapid spread to the entire upper surface of the ears, face, eyelids and thereafter to other parts of the body such as the neck, feet and perineum (Fig. 4).

Figure 3. Notoedric mange in a stray cat.

Figure 4. Notoedric mange lesions on a cat's face.

The skin becomes thickened, wrinkled and alopecic, and excoriation caused by the intense pruritus usually leads to secondary bacterial infection and peripheral lymphadenopathy. Untreated disease has occasionally resulted in toxaemia, anorexia, cachexia, and death (Figs. 5 and 6).

Clinical signs

Notoedric mange in cats is characterised by severe pruritus, initially with localised alopecia, erythema, scaling and crusting leading to lichenification. Common sites include the head, specifically the proximal edge of the pinnae and neck. Excoriation leading to secondary bacterial infection is very common. The disease can become more generalised, with lesions spreading over the body as it does in *Sarcoptes* infestations.

Diagnosis

Definitive diagnosis is by skin scraping, using the same technique as for *S. scabiei*, and although the mites are smaller, they are more readily identified in skin scrapings, occurring more superficially and in greater numbers. *N. cati* is morphologically similar to *S. scabiei* with similar short legs, but the dorsal surface of the mite is covered with concentric rings rather than the spines and triangular scales seen on *S. scabiei*, and the anus is located dorsally.

Control measures

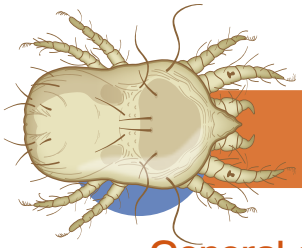
Products which are licensed for the treatment of sarcoptic mange and also indicated as safe for use in cats are likely to be effective against notoedric mange at the recommended dose for cats. One spot-on combination containing eprinomectin is licensed for the treatment of notoedric mange. Before treatment, infested cats should be washed with an anti-seborrhoeic shampoo to soften and remove skin crusts. The key to treating this disease is simultaneous treatment of all affected and contact animals, and regular cleaning/disinfection of the environment by vacuuming and washing bedding throughout the treatment period. It is advisable to skin scrape all treated animals regularly and to continue treatment until scrapes are negative and lesions resolve.

Risk to humans

Notoedres cati is a zoonotic parasite. The lesions associated with human infestations are intensely pruritic but the mite does not reproduce on human skin and the disease will resolve spontaneously within 2–6 weeks.

Figure 5. Hyperkeratotic lesions of notoedric mange in a cat. Courtesy of Emanuele Brianti.

Figure 6. Cachexia in a cat suffering from notoedric mange. Courtesy of Emanuele Brianti.



Cheyletiellosis

General comments

Cheyletiellosis is a form of dog and cat acariosis which is commonly called "walking dandruff" as the relatively large ***Cheyletiella*** mites (0.5 mm long) can be seen moving amongst the skin scales with the naked eye. There are three main *Cheyletiella* species affecting pets, including ***C. yasguri*** (dogs), ***C. blakei*** (cats) and ***C. parasitivorax*** (rabbits). They are not, however, strictly host-specific, so transmission of *Cheyletiella* species between the host species in a household is not uncommon, although it is unclear whether such inter-species transfers result in sustained infestations on the new hosts.

Cheyletiella mites are large (450–500 × 300–320 µm) (Fig. 1). They may be detritivorous (scale-eating) or prey on other mites, especially house dust mites.

Biology

All stages of the life cycle can be found at the skin surface as the mites are surface feeders and do not burrow into the skin surface, and they prefer to infest the dorsal skin of their host. Females lay eggs which attach to the hair by fibrillar strands (Fig. 2). White, elongated eggs hatch into six-legged larvae which develop into protonymphs, deutonymphs and finally new adults. The life cycle takes about 3 weeks. Mites can survive about 5–6 weeks off the host, feeding on small mites but they cannot breed off host.

Cheyletiellosis is common in large groups of dogs or cats. Although young pets are more likely to come into contact with infested animals and a contaminated environment (e.g., boarding or breeding kennels) and acquire the infestation, it is not unusual to see the condition, possibly in a milder clinical form, in adult dogs/cats or in animals that are debilitated by concurrent illness, such as internal neoplasia.

Epidemiology

The three *Cheyletiella* species listed above are widely distributed. Cheyletiellosis affects cats and dogs of both sexes, but is most commonly diagnosed in pet rabbits. Young animals seem to be particularly susceptible, and cheyletiellosis is more commonly seen in dogs and cats living in kennels and catteries. The disease is highly contagious because the mites are very mobile, and infestations are easily spread by direct contact between hosts and by fomites. Eggs on shed animal hair may be a source of infestation. *Cheyletiella* mites have also been reported to parasitise fleas, lice and flies, which provide another mode of transmission for mammalian infestations. *Cheyletiella* may survive for several weeks in kennels and they are thought to be predators of acarian dust mites. This explains how difficult it is to control cheyletiellosis in groups of animals.

Pathogenesis

Squamosis can be severe in affected animals and mites are usually present in large numbers, but occasionally the intensity of the pruritus is disproportionate to the parasite burden, perhaps due to hypersensitivity. An asymptomatic carrier state is also common, particularly in adults.

Figure 1. *Cheyletiella* sp. mite.

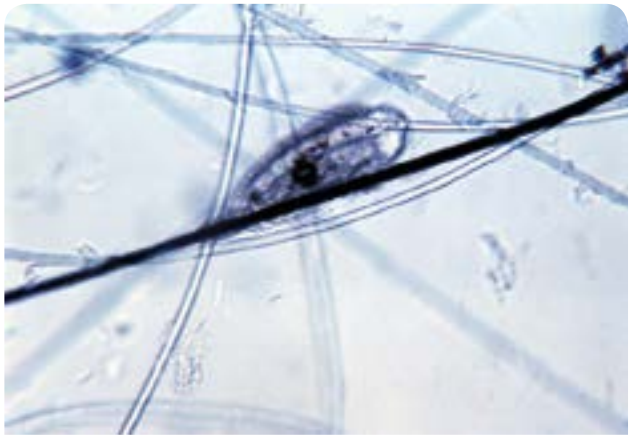


Figure 2. *Cheyletiella yasguri* egg.

Clinical signs

The primary clinical signs of cheyletiellosis are mild to moderate pruritus and skin scaling (Fig. 3). Erythema and crusted lesions may be seen in affected areas and the disease may appear as miliary dermatitis in cats. The degree of pruritus is very variable and does not appear to be proportional to the mite burden, so some hosts carrying large numbers of mites will only exhibit scaling with little or no pruritus, usually along the dorsal aspect of the trunk (Fig. 4), whilst others will be intensely pruritic. If the infestation is not treated in time, scaling may become severe and widespread, leading to hair loss.

Figure 3. Scales caused by cheyletiellosis.

Diagnosis

Diagnosis is made by mite identification. The easiest way to collect material for examination is to sit the patient on a dark surface and groom some of the scale from the skin surface. If the animal has cheyletiellosis, the dislodged material may appear to move ("walking dandruff"). This movement is visible as the pearly white, mobile, adult mites drag skin scales along, trapped in the long dorsal hairs on their body surfaces. The material dislodged can be collected in a petri dish or on a slide for microscopic examination at 40× magnification. Mites and eggs can also be harvested using sticky tape strips applied to the affected area and then stuck to a microscope slide. Superficial skin scrapings can also be performed and the material collected placed on a microscope slide with a drop of water under a cover slip. Individual mites can be examined by adding a drop of water to groomed debris on a microscope slide and covering it with a cover slip (Fig. 5): adults have four pairs of legs that protrude beyond the body margin and palps with powerful curved terminal claws (Fig. 6). The slightly hexagonal body has a "waist" just in front of the two pairs of hind legs and all of the legs have combs on the ends. Mite eggs may also be detected via coproscopy, because of the excessive grooming habits of some infested cats and dogs.

Figure 4. Dorsal scaling caused by cheyletiellosis in a puppy.

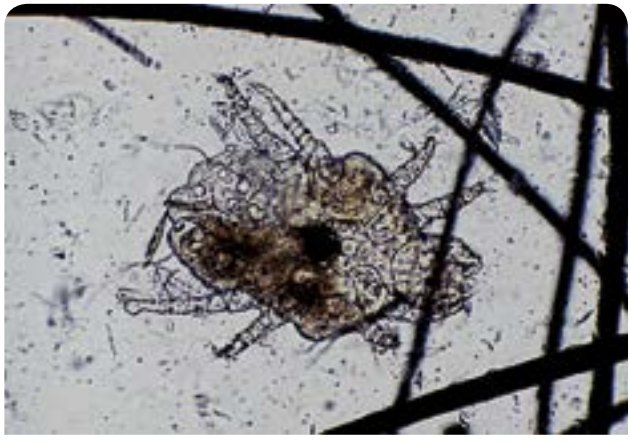


Figure 5. Magnification of an adult *Cheyletiella* spp. mite. Microscopic examination at ×40.

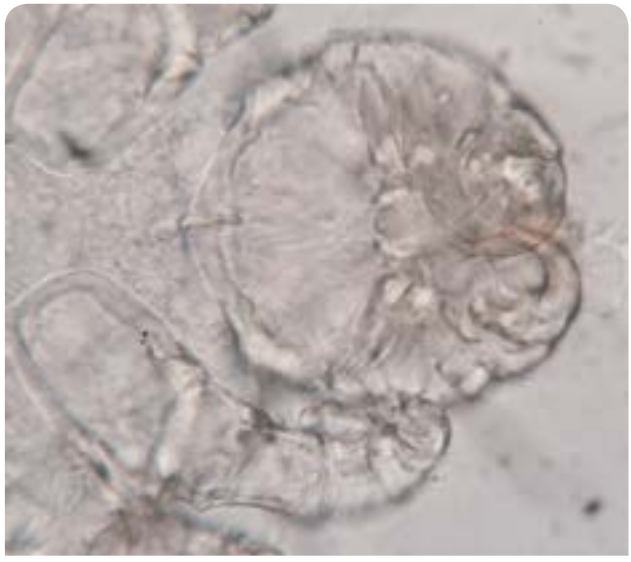


Figure 6. Anterior part of an adult *Cheyletiella* mite.

Figure 7. Human contamination in the owner of a cat with cheyletiellosis. Courtesy of the Parasitology Unit, Alfort Veterinary School.

Control measures

No treatments are specifically licensed for the treatment of cheyletiellosis but the mites have proven susceptible to many of the current topical acaricides licensed for use in dogs and cats. The presence of asymptomatic carriers, the highly contagious nature of the disease, with mites readily transferred between hosts, and the ability of the female mite to survive off-host make treatment of affected and contact animals a wise precaution.

Risk to humans

Humans in contact with pets having cheyletiellosis can acquire transient infestations which are seen as pruritic, papular lesions, often arranged linearly. Lesions are generally seen in areas that come into contact with the pet, such as the torso, arms and anterior surface of the thighs and they can occur even where clothing might been expected to provide protection (Fig. 7). Direct treatment seems unnecessary, provided that the primary hosts are effectively treated, as the mites do not reproduce on humans.

Canine demodicosis

General comments

Canine demodicosis is a parasitic skin condition caused by the characteristically cigar-shaped mite ***Demodex canis*** (family Demodicidae) in dogs (Fig. 1). The disease is caused by excessive multiplication of these host-specific, commensal mite, in hair follicules and sebaceous glands. The mite is a skin commensal believed to be transmitted from the dam when the young suckle. Signs of infestation are only seen when mite numbers increase significantly. Mites complete their entire life cycle on a single host.

Other species have been suggested (e.g., ***Demodex injai***, and ***Demodex cornei***), but some authors consider that they are only morphological variants of *D. canis*. Immunodeficiency is a predisposing factor which is probably hereditary in young dogs (under 2 years old) and acquired in adult dogs, following development of an underlying cause (e.g., excessive glucocorticoid therapy, Cushing's syndrome, diabetes mellitus and neoplasia). It is common but also under-diagnosed and can be very serious medically. Canine demodicosis can also have dramatic consequences in breeding units: bitches that have given birth to puppies with demodicosis must be removed from breeding, along with all their descendants.

D. canis has short legs, arranged in the shape sometimes described as resembling the Brandenburg cross. The male measures 150 µm in length and the female 200–300 µm. *Demodex* mites mainly feed on scale and sebum, the production of which they help increase. They never feed on blood and are incapable of living off their host.

Biology

The life cycle of *D. canis* lasts about 10–12 days and takes place entirely in the hair follicles and sebaceous glands. After mating, the males die off and fertilised females burrow into hair follicles, lay their eggs and then die (Fig. 2). After 2–3 days, eggs hatch out into free six-legged larvae which moult rapidly (Fig. 3), first into protonymphs and subsequently into eight-legged deutonymphs (Fig. 4). The deutonymphs climb back to the skin surface, probably moving up in the sebum, and infest new hair follicles. Deutonymphs make up the free infesting stage and become adults within 1–2 days (Fig. 5).

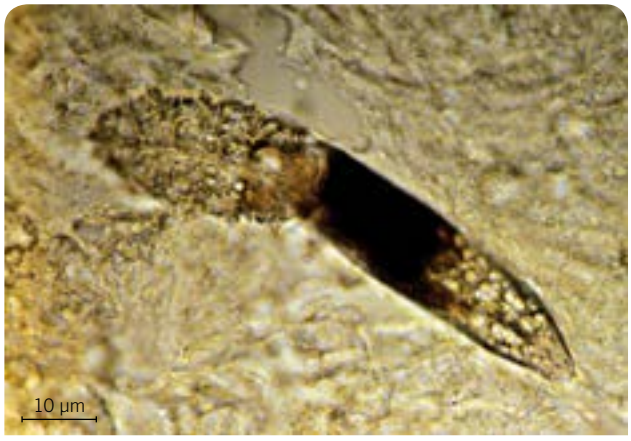


Figure 1. Adult *Demodex canis*.

Figure 2. *Demodex canis* egg. Courtesy of Parasitology Unit, Alfort Veterinary School.

Figure 3. *Demodex canis* larva. Courtesy of Parasitology Unit, Alfort Veterinary School.

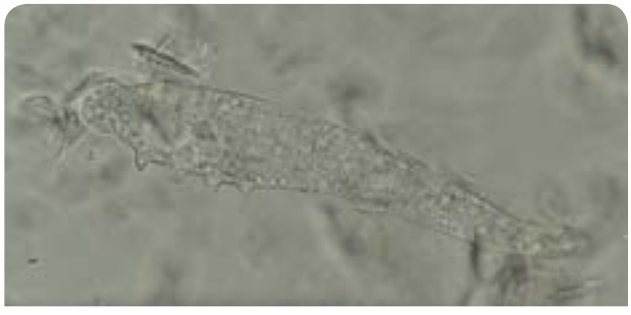


Figure 4. *Demodex canis* nymph. Courtesy of Parasitology Unit, Alfort Veterinary School.

Figure 5. Adult *Demodex canis*.

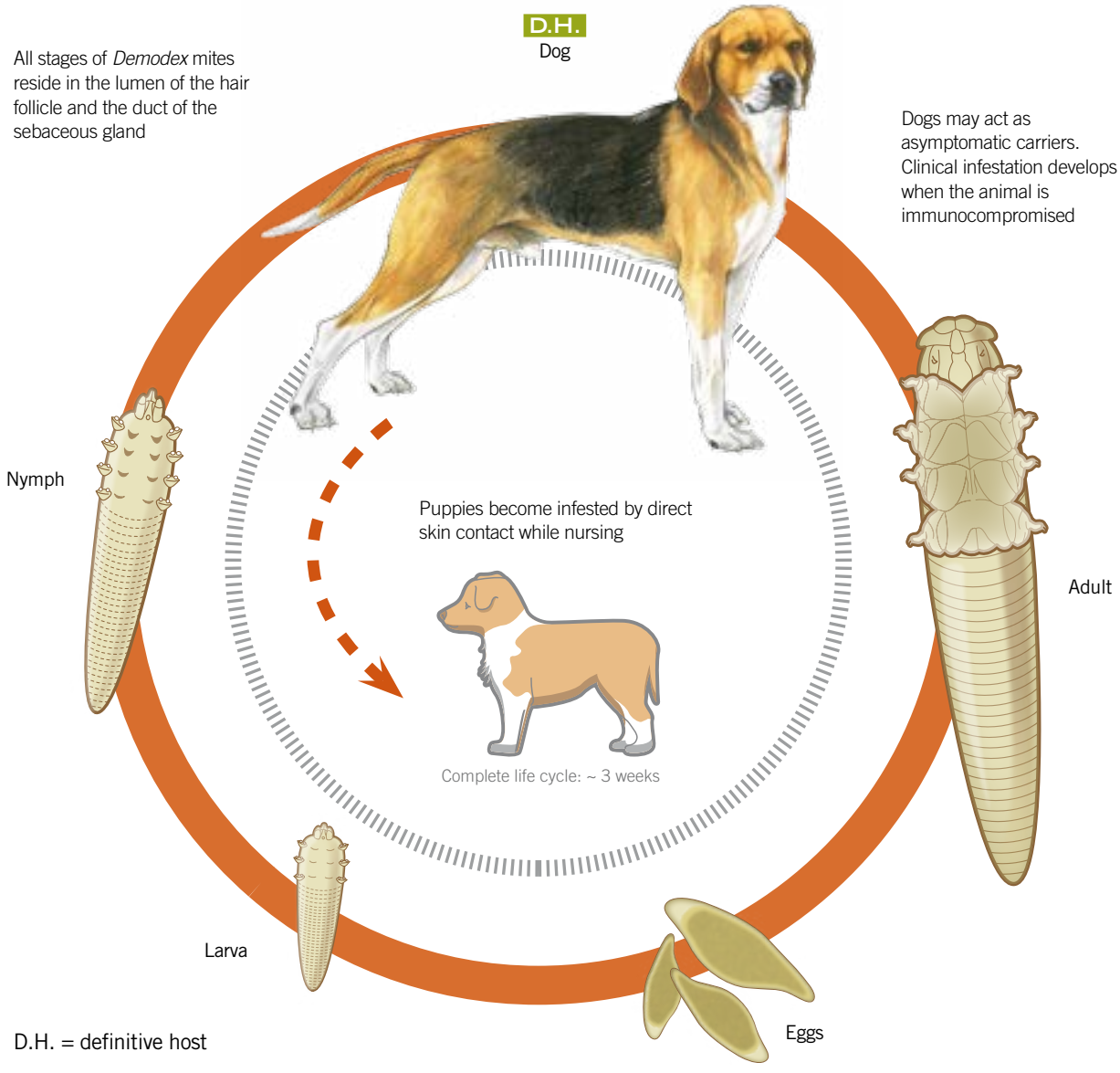
Epidemiology

Demodex sp. mites are highly host-specific but ubiquitous throughout the canine population. The infestation and disease is not transmissible between adult dogs and is not acquired from the environment.

About 50 % of adult dogs are asymptomatic carriers of *Demodex*. The only time that dog-dog transmission can occur is during the first 72 hours of life when the newborn puppy can acquire mites from the suckling bitch during washing and feeding. At no other time can mites be transmitted between dogs.

PDF

Life cycle of *Demodex canis*



Although demodicosis can occur in any breed of dog, there are clear breed predispositions which include the Staffordshire Bull Terrier, Old English Sheepdog, Boston Terrier, French Bulldog, Bernese Mountain Dog, German Pointer, Boxer, English Bulldog, English Bull Terrier, Pug, Cavalier King Charles Spaniel, Dobermann, Great Dane, Argentinian Mastiff, Dogue de Bordeaux, Jack Russell Terrier, Afghan Hound, Neapolitan Mastiff, Scottish Terrier, Shar Pei, Shih Tzu, Rottweiler, Newfoundland, West Highland White Terrier, Whippet and Yorkshire Terrier. This list is not exhaustive. Interestingly, breeds like the Poodle that almost never suffer from Generalised Juvenile-Onset Demodicosis (GJOD) seem to be predisposed to Adult-Onset Demodicosis (AOD) probably because of a breed susceptibility to hyperadrenocorticism, a common predisposing factor in the development of AOD.

Pathogenesis

Pathogenesis is complex and not fully understood. In addition to the mechanical and irritant effects of *D. canis*, antigenic effects and immunosuppression both contribute to pathogenicity. Substances released during moulting, metabolic products and products from the degradation of epithelial cells can act as antigens. Immunosuppression may arise through the production of immunosuppressive substances produced by the parasite itself. GJOD may be the result of a specific hereditary defect in cellular (T lymphocytic) immunity to *D. canis* and this host defect may allow *Demodex* to breed and produce immunosuppressive substances that can be detected in the serum of dogs with demodicosis. This leads to a vicious circle which enables the parasite to proliferate. Humoral immunity, on the other hand, seems to be stimulated. Plasma cell numbers increase in the spleen, lymph nodes and skin. IgG levels increase 2.5-fold and the number of circulating immune complexes also increases significantly.

The absence of cytotoxic T lymphocytes in localised demodicosis may allow spontaneous resolution. In generalised demodicosis, the immune defect involves both cytotoxic T lymphocytes and helper T lymphocytes and enables parasitic proliferation. In pyoderma associated with demodicosis, the immune defect involves cytotoxic T lymphocytes, helper T lymphocytes and B lymphocytes. Both type 1 and type 2 helper T lymphocytes seem to be involved in generalised demodicosis. A defect in interleukin 2 caused by dysfunction or inhibition of type 1 helper T lymphocytes has been reported and may be responsible for the condition becoming generalised.

In AOD, an underlying cause (e.g., long-term glucocorticoids, chemotherapy and other iatrogenic factors; spontaneous hyperadrenocorticism, lymphoma and other types of neoplasia; diabetes mellitus) has been identified in about 75 % of cases. Any form of immunosuppression is likely to promote the development of clinical demodicosis.

Clinical signs

Skin lesions are highly pleomorphic and vary according to breed and underlying factors. Two types of disease are recognised: localised and generalised. Localised demodicosis, whilst aesthetically undesirable, is normally benign and often self-resolving. Generalised demodicosis is defined as juvenile- or adult-onset and remains difficult to cure.

Localised demodicosis

- **Nummular** (coin-shaped) **demodicosis** involves a small number of areas (<5) of variably circumscribed erythema, scaling and hair loss (Fig. 6). The face (eyelids and lips), limbs and occasionally the trunk are most likely to be involved (Figs. 7–10). Pruritus is usually absent. Lesions regress spontaneously within a few weeks in 90 % of cases. Nummular demodicosis is common in short-coated breeds such as the Staffordshire Bull Terrier, Boston Terrier,

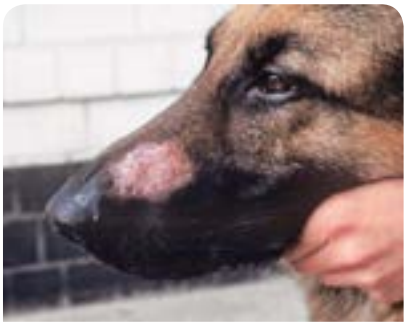


Figure 6. Nummular alopecia due to demodicosis.

Figure 7. Facial hyperkeratosis due to demodicosis.

Figure 8. Localised facial demodicosis lesion in a puppy.

French Bulldog, Boxer, German Pointer, English Bulldog, English Bull Terrier, Pug, Dobermann, Great Dane, Argentinian Mastiff, Dogue de Bordeaux, Jack Russell Terrier, Shar Pei, Rottweiler and Whippet.

- **Pododemodicosis** is localised, serious from the outset and often under-diagnosed (Fig. 11). Multiple feet are commonly involved. Various clinical forms can be seen:
  - Multiple periungual pododemodicosis is characterised by erythematous swelling, pruritus and pain around the nails. This is seen in the West Highland White Terrier, Scottish Terrier, Dobermann and Dachshund.
  - Interdigital and ventral pododemodicosis involves erythema, scaling and sometimes hyperpigmentation and/or furuncles in the interdigital region or in the area around the pads. This form is seen in the Dobermann and Shar Pei.
  - Digital ulceration and necrosis are seen in secondary chronic deep bacterial infections, mainly in the West Highland White Terrier and Scottish Terrier.
- **Aural demodicosis** is rare and presents as an erythemato-ceruminous otitis with profuse brownish discharge. It is usually found in association with demodicosis of other parts of the body. The authors have seen several cases in Neapolitan Mastiffs.

Generalised demodicosis

Demodicosis is considered to be generalised if at least five distinct body regions are affected, if one region of the body is completely affected or if two or more feet are affected. Generalised demodicosis is also clinically highly pleomorphic according to breed (Figs. 12 and 13).

Forms of generalised demodicosis

- **Multifocal alopecia:** a number of areas (>5) of variably circumscribed hair loss, or one area of complete alopecia, can be seen, along with mild pruritus, erythema and scaling (Fig. 14). The neck, trunk and limbs are mainly affected and it mainly occurs in short-haired breeds e.g., Staffordshire Bull Terrier, English Bull Terrier, English Bulldog, French Bulldog and Jack Russell Terrier. Extensive erythema and scaling around the dorsolumbar midline are common in the West Highland White Terrier and Scottish Terrier (Fig. 15). In the Pug, multifocal hair loss is associated with numerous comedones. A moth-eaten coat can be seen in the Shar Pei, and this must be distinguished from bacterial folliculitis, a common condition in this breed.

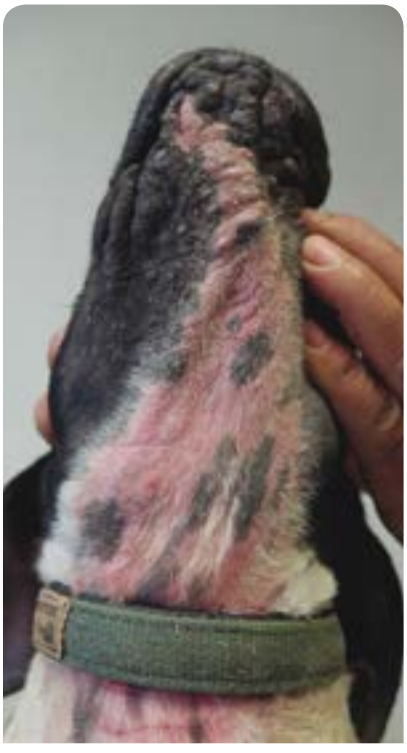


Figure 9. Erythema due to demodicosis. Courtesy of Parasitology Unit, Alfort Veterinary School.

Figure 10. Typical periocular alopecia due to demodicosis.

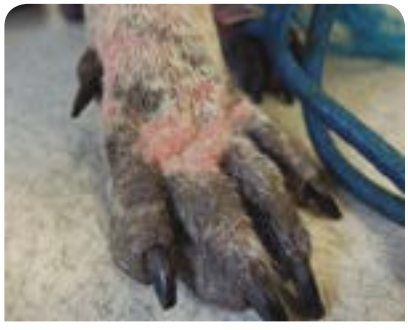


Figure 11. Lesion of pododemodicosis.

Figure 12. Generalised alopecia due to demodicosis.

Figure 13. Generalised demodicosis in a pug. Courtesy of Parasitology Unit, Alfort Veterinary School.

- **Scaling:** this form is striking because it presents simply with pityriasiform scaling, sometimes very noticeable. Clipping reveals well-circumscribed scaling lesions. Secondary infections (e.g., bacterial folliculitis) are common and cause pruritus. This distinctive form is seen in the Scottish Terrier and West Highland White Terrier.
- **Ulceration and crusting:** thick crusts are directly associated with the underlying furunculosis and/or cellulitis visible after clipping. These pruritic and painful lesions can be seen on the neck, trunk and limbs. Ulcers appear in various shapes and sizes, typically rounded and extensive with a jagged, friable border. This pattern is mostly seen in the West Highland White Terrier, Scottish Terrier and Cavalier King Charles Spaniel, but also in large, long-haired breeds, such as the Newfoundland, Bernese Mountain Dog and Leonberger.
- **Comedones** with variable alopecia: hundreds of comedones may be present, particularly in sparcely-haired regions such as the axillae, thorax and abdomen (Fig. 16). Secondary infection (folliculitis and/or furunculosis) and pruritus are common because hair follicles are obstructed by the comedones. This pattern is seen in the Pyrenean Sheepdog, Yorkshire Terrier, Pug and occasionally the West Highland White Terrier.
- **Follicular casts**: a follicular cast is a sheath of sebum that accumulates around the hair shaft. Large numbers of hairs are affected. Hair loss is rare but severe seborrhoea can be seen once the dog has been clipped. Pruritus resulting from secondary infection (e.g., cellulitis) is common. Long-haired breeds (e.g., Old English Sheepdog and Afghan Hound) are mainly affected.
- **Pustular demodicosis** is common and does not seem to vary with breed. Follicular and non-follicular pustules and haemorrhagic bullae (furunculosis and cellulitis) may occur anywhere on the body. Lesions are very pruritic, sometimes painful. In the Shar Pei, acne may be associated with demodicosis. Interestingly, cellulitis associated with demodicosis is never seen in this breed.
- **Haemorrhagic bullae**: these mainly occur in Neapolitan Mastiffs.

Systemic signs

Systemic signs are inconsistent. In generalised demodicosis with secondary bacterial infection, severe systemic signs may arise, including anorexia, pyrexia (40–41 °C), lethargy, dehydration and electrolyte disturbances. Septicaemia is not

Figure 14. Skin erythema due to demodicosis.

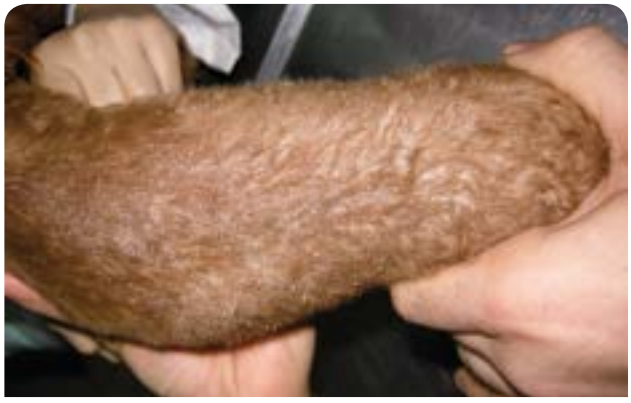


Figure 15. Demodicosis in a dog due to *Demodex* sp. Courtesy of Odile Crosaz, Parasitology Unit, Alfort Veterinary School.

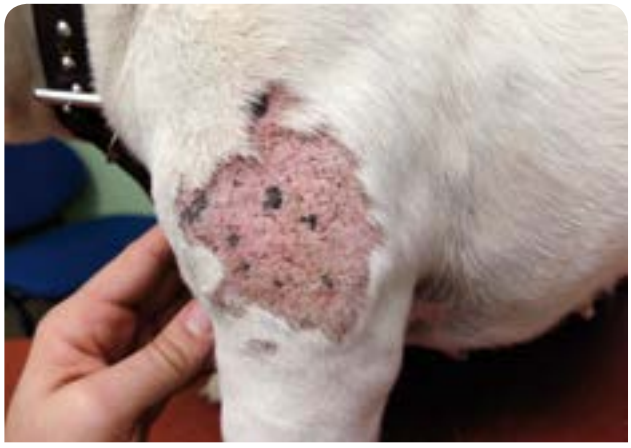


Figure 16. Comedones in a dog with demodicosis. Courtesy of Parasitology Unit, Alfort Veterinary School.

uncommon in cases of extensive, chronic demodicosis-associated cellulitis. In such cases, demodicosis can be a true dermatological emergency. Glomerulonephritis may also develop in chronic demodicosis. In old dogs, there will be signs associated with the underlying cause (e.g., hyperadrenocorticism and diabetes mellitus).

Diagnosis

Diagnosis is based on medical history, clinical signs and detection of the parasite at different stages of its life cycle.

- **Deep skin scrapings** (deep enough to see capillary "ooze") should be examined in chloral lactophenol, on a microscope slide under a cover slip (Fig. 17). Microscopic examination, at an initial magnification of ×40, then at ×100, reveals large numbers of *Demodex* mites: *D. canis* (eggs, larvae and adults). One *Demodex* mite is not enough to diagnose demodicosis since about 50 % of normal dogs with no clinical signs have *Demodex* mites. An appropriate history, indicative clinical signs and the presence of *Demodex* mites in skin scrapings are required to make the diagnosis (Fig. 18). Dogs with pododemodicosis sometimes need to be anaesthetised to obtain good skin scrapings. In cases of ulceration, crusting and haemorrhagic bullae, skin scrapings taken from ulcers and bullae respectively do not always reveal *Demodex* mites. In fact, there are no *Demodex* in ulcers since the mites live in pilosebaceous follicles; equally, mites in bullae are often lysed. In these cases, skin scrapings should be taken from the edge of the ulcer or bulla or from other types of lesions.
- Hair plucks from affected areas, mounted in chloral lactophenol, also reveal numerous mites in various stages of the life cycle, arranged around the hair shaft. Taking hair plucks is a very straightforward procedure and particularly useful when dealing with cases with follicular casts but it is not very sensitive.
- **Histopathological examination of skin biopsies** is useful but not the principal means of diagnosing demodicosis. *Demodex* mites will often be clearly seen in hair follicles and sebaceous glands, along with lymphocytic mural folliculitis and folliculitis lesions, furunculosis and cellulitis. The main indications for biopsy are pododemodicosis, demodicosis in the Shar Pei (the skin is thick in this breed because of dermal depots of mucin; hair follicles also penetrate further into the skin than in other breeds) and subclinical demodicosis. In pododemodicosis and Shar Pei demodicosis, perifollicular macrophagic granulomas, made up of histiocytes and giant cells, and pyogranulomas arranged around fragments of *Demodex* are commonly found. In subclinical demodicosis, early histopathological lesions include degeneration of the wall of the isthmus around the sebaceous glands, associated with histiocytes and giant cells. Lymphocytic mural folliculitis and perifollicular granulomas are seen later in the absence of *Demodex*.

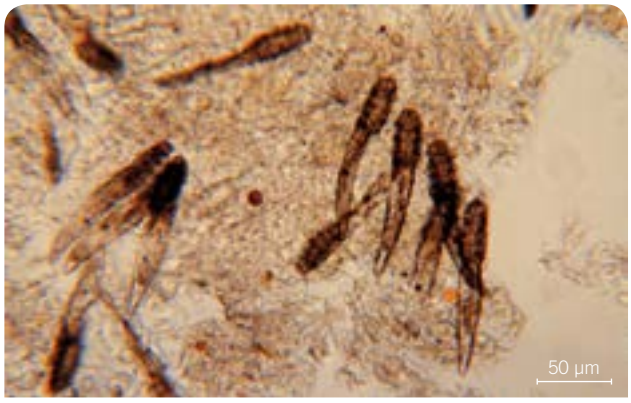


Figure 17. *Demodex canis* adults from a skin scraping.

Figure 18. *Demodex canis* adults.

Prognosis

Prognosis has improved considerably in recent years, thanks to the development of systemically-acting products (macrocyclic lactones and isoxazolines) effective against *Demodex*, some of which are licenced for use in dogs.

Control measures

Excellent communication between vet and owner is essential, as treatment can be long and expensive.

Indications

Treatment for generalised demodicosis, with or without secondary bacterial infection, pododemodicosis and aural demodicosis, is essential. Localised forms of demodicosis resolve spontaneously in 90 % of cases but they must be monitored carefully to determine which cases may subsequently need to be treated.

Preliminary measures

Skin scrapings taken from two or three representative sites give a baseline assessment of the mite population against which post-treatment mite populations can be measured.

A semi-quantitative list of the number of individuals in the different life cycle stages (adult, larvae and eggs) found in skin scrapings can be compiled (>50, 30–50, 10–30, <10). The presence of numerous adults, larvae and eggs suggests an extremely active form of the condition. Lesions must be clipped and cleaned, making topical acaricidal therapy and antiseptic treatment more effective and reducing the risk of toxic side effects. In pododemodicosis, the interdigital spaces and ventral pedal surfaces must be clipped carefully, then cleaned and washed with chlorhexidine.

Where treatment is necessary, the use of a licensed acaricide specific for *Demodex* spp. is recommended. Several acaricides have proven effective against *Demodex*: amitraz, milbemycin oxime, moxidectin, ivermectin, isoxazolines, etc. Whatever the choice of treatment, acaricidal treatment should be continued until clinical signs have resolved and two scrapes or plucks taken at monthly intervals after resolution of clinical signs are negative for mites.

Monitoring

Monitoring is essential. A dog with demodicosis needs to be cared for by its vet and its owner and it is to the vet's responsibility to motivate owners and give them good advice.

Treatment duration is often long, sometimes several months.

Each case should be reviewed clinically and parasitologically every month. The number of *Demodex* mites can be assessed semi-quantitatively at selected sites and the average number of different life stages can be recorded, along with counts of live and dead mites. The reduction in the number of larvae, eggs and ghost forms (clear, dead mites) allows treatment efficacy to be assessed. Clinical resolution (Fig. 19) normally comes before parasitological cure and cases should be reviewed every 2 months for a year after the parasitological cure. Relapse occurs in an average of 15 % of cases in the year following cessation of treatment.

Treatment of the underlying cause

In cases of AOD, it is important to treat the underlying cause when identified. This may involve stopping glucocorticoid therapy or treating hyperadrenocorticism.

Treatment of secondary infections

Secondary infections are very common and include pyoderma (bacterial folliculitis, furunculosis and cellulitis) and *Malassezia* dermatitis. These infections must be treated with systemic therapy based on the results of specific diagnostic tests: impression smears demonstrate the type of organism (e.g., cocci, bacilli or yeasts) and bacterial culture and sensitivity tests must always be carried out.

Risk to humans

Demodex species are host specific and there is no zoonotic risk associated with demodicosis.

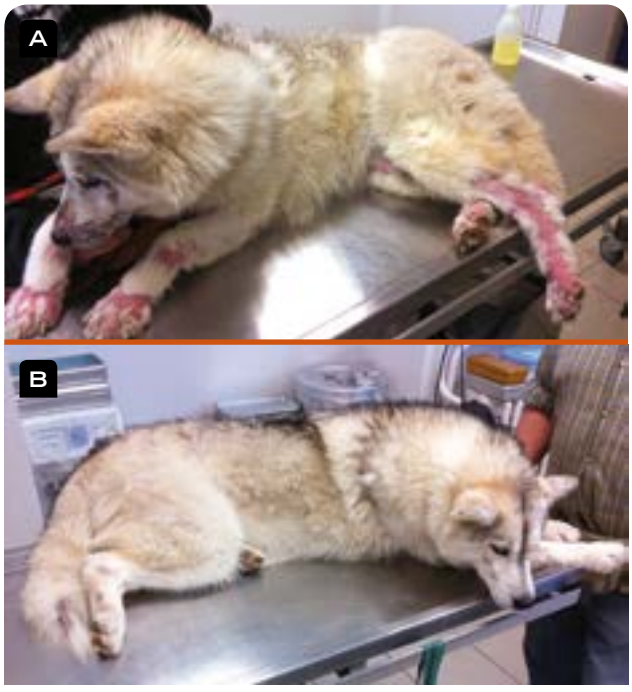


Figure 19. Demodicosis in a dog before treatment (A) and after (B). Courtesy of Parasitology Unit, Alfort Veterinary School.

Feline demodicosis

General comments

Demodicosis is a rare skin condition in the cat and only one species, ***Demodex cati*** (Hirst, 1919), had been described in this host species until recently. However, another species (***Demodex gatoi***) has been suggested in the last two decades. This new species is clearly morphologically, biologically, epidemiologically and molecularly different from *D. cati*. The existence of a third morphologically distinct species has recently been proposed, following further observation. However, this is still under debate and additional data are required to confirm its existence.

Demodex cati is a cigar-shaped mite with very short legs (Fig. 1). It resembles *Demodex canis*. The adult mites and nymphs are eight-legged and the larvae are six-legged. The opisthosoma (the mite's "tail") represents two thirds of the total length and is finely tapered. The adult female is 220 µm long and about 30 µm wide, and the adult male is smaller (182 µm × 20 µm) (Fig. 2). The egg is lemon-shaped and measures approximately 70 × 20 µm. *D. cati* mites live in the hair follicles, especially in the eyelids, face, chin and neck. There has been no specific work on the life cycle of *D. cati* but it is assumed to be similar to *D. canis* in the dog or *D. brevis* in humans. The eggs are laid in the follicular cavity, hatch to produce larvae then protonymphs, and finally male and female adults.

Demodex gatoi was first observed in a cat in Louisiana, USA (Fig. 3). Since then, its presence has been reported in other regions of the USA, and Europe. *D. gatoi* is smaller than *D. cati*, mainly due to the fact that the mite's opisthosoma is much shorter (half the full length of the mite) and blunt. The female and the male are about 100 and 90 µm long, respectively. *D. gatoi* inhabits the superficial skin layer (stratum corneum). Its life cycle has not been fully described and this mite species has biological and epidemiological features which are clearly different from *D. cati*. Molecular techniques have also confirmed that *D. cati* and *D. gatoi* are distinct species.

Figure 1. Adult *Demodex* sp. in a cat. Courtesy of Parasitology Unit, Alfort Veterinary School.

Figure 2. Adult *Demodex cati*. Skin scraping.

Figure 3. Adult *Demodex gatoi*. Skin scraping.

Epidemiology

Demodicosis is considered to be a rare skin condition in the cat. The epidemiology of *D. cati* and *D. gatoi* seems to differ significantly, so each mite is considered separately.

D. cati can be responsible for localised or generalised forms. There is no reported breed predisposition. The severity of the clinical signs is usually associated with concurrent primary conditions, such as diabetes mellitus, acquired immunodeficiency (FeLV, FIV infections), hyperadrenocorticism, etc. It is considered to be a non-contagious disease; the mite is probably acquired soon after birth through direct contact with the queen. In this respect, feline demodicosis shares many similarities with canine demodicosis.

D. gatoi is unique among *Demodex* mites as it lives very superficially in the keratin layer of the epidermis. It is responsible for a pruritic and contagious skin condition in cats. There is a breed predisposition, as *D. gatoi* has mainly been found in Cornish Rex, Burmese, Persian and Siamese cats. However, the disease does not appear to be associated with immunosuppression.

Pathogenesis

There is a known relationship between mite proliferation and the existence of potentially immunosuppressive systemic diseases in *D. cati*-infested cats, but very little information is available on the underlying immune mechanisms in the cat. Some studies suggest that humoral immunity does not play a major role. In humans, innate cutaneous immunity (Toll-like receptors, antimicrobial peptides, proteases) may play a key role, but this has not been investigated in the cat.

The proliferation of *D. gatoi* causes a pruritic and potentially contagious skin condition which does not seem to be associated with a primary immunosuppressive disease. Interestingly, some infected cats do not exhibit clinical signs. It has been suggested that affected cats may develop a hypersensitivity reaction to different mite allergens. Affected cats over-groom and this behaviour eliminates many mites, which may cause a diagnostic challenge. In contrast, infected asymptomatic cats may harbour very large numbers of parasites.

Clinical signs

Cats infested with *D. cati* may present quite variable clinical signs: alopecia, crusts, seborrhoea, scaling, papules, and miliary dermatitis (Fig. 4). Pruritus may be absent. The most commonly affected areas are the face, chin, neck and eyelids. Generalised forms have also described. Pyoderma is not often seen in generalised feline demodicosis, unlike in dogs. *D. cati* is often responsible for a ceruminous otitis externa which may or may not be associated with skin disease (Fig. 5). Depending on the clinical presentation, differential diagnosis includes *Otodectes cynotis* otitis externa and associated dermatitis, *Notoedres cati* infestation, dermatophytosis, allergic dermatitis, cutaneous lymphoma, bacterial pyodermatitis, pemphigus foliaceus.

Figure 4. Feline demodicosis due to *Demodex cati*.

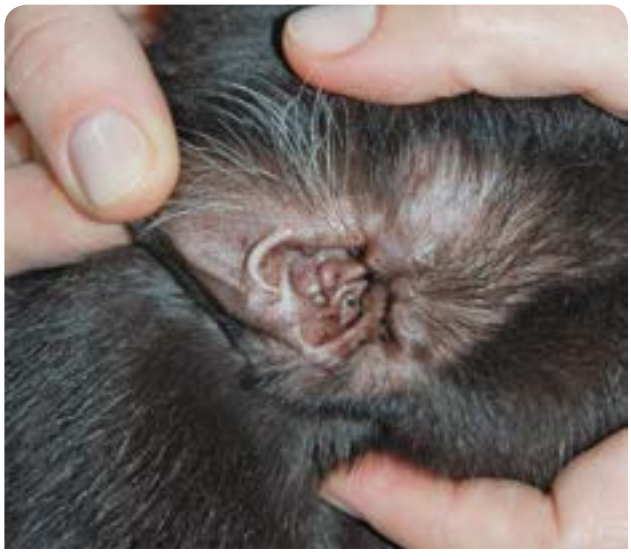


Figure 5. Otitis due to feline demodicosis caused by *Demodex cati*.

The primary clinical sign in cats infested with *D. gatoi* is pruritus and associated over-grooming. The cat presents with self-induced alopecia, especially on the easiest areas for the cat to reach (abdomen, flanks, inner thighs and forelimbs) (Fig. 6). Miliary dermatitis is uncommon. Differential diagnosis includes any type of pruritic contagious dermatitis: flea hypersensitivity, *Cheyletiella* sp. and *Felicola* sp. infections, trombiculosis, and food and contact allergies.

Diagnosis

In addition to medical history and clinical examination, *D. cati* infestations rely on detection of the mite in deep skin scrapings. A trichogram may be useful in areas that are difficult to scrape, as the mite inhabits hair follicles. Detection of mite eggs is also diagnostic. Biopsies are rarely used but can reveal mites in the hair follicles.

The mite population in cats infested with *D. gatoi* may be reduced, and the aetiological diagnosis made challenging, by over-grooming. Several superficial skin scrapings should be taken from different body areas, especially those which are difficult to groom. Cellophane tape may also be used and faecal flotation may reveal the ingested parasites. Identification is usually based on the typical morphology and superficial localisation of this mite.

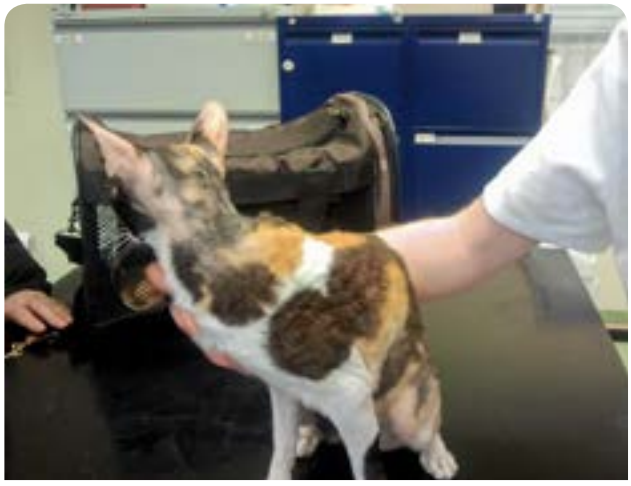


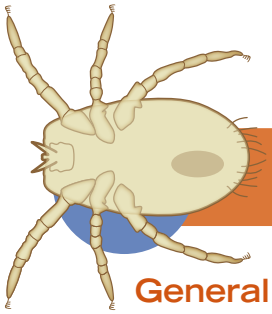
Figure 6. Feline demodicosis due to *Demodex gatoi*.

Control measures

Relatively little information on evidence-based medicine is available as far as the treatment of feline demodicosis is concerned. Classically, lime sulphur dips and amitraz were successfully used but lime sulphur is not widely available, especially in Europe, and amitraz must be used with caution in cats.

Risk to humans

Demodex species are host specific and there is no zoonotic risk associated with feline demodicosis.



Trombiculosis

General comments

Trombiculosis is a non-infectious, non-contagious ectoparasitosis caused by various species of mites belonging to the family Trombiculidae. It is common in cats, although many other species of mammals (including dogs, horses, cattle, sheep and humans), birds, reptiles and amphibians may be infested by these mites. Trombiculids are unique in that only the larval stage is parasitic because the nymphs and adults are free-living (Fig. 1). Depending on the geographical location, the larvae are known as harvest mites, chiggers, red bugs and scrub itch mites. They have also received very specific names in other languages. These names are either related to the mite's colour, its seasonal activity or the clinical signs of the condition it induces.

The most important species in veterinary medicine belong to the genus *Trombicula* which is subdivided into the sub-genera *Neotrombicula* and *Eutrombicula*. The main species are ***Neotrombicula*** (syn. ***Trombicula***) ***autumnalis*** in Europe, ***Eutrombicula alfreddugesi*** and ***Eutrombicula splendens*** in the Americas, and ***Eutrombicula sarcina*** in Australasia. However, other species have also been described and can be important locally. Consequently, trombiculosis can be considered to be a condition of mammals worldwide. Free-roaming cats are particularly exposed to the mites as the larval stages are found outdoors in specific biotopes (meadows, lawns, heathlands, corn field, wooded and marshy areas, etc.) depending on the mite species. Mite distribution in the environment is known to be quite patchy, so noticeable differences in prevalence can be seen, even within fairly limited areas. The factors responsible for this irregular distribution are largely unknown.

Only the larval stage has been described, as the nymphs and adults are never found on the animal. The six-legged *N. autumnalis* larva is very hairy, orange in colour and is 250–750 µm long, depending on the stage of repletion (Fig. 2). The dorsal shield or scutum is roughly pentangular and the chelicerae are flanked by stout, five-segmented palps. The larvae of *E. alfreddugesi* are very similar to those of *N. autumnalis* and they measure 150–600 µm in length and have a rectangular scutum. *E. splendens* is very similar to *E. alfreddugesi,* and is often sympatric.

Figure 1. Adult *Trombicula*.

Figure 2. *Neotrombicula autumnalis* larva.

Biology

The life cycle of *N. autumnalis* takes 2 to 12 months. The larvae, which are the only parasitic stage, can essentially be seen in summer and autumn so trombiculosis mainly occurs during those periods in temperate regions of north hemisphere. After the larval stage hatches in the environment, it climbs onto different plants, questing for a suitable host. After attachment to the skin, the larvae feed on tissues (Figs. 3–5). They pierce the skin with their mouth parts and inject histolytic saliva, so a mixture of blood and digested dermal tissues is ingested. This ingestion takes place through a canal known as stylostome, formed following the hardening of a specific salivary secretion; feeding behaviour reminiscent of ticks. Larvae are often attached in clusters which make their detection easier, as they appear as an "orange powder" in preferred sites (Fig. 6). Engorgement usually takes 24– 72 hours but may take up to 10 days. The larvae drop off into the environment after completing their

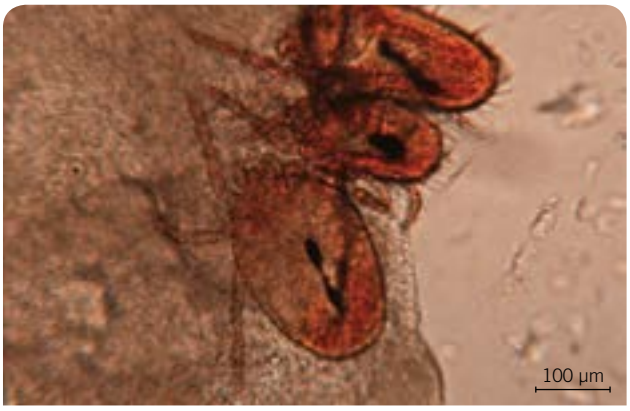


Figure 3. *Trombicula* mites attached to the skin. Courtesy of Parasitology Unit, Alfort Veterinary School.

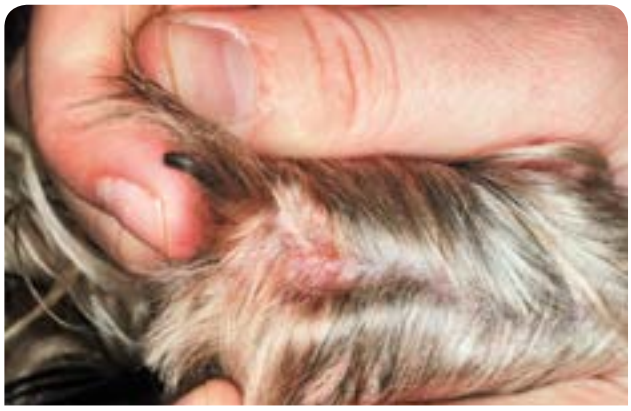


Figure 4. Attached *Trombicula* mites.

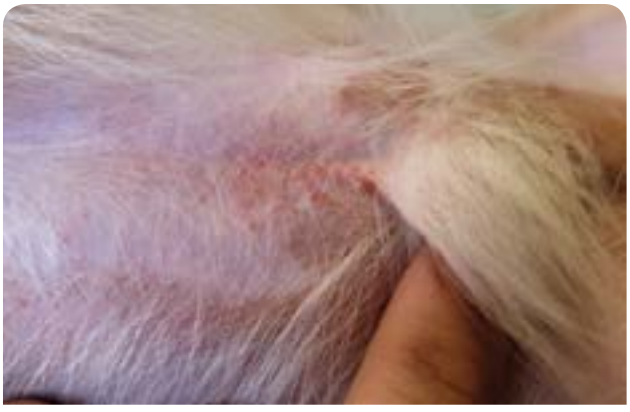


Figure 5. Trombiculosis in a dog.

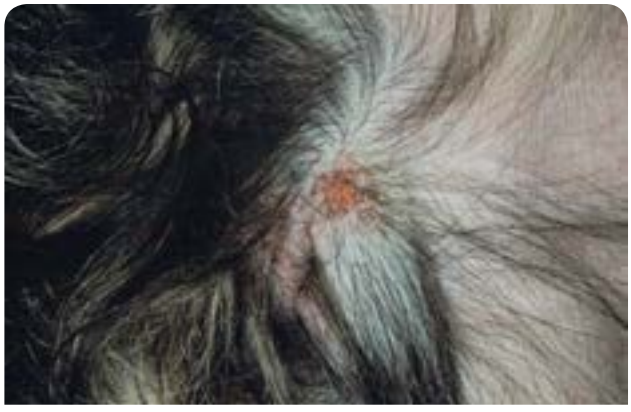


Figure 6. Several *Trombicula* mites attached.

meal. Life cycles of the other trombiculid species are very similar, but may differ in length according to local climatic conditions.

In Europe, several generations of trombiculids are produced between March and October and the risk of infestation persists until autumn. The larvae feed for 3–4 days before leaving the host to moult into nymphs, a process which takes 2–3 days. The immobile stage 1 nymph develops into stage 2 over 4– 5 days. The mobile second nymph stage feeds on small soil mites before moulting into a stage 3 nymph over 3– 4 days, and then into the adult in a further 3–4 days. Adult trombiculids mate and the females lay eggs after 8–10 days. They live for several weeks in the environment in the summer, before entering a resting stage at the end of autumn. They then become active again the following spring. Eggs hatch rapidly into hungry young larvae, the only parasitic stage, which actively seek out hosts on which to feed. They can fast for several months if necessary.

Epidemiology

The larval stage is parasitic on a wide range of mammals such as cats, dogs, rodents, rabbits, humans and birds and larvae will infest almost any animal entering their habitat, for example, horses being exercised, which they often attack on the legs. This mite is typically active in late summer and early autumn. Larval mites climb onto plants or other vantage points so that they can swarm onto a host when it comes close. Adult and nymphal mites have a free-living, predatory life cycle, feeding on other arthropods.

Infestation is exclusively from the external environment (gardens, undergrowth, hedges and copses) in summer and autumn, although it is not uncommon to see infestations in the spring, or even in winter, when weather conditions are favourable. There is no direct transmission. Trombiculosis is not a true zoonosis, as people are infested directly from the environment. Severe, often persistent pruritus, erythema and papules are seen on the limbs and trunk of infested people.

Pathogenesis

The mites pierce the skin with their needle-like chelicerae and inject saliva which breaks down cells and allows the mite to suck up the resultant fluid, feeding in this way for 2–5 days. Infestation is often noticeably pruritic and this may be caused by an allergic reaction to constituents of the mite saliva (proteases). Clinical signs may be exacerbated by self-inflicted injury and pruritus may continue for some days after mite removal.

Clinical signs

Infestation is usually pruritic and clumps of orange-coloured mites can sometimes be seen with the naked eye. Lesions on the feet are common, but the ventral body and face (including the ears) can also be affected (Figs. 7 and 8). Dermatological signs normally include local erythema, with papules, scales, crusts and pustules in some cases.

The interdigital spaces, pinnae (especially Henry's pocket), axillae, inguinal regions, interocular region, and lips are most often affected and larvae can even be found in surgical wounds. Pruritus increases as more parts of the body are affected, and can persist long after the larvae have left.

Diagnosis

Diagnosis is based on medical history, time of the year and clinical presentation. Skin scrapes may be required to identify the mites, which are characteristic of larval mites in having only three pairs of legs, but it is their orange appearance which allows them to be identified.

Material can be mounted onto a slide and examined microscopically at ×100 or ×400 magnification. Liquid paraffin or 10 % potassium hydroxide solution can also be added to the material and a cover slip placed on top. If skin scrapings are stored for some time before microscopic examination, gentle warming of the prepared slide under a lamp will encourage mite movement, but care must be taken not to overheat and kill the mites. If dry scrapings are taken, 10 % potassium hydroxide may be used instead of liquid paraffin to clear any debris on the slide but, besides being caustic, it also has the disadvantage of killing the mites, stopping the movement which can aid mite location under the microscope. Identification of a single mite is diagnostic. Lesions and clinical signs may persist for several days after mites have fed and left the host.

Control measures

A very few products are licensed for the treatment or prevention of trombiculosis, but several acaricidal treatments have been reported to be effective. Although individual larvae only feed for approximately 2–5 days, in heavy infestations or where infestation continues for a period of time, treatment may need to be repeated until all mites are killed and clinical signs resolved.

Risk to humans

Trombicula mites will infest humans directly from the environment but there is no risk of transfer from a pet to a human.

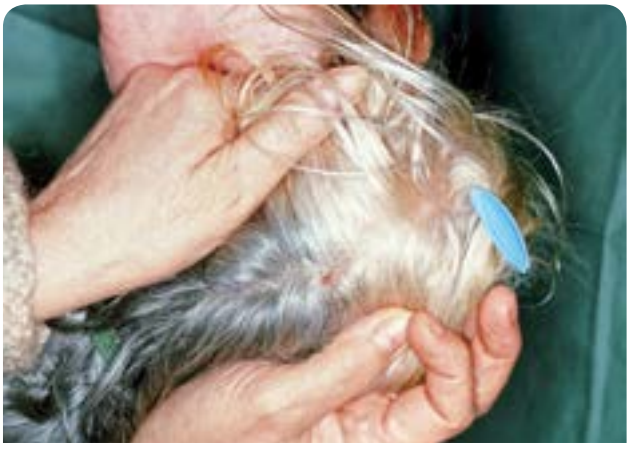


Figure 7. Trombiculosis on a dog's head.

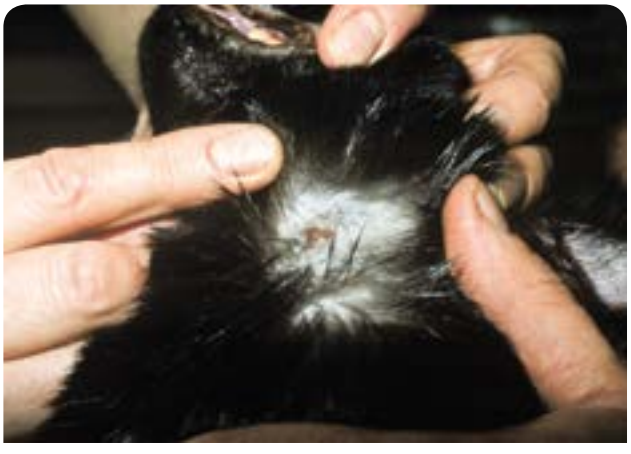
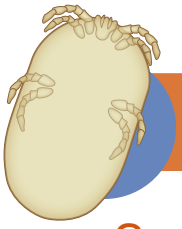


Figure 8. Trombiculosis in a cat.



Straelensiosis

General comments

Straelensiosis is a recently described skin condition caused by the larval form of the mite ***Straelensia cynotis*** (family Leeuwenhoekiidae, related to the Trombiculidae). These parasitic larvae were seen for the first time in 1968 in a wild dog in Eastern Europe. Cases were reported in central and south west France in the 1990s and have since been reported regularly in Europe. There have been no reports of transmission to humans.

Biology

S. cynotis larvae measure about 700 × 425 µm and strongly resemble *Neotrombicula autumnalis* larvae. Adults (which have not been described) are most probably free-living in the environment, in woods around fox earths and badger setts (Fig. 1). Nymphs and eggs can also be found in the environment. Only the larvae are pathogenic and their preferred hosts are foxes, and possibly small mammals. The dog is an accidental host which, when bitten, displays a severe cystic reaction at the point of attachment.

Infestation mainly occurs in areas where these parasites abound, for example near fox earths or badger setts. Straelensiosis is consequently seen more commonly in hunting dogs, notably terriers (e.g., Fox Terriers, Dachshunds and Jack Russell Terriers). Seasonal variation is not well understood and no age or sex predisposition has been reported.

Figure 1. Fox earth or badge sett, source of *Straelensia* mites for hunting dogs.

Pathogenicity is poorly understood. The larvae form a histosiphon at the level of the hair follicles (follicular ostium) in order to attach themselves. They then rapidly enter the dermis, where they become enclosed in a cyst, produced by the larvae themselves, and cause a very severe inflammatory reaction. This dermal cyst reaction to *S. cynotis* larvae seems to occur only in the dog and not in the fox, the parasite's usual host. It may afford protection against the parasite by preventing entry into the deep dermis.

Clinical signs

Skin lesions include the sudden appearance of papules, 1–5 mm in diameter, with nodules and distended ostia around the hair follicles (Fig. 2). These lesions, which may be haemorrhagic and pigmented, are usually numerous (sometimes more than 50 in total, with 2–6 per cm^2) and resemble encysted lead shot. They mainly occur on the face (forehead, bridge of the nose, lips and eyelids), pinnae, dorsal midline (neck, back, dorsal lumbosacral region, tail), medial and lateral limbs and occasionally over the whole body (Fig. 2). They are pruritic and often very painful, causing extreme skin hyperaesthesia and aggressive behaviour. Excoriations and thick crusts may be seen in chronic cases and systemic signs (e.g., lethargy and anorexia) are sometimes seen in severe, painful cases.

Diagnosis

Diagnosis is based on medical history, clinical signs and detection of parasitic larvae.

Superficial skin scrapings are often unrewarding.

The affected area should first be clipped under general anaesthesia. Very deep skin scrapings and papular incisions should then reveal the larvae. Selected papules should be raised and effectively scalped from underneath. Scrapings should be examined microscopically in chloral lactophenol, initially under ×40 magnification and subsequently under ×100 and ×250, with low to moderate lighting. U-shaped parasitic cysts with thick translucent walls can be seen (Figs. 3–5). The parasite is the rostrum located towards the base of the U.

The parasite can be identified by fine dissection of papules, preserved in 70 % alcohol, under a binocular dissecting microscope.

Histopathology of skin biopsies is indicative (Fig. 6). Affected hair follicles have a very dilated infundibulum containing a parasitic cyst made up of the shell of the thick basophilic cyst and the parasite. The parasite has a variably

Figure 2. Clinical straelensiosis in a dog. Courtesy of Françoise Gramatica.

calcified, striated external cuticle, a rostrum, a histosiphon (which pierces the end of the U-shaped cocoon) and fine silky strands. A neutrophil-rich liquid can be seen in the dermis at the end of the histosiphon. The thick, double-layered cyst is made up of vestiges of the external root sheath which exhibits severe trichilemmal keratinisation associated with severe pseudo-epitheliomatous hyperplasia. The adjacent dermis is rich in blood vessels and often mucinous. A severe inflammatory reaction, dominated by a variable number of eosinophils and plasma cells may be present, depending on the chronicity of the lesions. Evidence of eliminated cysts or pyogranulomatous reactions may also be seen.

Differential diagnosis includes sarcoptic mange, demodicosis, trombiculosis, calcinosis cutis, deep mycoses and certain multicentric tumours.

Prognosis and control measures

The prognosis is very guarded. There is no licensed treatment for this condition and therapeutic response is uncertain. Long-term application of amitraz has been proposed but results seem disappointing. Isoxazolines may be alternative treatments.

Prevention seems to be difficult and involves limiting contact between dogs and the parasite's usual hosts (e.g., foxes) in high risk areas during the hunting season.

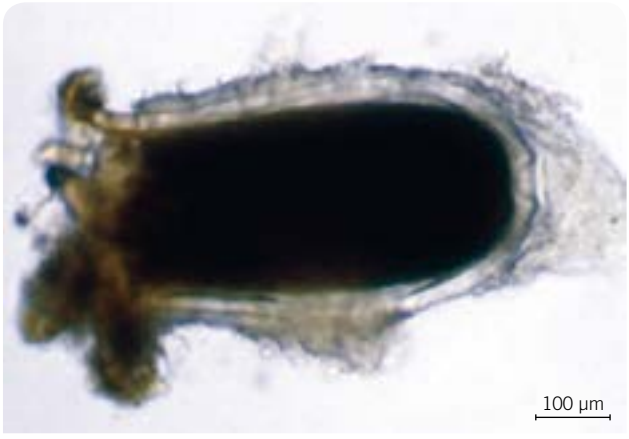


Figure 3. *Straelensia* nodule extracted from the skin.

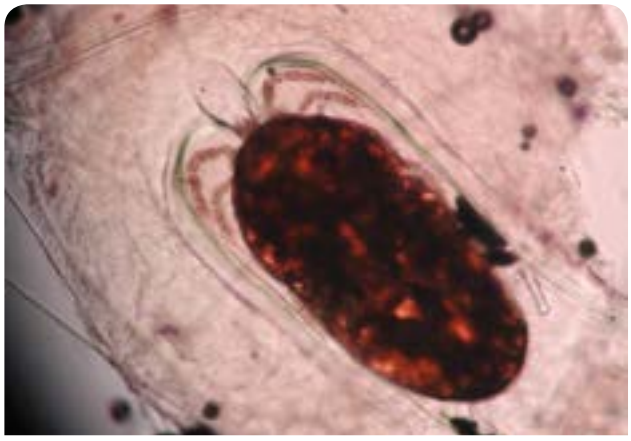


Figure 5. *Straelensia* mite in a cyst.

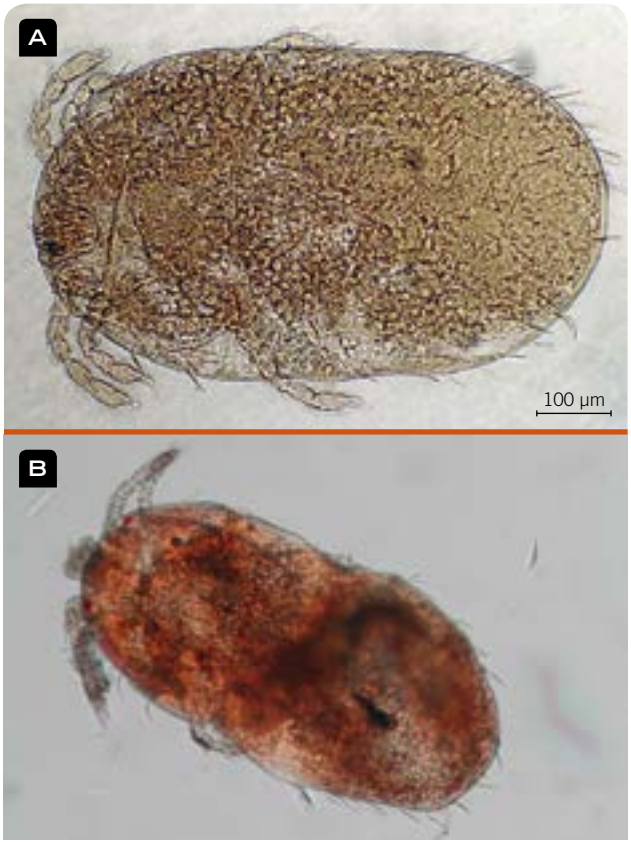


Figure 4. *Straelensia* mites extracted from a nodule.
(B) Courtesy of Françoise Gramatica.

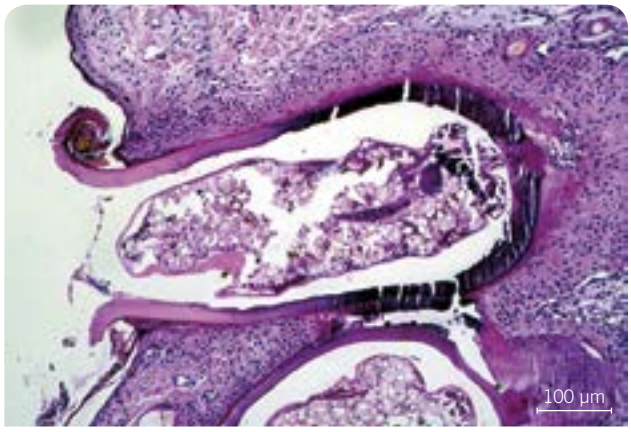
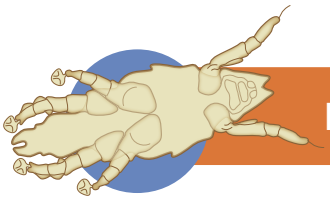


Figure 6. Histology of a skin biopsy showing a *Straelensia* cyst (×100 magnification).



Lynxacarosis

General comments

Lynxacarosis is caused by the development of the fur mite ***Lynxacarus radovskyi*** on the skin surface and coat in the cat. The parasite was first described in Hawaii (1974) and Puerto Rico (1977) and it seems to be quite common in tropical or warm regions. It has been observed in southern parts of the USA (Texas, Florida), Australia, New Zealand, New Caledonia, French Guyana, Caribbean Fiji, Malaysia, the Philippines and South America (several regions in Brazil). Distribution is probably much wider. Five species of *Lynxacarus* are already present in temperate countries, infesting wild carnivores (e.g., *L. mustelae* on mustelids).

Lynxacarus radovskyi (Tenorio, 1974) is a Listrophorida mite. Adult *Lynxacarus* sp. measure about 500 µm long, are elongated and laterally compressed, and the first third of the body is sclerotised (Fig. 1). The legs are relatively short and terminate in suckers, adapted to cling to the hairs. The different species of *Lynxacarus* are morphologically very close and the parasite typically exhibits the same life cycle as other listrophorids. The large and elongated eggs (about 200 µm long) are glued to the hairs distally (Fig. 2). They hatch into a six-legged larva then an eight-legged nymph, which moults into the adult stage. Details of the life cycle have not been studied.

Mite transmission is thought to occur mainly by direct contact, and the condition appears to be contagious. However, indirect contamination through fomites also seems possible.

Human contamination is unlikely, although there is one report of a papular rash on the arms of the owner of a highly infested cat that cleared when the cat was treated.

Clinical signs

Many cases remain asymptomatic. *Lynxacarus* mites are most commonly found on the tail and head, and in the perineal/perianal area. Mites can be found over entire body in heavy infestations. Distribution on the body is variable, and often generalised in long-haired cats.

The most common and indicative presentation is a dandruff-like condition, with a dull and dry coat associated with

Figure 1. Adult *Lynxacarus radovsky.*

the mites. This gives a "peppered" appearance due to the whitish colour of the mites, eggs and thin scales on the hairs.

Evidence of pruritus, alopecia and excoriations can be seen in some cats. Diffuse alopecia or "mangy" patches can also be seen. Alopecia is occasionally visible on the dorsal and lateral parts of the hind limbs, with evidence of self-induced injury.

Figure 2. *Lynxacarus* eggs.

The pruritus induced by the parasite is variable; usually mild in animals with light infestation, but possibly very intense in heavily infested individuals. Severe cases can be associated with feline miliary dermatitis

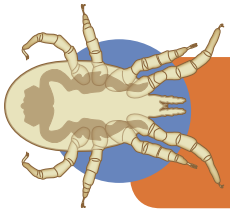
Other signs are probably caused by the excessive grooming induced by the parasite: gingivitis, gastrointestinal disturbances, rectal irritation or prolapses, and hairballs. Infested cats may also present with weight loss, anorexia, restlessness, and fever.

Diagnosis

The parasites can be detected by microscopic observation of samples obtained by combing, trichoscopy or the adhesive tape test. Different stages of mites and/or their eggs attached to the hair shafts can be seen. Diagnosis of heavy infestations is easy, but subclinical infestation is likely to be difficult to detect.

Control measures

A number of treatments have been shown to be effective and the parasite seems to be sensitive to all acaricides tested.



Dermanyssus infestation

General comments

Dermanyssus gallinae (De Geer, 1778) (Dermanyssoidea) is distributed worldwide and mainly affects birds. Infestation with *D. gallinae* is quite common in mammals and occurs when animals or humans associate with infested poultry or pigeons.

Biology

D. gallinae is known as the poultry red mite because it turns red after feeding on blood. Unfed mites are whitish or greyish. The fully engorged female mite is oval, around 1 mm long and is easily seen with the naked eye. All other developmental stages are smaller. Besides its colour, other distinctive morphological characters are obvious after clearing and microscopic examination: very prominent dorsal shield with a truncated posterior end, prominent anal plate on the posterior ventral surface, anus located on the posterior aspect of this plate and a pair of long, whip-like chelicerae.

The mite's life cycle has been extensively studied because of its significant economic impact on the poultry industry. The mites live in the poultry housing, especially in egg-laying hen houses, close to the avian hosts. The adult mites feed on blood at night and most of them return to the cracks and crevices after leaving their hosts. Up to seven eggs are laid at a time and these eggs hatch after 2 to 3 days, releasing the six-legged larvae, which do not feed. The larvae moult into the protonymph stage which feeds on blood (Fig. 1). The final moult takes place after another 2– 3 days, producing the adults (Fig. 2). The entire life cycle can be completed in 7 days under optimum conditions and the mites can survive for 5 months without feeding.

Only mammals which live close to poultry, ornamental birds and pigeons are affected. Humans can be bitten from the same sources, and present with pruritic papular dermatitis on the hands, forearms and legs.

Figure 1. *Dermanyssus gallinae* adult and nymphs (×40 magnification).

Clinical signs and diagnosis

Intense nocturnal pruritus, erythema, papules and alopecia are seen on the face, back and limbs. Self-induced excoriations may be seen. Differential diagnosis must include other living or non-living agents responsible for pruritic skin conditions.

D. gallinae is rarely observed on affected animals due to its feeding habits but the medical history will reveal access of the affected animal to a poultry house, or a recently converted poultry house.

Identification can be confirmed by gross examination with a magnifying glass, followed by microscopic examination. Tape strips are probably the best way to detect dermanyssids.

Differential diagnosis includes sarcoptic mange, cheyletiellosis, trombiculosis, louse infestation, flea allergy dermatitis and atopic dermatitis.

Prognosis is usually favourable.

Control measures

Treatment consists of removing the animal from the infested environment (to prevent reinfestation) and applying acaricidal washes, repeatedly if necessary. Thorough cleaning (e.g., steam cleaning combined with 5 % creosote water sprays) of infested buildings is essential to prevent recurrence.

Figure 2. Adult *Dermanyssus gallinae* (×100 magnification).

PARASITOLOGICAL DIAGNOSIS

General principles

Parasites, especially intestinal worms and ectoparasites, are often thought to be "easy" to treat, without any diagnosis by a vet. This common view probably explains why infestation rate is underestimated and treatment often fails.

The diagnosis of parasitic diseases is based primarily on an epidemiological and clinical suspicion. The climate, the period during which clinical signs appear, the animal's age and lifestyle, along with the expression of indicative clinical signs, can all raise the suspicion of a parasitic infection. Experimental confirmation is, however, necessary to identify the pathogenic agent(s). The laboratory diagnosis of parasitic diseases employs both direct and indirect techniques:

- Direct tests seek evidence of the parasite, or a fragment/element of the parasite (e.g., the whole parasite itself, eggs, DNA, etc.). New immunological tests have been developed that look for circulating or eliminated parasite antigens rather than antibodies. The most well-known test is undoubtedly the ELISA test for circulating *Dirofilaria immitis* (heartworm) antigens in dogs. Excreted *Giardia, Echinococcus* and recently *Toxocara*, *Ancylostoma* and *Trichuris* antigens can also be detected in faecal matter (coproantigens). Not only is the infestation diagnosed, but the presence of antigens indicates the viable and infectious nature of the parasite. Developments in molecular biology over the past few years have raised the possibility that PCR tests could be developed to identify parasite DNA, whether it be circulating, in tissues, or excreted in faeces.
- Indirect tests seek evidence of a host response to parasitic infection or infestation and can be specific (antibody detection) or non-specific (modification of blood chemistry, complete blood count [CBC] with noticeable eosinophilia during parasite migrations).

In this chapter, we will focus on parasitological diagnosis based on direct detection of the parasite (or fragment of the parasite) in a range of samples, for which some techniques have been developed, which are specific to parasitology. These are the most common methods because they are easy to perform, inexpensive and offer a rapid definitive diagnosis. However, they do require experienced users and may have a low sensitivity.

Coproscopy*

General comments

Even after the development of molecular biology and bioinformatics, diagnosis of endoparasites in animals and humans relies predominantly on coprological examination. Indeed, coproscopy (from the Greek words κόπρος = faeces and -σκοπία = exam), i.e., the analysis of faecal samples for the presence of parasites (adults or parts of them) and/or parasitic elements (PEs: eggs, larvae, cysts, oocysts) is the most widely used diagnostic procedure in veterinary parasitology.

Coproscopy can reveal the presence of parasites in several organ systems. Parasites which inhabit the digestive tract (e.g., ascarids, hookworms, cestodes, coccidia, etc.) produce PEs that leave the host's body in the faeces. Eggs and larvae of helminths such as lungworms can be coughed up from the respiratory tract into the pharynx and swallowed, exiting the body in the faeces and making coproscopy the diagnostic method of choice. Even ectoparasite eggs (e.g., mites and lice) may be licked from the skin, swallowed and found in faecal samples. Pseudoparasites (pollens, free-living nematodes, maggots, etc.) can also be observed in the faecal preparation, which may confuse the untrained observer. An accurate diagnosis of parasitism is based primarily on the diagnostician's awareness of the parasites prevalent in a particular region. However, with globalization and the movement of animals and humans, "exotic" and "unusual" parasites may be brought to the attention of veterinary practitioners. Therefore, when parasite-like material (pseudoparasite) is found in a faecal sample, correct identification is required by qualified and accredited parasitologists.

Before performing any of the coproscopic procedures described in the following sections, some important rules for technician safety and the accurate diagnosis of internal parasites should be considered. These rules can be summarised as follows: I) handle faecal samples carefully; II) clean up immediately after a coproscopic test is performed; III) keep good records of any observations on the appearance of the faecal sample and the presence of parasites.

Collection and preservation of faecal samples

It is important to observe the following instructions where possible:

Faecal material (FM) should be collected on a dry, clean surface, e.g., a plastic or cardboard sheet, etc. The total FM from which samples are taken should be the total faeces eliminated within a 24 hour period, if possible. Although this is scientifically desirable, it is unlikely to be possible for most owners.

Thorough homogenisation of the FM is important before performing the sampling procedure, as PEs are not evenly distributed throughout the faeces. The faecal sample should weigh at least 10 times the faecal aliquot to be examined. Larger faecal aliquots will result in more accurate diagnosis, although the faecal aliquot for small animals commonly corresponds to the entire faecal sample.

Particular care should be taken in handling FM as it can contain zoonotic agents, so good laboratory hygiene (e.g., the use of disposable gloves) is imperative.

Veterinary diagnosticians do not usually have the opportunity to collect faecal samples and must rely on samples brought in by clients. Regardless of who obtains them, it is important to have fresh faeces to work with. Faeces collected from litter boxes, gardens, kennel floors, etc. may be old and dry, so parasite eggs may have embryonated or larvated, oocysts may have sporulated, or pseudoparasites may be present. If fresh faeces cannot be submitted promptly, clients should be advised to refrigerate the sample for 1–3 days. Faeces should be submitted in a sealed glass or plastic container, clearly marked with the time and date of collection, species of animal, animal's name, owner's name, and any other information relevant to the case.

Coprological analysis can be performed on fresh faecal samples or samples stored at 4° C for 1–3 days, and/or preserved (fixed) faecal samples. The sample should not be frozen because freezing and other preservation methods will alter the results, but fresh FM should be submitted to the

*This chapter is inspired from Chapter 1–10 Coproscopy diagnosis. Laura Rinaldi et al., published in Parasitoses and Vector Borne Diseases of Cats, Ed. Merial, 2015.

laboratory packed with cold packs. Buffered formalin (5 % or 10 %) or sodium acetate-acetic acid-formalin (SAF) may be used to preserve samples if required. The use of preservatives/fixatives may incur additional shipping costs as many of these fluids are considered to be hazardous materials in some countries, and require very specific packaging and shipping conditions. However, fixatives have the added benefit of inactivating any other infectious organisms that may be present. Special fixatives, such as polyvinyl alcohol (PVA), are required to preserve protozoan trophozoites. The sample is best preserved by mixing faeces and fixative in a 1:4 ratio; that is, 1 part faeces with 3 parts fixative (formalin 5 % or 10 %, or SAF). The faeces must be completely homogenised with the fixative (Table 1) as soon as they are mixed.

In carnivores/omnivores, the type of diet can produce undesirable residues in the faeces which may influence the clarity of the resulting preparation, due to the flotation of small and/or large particles of debris. It is important to avoid feeding fatty foods to dogs and cats before a sample is collected for coproscopy, where possible. Either ether or ethyl acetate can be used as a fat-remover, but the former is toxic to humans and dangerous for the environment, and the latter is flammable and irritant to humans. Hemo-De is an alternative to ethyl acetate and is generally regarded as a safe compound to remove fats from faeces.

Macroscopic examination

Coprological diagnosis must start with a macroscopic examination of the faeces. Gross faecal characteristics, such as consistency (watery, soft, hard), age of sample (parasitological analysis may be wrong in old samples), colour and presence of blood or mucus in the sample should be noted. Alongside these observations, the faeces sample should be grossly examined for entire or part parasites, such as intact roundworms (*Toxocara, Toxascaris*) and/or of tapeworms proglottids (e.g., *Dipylidium caninum* and *Taenia* spp.). Proglottids can be motile and are identified by the number of genital pores on each segment (e.g., single pore for *Taenia* and double pores for *Dipylidium*). Larval stages of arthropods (e.g., flies) may also be found. Parasites should be identified morphometrically (using keys in the literature) or by using appropriate molecular techniques.

After gross examination is completed, the faeces should be examined by copromicroscopy, which can either be qualitative; only demonstrating the presence/absence of a particular PE (eggs, larvae, cysts, oocysts), or quantitative; providing quantification by faecal egg count (FEC). The FEC is expressed as eggs/larvae/oocysts/cysts per gram of faeces (EPG/LPG/OPG/CPG).

Microscopic techniques

Many copromicroscopic techniques have been developed since copromicroscopy was first used by C.J. Davaine in 1857, each with its own advantages and limitations. Although these techniques can be quantitative (FEC), diagnosis in dogs and cats is often only made qualitatively (presence or absence of a parasitic element). Coccidia are an exception and are often expressed in a semi-quantitative manner (e.g., 1+, 2+ and so on, correlating with the numbers seen per 10× field).

Techniques for the examination of faeces for helminth eggs/larvae and protozoan cysts/oocysts may vary from a simple direct smear to more complex methods involving concentration of PEs by either flotation or sedimentation. Flotation in centrifuge can also be performed, and novel multivalent devices, such as FLOTAC and its derivatives Mini-FLOTAC and Fill-FLOTAC, have been recently designed.

Although the direct smear was used for many parasites for many years, it has one great disadvantage and that is the small amount of faeces used, which often gives rise to false-negative results. To overcome this problem, some methods have been developed to concentrate parasitic material from a larger faecal sample into a smaller volume, which may then be examined microscopically. Concentration methods, which have greater analytical sensitivity than smears, include flotation and sedimentation, and the modified Baermann

Table 1. Examples of fixatives used in coproscopy.

Fixative	Composition
SAF	$C_2H_3NaO_2$ 1.5 g, $C_2H_4O_2$ 2 mL, CH_2O (40 %) 4 mL, H_2O 92 mL
Formalin 5 %	CH_2O (40 %) 5 mL, H_2O 95 mL
Formalin 10 %	CH_2O (40 %) 10 mL, H_2O 90 mL

technique. These methods are designed to separate the various parasitic stages from FM. Faecal sedimentation concentrates both faeces and eggs at the bottom of a liquid medium, usually tap water. In contrast, the principle of faecal flotation is based on the ability of a flotation solution (FS) to allow less dense material (including PEs) to rise to the surface. Finally, the modified Baermann technique is used to recover nematode larvae, many of which are unable to swim against gravity. There are many refinements and modifications of these techniques, but the same simple principle underlies them all.

Copromicroscopic examination for parasite eggs/larvae/oocysts/cysts is a fundamental part of the daily routine for most veterinary practices. Despite their limitations, direct smears are useful to detect motile protozoa (e.g., *Tritrichomonas* or *Giardia* trophozoites), and sedimentation techniques are useful for recovering heavy eggs (e.g., from *Physaloptera* spp.) or operculated eggs (e.g., fluke eggs) that do not float consistently or are distorted by the effect of FSs. Flotation techniques are most frequently used to recover parasite eggs, oocysts and cysts (see following sections).

Manuals of veterinary parasitology diagnostic are available and cover multiple animal species and techniques. The main techniques used for copromicroscopic examination in cats are described below.

Direct smear

The simplest method of microscopic faecal examination for parasites is the direct smear (Fig. 1), which consists of placing a small amount of faeces directly on a microscope slide. Some practitioners make direct smears using only the amount of faeces that clings to a rectal thermometer after taking the cat's temperature.

Several drops of saline solution or water are placed on the slide with an equal amount of faeces. The solution and faeces are then mixed together with a wooden applicator (or a pipette) until the solution is homogenous, and the solution is smeared over the slide in a thin film which should be thin enough to read print through. Finally, any large pieces of faeces are removed and a coverslip is placed over the smear which is examined under low microscopic power (×100).

Faecal smears can also be air-dried and stained for identification of intestinal protozoa (e.g., trichrome stain for *Giardia* and carbol-fuchsin, Giemsa or Ziehl-Neelsen for *Cryptosporidium*).

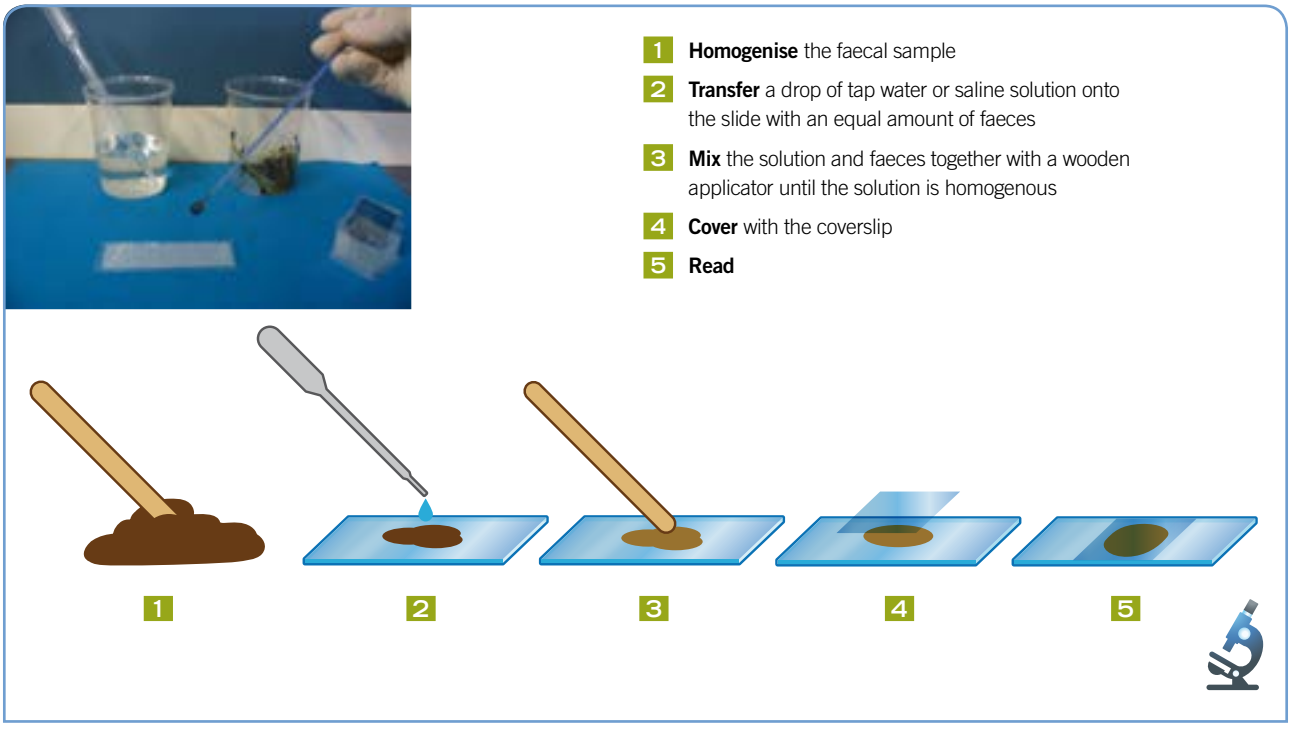


Figure 1. Direct smear.

Advantages

Rapid procedure and minimal equipment required. Useful for detecting protozoan trophozoites, which are distorted or destroyed by concentration media, and particularly heavy eggs that fail to float in these media.

Disadvantages

The small amount of FM required is not a good representative sample, and often provides false-negative results. It can also be difficult to identify the eggs as they may be partially covered by debris. Negative findings are inconclusive.

Flotation

Flotation techniques are the most common methods used to recover PEs. These procedures are based on differences in the specific gravity (s.g.) of parasite eggs, larvae, oocysts and cysts, faecal debris and FS (Fig. 2).

The average s.g. of many nematode eggs, including dog and cat ascarids, is between 1.05 and 1.24. The s.g. of the FS must be greater than that of the eggs to allow parasite eggs to float. Most of the FSs used in coproscopy (Table 2) are saturated and made by adding a measured amount of salt or sugar to a specific amount of water to produce a solution with the desired s.g. The salt or sugar (or a combination of both, depending on the FS) should be dissolved in the water by stirring on a magnetic stirrer. After preparing any FS, the s.g. must be confirmed with a density meter, allowing for the fact that the s.g. of the saturated solution will vary slightly depending on the ambient temperature. If the salt crystals in some FSs (in particular those based on NaCl) initially precipitate, but then later dissolve, more salt can be added to ensure that the solution remains saturated.

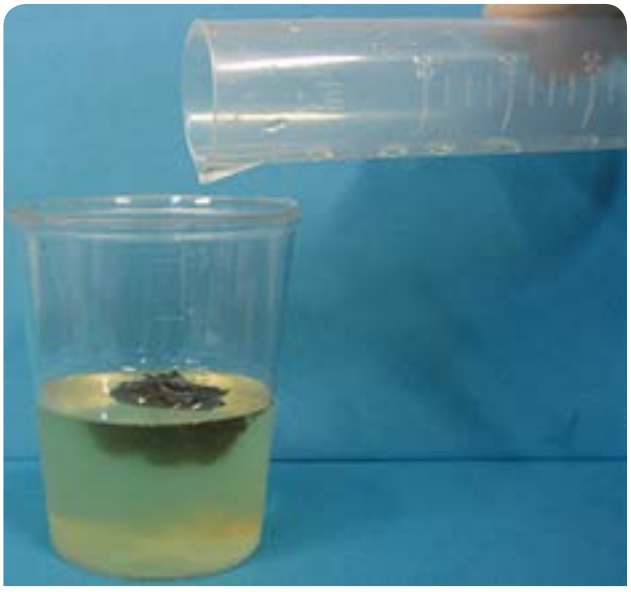


Figure 2. Flotation technique.

Table 2. Flotation solutions widely used in coproscopy.

Flotation solution	Composition	Specific gravity at saturation
Sucrose and formaldehyde	$C_{12}H_{22}O_{11}$ 454 g, CH_2O solution (40 %) 6 mL, H_2O 355 mL	1.200
Sodium chloride	NaCl 500 g, H_2O 1,000 mL	1.200
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$ 330 g, made up to 1,000 mL	1.200
Sodium nitrate	$NaNO_3$ 315 g, made up to 1,000 mL	1.200
Magnesium sulphate	$MgSO_4$ 350 g, made up to 1,000 mL	1.280
Sodium nitrate	$NaNO_3$ 250 g, $Na_2O_3S_2 \cdot 5\ H_2O$ 300 g, made up to 1,000 mL	1.300
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$ 685 g, H_2O 685 ml	1.350
Sodium chloride and zinc chloride	NaCl 210 g, $ZnCl_2$ 220 g, H_2O made up to 1,000 mL	1.350
Sucrose and sodium nitrate	$C_{12}H_{22}O_{11}$ 540 g, $NaNO_3$ 360 g, H_2O made up to to 1,000 mL	1.350
Sodium nitrate and sodium thiosulphate	$NaNO_3$ 300 g, $Na_2O_3S_2 \cdot 5\ H_2O$ 620 g, H_2O 530 mL	1.450
Sucrose, sodium nitrate and sodium thiosulphate	$C_{12}H_{22}O_{11}$ 1,200 g, $NaNO_3$ 1,280 g, $Na_2O_3S_2 \cdot 5\ H_2O$ 1,800 g, H_2O 720 mL	1.450

The FSs normally used for nematode and cestode eggs are mainly based on sodium chloride, whereas saturated solutions of zinc chloride or zinc sulphate are widely used for trematode eggs. Ideally, all PEs would float and still maintain their morphological integrity, and faecal debris would sink, in the chosen FS. The choice of FS is important and, in the authors' opinion, it is not given enough attention by the scientific community, despite the substantial effect that the FS can have on the diagnostic performance of any flotation technique. Only the s.g. or density of the FS is usually reported in manuals of diagnostic or in peer-reviewed literature. The efficiency of a FS in terms of the capacity to bring PEs to flotation is commonly believed to increase as the s.g. of the FS increases, but PEs should not be considered to be merely "inert elements". Instead, interactions between the elements within a floating faecal suspension (e.g., FS components, PE, fixative, ether and residues of the host's diet) might be complex. As a rule of thumb:

- Different FSs with the same s.g. do not produce the same results with respect to the same PE, even when the same technique is used.
- An FS which might be highly effective with respect to a particular PE and a particular technique, produces different results if the technique is changed.
- An FS which is effective with respect to a particular PE and a particular technique, using a fresh faecal sample, produces different results if the method of faecal preservation changes (e.g., frozen, preserved in formalin or SAF, or in other fixatives; see discussion above).
- An FS which is effective with respect to a particular PE and a particular technique, produces different results if the host's diet changes.
- It follows that each PE must be considered independently with respect to the FS, the technique and the method of faecal preservation used when copromicroscopic flotation is employed. What is known for a specific PE cannot be readily translated to a "similar" PE, or to the same PE when the technique or the faecal preservation method changes.

It is also important to note that the type of diet can produce undesirable residues and fats in the faeces which may influence the clarity of reading, due to the flotation of small and/or large particles of debris.

Simple (gravitational) faecal flotation

One method is as follows: a small quantity of the faecal sample (about 3 g) is placed in a 90 to 150 ml waxed paper cup then about 20 ml of FS are added. An emulsion is made by thoroughly mixing the solution with the faeces using a tongue depressor, until a faecal slurry is formed. This mixture of faeces and FS is filtered through a double layer of cheesecloth or gauze, or a tea strainer could also be used. The mixture is poured into a shell vial which is then filled to the top and slightly overfilled, so that a slight convex meniscus forms at the lip of the vial. If there is not enough fluid in the cup to fill the shell vial, a small amount of fresh flotation medium may be added. Finally, a glass coverslip is placed gently on top of the fluid and allowed to sit, undisturbed, for 10–20 minutes. The coverslip is then removed carefully and immediately placed on the microscope slide for examination (Fig. 3).

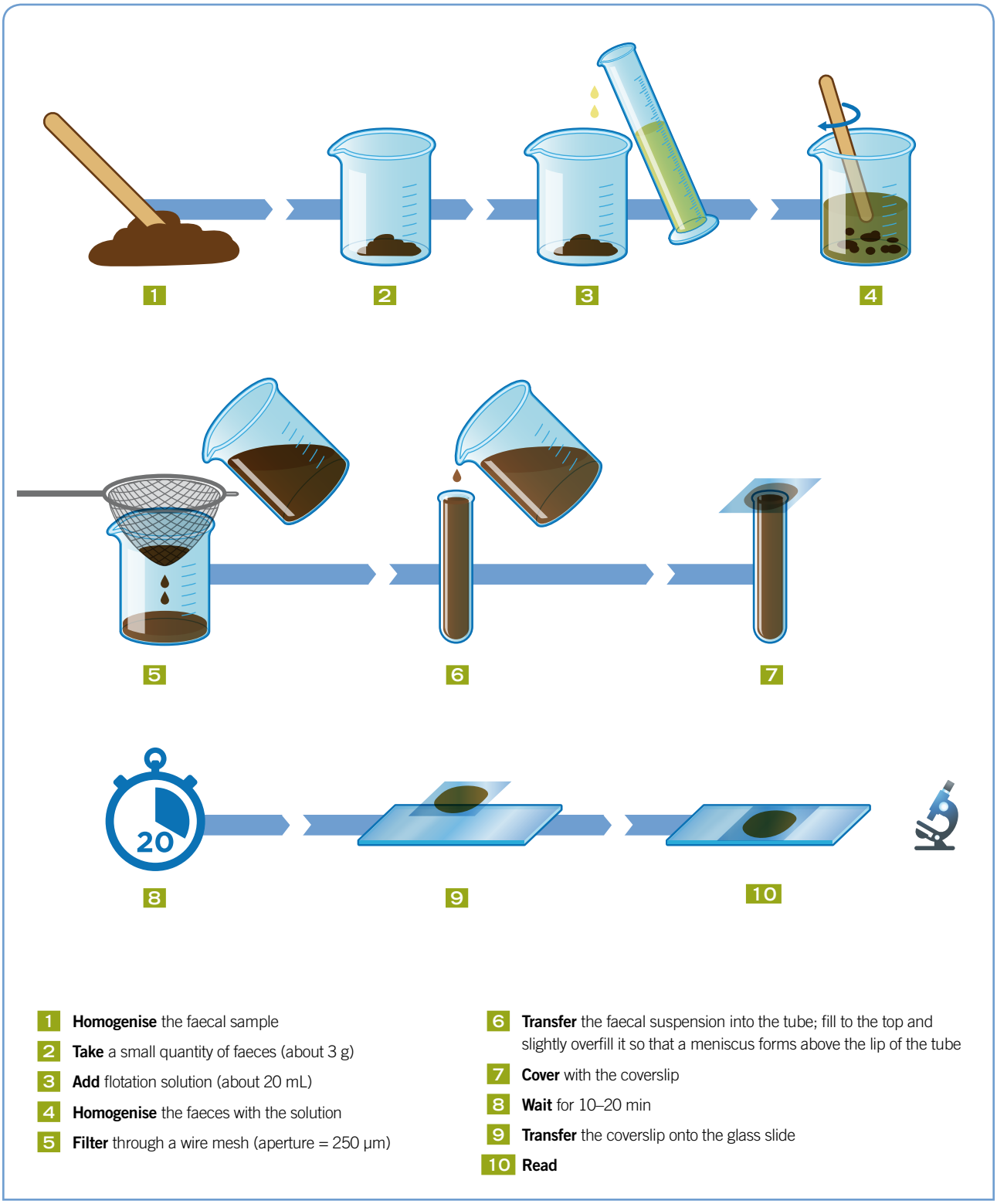
A plethora of flotation techniques, of varying degrees of difficulty (simple to complex), are described in the textbooks, diagnostic manuals and scientific literature, and there are several kits, complete with specific instructions, available commercially. These kits consist of prepared FS, disposable plastic vials, and strainers.

Advantages

Inexpensive and easy to perform.

Disadvantages

The slide must be examined promptly, otherwise, osmotic distortion may render the PEs difficult to identify, or crystallisation of the medium may obscure the microscopic field. Trematode eggs may be distorted because of the hypertonic effect exerted by the FS, as FSs have a very high s.g.



1 **Homogenise** the faecal sample
2 **Take** a small quantity of faeces (about 3 g)
3 **Add** flotation solution (about 20 mL)
4 **Homogenise** the faeces with the solution
5 **Filter** through a wire mesh (aperture = 250 µm)
6 **Transfer** the faecal suspension into the tube; fill to the top and slightly overfill it so that a meniscus forms above the lip of the tube
7 **Cover** with the coverslip
8 **Wait** for 10–20 min
9 **Transfer** the coverslip onto the glass slide
10 **Read**

Figure 3. Simple (gravitational) faecal flotation.

McMaster technique

The McMaster technique is widely used in large animals to detect and count strongyle eggs, primarily. Many variations of the McMaster technique are described in the literature, all of which use the traditional two-chambered device.

All flotation methods are qualitative in nature, unless a known amount of faeces is used, but any flotation method, even the gravitational type, can become quantitative if the faecal sample is weighed prior to use. There are several methods in which the quantity of faeces to be used is specified in the methodology and these include:

Modified McMaster method

3 g of faeces are put in a jar with FS to 45 mL. An emulsion is made by thoroughly mixing the solution with the faeces using a tongue depressor, until a faecal slurry is formed. This mixture is then poured through a wire mesh screen and the strained fluid caught in a bowl. The faeces filtrate is well stirred and withdrawn with a Pasteur pipette, and carefully placed into one counting chamber. After further stirring, a second sample is withdrawn and placed into the second chamber. All the eggs under the two separate grids are then counted (Fig 4). The EPG of the faeces is obtained by multiplying the total number of eggs under the two grids by 50 (the dilution factor). Alterations in the faeces to FS ratio will change the dilution factor, for example, 4 g of faeces to 30 mL of FS results in a dilution factor of 25 when both chambers are counted.

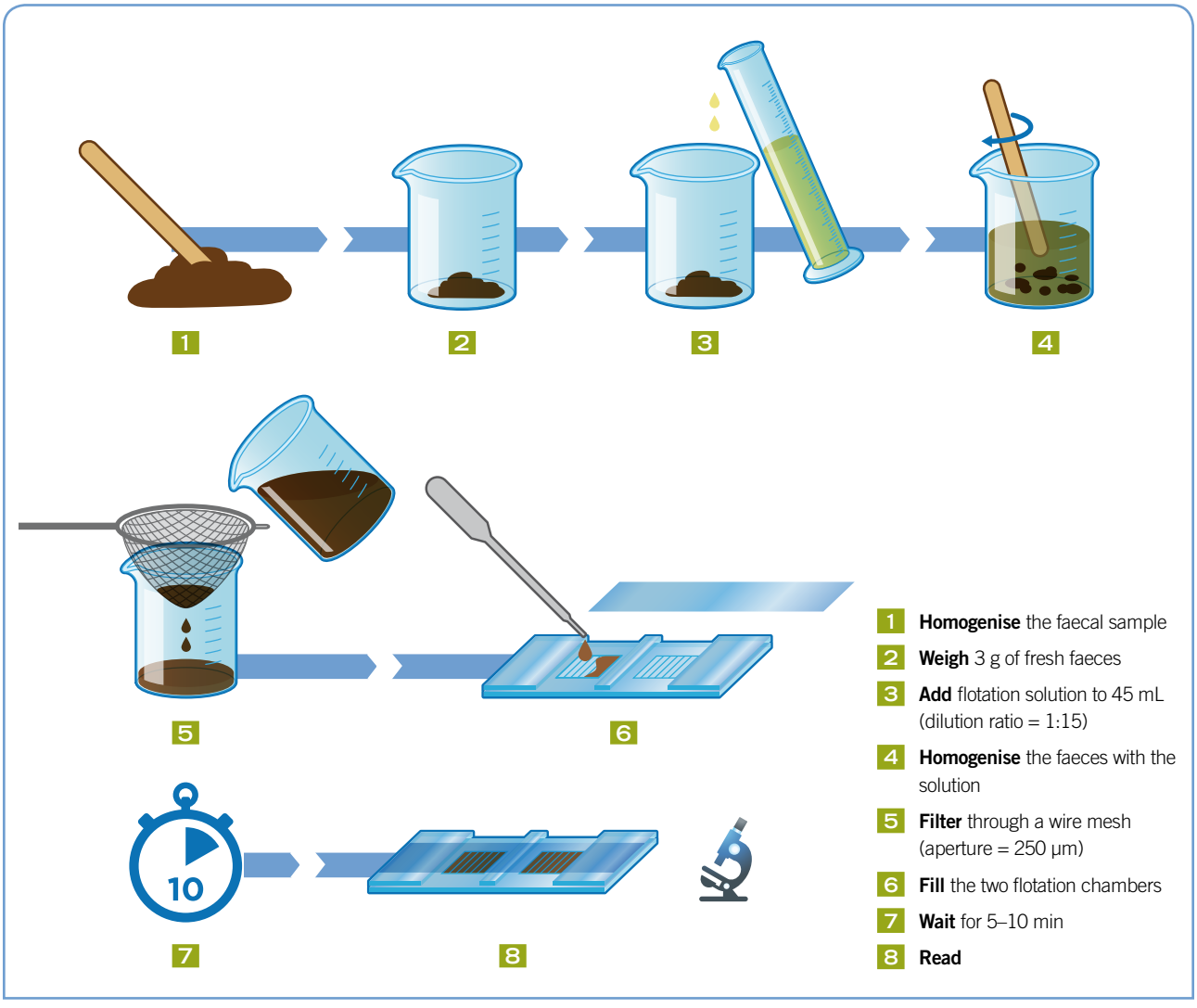


Figure 4. Modified McMaster technique.

Improved modified McMaster method

As above, 3 g of faeces are mixed thoroughly with water to 45 mL and poured through a wire mesh screen, and the strained fluid caught in a bowl. The strained fluid is stirred and a sample of it is poured into a centrifuge tube to within 10 mm of the top. The tube is centrifuged for 2 minutes at 170 x g and the supernatant is poured off and discarded. The tube is then filled with FS to the same level as before, the contents thoroughly mixed, and sufficient fluid is immediately withdrawn with a Pasteur pipette and carefully placed into one counting chamber. After further stirring, a second sample is withdrawn and placed into the other chamber. All the eggs under the two separate grids are then counted.

Advantages

It is considered by many authors to be the easiest quantitative test to perform.

Disadvantages

As a dilution technique, analytical sensitivity may be inappropriate when there are few PEs. A significant amount of fluid is discarded because the faeces are mixed with FS but only a small amount of this mixture is used to charge the slides, which may be cost-prohibitive in some cases.

Centrifugal faecal flotation

Numerous methods of centrifugal faecal flotation have been described and all have their attendant advantages and disadvantages. Some examples of common centrifugal flotation methods are described below.

Wisconsin method

3–5 g of faeces are placed into a polypropylene cup and mixed with water to 25 mL. A tongue depressor is used to macerate the sample and create a faecal slurry. This slurry is then passed through a tea strainer into a second cup, and the first container and strainer are rinsed with 8 mL of water. All of the fluid is then poured into two 15-mL conical end centrifuge tubes and centrifuged at 150 x g for 10 minutes (Fig. 5). The supernatant is decanted, care being taken not to lose the fine sediment of the pelleted material. The tubes are then filled approximately half way with FS, and the pellet is resuspended using an applicator stick. The tubes are then topped off with FS until a slightly convex meniscus is formed, and a 22 × 22 mm coverslip is placed on top. The

Figure 5. Centrifugal flotation.

samples are then centrifuged as before. The coverslips are removed, transferred to the slides and examined (Fig. 6).

Modified double centrifugation method (Cornell-Wisconsin method)

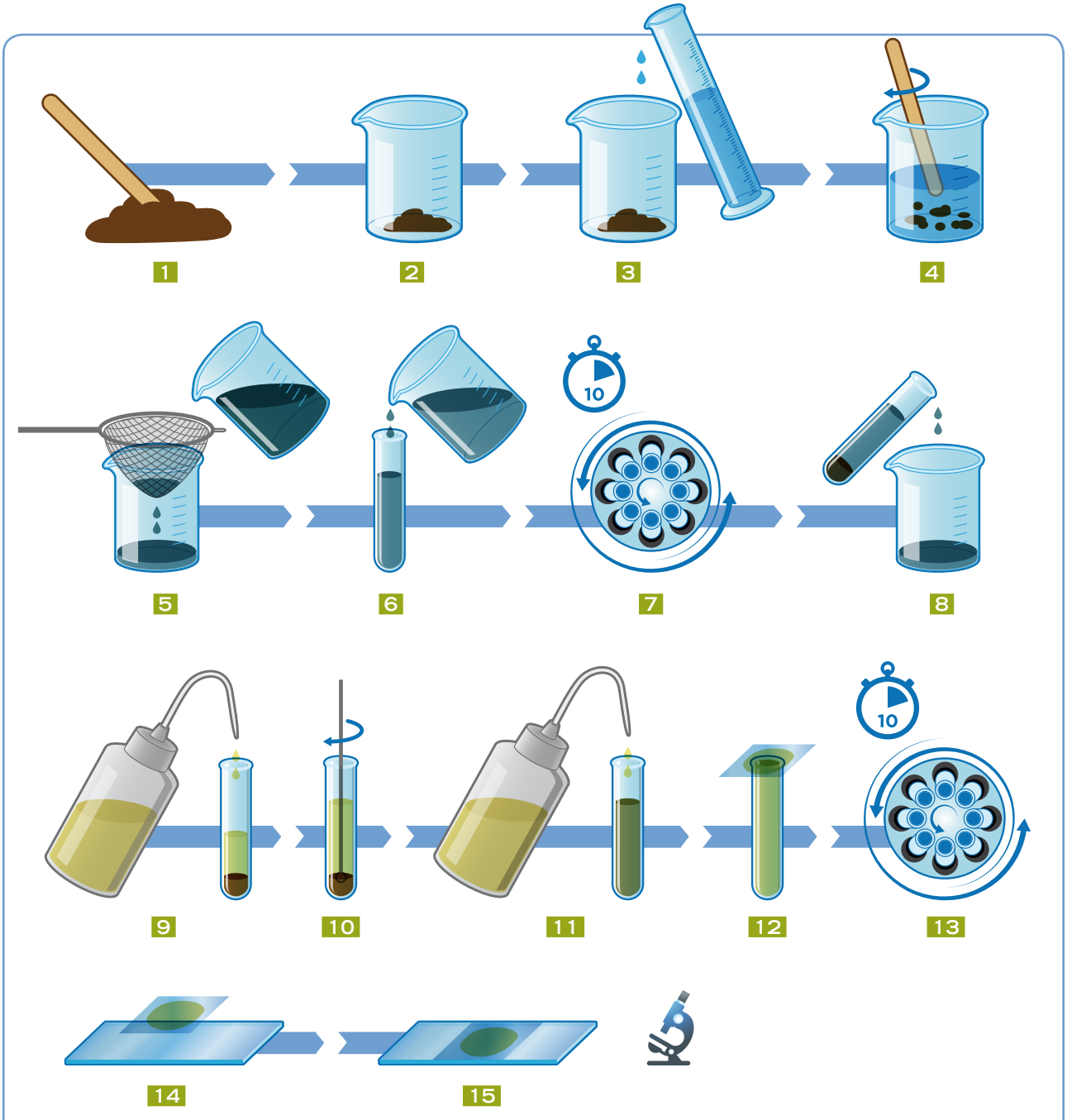
1–5 g of faeces (5 g in the original description) are mixed with 10–12 mL of water and the slurry strained through a tea strainer into a beaker. The mixing container is rinsed with 2–3 mL of water and this is also strained. The wet material in the strainer is pressed with a tongue depressor or spatula, to remove as much water as possible and then discarded. The strained faeces are placed into a 15 mL conical centrifuge tube and centrifuged at 264 x g for a minimum of 3 minutes (some manuals recommend 5–10 minutes). The supernatant is discarded, care being taken not to lose the fine sediment. The tube is filled about half full with the FS, the sediment is resuspended with an applicator stick, and then the tube is filled to the top with the same FS. The solution should be about level with the top of the tube (slightly convex meniscus) then a 22 × 22 mm coverslip is placed on top of the tube, ensuring that it makes contact with both the FS and the rim of the tube. The tube is then centrifuged for 5 minutes, as before (some manuals recommend 5–10 minutes). The coverslip is then removed and transferred to a slide for examination.

Advantages

Minimum detectable limit is 1 PE in 1–5 g of faeces. The water wash step helps to eliminate very light material, resulting in cleaner preparations.

Disadvantages

As a two-step technique, it requires slightly more time than single-step centrifugation.



1 **Homogenise** the faecal sample
2 **Weigh** 3–5 g of fresh faeces
3 **Add** tap water to 25 mL
4 **Homogenise** thoroughly with a stick
5 **Filter** through a wire mesh (aperture = 250 µm)
6 **Transfer** the faecal suspension into the tube
7 **Centrifuge** at 150 × g for 10 min
8 **Discard** the supernatant
9 **Fill** the tube approximately half full with FS
10 **Resuspend** the pellet using a wooden stick
11 **Top** the tube up with FS until a meniscus is formed
12 **Place** a 22 × 22 mm coverslip over the top of the tube
13 **Centrifuge** at 150 × g for 10 min
14 **Transfer** the coverslip onto the slide
15 **Read**

Figure 6. Wisconsin method.

FLOTAC techniques

These techniques use a FLOTAC apparatus and are based on the centrifugal flotation of a faecal suspension and subsequent translation (cutting) of the apical portion of the floating suspension.

Three methods can be used with the FLOTAC device (*basic*, *dual* and *double*), and these are variants of a single technique, but with different applications (Fig. 7).

The FLOTAC *basic technique* uses a single FS. This technique is recommended for use with faecal samples containing a low or very low number of PEs from a single parasite species (natural or experimental mono-infection), or with faecal samples containing a low or very low number of various types of PEs, which all have the same behaviour with respect to the FS used. With the FLOTAC *basic technique*, the reference units are the two flotation chambers (total volume 10 mL; corresponding to 1 g of faeces). The analytic sensitivity of the FLOTAC *basic technique* is 1 PE per gram.

The FLOTAC *dual technique* is based on the use of two different FSs that have complementary s.g. and are used in parallel on the same faecal sample. This technique is indicated for epidemiological surveys and routine diagnosis, to screen for a wide range of PEs with different characteristics in relation to the FS. With the FLOTAC *dual technique*, the reference unit is the single flotation chamber (volume 5 mL; corresponding to 0.5 g of faeces) and the analytical sensitivity of this technique is 2 PEs per gram.

The FLOTAC *double technique* is based on the simultaneous examination of two different faecal samples from two different hosts using a single FLOTAC apparatus. With this technique, the two faecal samples are assigned to their own single flotation chamber, using the same FS, and the reference unit for this technique is the single flotation chamber (volume 5 mL; corresponding to 0.5 g of faeces). The analytic sensitivity of the FLOTAC *double technique* is 2 PEs per gram.

The procedure is as follow: each faecal sample is diluted in tap water (dilution ratio 1:10). After homogenisation (the use of a hand blender is recommended), the resulting suspension is filtered through a wire mesh (aperture = 250 µm), then 6 mL from the filtered suspensionis placed into conical tubes. The tubes are centrifuged for 3 min at 170 × g at room temperature, then each supernatant is discarded, leaving only the sediment (pellets) in the tubes. Each tube is then filled with the chosen FS to the previous 6 mL level. The suspensions are homogenised thoroughly (before and between the fillings) and the two flotation chambers of the FLOTAC apparatus are filled. The FLOTAC apparatus is then closed and centrifuged for 5 min at 120 × g at room temperature. After centrifugation, simultaneous 45° rotation of the apparatus's translation disc and reading disc cuts (removal) the top portion of the suspension in both chambers (= translation) and the reading disc is examined under a microscope (Fig. 8).

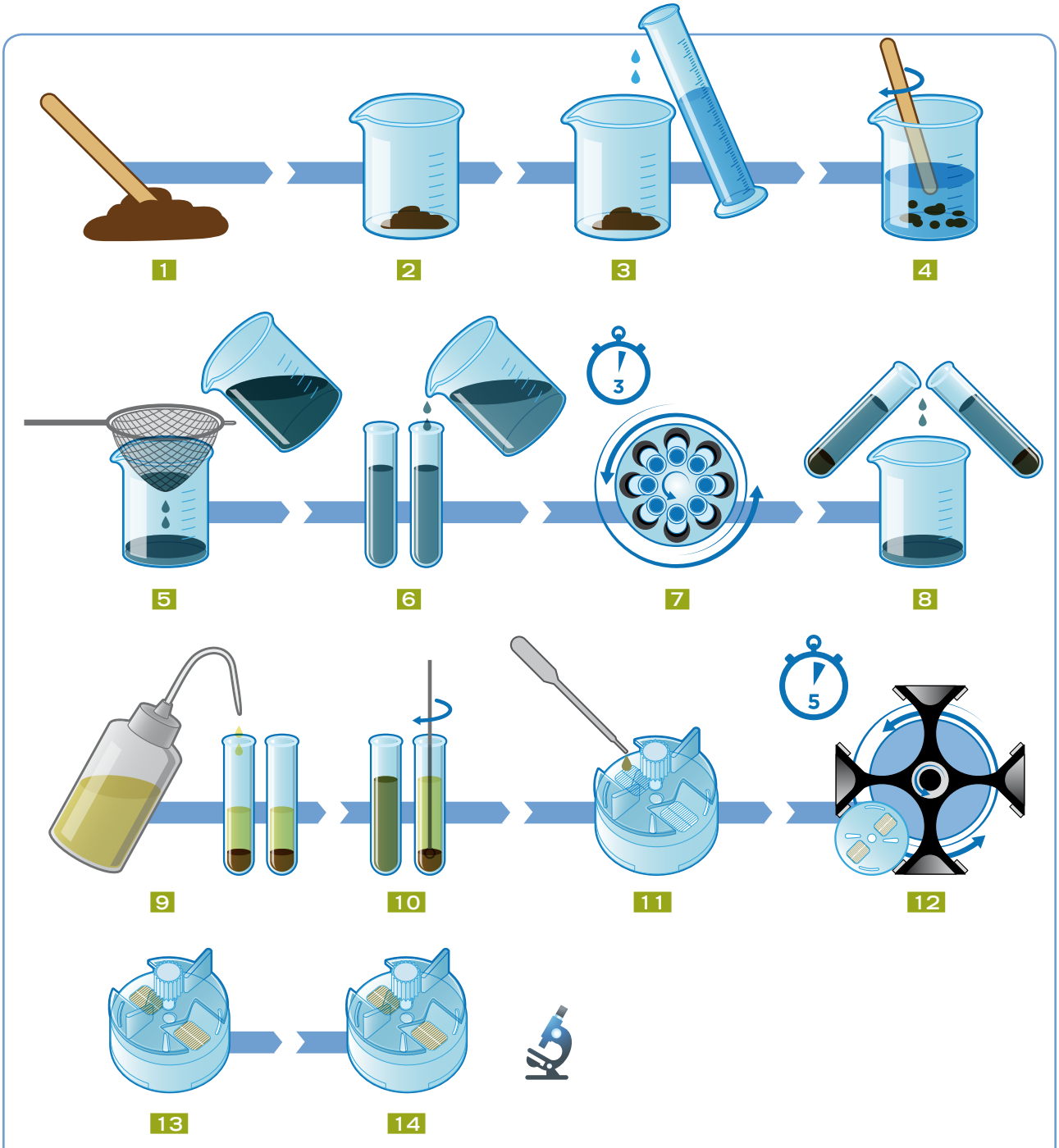
Advantages

The large amount of faecal suspension examined gives fewer false-negative results, so it is particularly suitable for situations of low parasite elements. Results obtained with the FLOTAC apparatus are easy to read.

Disadvantages

A certain level of laboratory infrastructure (e.g., large volume centrifuge or benchtop centrifuge with rotor for microtitre plates) is required for FLOTAC techniques, which is often not available in resource-constrained settings. It is more time-consuming than some other flotation techniques.

Figure 7. Devices in the "FLOTAC family": Mini-FLOTAC, FLOTAC and Fill-FLOTAC.



1 **Homogenise** the faecal sample
2 **Weigh** 10 g of fresh faeces
3 **Add** 90 mL of tap water (dilution ratio 1:10)
4 **Homogenise** the suspension thoroughly
5 **Filter** through a wire mesh (aperture = 250 µm)
6 **Transfer** two 6 mL aliquots of the filtered suspension into two conical tubes
7 **Centrifuge** at 170 × g for 3 min
8 **Discard** the supernatant
9 **Fill** the tubes with flotation solution to their previous 6 mL levels
10 **Resuspend** the pellet using a wooden stick
11 **Fill** the two FLOTAC flotation chambers
12 **Centrifuge** at 120 × g for 5 min
13 **Translate**
14 **Read**

Figure 8. FLOTAC *dual technique*.

Mini-FLOTAC technique

Mini-FLOTAC was developed to overcome the issue of limited facilities by eliminating the centrifugation step. Mini-FLOTAC is recommended for use in combination with Fill-FLOTAC, a disposable sampling kit, which consists of a container, a collector and a filter. Fill-FLOTAC facilitates the first four steps of the Mini-FLOTAC technique, i.e., sample collection and weighing, homogenisation, filtration and filling.

Briefly, 2 g of fresh faeces are weighed in a container and FS to 20 mL are then added (dilution ratio = 1:10). The suspension is homogenised thoroughly then filtered through a wire mesh (aperture = 250 µm). The suspension is mixed thoroughly and the two chambers of the Mini-FLOTAC filled (these four steps can be performed in the Fill-FLOTAC). The Mini-FLOTAC is left to stand for 10 minutes, then the top parts of the Mini-FLOTAC's flotation chambers are translated and read under the microscope (Fig. 9). The analytic sensitivity of the Mini-FLOTAC *basic technique* is 5 PEs per gram.

Advantages

It operates in a closed system and can be performed on fixed faecal samples. It can be used in place of the FLOTAC techniques in laboratories where the centrifugation step cannot be performed.

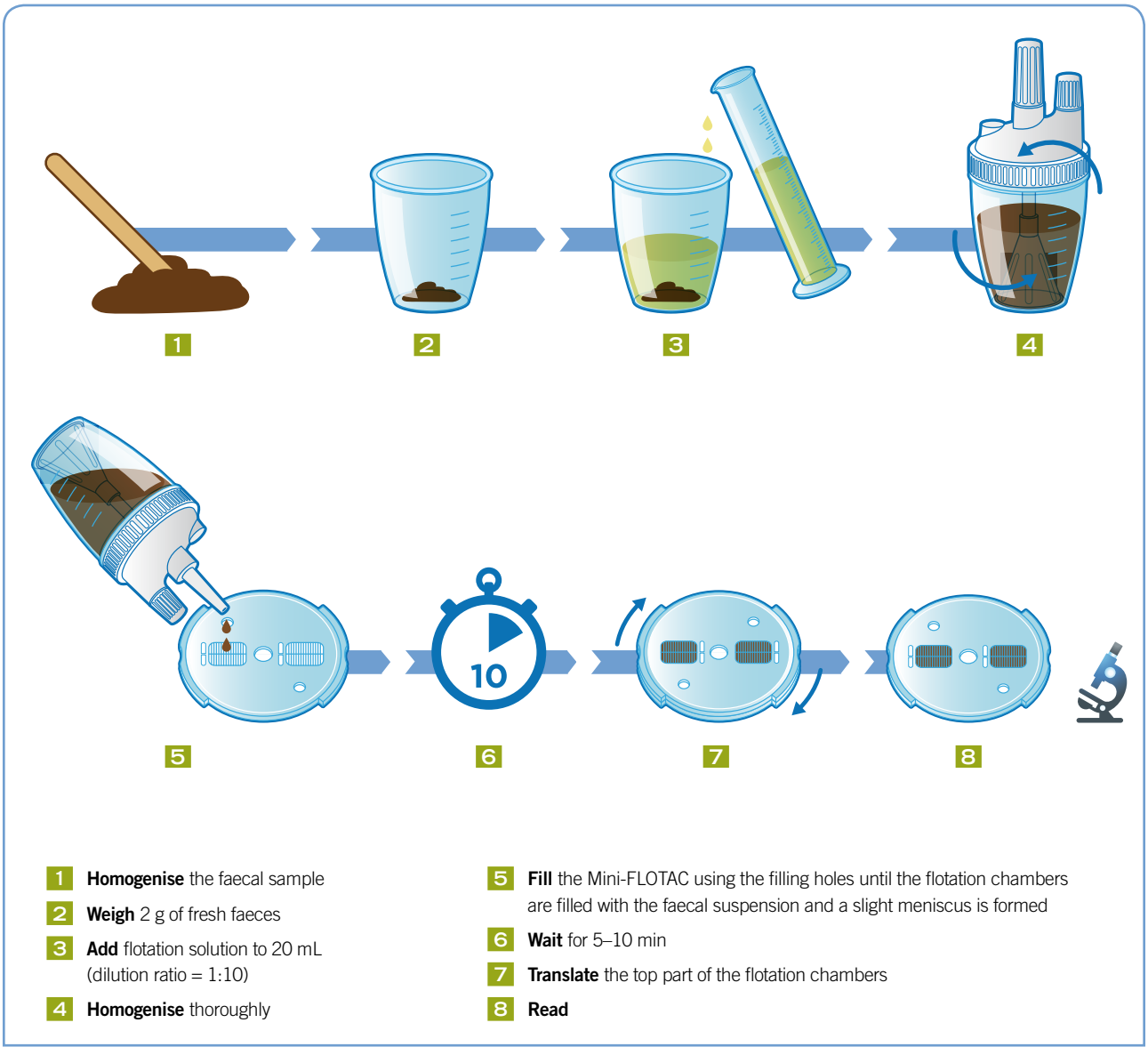
Mini-FLOTAC has been already validated in veterinary parasitology for the diagnosis of helminths (e.g., ascarids, hookworms, trichurids, gastrointestinal nematodes) in pets and livestock.

Disadvantages

Minimum detection limit of 5 EPG may not be appropriate in all situations.

Video 12.1
Detailed tutorial for the Mini-FLOTAC technique on fresh faeces.

Video 12.2
Detailed tutorial for the Mini-FLOTAC technique on fixed faeces.



1 **Homogenise** the faecal sample
2 **Weigh** 2 g of fresh faeces
3 **Add** flotation solution to 20 mL (dilution ratio = 1:10)
4 **Homogenise** thoroughly
5 **Fill** the Mini-FLOTAC using the filling holes until the flotation chambers are filled with the faecal suspension and a slight meniscus is formed
6 **Wait** for 5–10 min
7 **Translate** the top part of the flotation chambers
8 **Read**

Figure 9. Mini-FLOTAC technique.

Faecal sedimentation

A sedimentation procedure is used to isolate eggs or cysts whose s.g. is too high to float readily in common FSs or which would be severely distorted by FS (Fig. 10). One common method is as follows:

100 mL of water are mixed with 10 g of faeces and placed in a beaker or other container. The mixture of faeces and water is strained through a double layer of cheesecloth or gauze, or a tea strainer into a container. It is advisable to use a 250 mL conical container (height = 18 cm) or a 500 mL beaker (height = 12 cm). The mixture is allowed to sit for 20–30 minutes to 1 hour and then the supernatant is decanted. Water is then added to the previous level, the sediment is resuspended, and the sample allowed to rest again from 20–30 minutes to 1 hour. The supernatant is decanted again, and the sediment is removed and placed into a Petri dish for evaluation. Alternatively, a few drops of the remaining mixture can be placed on a microscope slide, a coverslip added and the preparation examined (Fig. 11).

Advantages

The benefit of the sedimentation procedure is to detect eggs that do not easily float if they are too heavy or too delicate to be concentrated by flotation, for example, so this procedure is mainly used for trematodes and some nematode (e.g., *Physaloptera* spp.) eggs. Large volumes of faeces can also be evaluated.

Disadvantages

It is more time-consuming to perform.

Figure 10. Sedimentation technique.

1. **Homogenise** the faecal sample
2. **Weigh** 3 g of fresh faeces
3. **Add** 100 mL of water
4. **Homogenise** thoroughly with a stick
5. **Filter** through a wire mesh (aperture = 250 µm)
6. **Allow** the mixture to rest for 1 hour
7. **Remove** 70 % of the supernatant
8. **Refill** the beaker with fresh water

Repeat steps 6–7–8 until the suspension is clear

9. **Remove** 90 % of the supernatant
10. **Stir** the remaining mixture
11. **Place** the entire amount in the Petri dish or place a few drops on the glass slide
12. **Read**

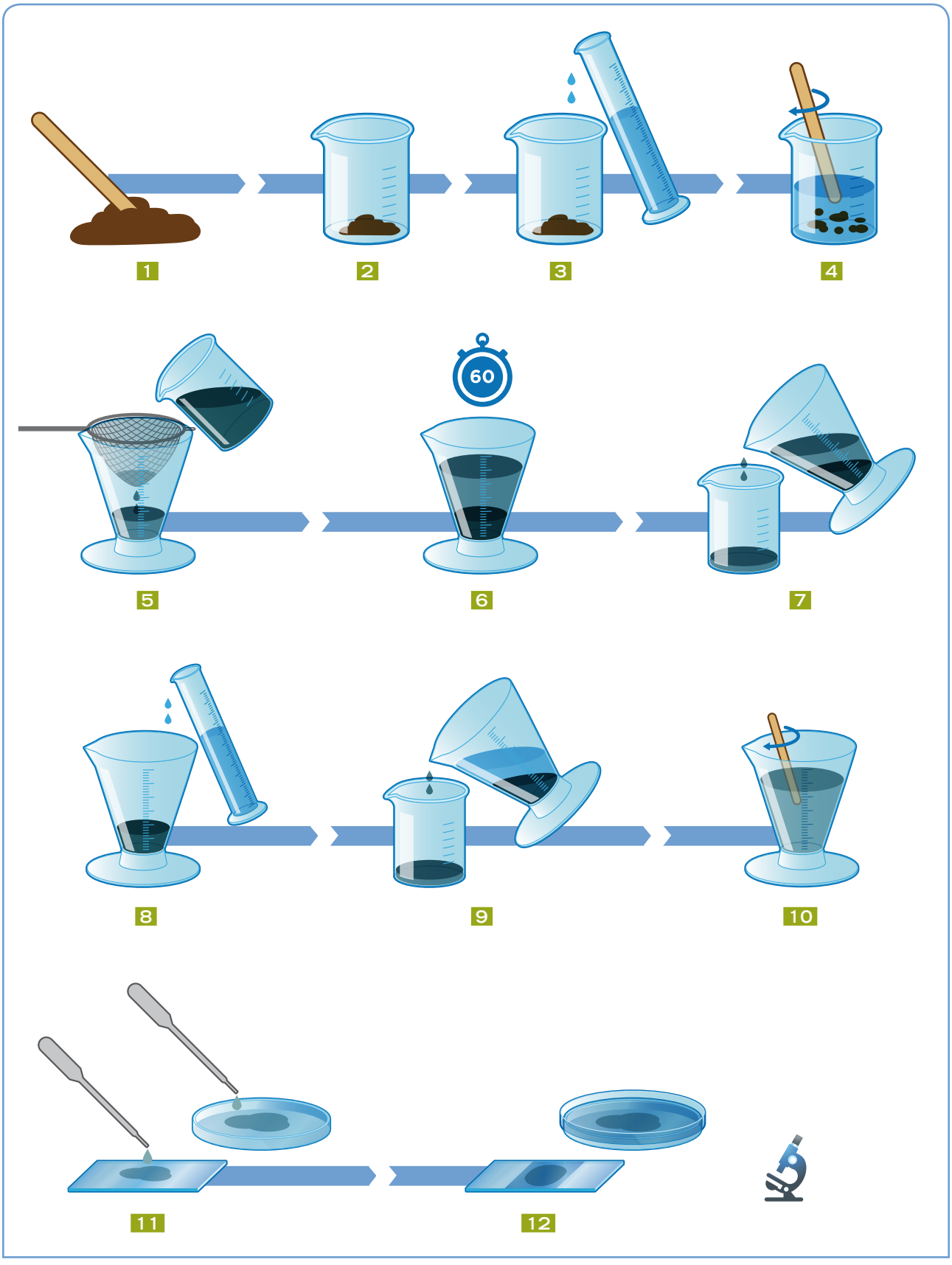


Figure 11. Faecal sedimentation.

Telemann technique

The Telemann technique concentrates eggs, cysts and larvae in samples with high fat concentrations. Briefly, about 1 gram of faeces is put in a beaker with 5 mL hydrochloric acid (15 %) and homogenised. The faecal suspension is filtered through a double layer of gauze and transferred into a 15 mL centrifuge tube. An equal amount of ether is added and the tube is plugged then shaken vigorously and centrifuged at 170 × g for 1 minute. Four layers are formed by the centrifugation: 1) ether; 2) plug of faecal debris; 3) acid; 4) sediment containing the PEs. The tube is placed in a horizontal position and the upper layers are removed, leaving only the sediment in the tube. The walls of the tube are cleaned with a cotton swab and, finally, a few drops of sediment are placed on a microscope slide, a coverslip is placed on top and the preparation is examined (Fig. 12).

Advantages

This technique is useful for samples with high fat concentrations.

Disadvantages

It is time-consuming to perform, and uses hazardous and toxic materials.

Baermann test

The Baermann test is used to isolate larvae from faecal samples. It depends on the ability of the larvae to migrate away from the faeces and into the surrounding water. The larvae settle out and are found at the bottom of the container. It requires equipment to hold the faecal sample in the water so that the larvae can migrate out and be collected. One method is as follows:

Place warm water (at approximately 25 °C) into a glass funnel with a stopcock or a clamp on a rubber hose over the end of the funnel. 5 or more grams of faeces are wrapped in two layers of gauze and placed in the water in the funnel. A support, such as a screen, tea strainer or sieve, is placed in the funnel, and the gauze-wrapped faeces are placed on it. The sample is allowed to rest for at least 8 hours, or preferably overnight. The clamp is then released and the first 10 mL of fluid collected in a centrifuge tube. This is spun for a minimum of 3 minutes at 170 × g, the supernatant is discarded, and the sediment examined (Fig. 13). Identification of the larvae often requires that they be killed in an extended position. This is easily achieved by judiciously warming the droplet of water before applying the coverslip. As an alternative to heating, a drop of Lugol's solution may be added at the edge of the coverslip. This both relaxes and stains the larvae.

The sluggishness of some larvae is a problem with this method, so fresh samples are absolutely essential. Another issue may be the sloped sides of the funnels, so modified funnels with vertical sides have been produced to try to overcome this.

This technique is considered to be the gold standard for lungworm detection.

Advantages

The larvae recovered are not distorted and easier to identify because no flotation medium is used.

1. **Homogenise** the faecal sample
2. **Weigh** 1 g of fresh faeces
3. **Add** hydrochloric acid 15 % (about 5 mL)
4. **Homogenise** thoroughly with a stick
5. **Filter** through a double layer of gauze
6. **Transfer** the faecal suspension into the tube
7. **Add** an equal amount of ether
8. **Shake** the tube vigorously
9. **Centrifuge** at 170 × g for 1 min
10. **Check** that four layers have been formed after centrifugation
11. **Discard** the supernatant
12. **Transfer** 2–3 drops of the sediment onto the microscope slide
13. **Cover** with the coverslip
14. **Read**

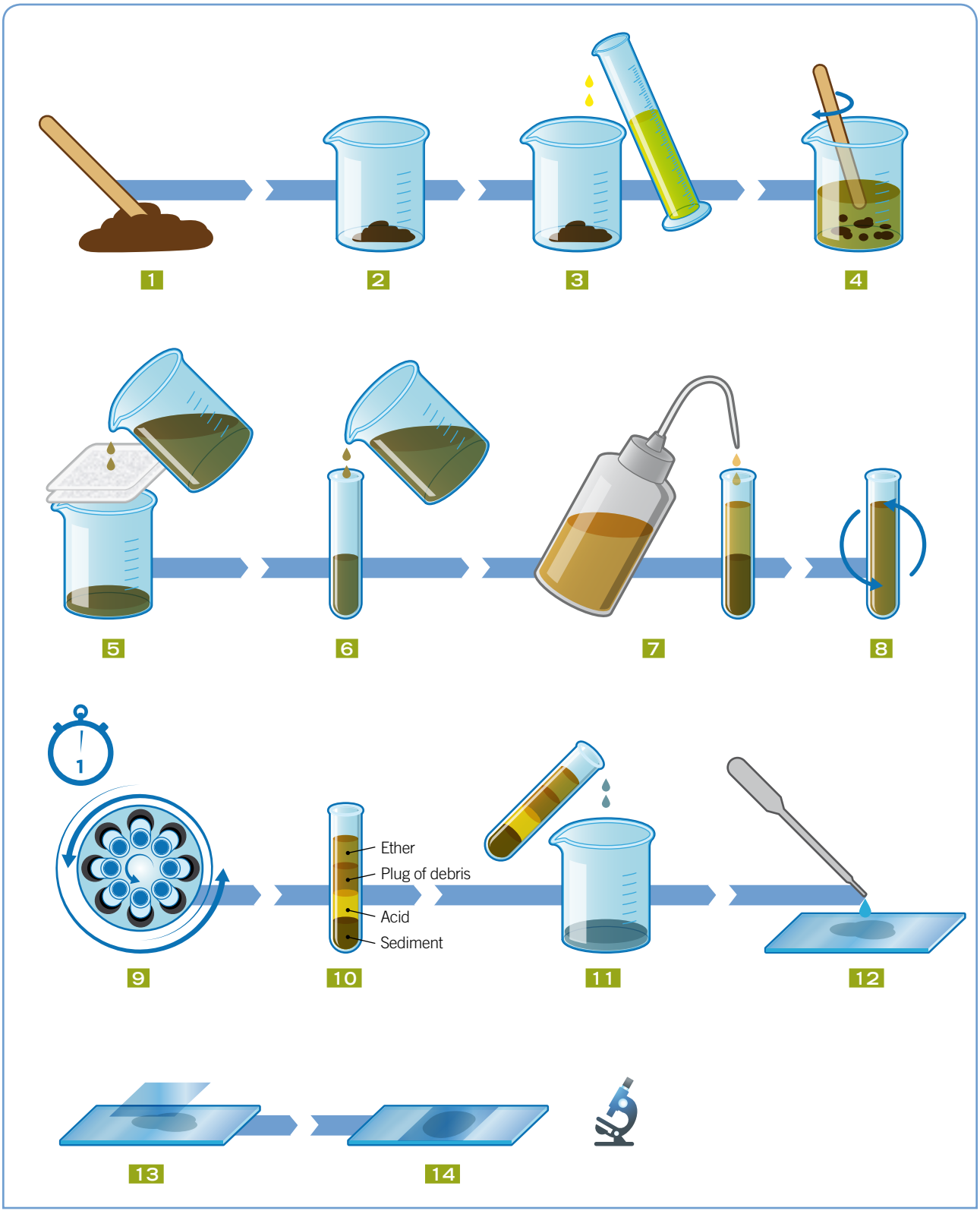
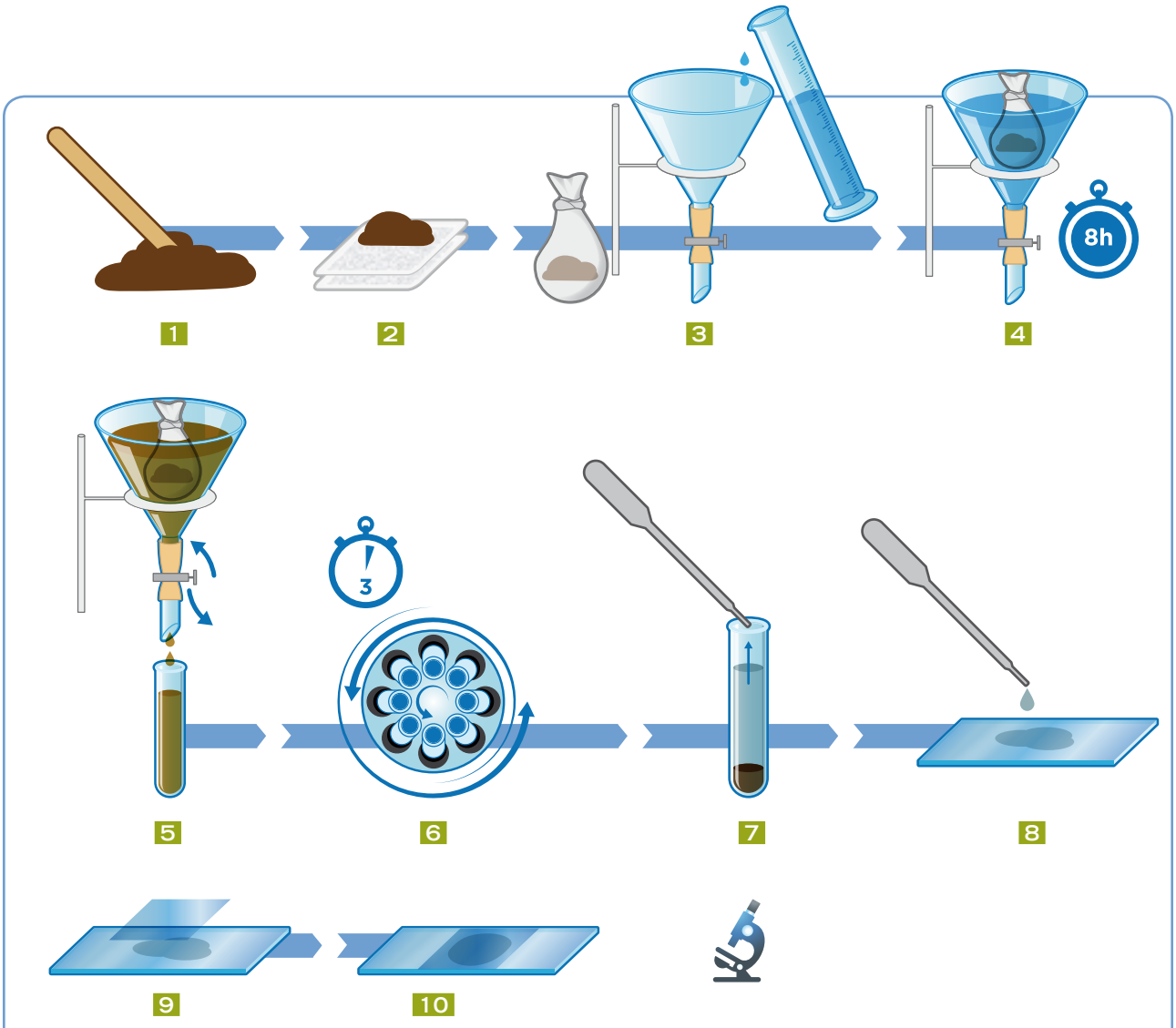


Figure 12. Telemann-Rivas method.



1. **Homogenise** the faecal sample
2. **Weigh** 5–10 g of faeces
3. **Add** water (preferably warm water at 25 °C) into a glass funnel that has a stopcock or a clamp on a rubber hose over the end of the funnel. Take 5 or more grams of faeces, wrap the faeces in 2 layers of gauze and place in the water in the funnel
4. **Allow** the sample to rest for at least 8 hours, preferably overnight
5. **Release** the clamp and collect the first 10 mL of fluid in a centrifuge tube
6. **Centrifuge** at 170 × g for 3 min
7. **Aspirate** the supernatant
8. **Transfer** 2–3 drops of sediment onto the microscope slide
9. **Cover** with the coverslip
10. **Read**

Figure 13. Baermann technique.

Disadvantages

Samples in which larvae may be dead or dying are unlikely to yield positive results, and the requirement for the sample to rest undisturbed for 8+ hours means results are not usually available until the next day.

Discussion

Correct techniques are essential for the accurate diagnosis of intestinal parasites in dogs, cats and other pets. Veterinarians and their staff should re-evaluate their attitude of "it is only a faecal sample" and use these important techniques better in their routine diagnostic plans.

Numerous different factors may influence the performance of copromicroscopic techniques based either on flotation (e.g., simple flotation, McMaster, Wisconsin, FLOTAC, etc.) or on sedimentation. These factors include the choice of fixative used for faecal preservation (e.g., formalin 5 % or 10 %, or SAF), the duration of faecal preservation before analysis, the animal's diet, selection of the FS, and the concurrent use of ether. As mentioned in the previous sections, FS and the method of faecal sample preservation have a major influence on the analytical sensitivity, precision and accuracy of any copromicroscopic technique based on flotation, be it qualitative or quantitative.

In human medicine, it is widely accepted that diagnostic methods must be accurate, simple and affordable to be useful. They must also provide a result quickly enough to implement effective control measures, especially treatment.

Although a faecal examination is considered a routine procedure in many veterinary clinics, little thought is often given to performing the procedure correctly.

While copromicroscopy is considered to be highly specific, its performance depends not only on the level of infestation, but also on the detection limit of the technique employed. In addition to the performance characteristics (e.g., sensitivity, specificity, reproducibility, positive and negative predictive values) of a method, the operational features (e.g., simplicity, ease of use, user acceptability) should be considered whenever a diagnostic test is evaluated. Athough coprological examinations should be performed by veterinarians or trained veterinary technicians in small animal practice, the task is commonly assigned to the newest staff member, often with very little instruction or emphasis on its importance. Accurate evaluation of faecal samples is important and must be taken seriously by all members of the clinical practice.

There is a clear lack of standardisation of copromicroscopic techniques and each lab usually uses its own method, mostly based on "lab traditions" rather than on the technique's performance or operational characteristics. However, it is important to emphasise that various factors may influence the performance of any copromicroscopic technique, so interlaboratory validation is strongly recommended.

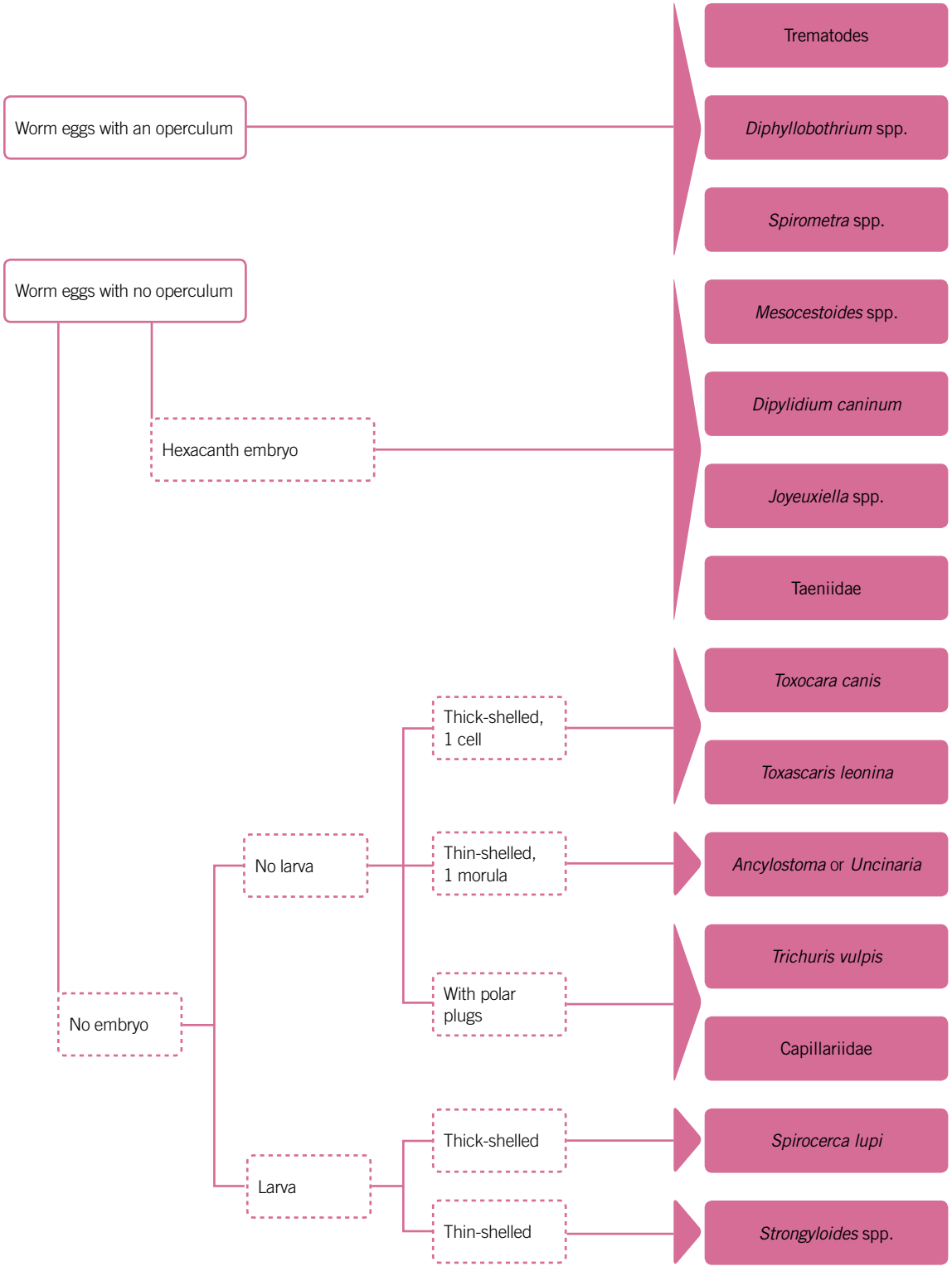
The results of any copromicroscopic technique also depend on the "human factor" (the experience of the personnel performing the test) so reliable identification of parasitic infections requires in-depth training for the specimen preparation, and expertise and experience for the subsequent microscopic examination.

The method of copromicroscopy to be chosen also depends on what the information is going to be used for.

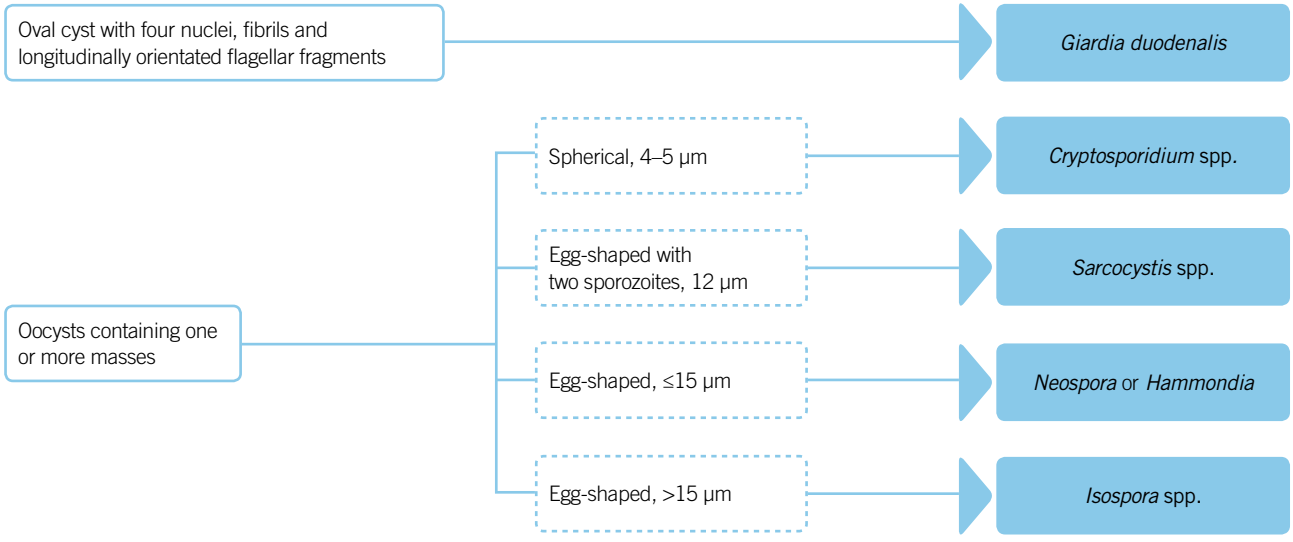
Identification of parasite eggs, cysts and larvae in dogs

Microscopic coproscopy

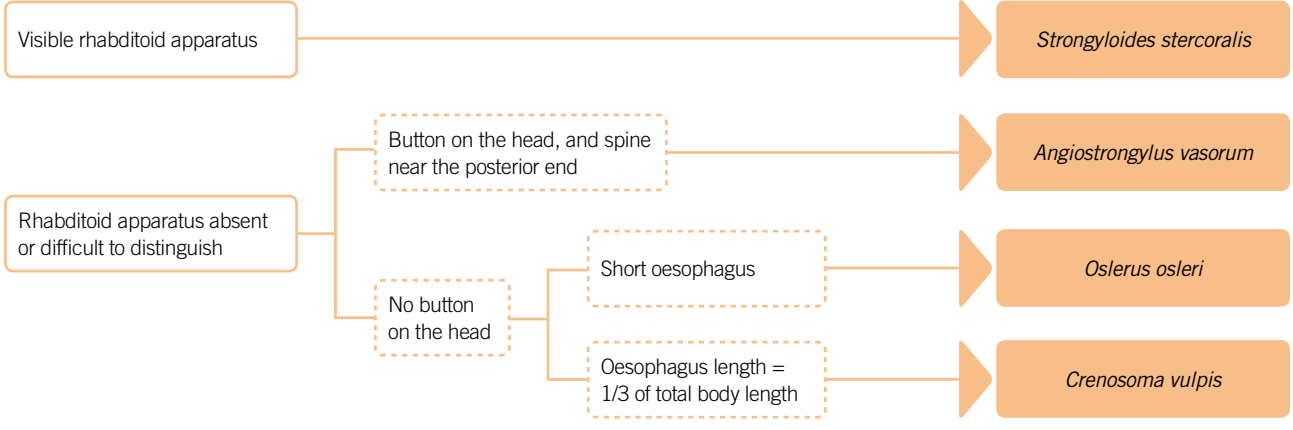
Major helminth eggs in dogs



Major protozoan cysts or oocysts in dogs

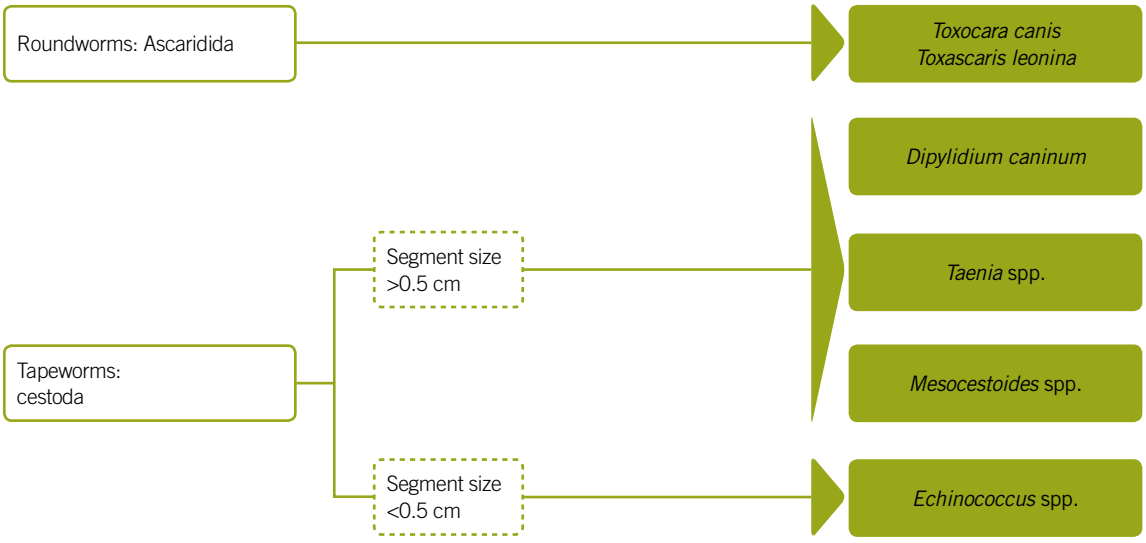


Major helminth larvae in dogs



Macroscopic coproscopy

Major visible worms in dogs



Microscopic coproscopy in dogs

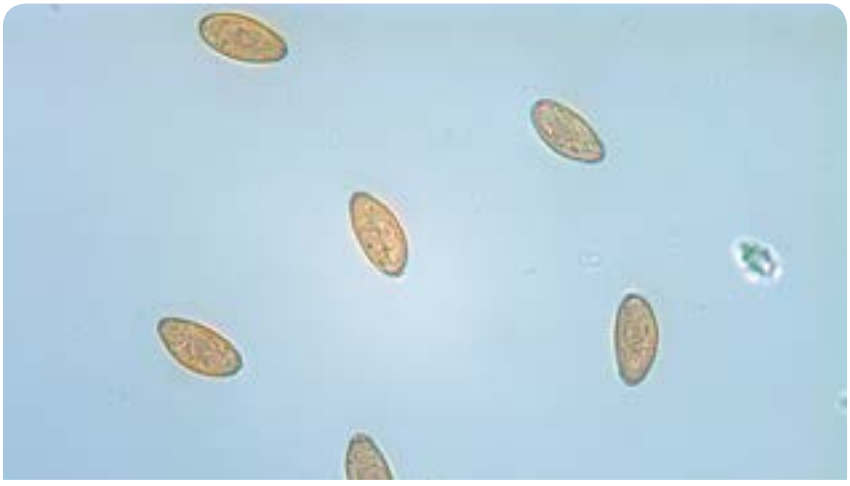


Figure 14. *Opisthorchis* eggs.

Opisthorchiidae

Trematode egg

Description: small ovoid egg; thick shell with an operculum at one pole and a spine at the other (Fig. 14).

Contents: an embryo.

Size: 30 × 20 µm.

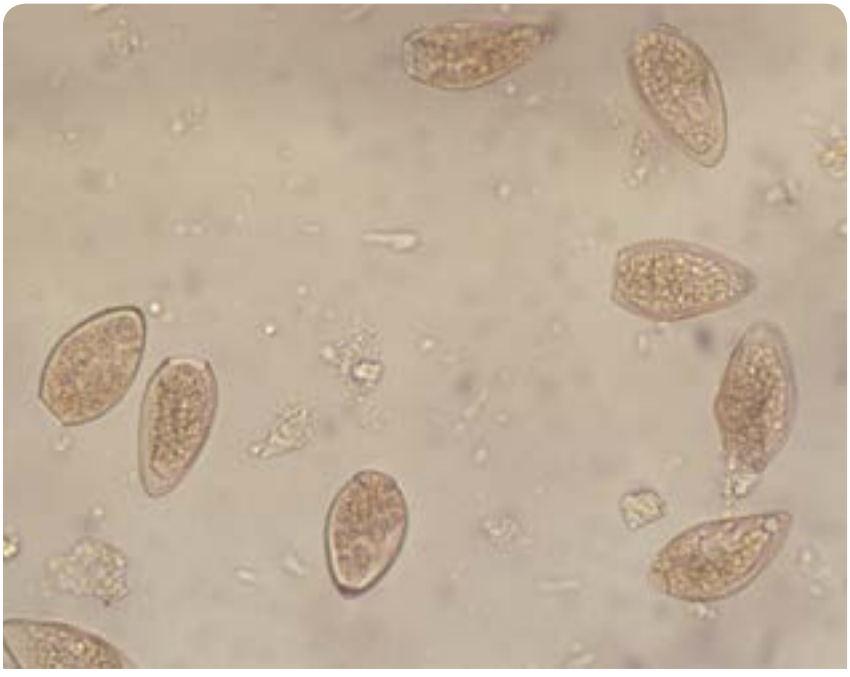


Figure 15. *Diphyllobothrium* or *Spirometra* eggs.

Diphyllobothrium latum

Cestode egg

Description: medium-sized, spherical to ovoid egg; thin shell with an operculum at one pole (Fig. 15).

Contents: a light brown syncytium that fills the entire egg.

Size: 60 × 45 µm.

Spirometra

Cestode egg

Description: medium-sized, ovoid to almost spherical egg; smooth, thin shell with an operculum at one pole (Fig. 15).

Contents: a light brown syncytium that fills the entire egg.

Size: 70 × 60 µm.

Figure 16. *Dipylidium* eggs.

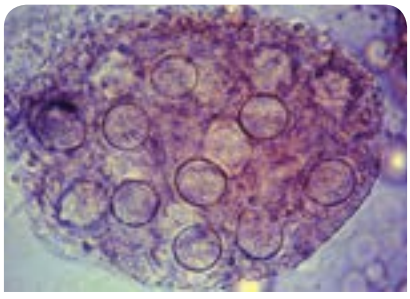


Figure 17. *Dipylidium* oviferous capsule.

Dipylidium caninum

Cestode egg

Description: small egg; smooth, thin shell (Fig. 16).

Contents: a hexacanth embryo.

Size: 50 × 40 µm.

Comment: *Dipylidium* spp. eggs are grouped in clusters of 20 inside a thin shell: the egg packet or oviferous capsule (Fig. 17).

Joyeuxiella

Cestode egg

Description: small egg; smooth, thin shell; cannot be differentiated from *Dipylidium caninum* using only a microscope (Fig. 18).

Contents: a hexacanth embryo.

Size: 50 × 40 µm.

Comment: oviferous capsule containing only one egg.

Figure 18. *Joyeuxiella* eggs.

Taeniidae

Cestode egg

Description: small, globular egg with a unique, thick envelope; radially striated (Fig. 19).

Contents: a hexacanth embryo.

Size: 30–40 × 20–30 µm.

Comment: eggs are excreted in an oviferous segment; no morphological differences between *Taenia* and *Echinococcus*.

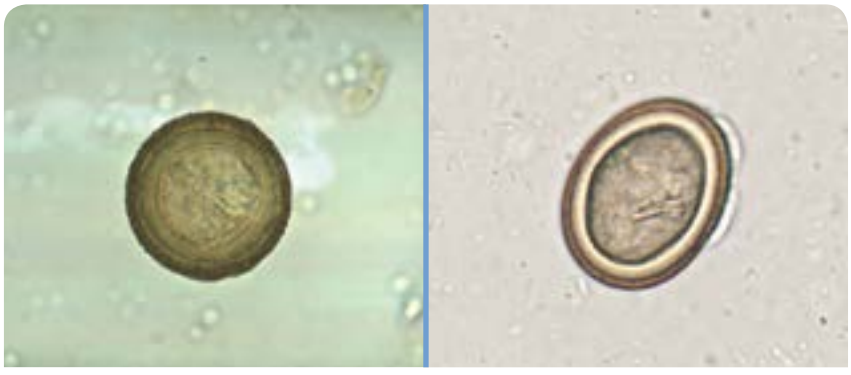


Figure 19. Taeniidae eggs.

Mesocestoides

Cestode egg

Description: small, globular egg; smooth, thin shell (Fig. 20).

Contents: a hexacanth embryo.

Size: 50 × 40 µm.

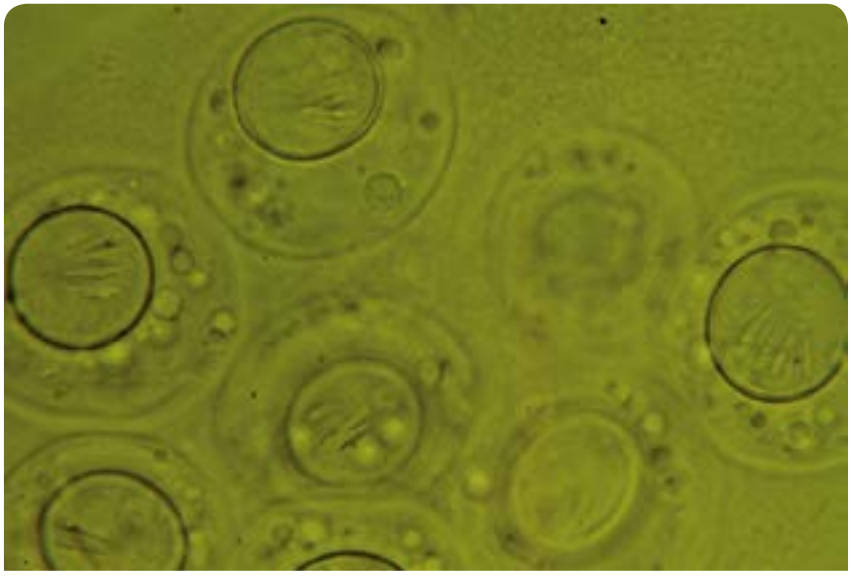


Figure 20. *Mesocestoides* eggs.

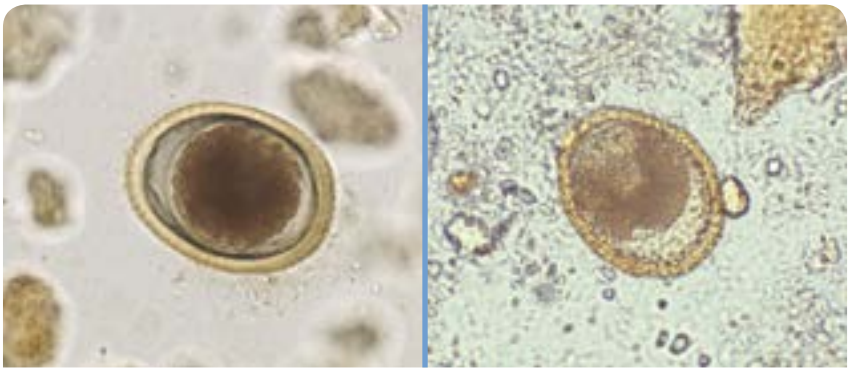


Figure 21. *Toxocara canis* eggs.

Toxocara canis

Nematode egg

Description: medium-sized, globular egg; thick-shelled with an alveolar external layer (thimble-like) and smooth internal layer (Fig. 21).

Contents: a single, dark-brown cell that almost fills the entire egg.

Size: 70–90 × 65–75 µm.

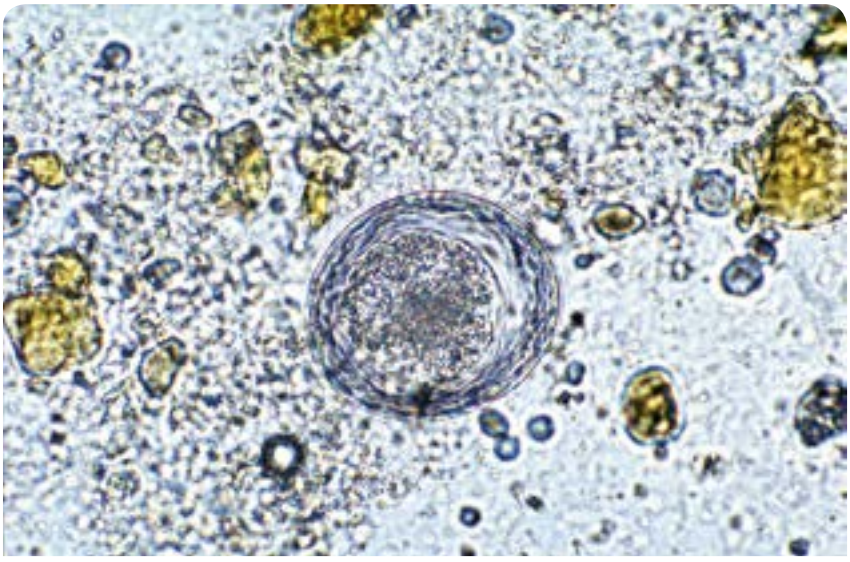


Figure 22. *Toxascaris leonina* egg.

Toxascaris leonina

Nematode egg

Description: medium-sized, subglobular egg; thick, smooth outer shell with concentric layers (Fig. 22).

Contents: a single, light-brown cell that does not fill the entire egg.

Size: 75–85 × 65–75 µm.

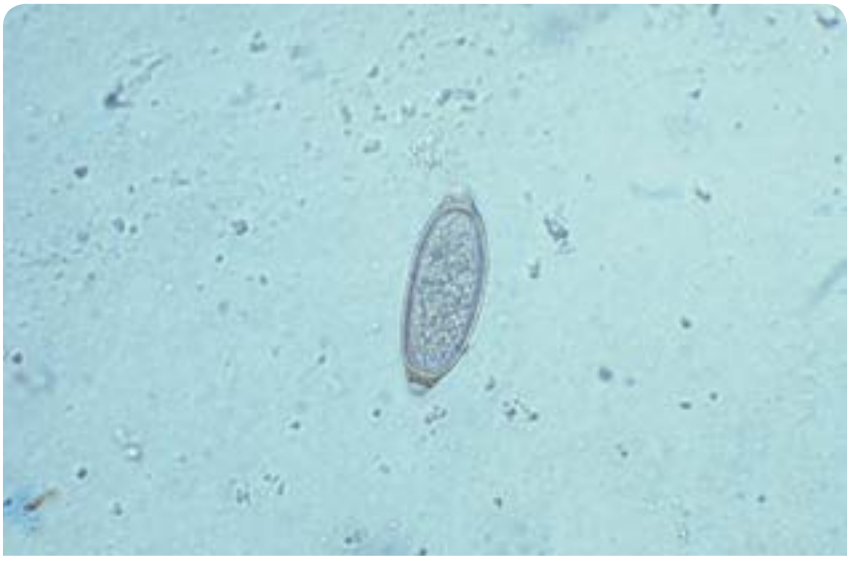


Figure 23. *Capillaria aerophila* egg.

Capillaria

Nematode egg

Description: medium-sized, quite narrow beige to brown egg; smooth shell of medium thickness; elongated lemon shape with a flattened polar plug at each pole (Fig. 23).

Contents: one cell.

Size: 55–70 × 30 µm.

Comment: differentiation from *Trichuris* eggs is based on the protruding appearance of the polar plugs, and globular shape, of *Trichuris* compared to *Capillaria*.

Ancylostoma and *Uncinaria*

Nematode egg

Description: ovoid, medium-sized strongyle-like egg; thin, smooth shell (Fig. 24).

Contents: a morula with four to eight large blastomeres.

Size: 55–65 × 40–45 µm.

Comment: *Ancylostoma* eggs are smaller (55–65 × 40 µm) than *Uncinaria* eggs (65–80 × 45–50 µm), but it is difficult to distinguish between them in practice.

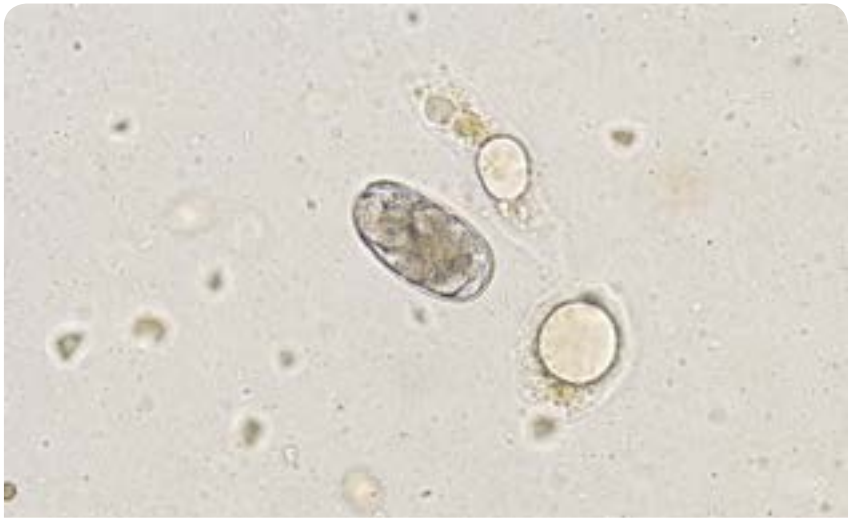


Figure 24. *Ancylostoma* or *Uncinaria* egg.

Spirocerca lupi

Nematode egg

Description: small, elongated egg; smooth shell with parallel sidewalls (Fig. 25).

Contents: a clearly visible first stage larva, often folded twice.

Size: 35 × 10–15 µm.

Figure 25. *Spirocerca lupi* egg.

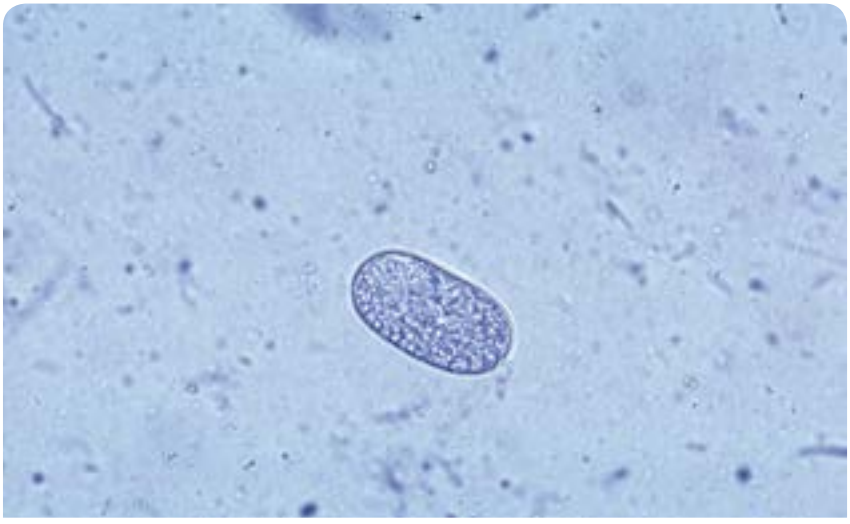


Figure 26. *Strongyloides* egg.

Strongyloides

Nematode egg

Description: small, rectangular small egg with parallel sidewalls; thin-shelled; light coloured (Fig. 26).

Content: a larva, not always clearly visible.

Size: 35–50 × 25–30 µm.

Comment: stage 1 larvae are expelled in carnivores, whereas eggs are excreted in herbivores and Suidae.

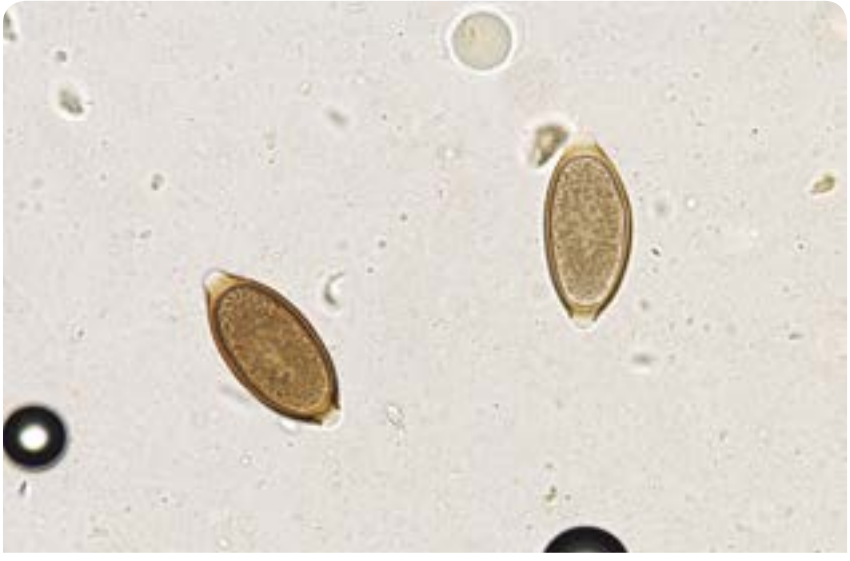


Figure 27. *Trichuris vulpis* eggs.

Trichuris vulpis

Nematode egg

Description: medium-sized, orange-brown egg; smooth, thick shell; elongated lemon shape with a protruding polar plug at each pole (Fig. 27).

Contents: a single cell.

Size: 60–85 × 40–45 µm.

Giardia

Protozoan cyst

Description: egg-shaped to oval cyst; smooth, thin shell (Fig. 28).

Contents: two to four flagella, the cellular nucleus and drumstick-like residual bodies of which are visible and feature a central S shape (similar to the Ying/Yang symbol).

Size: small (7–10 × 8–12 µm).

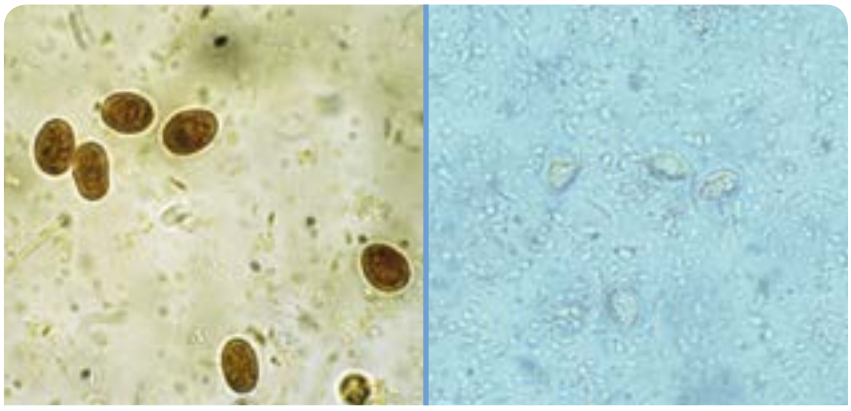


Figure 28. *Giardia* cysts.

Sarcocystis

Protozoan cyst

Description: sporocyst with four sporozoites; very thin shell element; smooth, elongated shape with rounded poles (Fig. 29).

Contents: four infective, banana-shaped cells: the sporozoites.

Size: 12 × 8 µm to 20 × 16 µm according to the species.

Comment: excreted as oocysts similar to *Isospora*, but directly sporulated and often producing sporocysts in the digestive tract, therefore possible to detect by coproscopy.

Figure 29. *Sarcocystis* cyst.

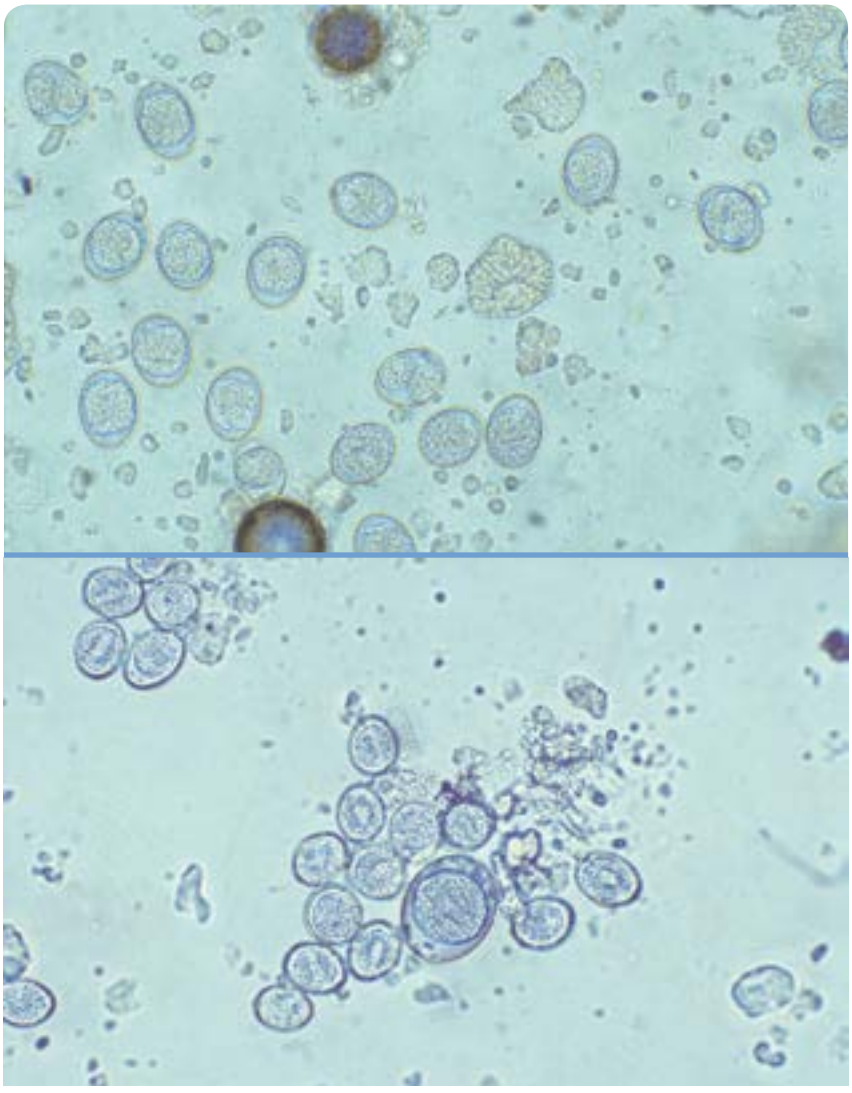


Figure 30. *Isospora* oocysts.

Isospora

Protozoan cyst

Description: ovoid oocyst; thin-shelled; slightly more pointed at one end and more rounded at the other (Fig. 30).

Contents: a rounded cell with granular content when hatched; two sporocysts that contain four sporozoites each after sporulation in the external environment.

Size: medium-sized (20–40 × 15–35 µm); *Isospora canis*: 38 × 30 µm; *Isospora ohioensis*: 23 × 19 µm.

Cryptosporidium parvum

Protozoan cyst

Description: spherical to egg-shaped oocyst, with a relatively thick shell compared to other coccidia (Fig. 31).

Contents: clearly visible oocyst residual body, and four vermiform sporozoites hardly visible with light microscopy.

Size: small (5 × 4 µm).

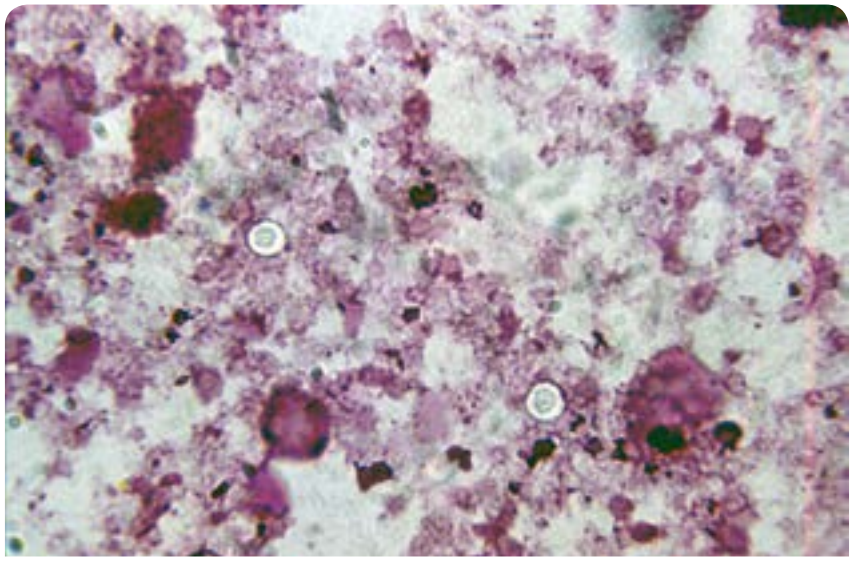


Figure 31. *Cryptosporidium parvum* oocysts.

Hammondia and *Neospora*

Protozoan cyst

Description: egg-shaped oocyst; smooth, thin shell with rounded ends (Fig. 32).

Contents: a single granular, spherical cell before sporulation, then two sporocysts containing four sporozoites each on sporulation in the external environment.

Size: 12–15 × 10–13 µm.

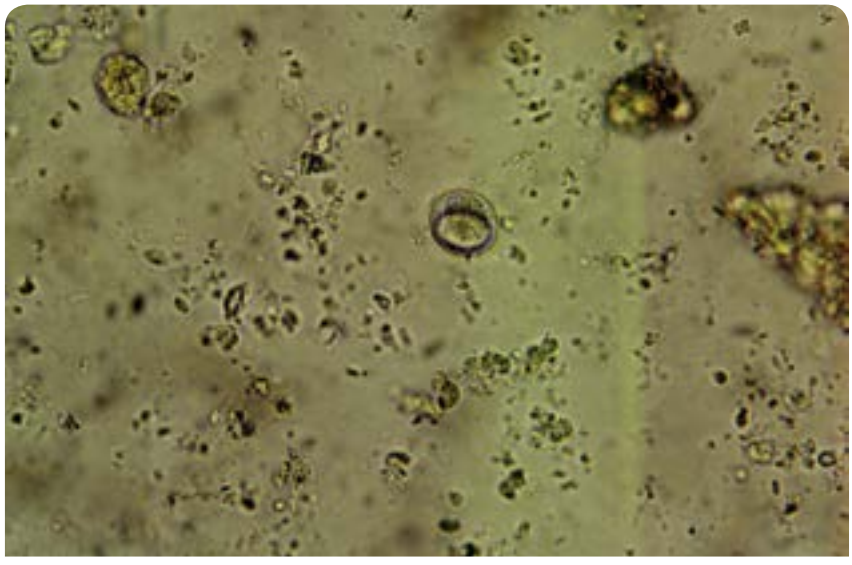


Figure 32. *Neospora* oocyst.

Figure 33. *Strongyloides stercoralis* L1.

Strongyloides stercoralis

Nematode larva

Description: thin, rhabditiform stage 1 larva; club-shaped anterior end; short buccal cavity; sharp, pointed tail. Large genital primordium clearly visible (Fig. 33).

Size: 280–380 × 15–18 µm.

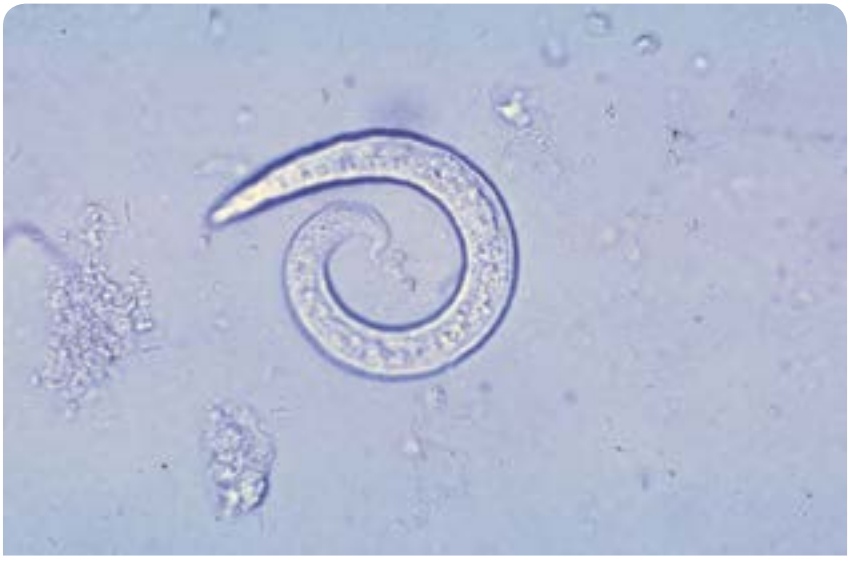


Figure 34. *Oslerus* L1.

Oslerus osleri

Nematode larva

Description: strongyle-like stage 1 larva; undulating, pointed tail with two curls;. oesophagus hardly visible (Fig. 34).

Size: 250–350 µm long.

Angiostrongylus vasorum

Nematode larva

Description: thin, strongyle-like stage 1 larva; button on the head; undulating, pointed tail with a dorsal notch (Fig. 35).

Size: 330–360 µm long.

Figure 35. *Angiostrongylus* L1.

Crenosoma vulpis

Nematode larva

Description: strongyle-like stage 1 larva; elongated caudal end with no dorsal notch; no button on the head; oesophagus clearly visible and strongyle-like, measuring up to 1/3 of the entire length of the larva (Fig. 36).

Size: 265–330 µm long.

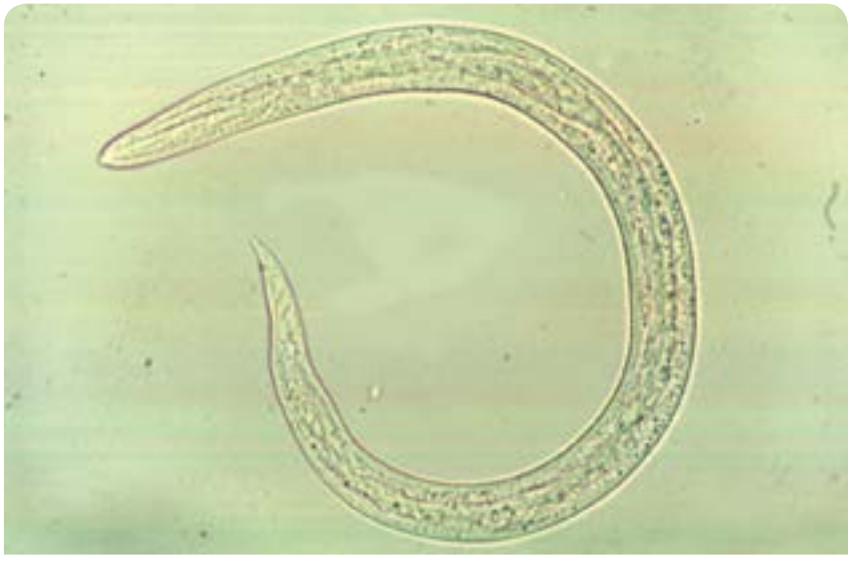


Figure 36. *Crenosoma vulpis* L1.

Macroscopic coproscopy in dogs

Figure 37. Adult ascarids, hookworms and whipworms.

Toxocara canis and *Toxascaris leonina*

Nematode

If numerous adults are present in young dogs' intestines, they can be eliminated in faeces or vomit.

Description: large, white, rounded worms; up to 10 cm in length and 2–3 mm in diameter; often intertwined with one another and forming ascarid clusters (Fig. 37).

Figure 38. *Dipylidium* proglottids on dog faeces.

Dipylidium caninum

Cestode

Description: strobila made up of whitish segments longer than they are wide; relatively narrow and barrel-shaped.

Expelled one at a time (2–4 × 6–10 mm) or in clusters of segments (Figs. 38 and 39).

Elements attach to fur and look like a rice or semolina grains when drying.

Can be found in the animal's direct environment: bed, chairs, etc.

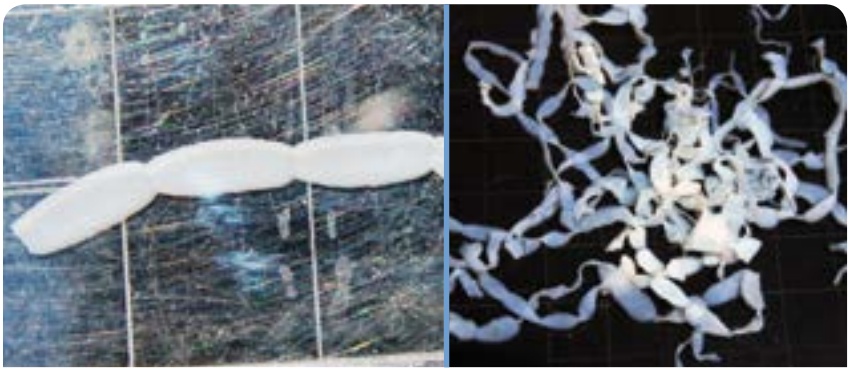


Figure 39. *Dipylidium* proglottids.

Taenia spp.

Cestode

Description: strobila made up of rectangular, whitish segments, longer than they are wide. Expelled as single element or in clusters (Fig. 40).

Size: 8–15 × 5–6 mm for each oviferous segment.

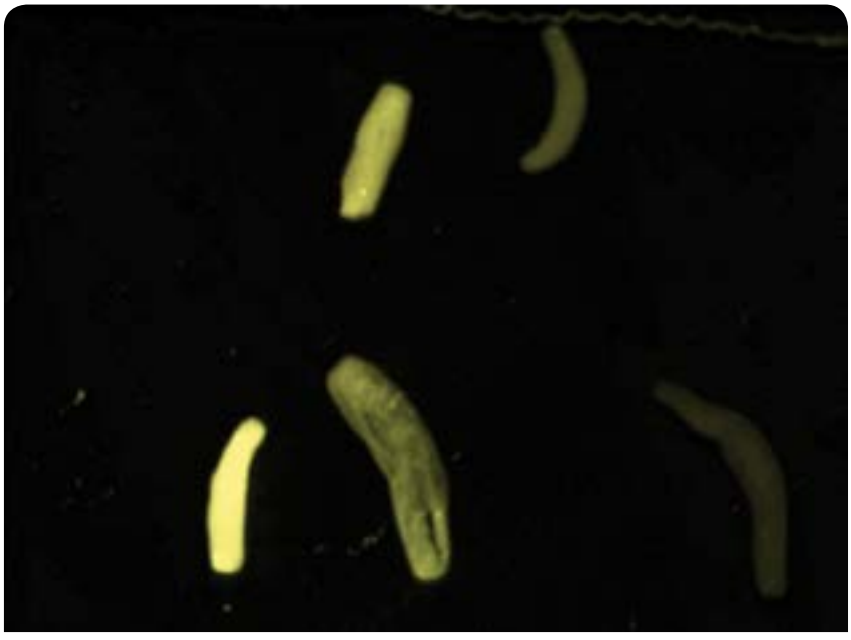


Figure 40. *Taenia* proglottids.

Mesocestoides (segment)

Cestode

Description: small whitish segments with rounded sidewalls; central spot representing the parauterine organ (Fig. 41).

Size: 5–6 × 3–4 mm.

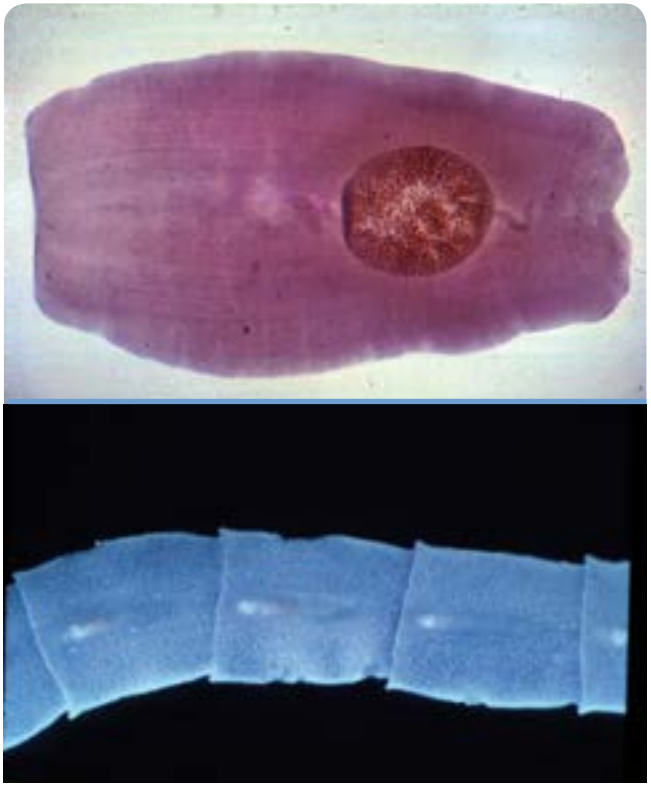


Figure 41. *Mesocestoides* segments.

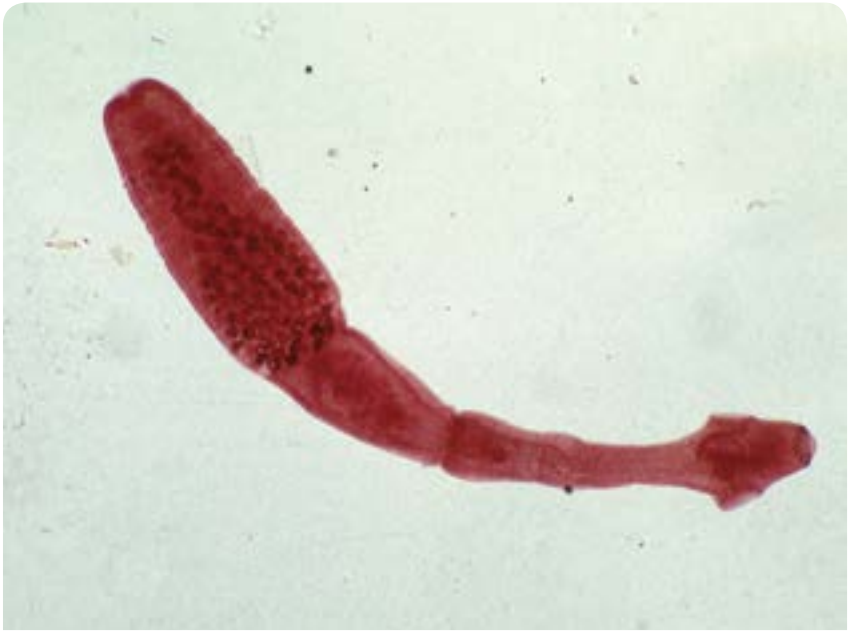


Figure 42. *Echinococcus granulosus*.

Echinococcus granulosus

Cestode

Description: one rectangular oviferous segment per cestode; 4–6 mm long and 1 wide; difficult to identify macroscopically. Oviferous segment represents more than half of the total body length and contains a longitudinally elongated uterus (Fig. 42).

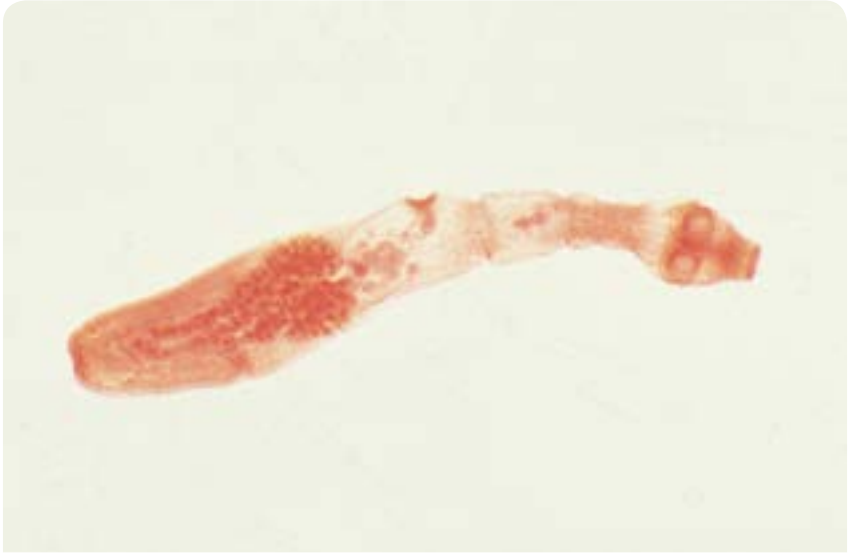


Figure 43. *Echinococcus multilocularis*.

Echinococcus multilocularis

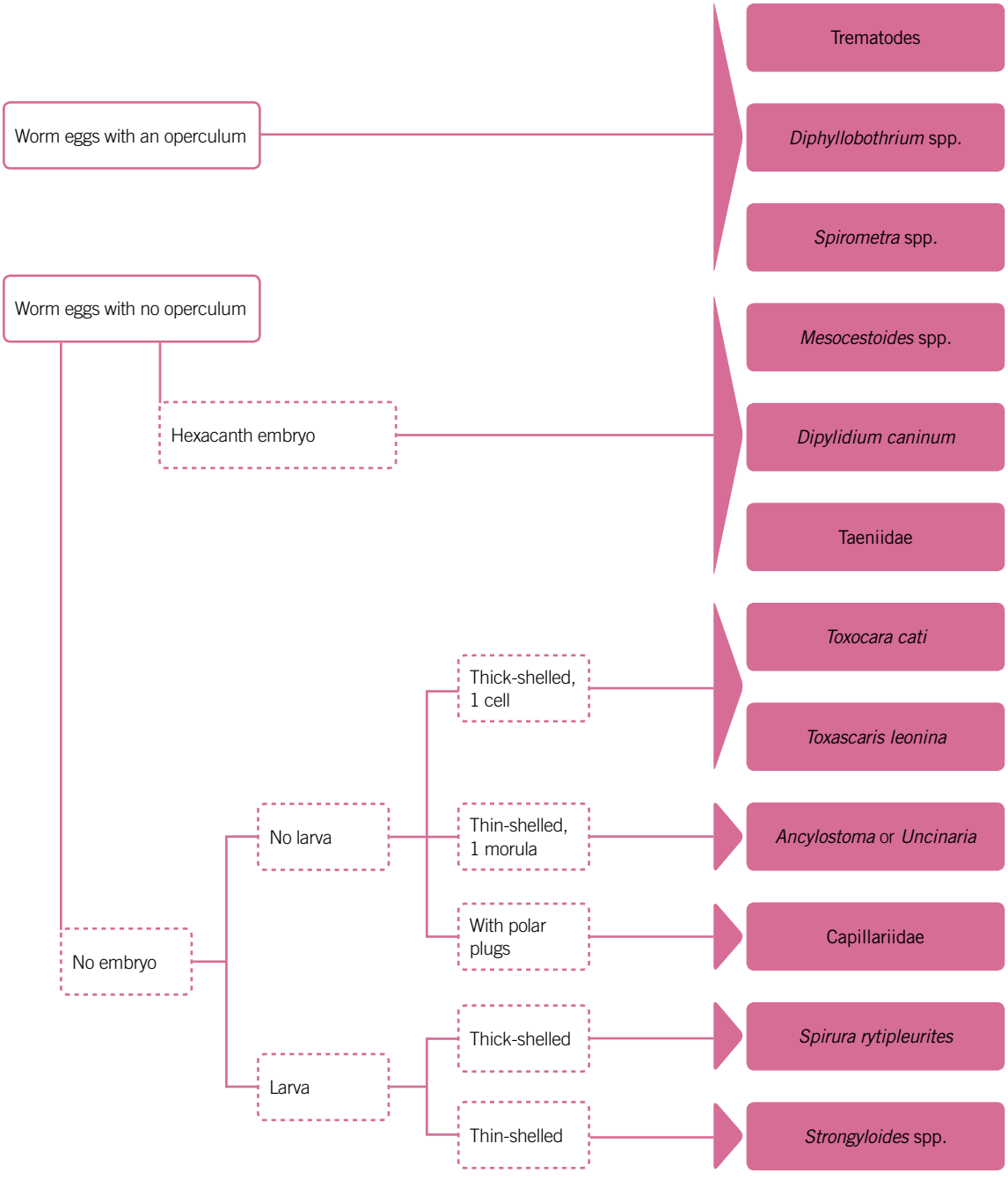
Cestode

Description: one rectangular oviferous segment per cestode; 2-3 mm long and 1 wide; difficult to identify macroscopically. Oviferous segment represents less than half of the total body length and contains a sacculated uterus (Fig. 43).

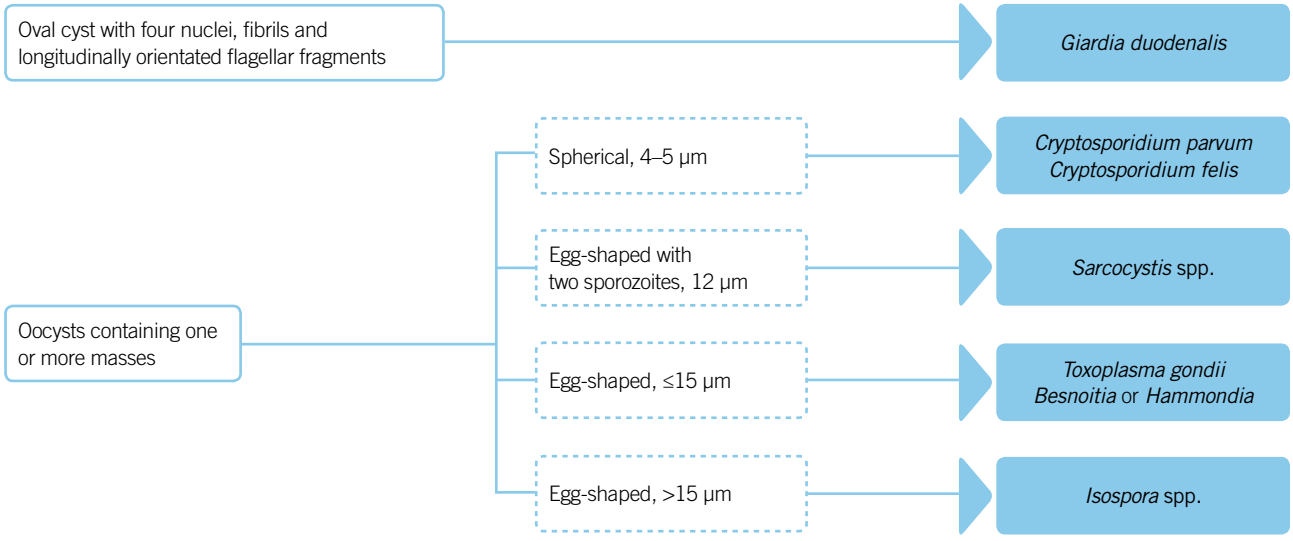
Identification of parasite eggs, cysts and larvae in cats

Microscopic coproscopy

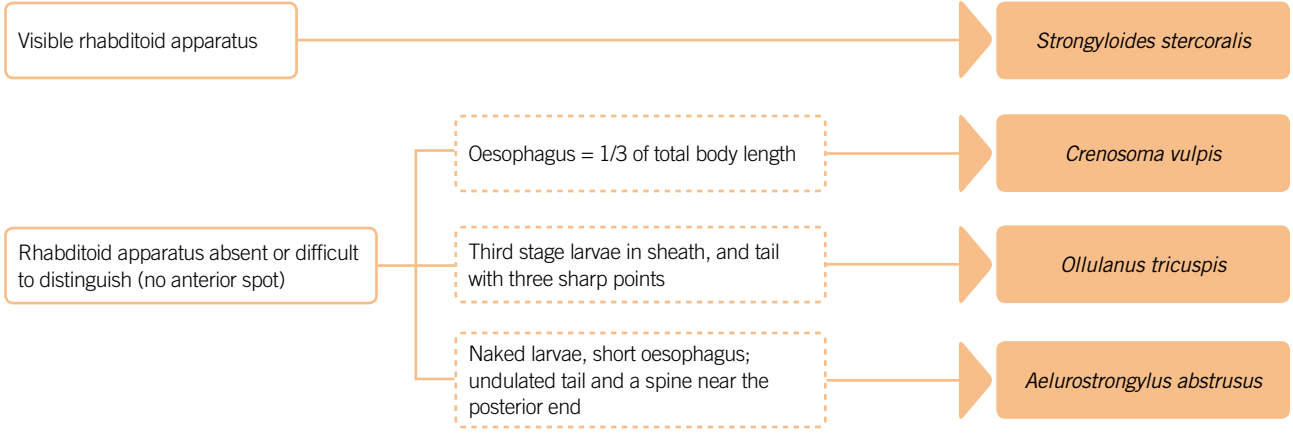
Major helminth eggs in cats



Major protozoan cysts or oocysts in cats



Major helminth larvae in cats



Macroscopic coproscopy

Major visible worms in cats

Microscopic coproscopy in cats

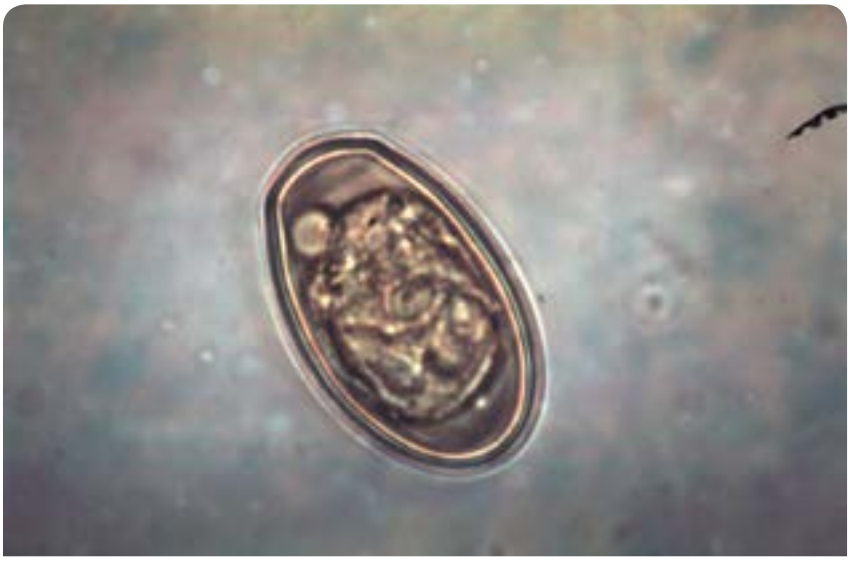


Figure 44. *Opisthorchis egg.*

Opisthorchiidae: *Clonorchis* and *Metorchis*

Trematode egg

Description: small ovoid egg; thick shell with an operculum at one end and a polar spine at the opposite end (Fig. 44).

Contents: an embryo.

Size: 30 × 20 µm.

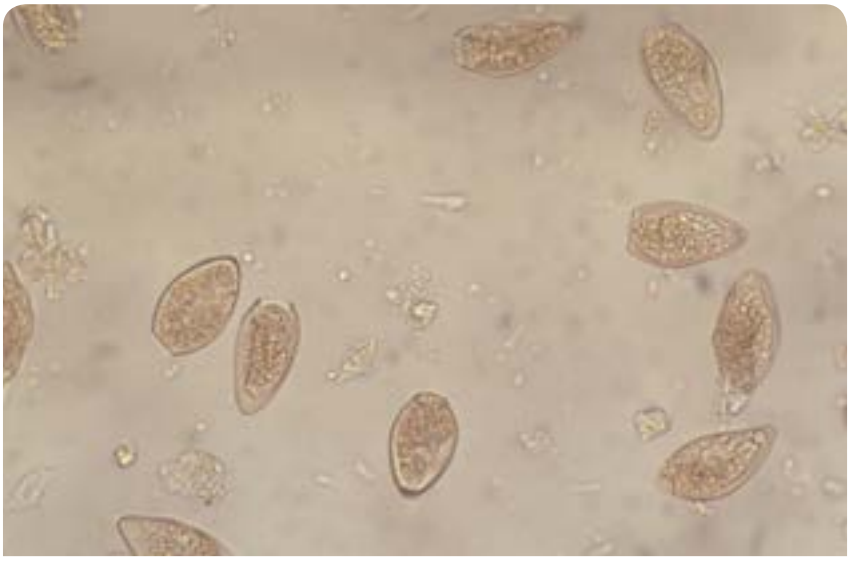


Figure 45. *Spirometra* or *Diphyllobothrium* eggs.

Diphyllobothrium or *Spirometra*

Cestode egg

Description: medium-sized ovoid egg; smooth shell with an operculum (Fig. 45).

Contents: light golden-yellow syncytium which fills the entire shell.

Size: 60 µm long × 45 µm wide.

Dipylidium caninum

Cestode egg

Description: small egg; thin, smooth shell (Fig. 46).

Contents: a hexacanth embryo.

Size: 40 × 50 µm.

Comment: *Dypilidum* spp. eggs are grouped in clusters of about 20 in egg capsules, encased by a thin shell: the oviferous or egg-bearing capsule. Size: 200 × 400 µm.

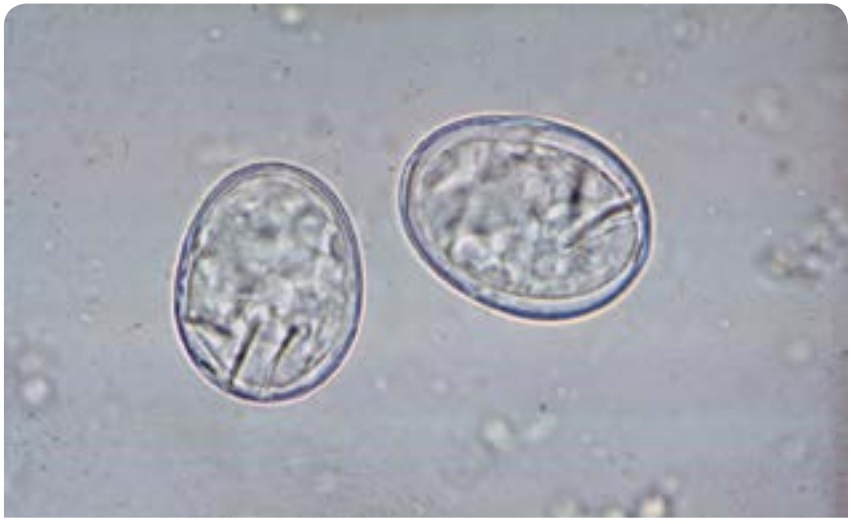


Figure 46. *Dipylidium* eggs.

Taeniidae

Cestode egg

Description: small globular egg; unique, thick envelop; radially striated (Fig. 47).

Contents: a hexacanth embryo.

Size: 30–40 × 20–30 µm.

Comment: eggs are eliminated in an ovigerous segment. Genus and species diagnosis is not possible (*Taenia* or *Echinococcus*).

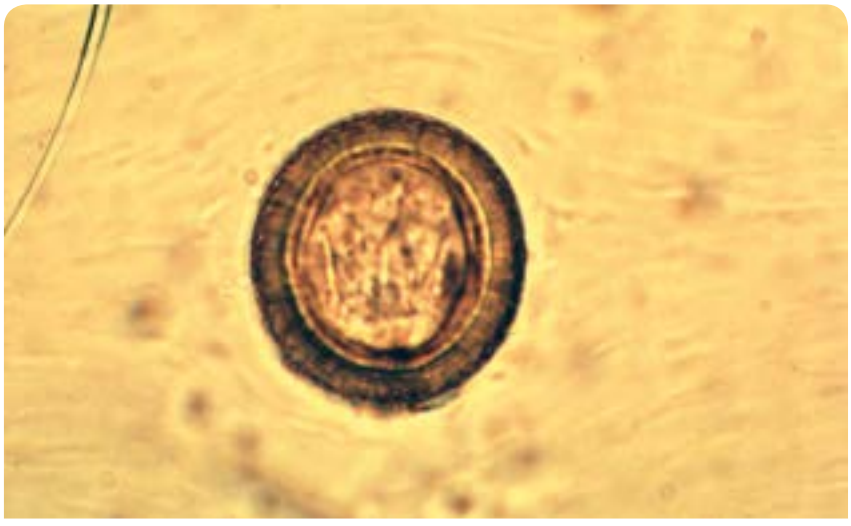


Figure 47. Taeniidae egg.

Mesocestoides

Cestode egg

Description: small, globular egg; thin, smooth shell (Fig. 48).

Contents: a hexacanth embryo.

Size: 40 × 50 µm.

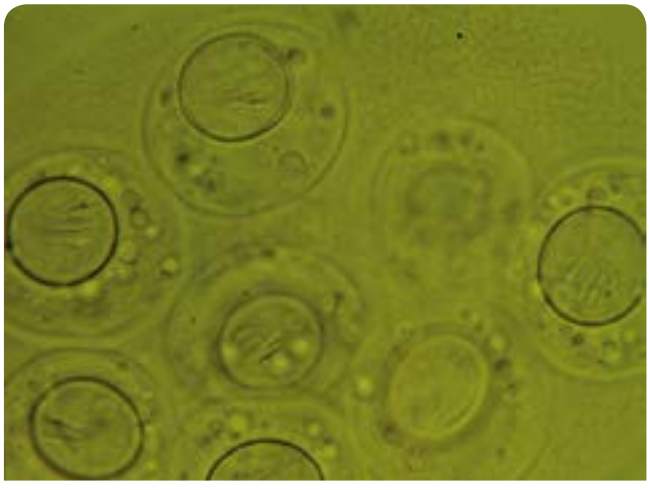


Figure 48. *Mesocestoides* eggs.

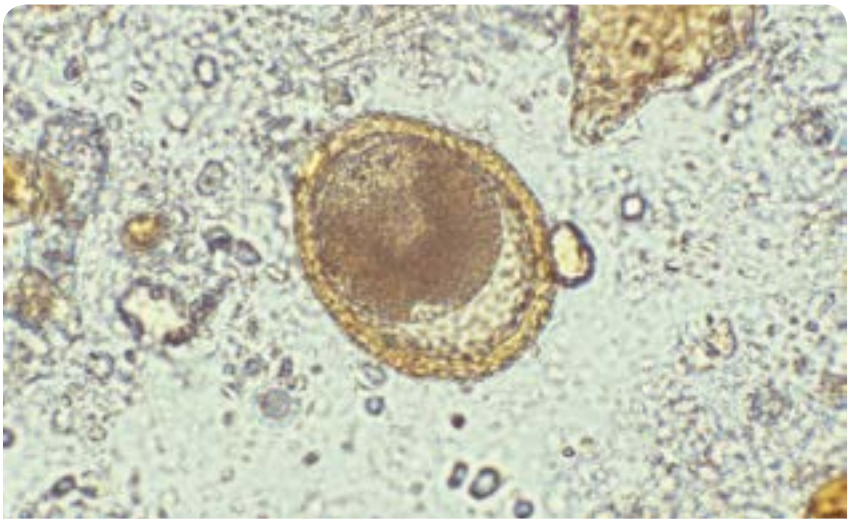


Figure 49. *Toxocara cati* egg.

Toxocara cati

Nematode egg

Description: medium-sized, globular egg; thick-shelled with an alveolar external layer (thimble-like) and smooth internal layer (Fig. 49).

Contents: a single, brownish-black cell filling almost all the shell.

Size: 70–90 × 65–75 µm.

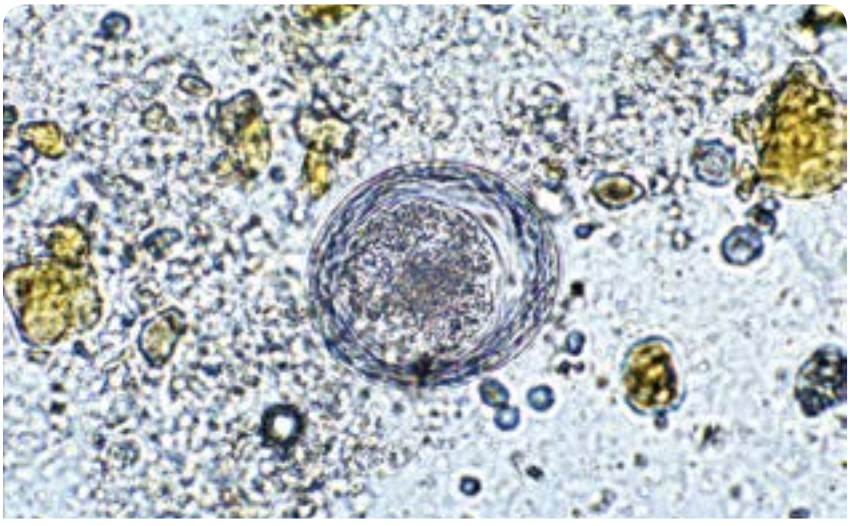


Figure 50. *Toxascaris leonina* egg.

Toxascaris leonina

Nematode egg

Description: medium-sized, subglobular egg; smooth, thick shell with concentric layers (Fig. 50).

Contents: a single, yellowish-brown cell filling only part of the shell.

Size: 75–85 × 65–75 µm.

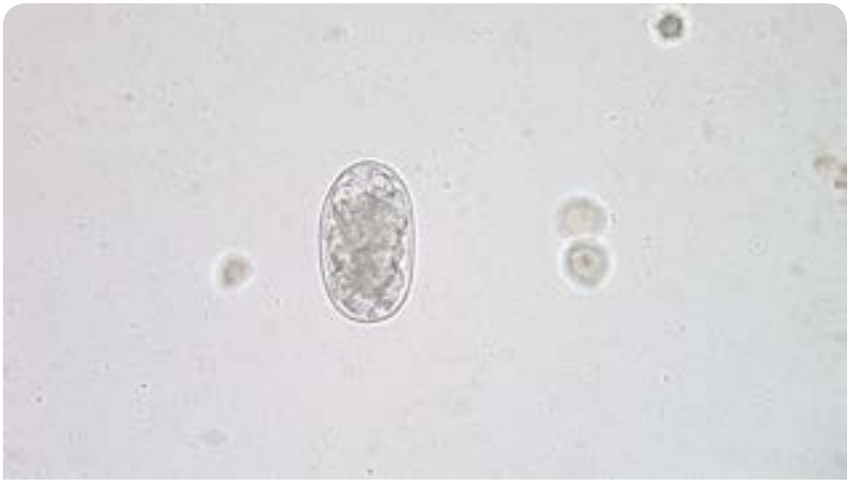


Figure 51. *Ancylostoma* egg.

Ancylostoma or *Uncinaria*

Nematode egg

Description: ovoid, medium-sized strongyle-like egg; thin, smooth shell (Fig. 51).

Contents: a morula with four to eight large blastomeres.

Size: 55–65 × 40–45 µm.

Comment: *Ancylostoma* eggs are smaller (55–65 × 40 µm) than *Uncinaria* eggs (65–80 × 45–50 µm), but it is difficult to distinguish between them in practice.

Capillaridae

Nematode egg

Description: medium-sized light-coloured egg; relatively straight, smooth and semi-thick shell; elongated (lemon-shaped) with a flattened polar plug at each end (Figs. 52 and 53).

Contents: a single cell.

Size: 55–70 × 30 µm.

Comment: the identification of *Trichuris* eggs is not relevant to domestic cats in Europe. The only known *Trichuris* in Felidae were found in wild animal species, especially in South America.

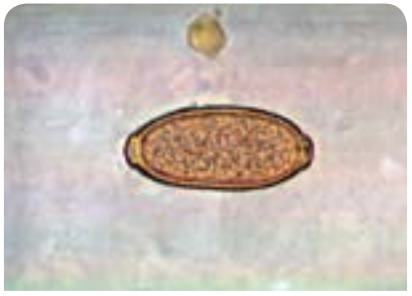


Figure 52. *Capillaria plica* (urine) egg.

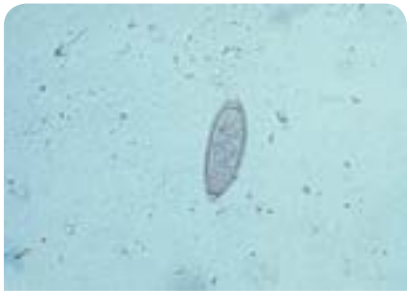


Figure 53. *Capillaria aerophila* egg.

Spirura rytipleurites

Nematode egg

Description: elongated egg; thick, smooth shell (Fig. 54).

Contents: a well-developed larva.

Size: 45–55 × 25–35 µm.

Figure 54. *Spirura* egg.

Strongyloides

Nematode egg

Description: small, rectangular small egg with parallel sidewalls; thin-shelled; light coloured (Fig. 55).

Content: a larva, not always clearly visible.

Size: 35–50 × 25–30 µm.

Comment: stage 1 larvae are expelled early in carnivores, whereas eggs are excreted in herbivores and Suidae.

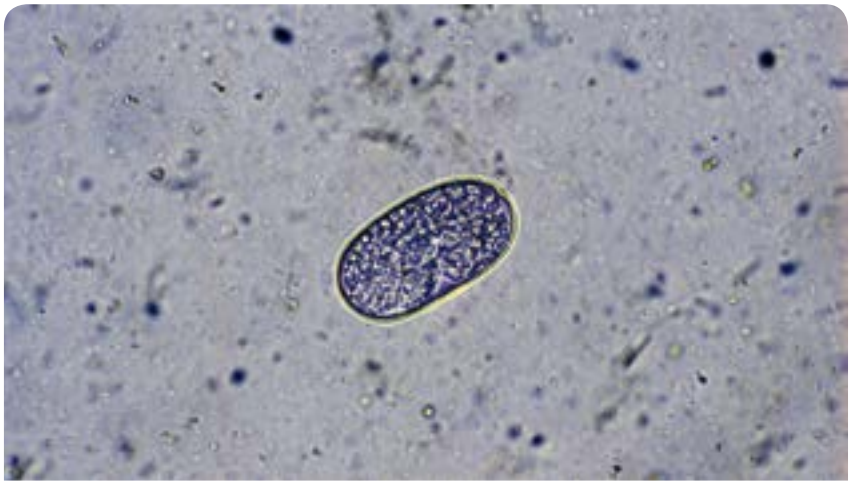


Figure 55. *Strongyloides* egg.

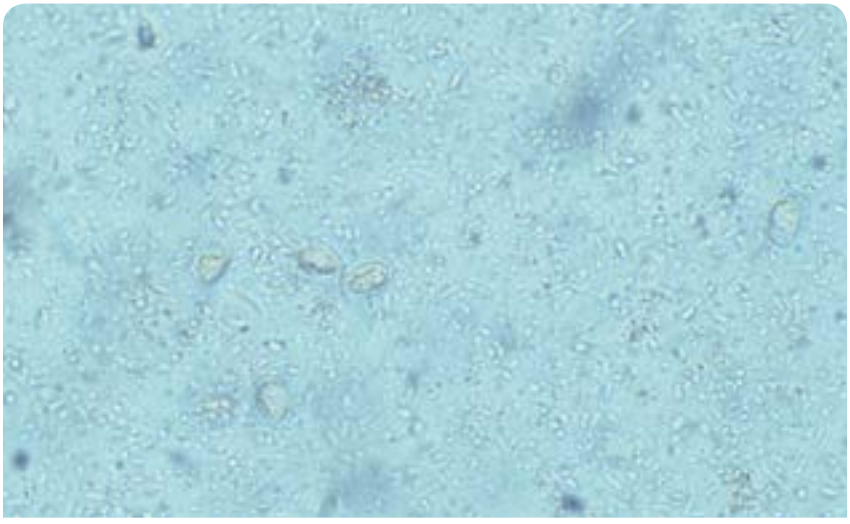


Figure 56. *Giardia* cysts.

Giardia

Protozoan cyst

Description: egg-shaped to oval cyst; smooth, thin shell (Fig. 56).

Contents: two to four flagella, the cellular nucleus and drumstick-like residual bodies of which are visible and feature a central S shape (similar to the Ying/Yang symbol).

Size: small (7–10 × 8–12 µm).

Comment: Iugol staining.

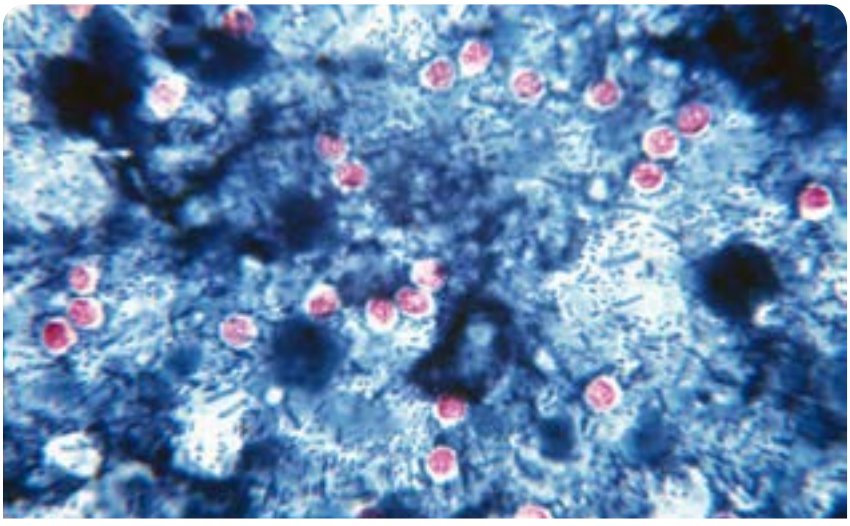


Figure 57. *Cryptosporidium* cysts (Ziehl-Neelsen staining).

Cryptosporidium

Protozoan cyst

Description: spherical to egg-shaped oocyst; relatively thick shell compared to other coccidia (Fig. 57).

Contents: clearly visible oocyst residual body, and four sporozoites hardly visible with light microscopy.

Size: small (5 × 4 µm).

Comment: Ziehl-Nielsen staining.

Figure 58. *Sarcocystis* oocyst.

Sarcocystis

Protozoan cyst

Description: sporocyst contains four sporozoites; very thin and smooth sidewalls; elongated shape with rounded ends (Fig. 58).

Contents: four infectious banana-shaped cells: the sporozoites.

Size: 12 × 8 µm to 20 × 16 µm according to the species.

Comment: cysts that are hatched with a similar shape to that of an *Isospora* oocyst, although sporulated and often delivering sporocysts into the digestive tract, therefore visible on coproscopy.

Toxoplasma, Hammondia and *Besnoitia*

Protozoan cyst

Description: egg-shaped oocyst; smooth, thin shell with rounded ends (Fig. 59).

Contents: a single granular, spherical cell before sporulation, then two sporocysts that contain four sporozoites each at sporulation in the external environment.

Size: 12–15 × 10–13 µm.

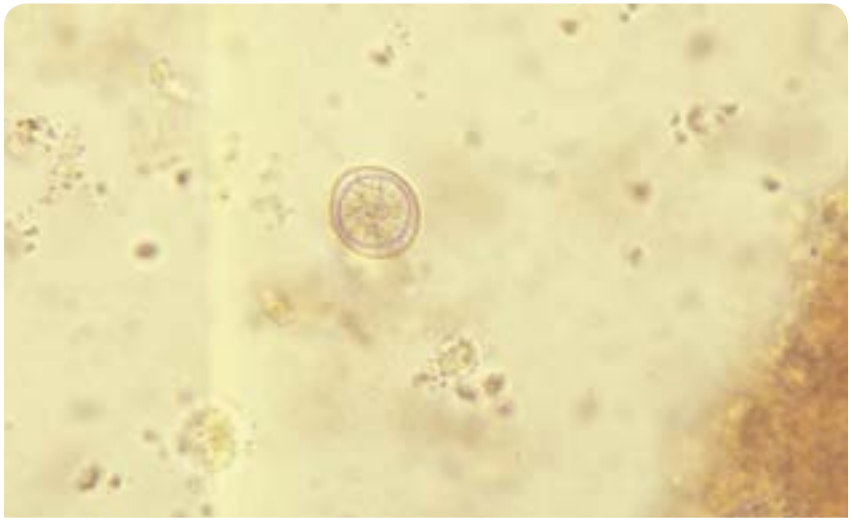


Figure 59. *Toxoplasma, Hammondia* or *Besnoitia* oocyst.

Isospora

Protozoan cyst

Description: thin-shelled, ovoid oocyst with one end being more pointed than the other (Fig. 60).

Contents: a single rounded cell with granular contents when hatching; two sporocysts that contain four sporozoites each after sporulation in the external environment.

Size: *Isospora felis*: 40 × 30 µm; *Isospora rivolta:* 25 × 20 µm.

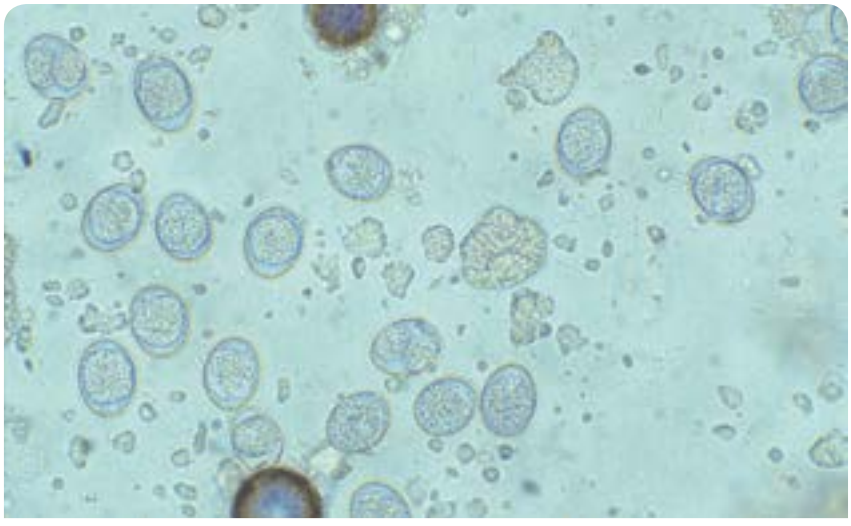


Figure 60. *Isospora* oocysts.

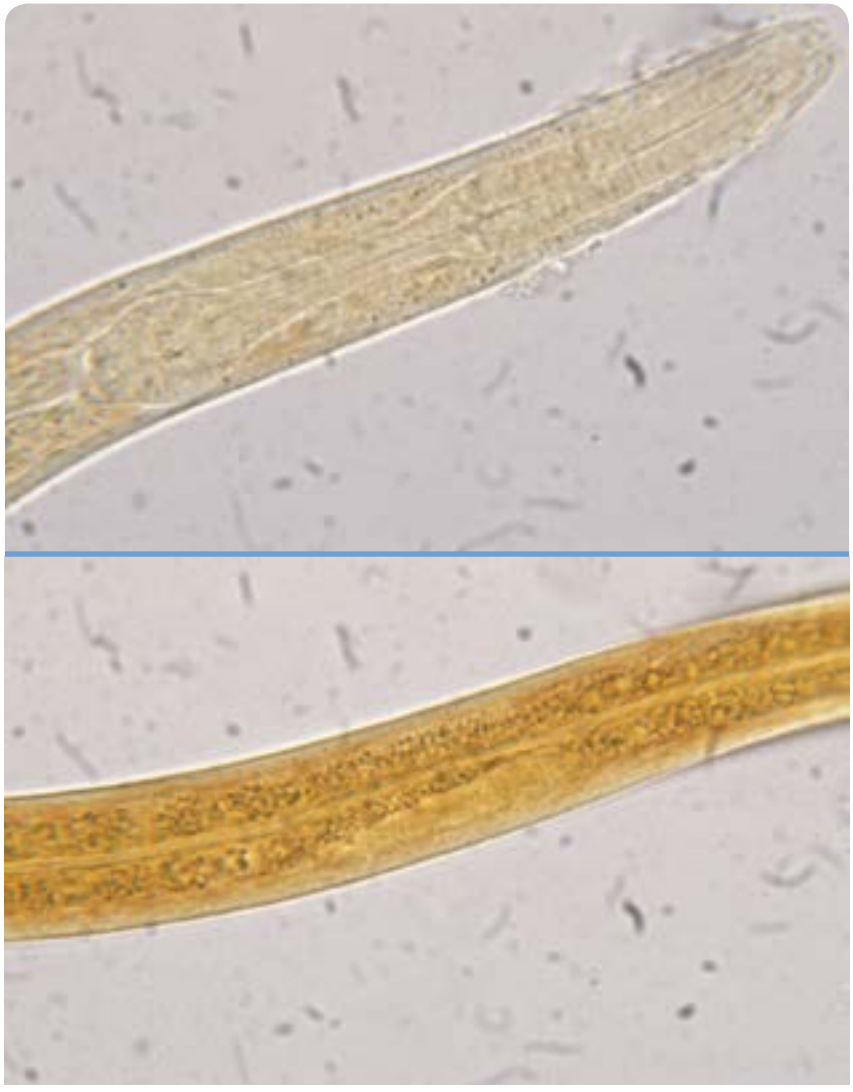


Figure 61. *Strongyloides* L1.

Strongyloides

Nematode larva

Description: thin, rhabditiform stage 1 larva; club-shaped anterior end; short buccal cavity; sharp, pointed tail. Large genital primordium clearly visible (Fig. 61).

Size: 280–380 × 15–18 µm.

Figure 62. *Crenosoma vulpis* L1.

Crenosoma vulpis

Nematode larva

Description: strongyle-like larva 1; elongated posterior end; no dorsal spine or cephalic plug. Strongyle-like oesophagus clearly visible and measuring a third of the total length (Fig. 62).

Size: 265–330 µm.

Aelurostrongylus abstrusus

Nematode larva

Description: thin-walled, strongyle-like larva 1 with undulating, pointed tail at the posterior end and a dorsal spine (Fig. 63).

Size: 360–400 µm.

Figure 63. *Aelurostrongylus abstrusus* L1.

Macroscopic coproscopy in cats

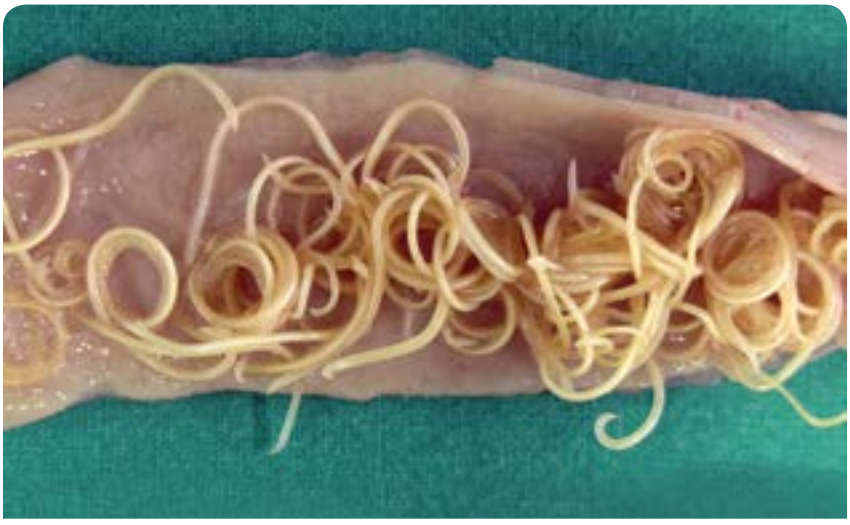


Figure 64. Toxocarosis (*Toxocara cati*) in a cat.

Toxocara cati – *Toxascaris leonina*

Nematode

When adults are numerous in a kitten intestine, they can be expelled in faeces or vomit.

Description: large, white, rounded worms measuring up to 10 cm long, with a diameter of 2–3 mm; often intertwined with one another and forming ascarid clusters (Fig. 64).

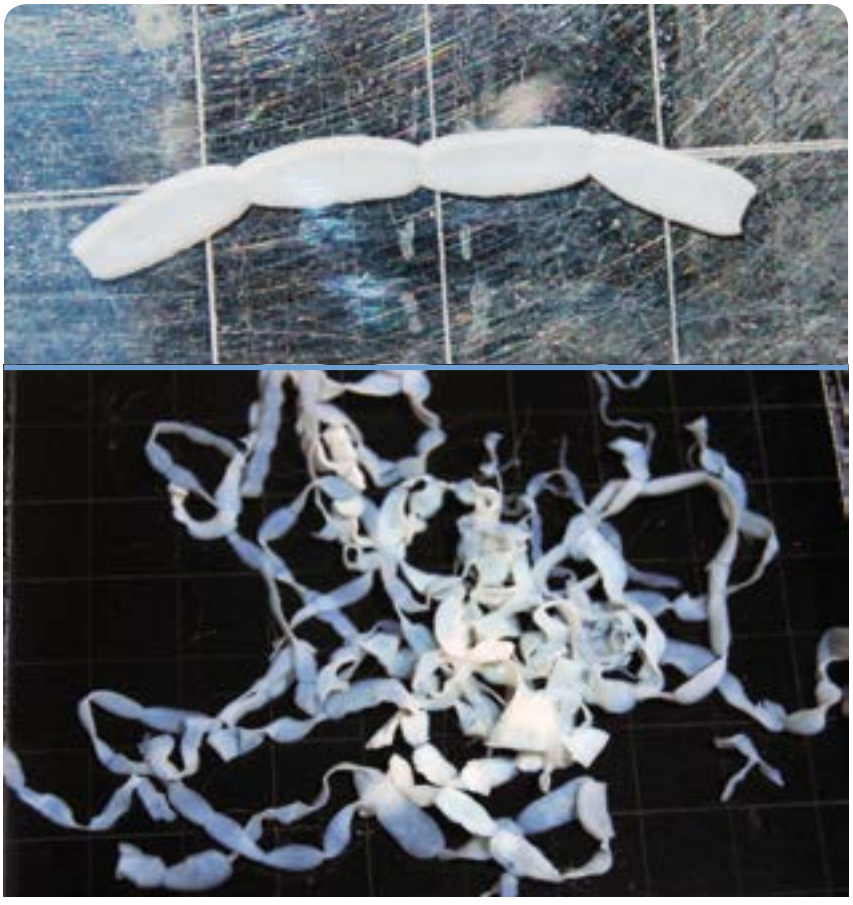


Figure 65. *Dipylidium* proglottids.

Dipylidium caninum (strobila)

Cestode

Description: strobila made up of whitish segments, longer than they are wide; relatively narrow and barrel-shaped. Expelled one at a time (2–4 × 6–10 mm) or in clusters of segments (Fig. 65). Elements attach to fur and look like a rice or semolina grains when drying. Can be found in the animal's direct environment: bed, chairs, etc.

Taenia taeniaeformis

Cestode

Description: strobila made up of rectangular, whitish segments, longer than they are wide. Expelled as single element or in clusters (Fig. 66).

Size: 8–15 × 5–6 mm for each oviferous segment.

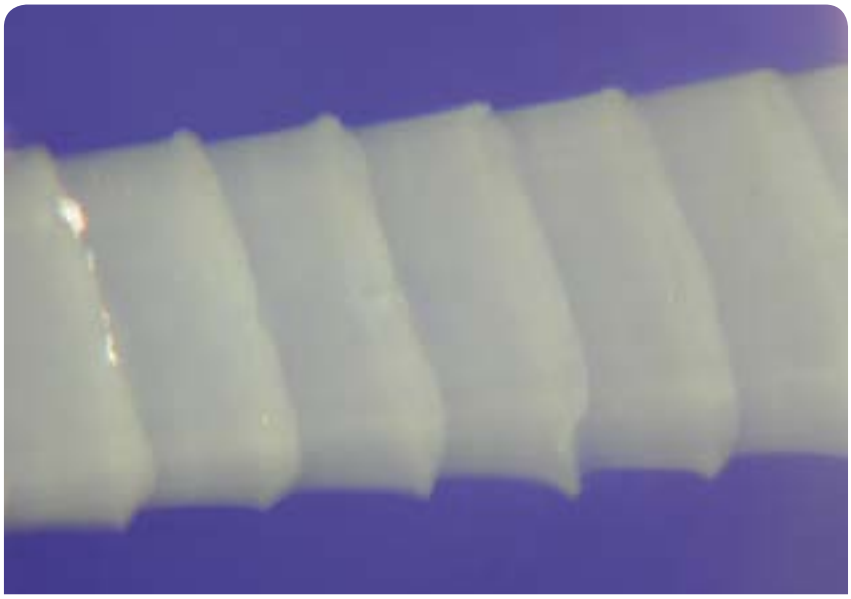


Figure 66. *Taenia taeniaeformis* strobila.

Mesocestoides

Cestode

Description: small whitish segments with rounded sidewalls; central spot representing the parauterine organ (Fig. 67).

Size: 5–6 × 3–4 mm.

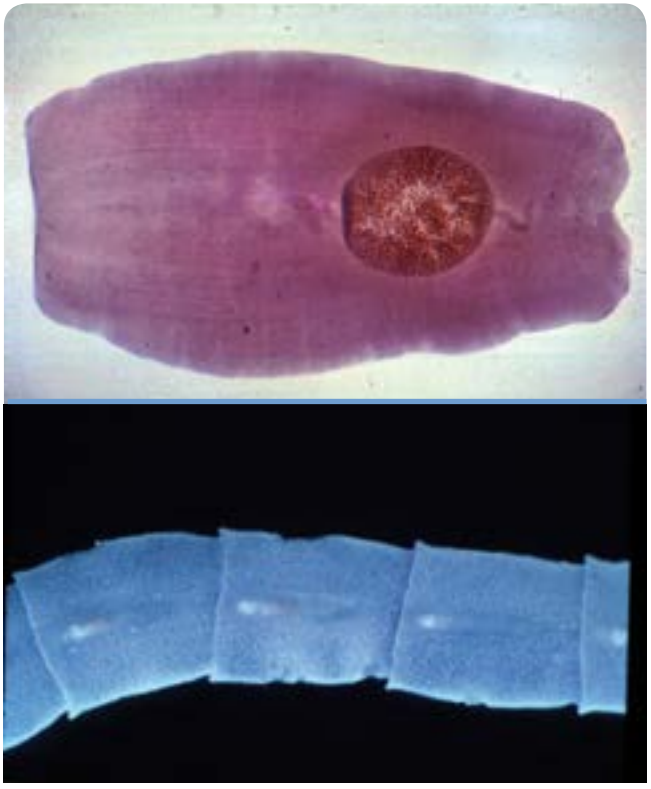


Figure 67. *Mesocestoides* segments.

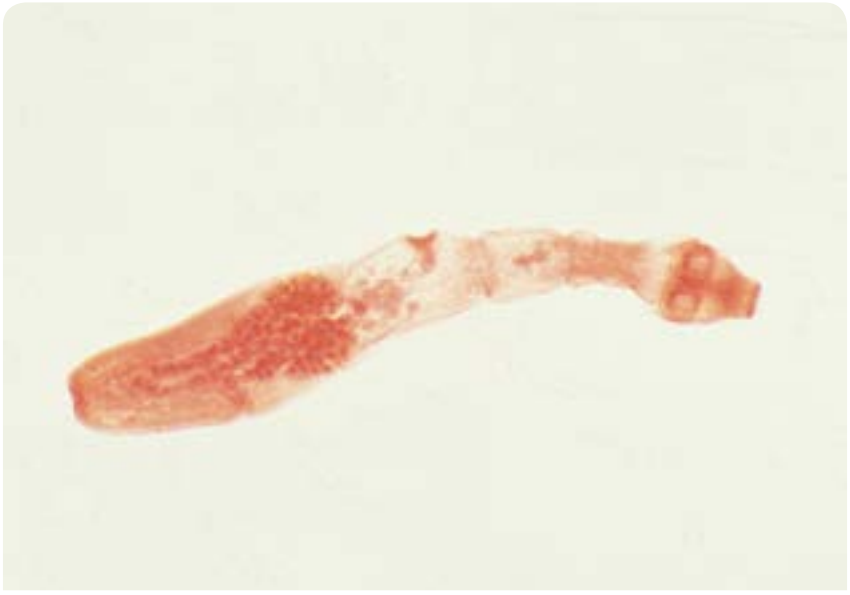


Figure 68. *Echinococcus multilocularis*.

Echinococcus multilocularis

Cestode

Description: rectangular oviferous segment; 2–3 mm long and 1 mm wide; one per cestode; difficult to identify macroscopically (Fig. 68).

Blood and lymph examination

Freshly prepared smears can be used to diagnose a number of parasitic infections and, even though the presence of parasites in the blood can be rare, the ease and rapidity of the method makes it a useful support for final diagnosis and it should always be performed.

Blood parasites that can potentially be identified in a blood smear are: *Trypanosoma* sp., *Babesia* sp., *Cytauxzoon felis*, and the microfilaria of several species of filarial nematodes. A variation in the number of parasites in the blood with time has been described for some species. *Dirofilaria* sp. microfilariae are more active at twilight when the mosquito vector is active. Some haemotropic bacteria, such as vector-borne Rickettsiae (*Ehrlichia* sp. or *Anaplasma* sp. morulae) may also be identified on blood smears.

Most of the parasites seen in a blood smear are vector-borne and transmitted by haematophagous arthropods. This transmission mechanism concentrates the parasites in the peripheral capillary blood. It is therefore advisable to use blood from peripheral areas of the body, rather than from the central circulation. Blood can be sampled from the ear pinna or by scarification in dogs and cats.

Blood smear for parasitic examination

The drop of blood is placed on a microscope slide that has been washed with ethanol. A second microscope slide is placed in the blood, inclined at 2° to 45°, and pushed slowly to spread the blood across the slide to form a layer one cell thick (Fig. 1). Microscopic observation of the smear starts on the border at the tail of the smear where the heaviest, parasitised cells are located. Several staining methods are available for blood smears but the most common is May-Grünwald-Giemsa stain.

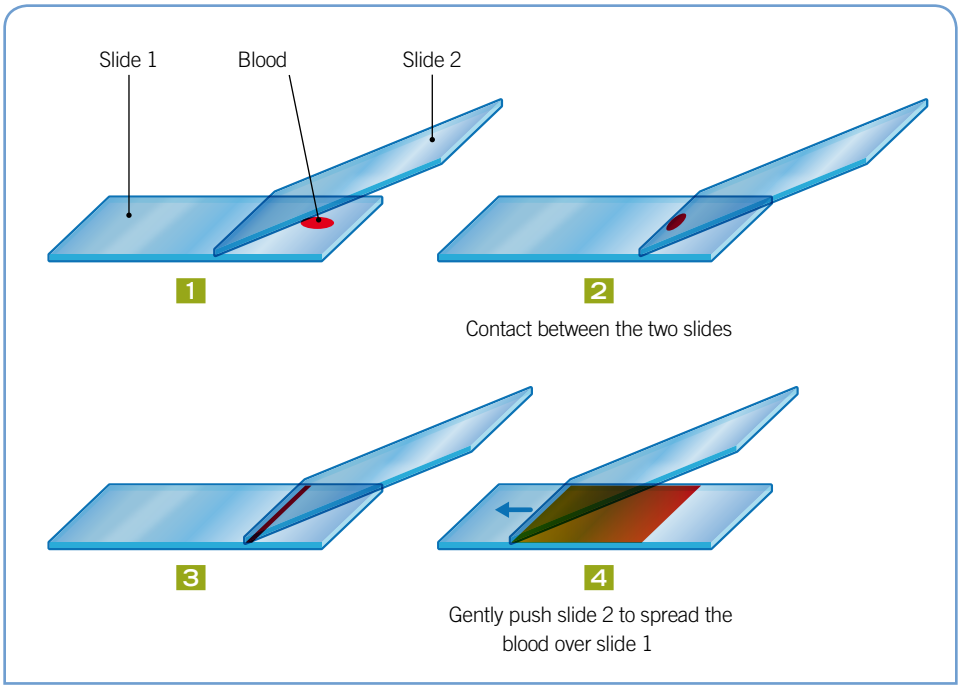


Figure 1. Blood smear technique.

Knott's test

This method concentrates the microfilaria present in the blood: 1 mL of blood is diluted in 9 mL of 2 % formalin and the solution is agitated for several minutes. It is then centrifuged for 5 minutes at 1,500 rpm. The supernatant is removed and one drop of methylene blue (1 %) is added to the remaining liquid. The acid phosphatase patterns of microfilaria can be stained and used to identify species of filarial nematodes (commercial kits are available). The number of areas of acid phosphatase activity seen in the body of the filaria varies from one species to another (see Table 1 in *Cardiopulmonary dirofilariosis*, page 129).

Lymph examination

Lymph node aspiration is a useful tool for diagnosing the microorganisms which parasitise lymphocytes, monocytes and macrophages, especially *Leishmania infantum*.

Insert a fine needle (19– 22 gauge), attached to a 2 mL syringe, into the lymph node. No local anaesthetic is required. While applying constant suction and keeping the needle in the node, repeatedly advance and withdraw the needle in multiple directions until a small amount of aspirate appears in the hub of the needle. A large volume of aspirate is not required and may reduce the yield by diluting the material aspirated. Release suction before removing the needle from the node. Remove the needle and then fill the syringe with air (the aspirated material must remain in the needle). Replace the needle and express the aspirate onto the slide. Only a small drop of aspirate is required.

Dermatological examination

External examination of the animal's skin and fur enables several ectoparasites to be detected and identified. These includes fleas, lice, mites and ticks which can cause parasitic diseases, but also transmit vector-borne pathogens. Some ectoparasites are visible to the naked eye, and others require microscopic identification (see *Ectoparasites of dogs and cats under the microscope*, page 345).

Skin scrapings

A skin scraping may be superficial or deep. In the first case, it consists of sampling the first layers of the epidermis to collect ectoparasites that remain on the surface of the skin (*Cheyletiella* mites). Deep skin scrapings are recommended for *Demodex* mites which live deep in the hair follicle, and for *Sarcoptes scabiei* females that burrow into the deeper layer of the epidermis.

Scraping is carried out with a blunt scalpel blade (Fig. 1). Lactophenol (or paraffin oil, which does not kill *Demodex*) may be applied to the blade to make sure debris and parasites adhere, and is also placed on the slide and mixed with the collected material before being covered with a coverslip. Superficial skin scrapings are taken from large body areas. Deep skin scrapings are usually performed in an area of hair loss or skin lesions. The skin is squeezed before or during the scraping to promote extrusion of mites from hair follicles (*Demodex*) or burrows (*Sarcoptes*).

Trichogram (microscopic examination of plucked hair)

A trichogram consists of plucking several hairs using clamp scissors (Fig. 2). It enables whole hair follicles to be collected, which is useful for the observation of *Demodex* mites, especially in areas where skin scraping is difficult or especially painful (from sites close to the eyes, for example).

Scotch tape test

The use of a clear sticky tape enables debris to be collected from the surface of the skin. The sticky side of the tape is pressed down onto the skin several times and then pressed onto a slide where it serves as a coverslip. This technique is especially useful for the detection of *Cheyletiella* and *Lynxacarus* mites.

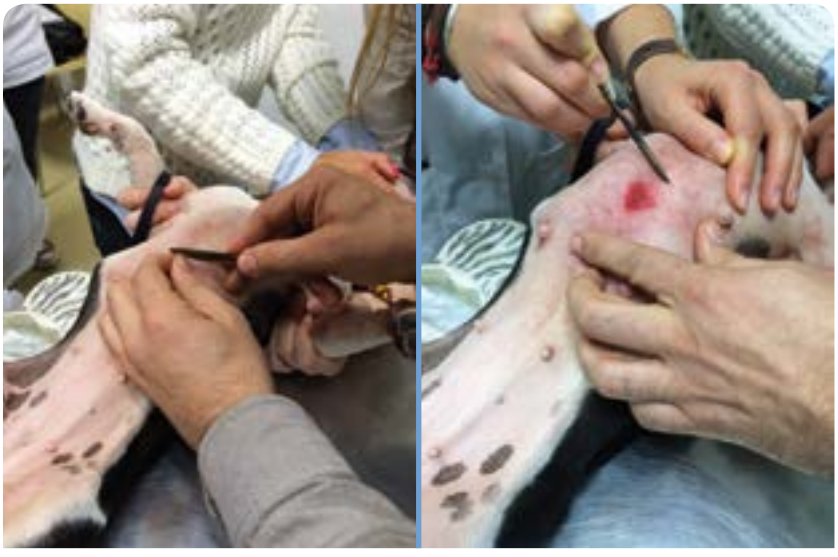


Figure 1. Skin scraping being taken from a skin lesion in a dog with suspected demodicosis.

Figure 2. Hairs being plucked from a skin lesion in a dog with suspected demodicosis.

Tick extraction

Ticks are the biggest arthropods found on animals and they are visible to the naked eye. Methodical examination of the dog/cat fur by thumb palpation is still required to locate them and unengorged immature stages may be difficult to see and identify. When extracting the tick, it is important to avoid leaving the tick rostrum in the skin. Tweezers should therefore be used to carefully twist the tick until the whole rostrum is detached, or a specific tick remover can be used (Fig. 3). Once removed, the tick can be identified using a diagnostic key (see *Diagnose of the main tick genera infesting dogs, cats and humans*, page 249).

Flea combing

It may be difficult to see fleas on an infested animal, especially animals with a long, dark coat. All debris can be collected from the coat with a specific comb with very fine teeth, called a flea comb. Flea faeces may be collected in the absence of fleas and these may be differentiated from other debris by placing them onto damp white paper or tissue where dilution of the faeces shows as red traces of digested blood.

Figure 3. A tick being extracted from a dog using a tick remover.

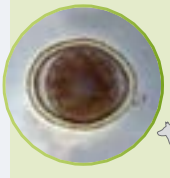
Collection of ear wax

Ear wax collection is the examination of choice to diagnose *Otodectes cynotis*, which causes ear mange. Ear mites can often be seen during otoscopic examination. The typical dark brown smelly debris can be collected, mounted in lactophenol and examined under the microscope.

ENDOPARASITES OF DOGS AND CATS UNDER THE MICROSCOPE

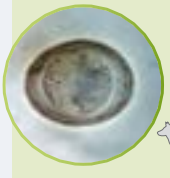
NEMATODES EGGS

ROUNDWORMS (ASCARIDS)



Toxocara canis, T. cati

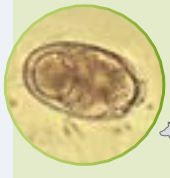
- The most common gastrointestinal parasite in dogs and cats
- **Eggs:** Large • Globular • Thick-shelled
  - Alveolar external layer (thimble-like)
  - Smooth internal layer
- Contents: a single dark-brown cell that almost fills the entire egg



Toxascaris leonina

- **Eggs:** Large • Globular
  - Thick-shelled
  - Smooth outer shell with concentric layers
- Contents: a single light-brown cell that does not fill the entire egg

HOOKWORMS



Ancylostoma caninum,
A. braziliense, A. tubaeforme
Uncinaria stenocephala

- **Eggs:** Strongyle-type • Ovoid, medium-sized
  - Thin, smooth shell
- Contents: a morula with 4 to 8 large blastomeres
- *Ancylostoma* eggs are smaller (55–65 × 40 µm) than *Uncinaria* eggs (65–80 × 45–50 µm), but difficult to distinguish between them in practice

WHIPWORMS

Trichuris vulpis

- **Eggs:** Medium-sized egg • Smooth, thick shell
  - Elongated, lemon shape
  - Protruding polar plug at each pole
  - Orange-brown colour
- Contents: a single cell

FIRST STAGE NEMATODE LARVAE

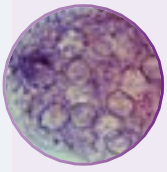
LUNGWORMS

Angiostrongylus vasorum,
Aelurostrongylus abstrusus

- **Larvae:** First stage larvae • Strongyle-like and thin • A button on the head (*Angiostrongylus*) and an undulating pointed tail with a dorsal notch when examined using the Baermann technique.

CESTODES EGGS

DIPYLIDIUM



Dipylidium caninum

- **Eggs:** Grouped in clusters of 20 encased in a thin shell: **the oviferous capsule** • Small eggs • Smooth, thin shell
- Contents: a hexacanth embryo (characteristic of cestode eggs)

TAENIIDAE

Taenia hydatigena, T. taeniaeformis,
T. pisiformis, T. multiceps, T. serialis
Echinococcus granulosus,
E. multilocularis

- **Eggs:** Excreted in a fragile oviferous segment • Small and globular • Unique, thick envelope • Radially striated • Taeniidae eggs are immediately infective • Major zoonotic risk from *Echinococcus* eggs
- Contents: a hexacanth embryo (characteristic of cestode eggs)

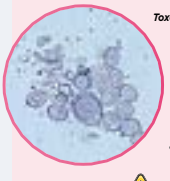
PROTOZOAN CYSTS

GIARDIA

Giardia duodenalis

- **Cysts:** Egg-shaped to oval-shaped • Small • Smooth, thin shell
- Contents: 2 to 4 flagella with visible cellular nuclei and drumstick-like residual bodies, forming a central S-shape (similar to the Ying/Yang symbol)

COCCIDIA



Toxoplasma
Hammondia, Besnoitia, Isospora

- **Oocysts:** Ovoid • Smooth, thin shell with rounded ends (one of the two ends being more pointed in *Isospora* spp.)
- Contents: a single granular, spherical cell before sporulation, then 2 sporocysts containing 4 sporozoites each on sporulation in the external environment

⚠ Zoonosis

Coprological techniques

- Simple and inexpensive methods.
- Sedimentation of parasite elements in a low density solution (water) or, more commonly, concentration of those elements on the surface of a high density solution using flotation methods.
- Poor sensitivity for cestode eggs.

Equipment: Mortar, pestle, measuring cylinder, Pasteur pipettes, mixer, test tubes, sieve, tea strainer, slides, coverslips, microscope with 4×, 10×, 40× and 100× objective lenses (immersion objective), gloves, gauze, plastic pipettes.

Sampling: Fresh faeces (refrigeration at +4 °C is the most suitable if preservation is required).

Procedure for the classic flotation method

1. Reduce faeces sample to a uniform consistency.
2. Dilute 5 g of faeces in 75 mL of dense solution in a measuring cylinder.
3. Sieve the mixture in a tea strainer.
4. Fill a test tube to the brim with the mixture obtained, so that a convex meniscus is formed, then cover the tube with a coverslip.
5. Leave to stand for about 10–15 minutes.
6. Remove the coverslip, which will potentially have parasite elements stuck to it, and place it on a slide. View under the microscope.

Example of high-density solutions

33 % solution of zinc sulphate (D = 1.18).

Saturated solution of NaCl (D = 1.20).

Magnesium sulphate: 35 % saturated solution of magnesium sulphate (D = 1.28).

Cleaning and setting up the microscope

1. Dust eye pieces, condenser and objective lenses with lens tissue.
2. Turn light on with power on low.
3. Increase brightness until white light is visible.
4. Turn condenser up as far as possible.
5. Use low power, preferably ×10, or ×4.
6. Place slide on stage.
7. Rack up until slide is in focus.
8. Close one eye and focus the eye piece, then do the same with the other eye piece.
9. Move the condenser up or down and adjust the diaphragm opening to the appropriate brightness.

Examining for endoparasites

- Move the condenser until definition is optimal.
- Use the lowest objective lens (×4 or ×10) to examine the slide and increase the objective lens magnification to examine something more closely.
- Ensure that your technique allows you to view the whole slide.
- The magnification of the objective lens is multiplied by the magnification of the ocular lens, usually ×10, so that the overall magnification of the parasite element is ×40 to ×100.

ECTOPARASITES OF DOGS AND CATS UNDER THE MICROSCOPE

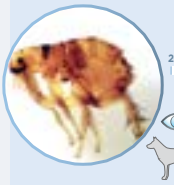
INSECTS

FLEAS

Ctenocephalides felis

- Most common flea on dogs and cats
- Orange to dark brown, wingless
- Laterally compressed body
- Long head, 6 notches bearing setae on dorsal border of hind tibia
- Possible pathogen transmission:
  - *Bartonella henselae* (cat scratch disease) ⚠
  - *Dipylidium caninum* (tapeworm)

Eggs



Ctenocephalides canis

- Seen on dogs, less common than *C. felis*
- Similar to *C. felis* in size and appearance
- Short head, 8 notches bearing setae on dorsal border of hind tibia
- Possible pathogen transmission

Eggs

LICE

Linognathus setosus
Sucking louse

- Small pointed head with terminal mouthparts
- Bluish-black colour

Eggs: 1 mm

Trichodectes canis
Chewing louse

- Head is wider than it is long
- Wide yellowish body
- Antennae in 3 segments

Eggs: 1 mm

Felicola subrostratus
Chewing louse

- Wide body
- Triangular head, pointed anterior end

Eggs: 1 mm

ACARIDS

MITES

Sarcoptes scabiei
Dog sarcoptic mange mite

- Seen in deep skin scrapings, difficult to find
- Best results from non-excoriated sites e.g., edges of the ears, elbows, hocks
- White oval body
- 4 pairs of short legs: anterior legs have suckers, posterior legs do not extend beyond body
- Many transverse ridges and triangular scales on dorsum

Notoedres cati
Cat mange mite

- Smaller than *S. scabiei*
- Many concentric striations on dorsum

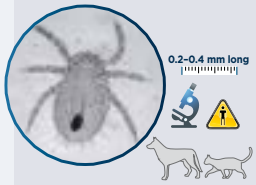
Eggs

C. yasguri *C. blakei*

***Cheyletiella* spp.**
Fur mite, Walking dandruff

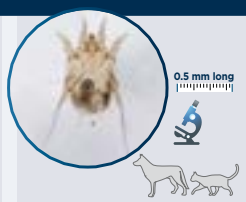
- Saddle-shaped with a "waist"
- 4 pairs of legs
- Found by direct examination of coat brushings with magnifying glass
- Confirmed microscopically by superficial skin scrapings or adhesive band test

Eggs



Neotrombicula autumnalis
Harvest mite, Chigger

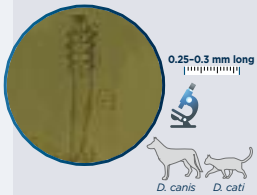
- 3 pairs of legs (larva is the only parasitic stage)
- Bright red, pin-head dots, typically found between paws, on ears, or on eyelids
- Seasonal; seen in autumn
- Confirmed microscopically by superficial skin scrapings



Otodectes cynotis
Ear mite

- Visible *in situ* with otoscope (poor sensitivity)
- Adults and eggs seen microscopically in ear cerumen
- Large white oval body
- 4 pairs of projecting legs (4th pair reduced in females)
- Whip-like setae attach terminally to 3rd and 4th pairs of legs
- Adult mites occasionally seen in skin scrapings from other body areas

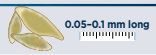
Eggs



D. canis *D. cati*

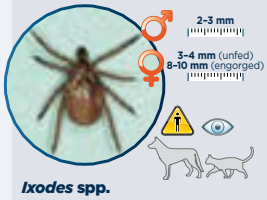
***Demodex* spp.**

- Seen in deep skin scrapings from areas of comedomes
- Squeeze skin to extrude mites from follicles
- Take hair plucks from affected areas
- Long cigar-shaped body
- 4 pairs of atrophied legs



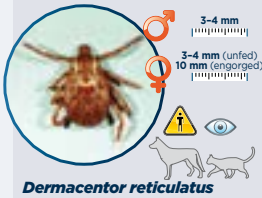
Eggs

TICKS



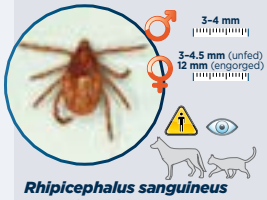
***Ixodes* spp.**
I. ricinus Forest tick
I. hexagonus Hedgehog tick

- 4 pairs of legs, long mouthparts
- Engorged female: light grey, bean-shaped
- Male: legs visible due to small abdomen
- Nymph: 4 pairs of legs, < 2.0 mm long
- Larva: 3 pairs of legs, yellowish, < 1.0 mm long
- Most common species found in Northern, Western and Continental Europe. Found in forest areas and shaded parks.
- Possible disease transmission:
  - Anaplasmosis
  - Lyme disease ⚠
  - Rickettsiosis ⚠
  - Tick-borne encephalitis ⚠



Dermacentor reticulatus
Ornated dog tick

- 4 pairs of legs
- Rectangular base of capitulum (mouthparts including their base)
- Ornate scutum (chitinous plate on dorsum)
- Eyes, festoons (notches on posterior border of body)
- IV coxae (proximal leg section) larger than I to III
- Commonly found in Western Europe
- Prefers parks, gardens, rural areas, river banks
- Possible disease transmission:
  - Babesiosis
  - Rickettsiosis ⚠



Rhipicephalus sanguineus
Brown dog tick

- 4 pairs of legs
- Hexagonal base of capitulum
- Inornate scutum
- Eyes, festoons
- Common in Mediterrnean countries and Southern Continental Europe
- Endophile tick living in kennels, villages, gardens; likes warm, humid or arid climates
- Possible disease transmission:
  - Babesiosis
  - Ehrlichiosis
  - Rickettsiosis ⚠

Visible with naked eye ⚠ Zoonosis Visible under the microscope

Cleaning and setting up the microscope

1. Dust eye pieces, condenser and objective lenses with lens tissue.
2. Turn light on with power on low.
3. Increase brightness until white light is visible.
4. Turn condenser up as far as possible.
5. Use low power, preferably ×10, or ×4.
6. Place slide on stage.
7. Rack up until slide is in focus.
8. Close one eye and focus the eye piece, then do the same with the other eye piece.
9. Move the condenser up or down and adjust the diaphragm opening to the appropriate brightness.

Examining for ectoparasites

- Move the condenser until definition is optimal.
- Use the lowest objective lens (×4 or ×10) to examine the slide and increase the objective lens magnification to examine something more closely.
- Ensure that your technique allows you to view the whole slide.
- The magnification of the objective lens is multiplied by the magnification of the ocular lens, usually < ×10, so that the overall magnification of the parasite element is ×40 to ×100.

TREATMENT AND PROPHYLAXIS

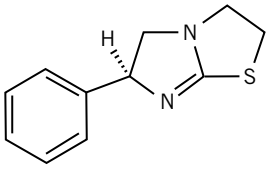
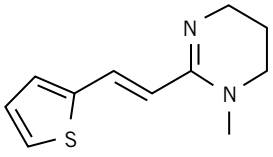
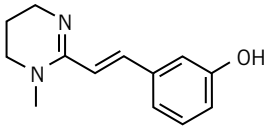
Anthelmintics

Anthelmintics suitable for dogs and cats belong to various chemical groups and they have traditionally been administered orally, although spot-on formulations have recently been introduced (especially for use in cats). Consideration must also to be given to the development of parasiticidal drugs with both internal and external antiparasitic effects which meet the demand for prevention of both endo- and ectoparasitoses in carnivores.

Dog and cat anthelmintics have no persistent effect, which means that a single administration will kill the parasites present in the host at the time of treatment, but will not protect the host against reinfestation.

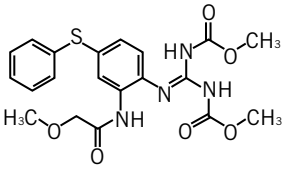
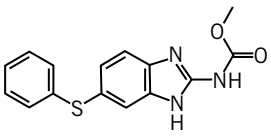
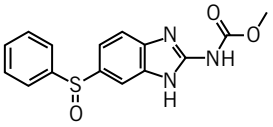
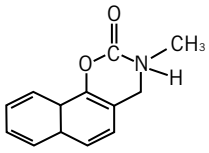
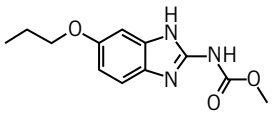
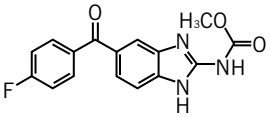
Anthelmintic groups and molecules

Table 1. Anthelmintics available for dogs and cats.

Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against helminths	Prevention of cardiopulmonary dirofilariosis
Imidazothiazoles	Levamisole		Cholinergic agonist that selectively causes depolarisation and spastic paralysis of nematode muscle cells	Roundworms, hookworms, and nematodes of the respiratory and urinary systems	No
Pyrimidines	Pyrantel		Cholinergic agonist that selectively causes depolarisation and spastic paralysis of nematode muscle cells	Roundworms, hookworms	No
	Oxantel			Whipworms	No

▶

Table 1. Anthelmintics available for dogs and cats (continuation).

Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against helminths	Prevention of cardiopulmonary dirofilariosis
Benzimidazoles and probenzimidazoles	Febantel (pro-fenbendazole)		Inhibit tubulin polymerisation by binding to the α-tubulin in nematodes and some cestodes	Roundworms, hookworms, whipworms (single administration)	No
	Fenbendazole			Roundworms, hookworms, whipworms, *Taenia* spp. (repeated administration required)	No
	Oxfendazole			Roundworms, hookworms, whipworms, *Taenia* spp., *Dipylidium caninum* (repeated administration required)	No
	Mebendazole			Roundworms, hookworms, whipworms, *Taenia* spp. (repeated administration required)	No
	Oxibendazole			Roundworms, hookworms, whipworms, *Taenia* spp. (repeated administration required)	No
	Flubendazole			Roundworms, hookworms, whipworms, *Taenia* spp. (repeated administration required)	No

▶

Table 1. Anthelmintics available for dogs and cats (continuation).

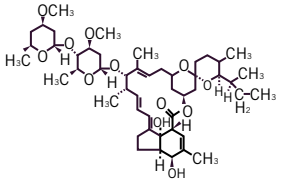
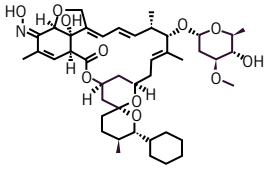
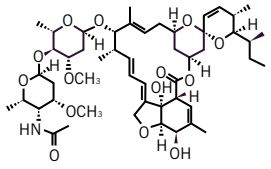
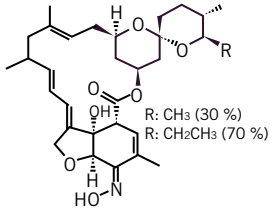
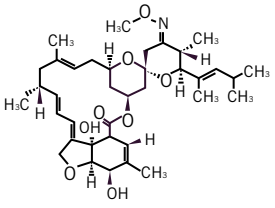
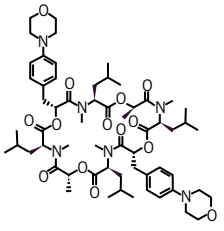
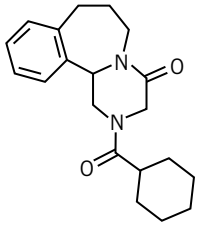
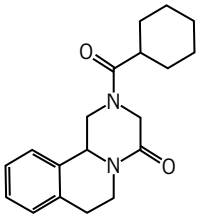
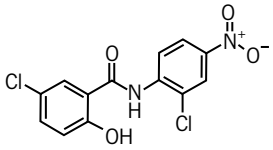
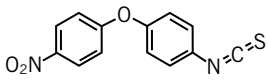
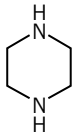
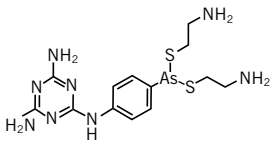
Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against helminths	Prevention of cardiopulmonary dirofilariosis
Macrocyclic lactones (avermectins and milbemycins)	Ivermectin	 22,23-dihydroavermectin B1a	Open chloride channels in the neuronal synapses by binding to glutamate receptors. Cause hyperpolarisation and block muscle cell stimulation	No licensed product for gastro-intestinal nematodes. Active against most nematodes at a subcutaneous dose of 200 µg/kg	Yes (dose of 6 µg/kg)
	Selamectin			Roundworms (*Toxocara canis, T. cati*), hookworms (*Ancylostoma caninum, A. tubaeforme*)	Yes
	Eprinomectin			Roundworms (*Toxocara cati, Toxascaris leonina*), hookworms (*Ancylostoma tubaeforme, A. braziliense, A. ceylanicum*), *Capillaria plica*	Yes
	Milbemycin oxime	 R: CH_3 (30 %) R: CH_2CH_3 (70 %)		Roundworms, hookworms (*Ancylostoma*), whipworms, *Thelazia callipaeda, Spirocerca lupi*	Yes
	Moxidectin			Roundworms, hookworms, whipworms, *Angiostrongylus vasorum, Spirocerca lupi, Crenosoma vulpis, Thelazia callipaeda*	Yes
Octadepsipeptides	Emodepside		Bind to a group of G protein-coupled receptors called latrophilins in neuromuscular junctions in nematode muscle cells	Roundworms, hookworms (including migrating larvae) and whipworms	No

Table 1. Anthelmintics available for dogs and cats (continuation).

Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against helminths	Prevention of cardiopulmonary dirofilariosis
Isoquinolines	Epsiprantel		Causes muscular contraction in cestodes and some trematodes	*Taenia* spp., *Echinococcus* spp., *Dipylidium caninum*	No
	Praziquantel			*Taenia* spp., *Echinococcus* spp., *Dipylidium caninum*	No
Other chemical groups	Niclosamide		Inhibits oxidative phosphorylation and blocks glucose absorption by cestodes	*Taenia* spp. (at 80 to 150 mg/kg)	No
	Nitroscanate		Inhibits the respiratory chain (ATP synthesis) in parasite cells	Roundworms, hookworms, *Taenia* spp., *Dipylidium caninum*	No
	Piperazine		Blocks the acetylcholine and GABA activity at the neuromuscular junction. Ascarids are then eliminated by intestinal peristalsis	Roundworms	No
	Melarsomine		Effects on heartworms have not been completely defined but include alterations in glucose uptake and metabolism, glutathione reductase inhibition, and alterations in the structure and function of the parasite's intestinal epithelium	*Dirofilaria immitis* (adults)	No

Molecules active against nematodes

Piperazine salts

Piperazine salts (adipate, hydrate and citrate) have long been used for the treatment of roundworms (*Toxocara* and *Toxascaris*) but they have a narrow spectrum of activity and are less effective than more modern products. They act by blocking acetylcholine, which affects neurotransmission and leads to flaccid paralysis of the roundworm. The standard dose is 200 mg/kg/day for 3 days. Piperazine salts can also irritate the gastrointestinal tract and cause vomiting or diarrhoea, which is another drawback in addition to their narrow spectrum. Their use, however, can still be indicated in kittens and puppies with heavy infestations as piperazine salts induce roundworm paralysis (not lysis), so the risk of an anaphylactoid reaction is reduced.

Levamisole and pyrantel

Levamisole (imidazothiazole) and **pyrantel** (tetrahydropyrimidine) are nematicidal products whose activity arises from their cholinomimetic effect and which cause spastic paralysis of the nematodes

Pyrantel is not absorbed by the gastrointestinal tract mucosa so its spectrum of activity is limited to nematode species or stages which are present in the lumen of the stomach or intestines. The basic dose of pyrantel is 5 mg/kg of the native molecule (or 14.5 mg/kg pyrantel pamoate) for dogs, and 20 mg/kg (or 58 mg/kg pyrantel pamoate) for cats. Levamisole, which is absorbed by the gastrointestinal tract mucosa and diffuses into the tissues well, is active against many nematodes of the intestinal, respiratory, circulatory and urinary systems. The basic dose of levamisole is 7.5 mg/kg for dogs and cats.

Both levamisole and pyrantel are totally effective against roundworms (*Toxocara* and *Toxascaris*) and hookworms (*Ancylostoma* and *Uncinaria*), but not against whipworms (*Trichuris*). Pyrantel has a wider spectrum for whipworms when it is associated with febantel (probenzimidazole). Levamisole is effective against spiruroids, but pyrantel is not. **Oxantel** is a tetrahydropyrimidine that is used in combination with pyrantel (and praziquantel) in some countries. It is active against whipworms.

Benzimidazoles and probenzimidazoles

Benzimidazoles and probenzimidazoles are anthelmintics that are widely used in veterinary medicine, particularly because of their very low toxicity (therapeutic index between 20 and 40, compared to a therapeutic index of 4 for levamisole). They inhibit tubulin polymerisation by binding to the β-tubulin in nematodes, and to some cestodes. They kill helminths by disrupting the cytoskeletons of various cells, mainly in the gastrointestinal epithelium in nematodes. This effect on the cellular organisation of the helminth is progressive and the worms die slowly. This is why the contact time between the parasite and the benzimidazole is an important factor, and explains why treatments sometimes continue for several days (an average of 3 to 5). Prolonged treatment with benzimidazoles is effective against migrating larvae and whipworms in dogs**.** The following molecules are frequently used in domestic carnivores: **mebendazole**, **fenbendazole**, **oxfendazole**, **oxibendazole**, **flubendazole** and **febantel** (profenbendazole).

All of these molecules are very effective against adult roundworms and hookworms in the intestines. Repeated administration is required to be effective against whipworms: mebendazole (25 mg/kg/day for 5 days), febantel (30 mg/kg/day for 3 days), oxfendazole (11.3 mg/kg/day for 3 days), fenbendazole (50 mg/kg/day for 3 days, although a single dose seems to be enough), and flubendazole (22 mg/kg/day for 3 days).

Synergy can occur when benzimidazoles are combined with other anthelmintics. For example, a single dose of a febantel-pyrantel combination is highly effective against whipworms at doses lower than those products which contain the same active ingredients but which are not combined with another, e.g., 15 mg/kg febantel (instead of 30 mg/kg) plus 5 mg/kg pyrantel as pyrantel embonate (instead of 14.5 mg/kg, as pyrantel pamoate).

The efficacy of benzimidazoles against roundworm (*Toxocara canis* and *T. cati*) larvae requires good tissue diffusion, which may vary according to the molecule, and doses higher than those normally prescribed. Fenbendazole and oxfendazole (the sulfoxide metabolite of fenbendazole) are the two most larvicidal molecules, although they are still not totally effective. Several larvicide protocols using fenbendazole, oxfendazole and mebendazole

have been published and the protocol usually recommended for breeding bitches is the daily administration of 50 mg/kg fenbendazole during the last third of gestation and the first 15 days of lactation. Infestation of the puppies *in utero* and through the milk is therefore avoided. Such measures are only possible under certain conditions in special breeding facilities.

In addition to their anthelmintic activity, some benzimidazoles are effective against some flagellate protozoa, especially *Giardia duodenalis*. Fenbendazole and oxfendazole, administered at the anthelmintic dose for 3 days, can therefore be used to treat giardiosis in dogs and cats.

Nitroscanate

Nitroscanate is a phenyl isothiocyanate that inhibits the respiratory chain of parasite cells (inhibition of ATP synthesis). Administered at 50 mg/kg, nitroscanate is very effective against roundworms and hookworms but not totally effective against whipworms. It is tolerated well by dogs, in spite of possible vomiting due to gastric irritation which can be avoided by administering the drug in the morning with one third of the dog's daily ration. Nitroscanate is not tolerated well by cats, so it is not licenced for use in cats.

Macrocyclic lactones

Macrocyclic lactones of the avermectin/milbemycin group are effective against most nematodes found in carnivores. These molecules are considered endectocides as they are also active against external arthropod parasites, such as lice, Diptera, mange mites, *Demodex* spp., and some fleas. Macrocyclic lactones act by opening chloride channels in the neuronal synapses by binding to glutamate receptors. They cause hyperpolarisation and block the muscle cell stimulation, leading to flaccid paralysis and death of the nematodes and arthropods. Macrocyclic lactones are not effective against flat worms (cestodes or trematodes).

The larvicidal activity of macrocyclic lactones is no greater than that of benzimidazoles for migrating roundworm and hookworm larvae in dogs and cats, and they are generally less effective against whipworms.

Ivermectin for prevention of cardiopulmonary canine dirofilariosis (in a single dose of 6 µg/kg every month), treatment of intestinal nematodes in dogs and topical treatment of otoacariosis in cats. Various scientific publications have reported the activity of ivermectin, at a subcutaneous dose of 200 µg/kg, against most nematode parasites: roundworms, hookworms (*Ancylostoma* and *Uncinaria*, whipworms, spiruroids, *Oslerus osleri*, and *Angiostrongylus vasorum*). Signs of intoxication have been observed in some dogs, especially Collies and similar breeds (Australian Shepherds, Old English Sheepdogs, Shetland Sheepdogs, etc.) at doses as low as 50 µg/kg. These dogs have a mutation in the MDR1 gene that affects the blood-brain barrier and makes it more permeable to ivermectin.

Milbemycin oxime licenced for the prevention of cardiopulmonary canine dirofilariosis (in a single dose of 500 µg/kg every month) and for the treatment of intestinal nematodes in dogs and cats (in combination with praziquantel). Milbemycin oxime is effective against roundworms, hookworms (*Ancylostoma* spp.) and whipworms.

Selamectin is an avermectin administered as a spot-on application at 6 mg/kg bodyweight. The product is absorbed through the skin into the bloodstream and it has both a residual action on some external parasites (fleas, *Sarcoptes* spp., *Otodectes*) and an anthelmintic effect. It is effective against: *T. canis*, *T. cati*, *Ancylostoma caninum* and *A. tubaeforme*, but it is not effective against *Uncinaria stenocephala, Toxascaris leonina* or *Trichuris vulpis*.

Moxidectin is now used as a spot-on, like selamectin, for dogs, cats and ferrets. The dosage is 1 mg/kg for cats and ferrets, and 2.5 mg/kg for dogs. Moxidectin is effective against gastrointestinal nematodes: *T. canis*, *T. cati*, *T. leonina, A. caninum, A. tubaeforme, U. stenocephala* and *T. vulpis*. The molecule is also licenced for the treatment and prevention of canine angiostrongylosis, the prevention of cardiopulmonary dirofilariosis and spirocercosis and the treatment of *Crenosoma vulpis* or *Dirofilaria repens* infestation.

Eprinomectin may be used as a spot-on in cats (combined with fipronil, s-methoprene and praziquantel) and is effective against the following gastrointestinal nematodes: *T. cati*, *T. leonina, A. tubaeforme*, *A. braziliense* and *A. ceylanicum*. Eprinomectin may also be used to prevent cardiopulmonary dirofilariosis and treat *Capillaria plica* infestation.

Emodepside

Emodepside is an octadepsipeptide that works by binding to a group of G protein-coupled receptors called latrophilins in the neuromuscular junctions in nematode muscle cells. There is also some evidence that it interferes with the potassium channels in neuronal membranes. Administered at 1 mg/kg orally in dogs or as a spot-on in cats, emodepside is effective against roundworms (both adults and migrating larvae), hookworms and whipworms. Emodepside may cause neurological toxicity in dogs that are homozygous for the MDR1 mutation (mutant/mutant).

Molecules active against cestodes

Arecoline hydrobromide

Arecoline hydrobromide is a cestodicide with parasympathomimetic activity which provokes intestinal hypermotility and causes the cestode scolex to detach. Doses of 2 mg/kg are prescribed for *Taenia* spp. and *Dipylidium caninum*, but 4 mg/kg is required to eliminate *Echinococcus* tapeworms. Arecoline hydrobromide is tolerated rather poorly by dogs, causing salivation, diarrhoea and stomach pain, and its efficacy is inconsistent. It is also contraindicated in gestating dogs and in cats and is no longer marketed in Europe, although it was used for many years to screen for *Echinococcus granulosus* in dogs.

Benzimidazoles and probenzimidazoles

Repeated administration of benzimidazoles and probenzimidazoles is effective against *Taenia* spp. cestodes. No activity has been reported against *Echinococcus* spp. tapeworms at normal doses, and only oxfendazole (11.3 mg/kg for 3 days) is effective against *D. caninum*.

Niclosamide

Niclosamide is a specific cestodicide that inhibits oxidative phosphorylation and blocks glucose absorption by the cestode. It is not absorbed in the gastrointestinal tract and is tolerated well. It is active at 125 mg/kg for *Taenia* spp. cestodes but is often insufficiently active against *D. caninum*. A dose of 150–200 mg/kg is required for complete efficacy against the latter. This does not always correspond to the dosage recommended for commercial veterinary products. Higher doses, of around 500 mg/kg, are active against *Echinococcus* spp.

This active ingredient is rarely used alone but is usually combined with a nematicide (oxibendazole or pyrantel) to create a broad-spectrum product.

Nitroscanate

Nitroscanate is effective against *D. caninum* but it is not active against *Echinococcus* spp. cestodes at 50 mg/kg. However, three doses of 250 mg/kg in 24 hours are effective.

Praziquantel

Praziquantel is a specific cestodicide whose mode of action is not well understood, but it causes muscular contraction of cestodes and some trematodes (notably *Clonorchis* spp. and *Opisthorchis* flukes), causing them to detach and die. Praziquantel is thought to act by opening calcium channels in various types of cells, especially muscle fibres. It is not effective against nematodes and has only a narrow spectrum for platyhelminthes. It is active after oral administration at 5 mg/kg against all Cyclophyllidea cestodes, especially *D. caninum*, *Taenia* spp. and *Echinococcus* spp. It also has some ovicidal activity. Praziquantel is the only cestodicide effective against *Diphyllobothrium latum* and *Spirometra* spp. at 40 mg/kg. It can be administered orally (combined with nematicide molecules), injected, or even in a spot-on formulation, combined with nematicide molecules or ectoparasiticides.

30–40 mg/kg praziquantel is effective against most trematodes found in domestic carnivores. This is 6 to 8 times greater than the dose usually recommended for cestode control (5 mg/kg). It is also used in human medicine to treat schistosomosis (bilharziosis) and Southeast Asian and Chinese liver fluke diseases (opisthorchiosis and clonorchiosis respetively).

Epsiprantel is closely related to praziquantel. It is used at 5 mg/kg to treat tapeworms in dogs and cats.

Selection criteria

Various criteria must be taken into consideration when a deworming programme is proposed, especially in dog and cat breeding facilities.

Age

Puppies can be infested by *T. canis* before they are born or as soon as they begin to suckle **so** adult roundworms can appear when puppies are 10 days old. *T. cati* infestation does not occur in kittens *in utero*, but begins when the kittens start to suckle. The first intestinal roundworms appear a little later, when the kittens are about 3 weeks old.

Puppies should be treated with appropriate anthelmintics when they are 2 weeks of age, continuing at fortnightly intervals until 2 weeks after weaning, and then monthly until 6 months of age. As prenatal infestation does not occur in kittens, fortnightly treatment can begin at 3 weeks of age and be repeated fortnightly until 2 weeks after weaning, then monthly for 6 months. An anthelmintic which is active against migrating larvae is recommended until puppies and kittens are weaned. Thereafter, any anthelmintics are suitable, including those that do not cross the intestinal barrier, such as pyrantel. The choice will also depend on what other parasites are present if coproscopy indicates that the animal is infested with whipworms, tapeworms (*Dipylidium*) or *Giardia*, for example.

There is less risk of reinfestation when weaned puppies and kittens go to live with their new owners. Systematic deworming is recommended when animals are purchased and 1 month later. This can easily be done when the young animals are presented in consultation for their vaccinations.

Toxocara spp. infestation can occur in older dogs and cats, and is extremely unlikely to be associated with clinical signs; therefore it is difficult to detect whether a dog is infested unless regular faecal examinations are conducted. These parasites are prolific egg-layers and just a few worms can produce a large number of eggs. Continued, regular treatment of dogs and cats using a suitable anthelmintic is therefore recommended by ESCCAP (European Scientific Counsel Companion Animal Parasites: www.esccap.org) if regular diagnostic testing is not undertaken. Either a broad- or narrow-spectrum anthelmintic can be chosen, according to the risk of multiple worm infestations. The prepatent period for *Toxocara* spp., after ingestion of larvae by predation of paratenic hosts (rodents) or infective eggs from the environment, is a little over 4 weeks so monthly treatment minimises the risk of patent infestations and is recommended in high-risk scenarios, such as in pets living in a family with small children and sharing the garden (or similar situations). Annual or twice yearly treatments do not prevent patent infestation within a population to any significant extent, so a treatment frequency of at least 4 times per year is the general recommendation.

Fleas, the intermediate hosts of *D. caninum*, are abundant on some dogs and cats, so it may be advantageous to choose a product which is active against this cestode.

Breeding bitches can transmit *T. canis* to their puppies *in utero* or during lactation so they should be dewormed 1 to 2 weeks before the end of gestation, immediately after birth of the puppies, and then every 2 weeks until the puppies are weaned. An anthelmintic with a larvicidal effect against roundworms should be used (benzimidazoles, emodepside or avermectin/milbemycin). Special protocols might be required in breeding facilities.

Breeding cats (queens) should be dewormed when their kittens are born, then every 15 days until the kittens are weaned, with an anthelmintic which is active against larvae. Queens can be dewormed 1 month after each oestrus since encysted roundworm larvae can be reactivated at this time.

Environment and lifestyle

Urban and rural cats and dogs may host different parasites, and animals living in urban areas, with no access to gardens, peri-urban parks or woodland, may host less various parasites. These urban animals should be dewormed following faecal examination and treated with products which are active against these parasites (and fleas).

In rural areas, domestic carnivores spend more time outdoors, especially hunting dogs and farm cats, and many parasites can be found in these animals so polyparasitism is common. For example, dogs might be infested with *Ancylostoma* (*Uncinaria*)/*Trichuris* and/or *Taenia* species (if they have access to ruminant or rabbit viscera). Cats are often infested by *Taenia taeniaeformis* or *Mesocestoides* spp. tapeworms by eating rodents (rats and mice).

E. granulosus infestation in dogs is mainly associated with lifestyle: sheepdogs and dogs who have access to viscera from slaughterhouses are at the greatest risk. Ensuring that shepherds are aware of the parasite cycle, and systematic deworming of sheepdogs (with praziquantel), is recommended, especially when moving between summer and winter pastures.

In areas where *E. multilocularis* is enzootic, and foxes are the main definitive hosts and voles the intermediate hosts, dogs that might eat rodents should be treated at four-weekly intervals with an effective anthelmintic containing praziquantel. Cats, however, are epidemiologically insignificant sources of eggs as they are poor hosts for this worm, although they do occasionally become infested and pass eggs. Although it is common to find eggs in the fur of infested dogs, no eggs have been recovered to date from the coat of an infested cat. Due to this small risk of cats being infested, it is reasonable to recommend treatment in high-risk situations, such as prior to entry into countries where the parasite is not present.

Spectrum of activity

Clinical suspicion and faecal examination should be used more when deciding whether to use anthelmintics to treat carnivores. Faecal examination is inexpensive and easy to perform at the veterinary practice, and can prevent healthy animals being treated, and enable treatment to be adapted to each situation.

A good example of this is *D. caninum*. This cestode is not a major pathogen and infestation is tolerated well by the animal. The segments are numerous and clearly visible. They are 4–6 mm long and either mobile and whitish, or dry and white. They can be seen in the perianal region or on the animal's hind legs, or in its immediate surroundings. Diagnosis is simple and treatment can be restricted to animals known to be infested.

Finally, one of the most prevalent parasites is the flagellate *G. duodenalis*. The presence of cysts should be actively investigated and, when they are identified, this parasite should be included in any treatment for internal parasites.

Ease of administration

Pet owners demand products that are not only effective, but easy to use. Considerable progress has been made in the development of veterinary products to control both external and internal parasite infestations.

Injectable forms are useful in breeding facilities and oral pastes are easy to administer, especially to young or small animals. Tablets are also easy to administer to medium-sized and large dogs, but small dogs and cats, which may be less obliging, are generally resistant to this form of product and pill size is a determining factor. Chewable worming tablets are now available and these formulations are easily administered to dogs and cats.

Water-dispersible liquids, and tablets that can be mixed with feed, make it easier to administer a drug but they are only effective if they do not reduce the palatability of the food and are correctly and completely consumed. They are not suitable for group treatment because it is difficult to know how much each animal has received. Transdermal drug delivery systems, such as systemic spot-on formulations, have the advantage of being easy for pet owners to administer.

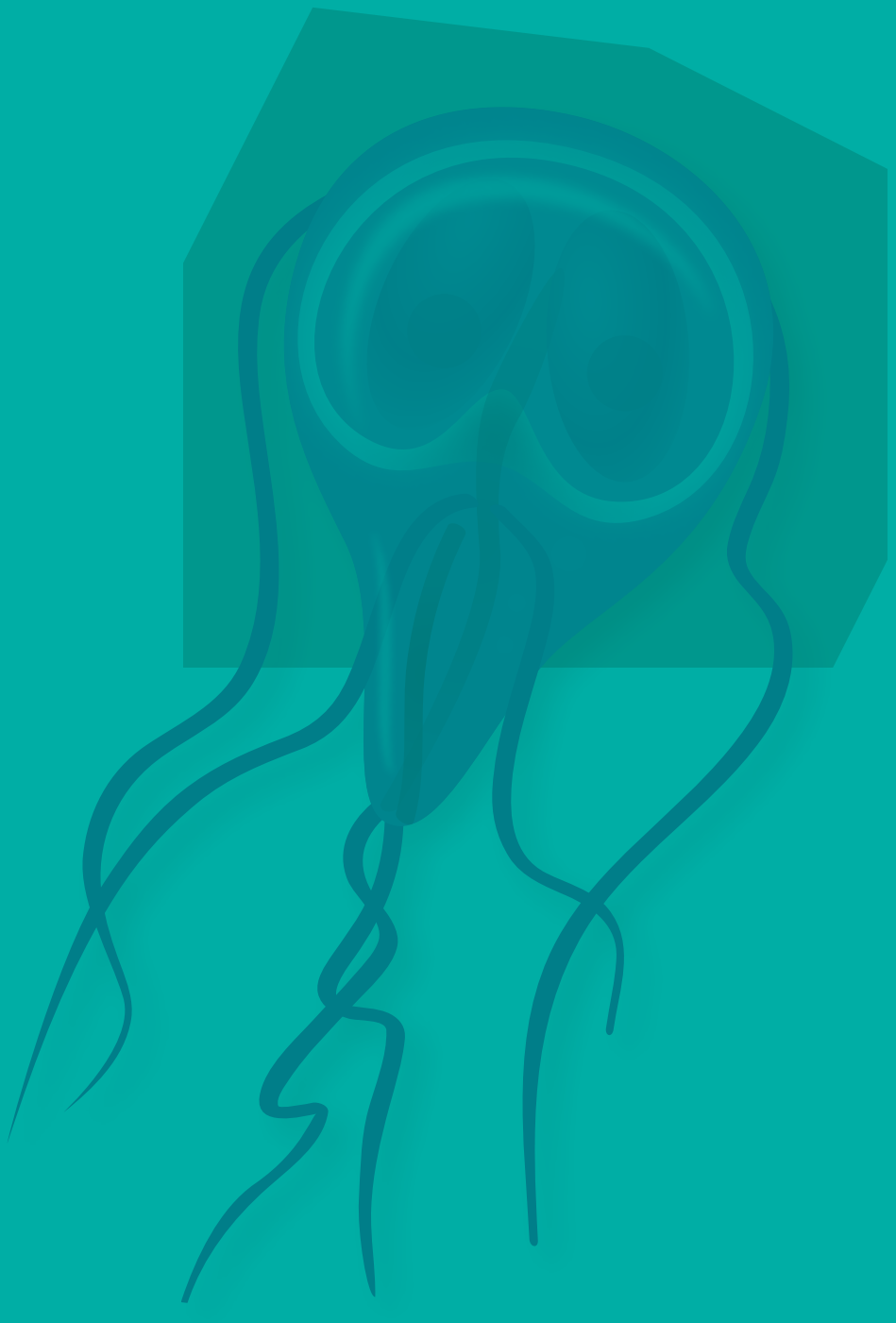
Regardless of the drug delivery system, most pet owners prefer one-step administration protocols to repeated treatments. The choice depends on the commercial products available and the internal parasites targeted. Certain diseases, such as giardiosis, still require repeated administrations.

Conclusion

Deworming dogs and cats is now very common and it is often done systematically, without prior diagnosis. In this context, the anthelmintic is selected based on epidemiological factors, such as the age and the lifestyle of the animal. Product choice also depends on the size of the animal and owner compliance with the treatment regime.

Systematic deworming has the advantage of limiting new infestations but it is still possible for animals to be infested outside the classic deworming periods, and treatment can fail due to the presence of parasites (e.g., protozoa, cestodes or less common nematodes) that are not covered by the chosen anthelmintic.

Breeding facilities must remember that regular worming is necessary, but not the only precaution required, as effective cleaning and disinfection are essential.



Antiprotozoals

Antiprotozoals belong to a wide range of chemical groups and classification of these compounds is complex. Each of the groups usually displays a relatively narrow spectrum of activity. Most antiprotozoals are administered orally and are used to control gastrointestinal parasites (coccidia or intestinal flagellates like *Giardia duodenalis*). Others, particularly those targeting haemoprotozoan or *Leishmania* infections, are given parenterally. The number of products licenced to treat protozooses in dogs and cats is limited and vets usually have to use antiprotozoals off-label. Antiprotozoal dosage regimes are also often based on published clinical trials (rather than on full evaluation). Antiprotozoal use in dogs and cats is usually extrapolated from their use in humans or farm animals. Treatment of many protozooses reduces or eliminates clinical signs but does not result in a parasitological cure. Relapses may occur as a result, and clinical and experimental follow-up of these animals is highly recommended.

Antiprotozoals active against intestinal coccidia

Sulfonamides

Sulfonamides have long been used for the treatment of coccidioses in animals, including domestic carnivores. These synthetic drugs are competitive inhibitors of the enzyme dihydropterate synthetase (DHPS) involved in folate synthesis. They inhibit the conversion of dihydrofolic acid into tetrahydrofolic acid, an important cofactor in amino acid synthesis. Sulfonamides are active against first- and second-stage coccidian meronts as they are coccidiostatic at low doses and coccidiocidal at higher doses. Daily sulfonamide administration for 5–7 days controls diarrhoea effectively, but does not prevent oocyst excretion. Synergy between sulfonamides and specific diaminopyrimidines (such as pyrimethamine, ormethoprim and trimethoprim) makes these drugs much more effective than sulfonamides alone. The following molecules, or combination of molecules, have been used in dogs and cats with coccidiosis: sulfadimethoxine (at 55 mg/kg for the first day and 27.5 mg/kg per day for the following 4 days), sulfaguanidine (150 to 200 mg/kg per day for 5 days), sulfadimethoxine and ormethoprim (55 mg/kg sulfadimethoxine plus 11 mg/kg ormethoprim per day for up to 23 days), sulfadiazine and trimethoprim, and sulfamethoxazole and trimethoprim (30 mg/kg once or twice daily for 14 to 21 days).

Triazones

Triazones inhibit nuclear division in parasites and are active against all intracellular stages of coccidia. **Toltrazuril** is a symmetrical triazone licenced for use in birds, cattle, sheep, pigs and dogs (when combined with emodepside). **Diclazuril** is an asymmetrical triazone with a broad spectrum of activity against coccidia in birds and mammals (including rabbits) at low concentrations (0.5–2 ppm in feed). Toltrazuril and diclazuril are currently the drugs of choice against feline cystoisosporosis but are not actually licensed for use in this species. In dogs, the combination of toltrazuril/emodepside (9 mg/0.45 mg/kg) is licenced for coccidian and ascarid coinfections. Oral formulations designed for mammals are suitable for off-label use of toltrazuril or diclazuril in dogs and cats, but the solution administered to poultry in their drinking water is not. A single application of toltrazuril (9–20 mg/kg) or diclazuril (2.5–5.0 mg/kg) significantly reduces oocyst shedding in excreting animals.

Amprolium

Amprolium is a competitive thiamine antagonist which acts on first generation meronts. It has been used as poultry feed additive to prevent coccidiosis and it can also be used to treat intestinal coccidiosis in birds, cattle, sheep, rabbits and domestic carnivores. It usually has a wide safety margin in dogs but cases of neurological disease have been reported. The following doses are recommended by CAPC (Companion Animal Parasite Council: www.capcvet.org): 110–200 mg/kg daily for 7–12 days in dogs; 60–100 mg/kg daily for 7 days in cats.

Paromomycin

Paromomycin is an aminoglycosid antibiotic which is not absorbed in the gastrointestinal tract. Paromomycin at 150 mg/kg once a day for 5 days is recommended off-label to treat cryptosporidiosis in dogs and cats. Activity has also been reported against *G. duodenalis*, *Balantidium* spp. and *Leishmania infantum*.

Nitazoxanide

Nitazoxanide is a synthetic nitrothiazolyl-salicylamide derivative which, was only approved for use in human cryptosporidiosis until recently, but a regime has been approved to treat adult animals (100 mg per animal every 12 hours for 5 days in animals 24 to 47 months old, and 200 mg per animal every 12 hours for 5 days in animals 4 to 11 years old).

Antiprotozoals active against *Toxoplasma gondii* and *Neospora caninum*

Clindamycin

Clindamycin is a macrolide antibiotic that acts by inhibiting protein synthesis. It has been used to treat systemic toxoplasmosis in dogs and cats (at 12.5 mg/kg every 12 hours for 4 weeks). Clindamycin also has some activity against *Entamoeba histolytica*, and is effective against *N. caninum* tachyzoites in cell cultures. The combination of clindamycin and sulfonamide is highly effective against neosporosis. The multidrug combination of pirithrexim, clindamycin, diclazuril, robenidine and pyrimethamine is also experimentally active against neosporosis.

Spiramycin

Spiramycin is active against *T. gondii* and it can be used to treat acute prenatal toxoplasmosis. **Clarithromycin** is used in combination with a sulfonamide with good activity in human toxoplasmosis.

Ponazuril

Ponazuril is an antiprotozoal and an active metabolite of toltrazuril. It is approved to treat equine protozoal myeloencephalitis caused by *Sarcocystis neurona*, but it is also experimentally effective against *N. caninum* and *T. gondii*.

Sulfamethoxazole

Sulfamethoxazole (combined with trimethoprim) can be used to prevent toxoplasmosis and pneumocystosis in immunocompromised human patients.

Antiprotozoals active against intestinal flagellates

Nitroimidazoles

Nitroimidazoles include dimetridazole, ornidazole, metronidazole, ronidazole and tinidazole. These drugs interfere with RNA synthesis and are very active against flagellates of the trichomonad group. **Metronidazole** has long been used to treat giardiosis in dogs and cats (at 25 mg/kg every 12 hours for 5 days). According to CAPC, the combination of metronidazole and fenbendazole may result in better resolution of clinical disease and cyst shedding. Gastrointestinal and neurological toxicity after long-term therapy or acute high doses of metronidazole has been reported in some puppies and kittens. **Ronidazole** at 30–50 mg/kg/day for 14 days is currently considered to be the treatment of choice for feline trichomonosis. Modified-release formulations that deliver ronidazole to the site of action in the large intestine have improved efficacy. A reversible neurotoxicity similar to that seen with metronidazole has been reported in some cats.

Benzimidazoles and probenzimidazoles

Benzimidazoles and probenzimidazoles are basically anthelmintic drugs, but some of these molecules (such as **albendazole**, **fenbendazole**, **mebendazole**, and **febantel**) are also active against *G. duodenalis*. The mechanism of action against *Giardia* is thought to be directed against the ventral disc microtubules. Prolonged treatment (for at least 5 days) is required. Fenbendazole (50 mg/kg/day for 5 days) is often used in dogs and cats. Another option is febantel in combination with pyrantel/praziquantel (at the standard deworming dose, once daily for 3 days).

Antiprotozoals active against *Leishmania infantum*

Pentavalent antimonial compounds

Pentavalent antimonial compounds have been used extensively to treat leishmaniosis in humans and dogs. They bind to polypeptides, inhibit DNA topoisomerase enzymes and fatty acid β-oxidation in amastigotes inside macrophages. Pentavalent antimonial compounds include **meglumine antimonate** and **sodium stibogluconate**. These drugs may cause significant toxic effects, such as arthralgia, nephrotoxicity and pancreatitis. Numerous pharmacokinetic studies have shown that meglumine antimoniate administration by intramuscular (IM) or subcutaneous (SC) injection maintains sustained drug plasma levels more effectively than intravenous injections. Different dosages of meglumine antimoniate have been proposed but the most widely accepted regime is 75–100 mg/kg/day for 4–8 weeks (SC).

Allopurinol

Allopurinol is a pyrazolpyrimidine which inhibits xanthine oxidase, the enzyme which catalyses the oxidation of hypoxanthine to xanthine, and of xanthine to urate/uric acid. Allopurinol is now commonly used twice or three times daily in doses of 10–20 mg/kg orally for 6–18 months in dogs with leishmaniosis. Some treatment side effects have been reported, including the development of xanthine urolithiasis.

Miltefosine

Miltefosine is an alkylphospholipid which is thought to interact with lipids (phospholipids and sterols), inhibit cytochrome c oxidase, and cause apoptosis-like cell death. It is considered to be a broad-spectrum antimicrobial drug, active against pathogenic bacteria and fungi as well as the trematode species *Schistosoma mansoni*. Miltefosine has been tested at 2 mg/kg/day for 4 weeks in dogs with natural *L. infantum* infection and has exhibited similar therapeutic effectiveness to antimonial compounds. Miltefosine is licenced to treat canine leishmaniosis in some European countries. Side effects, including vomiting, diarrhoea and anorexia of varying severity, have been reported when the drug is administered with food but these are quick to resolve.

Antifungals

Antifungals (especially amphothericin B) have been proposed as second line drugs for the treatment of *L. infantum* infection in dogs and humans. **Amphotericin B** is not accepted well because of its nephrotoxicity and the invasive intravenous method of administration. The development of lipid and lipoposome formulations has reduced nephrotoxicity but these formulations are very expensive. The drug is not recommended for use in dogs to avoid resistance and reserve it for use in humans.

Antiprotozoals active against trypanosomes

A very large number of molecules in different chemical groups have been tested to treat trypanosomosis in cattle. Some of these have also been used to treat *Trypanosoma brucei*, *T. congolense*, *T. evansi* and *T. cruzi* infections in dogs.

Suramin

Suramin is a complex molecule with a urea functional group in its centre and it was one of the first antitrypanosomal drugs developed. It inhibits enzymes in the glucose metabolic pathway and has been used at 7–10 mg/kg (2–3 weekly treatments) in dogs. Suboptimal doses may give rise to suramin-resistant isolates.

Benznidazole

Benznidazole belongs to the nitroimidazole group and is the drug of choice to treat *T. cruzi* infection in humans and animals, including dogs. It produces free radicals, to which *T. cruzi* is particularly sensitive given its limited capacity for detoxification. Side effects in dogs include apathy, hypertonia and hyperreflexia of the hind limbs, and loss of balance.

Phenanthridines

Phenanthridines have been exclusively used to treat animal trypanosomosis and they interfere with nucleic acid synthesis by intercalative DNA binding. **Quinapyramine** (5 mg/kg SC) is highly active against the *T. congolense* and *T. brucei* isolates that can be detected in dogs.

Arsenicals

Arsenicals (**tryparsamide** and **melarsomine**) have been used to treat canine trypanosomosis in Africa. Melarsomine can be used in dogs at 0.25 mg/kg IM for 4 days.

Aromatic diamidine

Aromatic diamidines include several molecules that may be used to treat trypanosomosis, leishmaniosis, babesiosis and sometimes pneumocystosis. **Pentamidine** is mainly used in human medicine, but several publications report its administration in cases of canine trypanosomosis (3–4 mg/kg in three daily IM injections). **Diminazene** has been used as a trypanocidal drug in livestock since 1955. The main biochemical mechanism of diminazene's trypanocidal activity is thought to be binding to kinetoplast DNA, inducing complete and irreversible loss of kDNA in certain trypanosome strains.

Isometamidium chloride

Isometamidium chloride is a phenanthridine derivative with a narrow therapeutic index which has been marketed for over 30 years as both a prophylactic and a therapeutic trypanocidal drug. It is used curatively at lower doses (0.25–0.5 mg/kg), and prophylactically at higher doses (0.5–1 mg/kg). 2 to 4 months' protection is possible. The preventive effect of the drug is increased if animals receive curative treatment with another molecule, such as diminazene, at least 2 weeks before.

Imidocarb dipropionate

Imidocarb dipropionate can be used to treat trypanosomosis in dogs (0.5 mg/kg IM).

Antiprotozoals active against piroplasms

Imidocarb dipropionate

Imidocarb dipropionate is a carbanilide derivative. Its mode of action is unclear, though two mechanisms have been proposed: interfering with polyamine production or utilisation, or preventing inositol from entering the erythrocytes containing the parasites. It is also generally accepted that imidocarb dipropionate has an anticholinesterase and anti-inflammatory effect. It remains the drug of choice to treat piroplasmosis in cattle, horses and dogs. The recommended dose for canine babesiosis ranges from 4 to 6.6 mg/kg IM or SC, repeated after 2 weeks. 5–6 mg/kg of Imidocarb diproprionate in a single injection IM or SC provides protection against severe disease for approximately 4 weeks, but does not protect against *Babesia canis* infection. Side effects are related to the anticholinesterase effect and include hypersalivation, tachycardia, dyspnoea, vomiting and diarrhoea. **Quinuronium sulfate** is another carbanilide derivative which has been the drug of choice to treat bovine babesiosis for many years and is active against the large *Babesia* spp. of pigs, horses and dogs. It has a low therapeutic index and may stimulate the parasympathetic nervous system.

Phenamidine

Phenamidine is mainly used to treat canine and equine babesiosis, especially in countries where imidocarb dipropionate is unavailable. The recommended dose is 15–20 mg/kg SC, but a second dose is sometimes required after 48 hours. Side effects in dogs include injection site pain, hypotension, tachycardia and vomiting.

Diminazene

Diminazene is highly active against bovine, ovine, porcine, equine and canine piroplasms; small piroplasms are generally more refractory to treatment than large ones. A low dose (1.75 mg/kg twice at a 24-hour interval) is recommended to reduce or prevent neurotoxic side effects in dogs (ataxia, opisthotonus, nystagmus, extensor rigidity, coma, and even death).

Pentamidine

Pentamidine is active against *B. canis* and *B. gibsoni* infection in dogs. A dose of 16.5 mg/kg IM once, or twice at a 24-hour interval, is usually recommended. Common side effects are vomiting, nausea, hypotension, tachycardia, and pain at the injection site.

Atovaquone

Atovaquone is a naphthoquinone with broad-spectrum activity against *Pneumocystis*, *Plasmodium*, *Babesia* and *T. gondii*. It is structurally similar to the inner mitochondrial protein ubiquinone. It can be used in dogs at 13 mg/kg orally every 8 hours for 10 days. It has been reported to be highly effective against *Theileria annae* infection

Tetracyclines

Tetracyclines are broad-spectrum antibiotics that are active against a wide range of microorganisms, including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae and protozoa (*Plasmodium*, *Balantidium*, *Theileria* and *Entamoeba*). Tetracyclines inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor site. **Doxycycline** can be useful to treat small *Babesia* infections. A dose of 10 mg/kg/day orally for 4 weeks is recommended in dogs.

Primaquine diphosphate

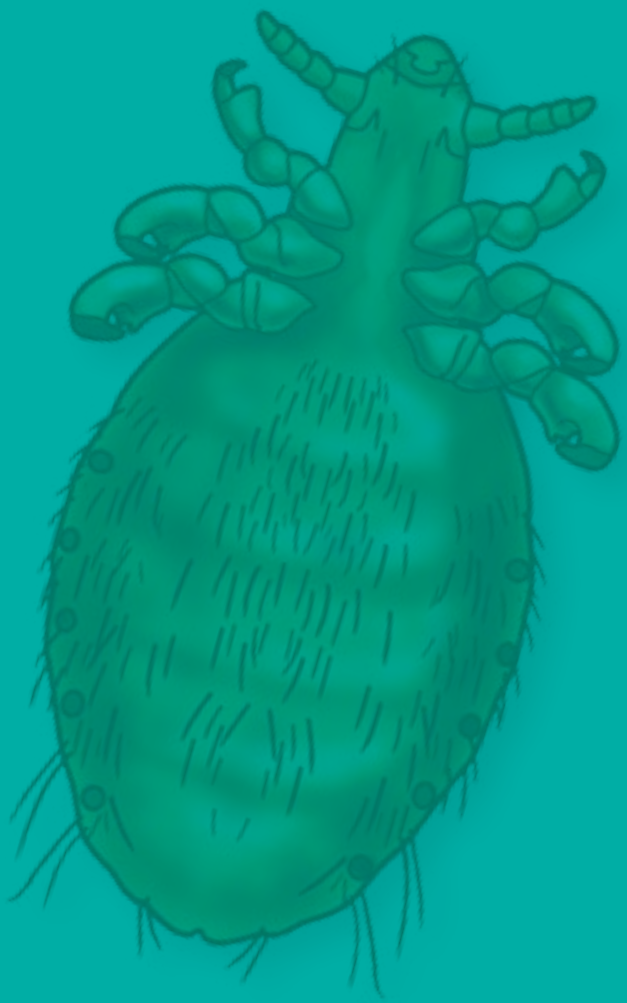
Primaquine diphosphate is a pyridine derivative very active against the tissue stages of *Plasmodium*. The drug is also active against *B. felis*. The maximum tolerated basal dose is 1 mg/kg; higher doses cause mortality.

Antiprotozoals active against *Hepatozoon* spp.

The following drugs have been proposed to treat hepatozoonosis in dogs and cats: **toltrazuril** (5 mg/kg orally, every 12 hours for 5 days), **imidocarb diproprionate** (5 mg/kg SC in a single dose) and a combination of **clindamycin** (10 mg/kg every 8 hours), **trimethoprim** (15 mg/kg every 12 hours) and **pyrimethamine** (0.25 mg/kg/day) orally for 14 days. These drugs or combinations may reduce clinical signs, but they usually fail to prevent relapses. Oral **ponazuril** (10 mg/kg every 12 hours for 14 days) has also been proposed.

Oral **doxycycline** (5 mg/kg for 10 days) has been reported to be active against *Hepatozoon* spp. in cats.

Primaquine appears to be effective against *H. canis* infection.



Ectoparasiticides

Companion animals are commonly treated with ectoparasiticides, which represent 75 % of all antiparasitic drugs used in dogs and cats. This is due to the prevalence of ectoparasites which cause the most common diseases in carnivores.

Ectoparasiticides applied directly onto the animal are veterinary drugs. They must be registered as veterinary medicines by the health agencies in each country in order to be granted with marketing authorisation. On the other side, insecticides or acaricides which are not applied to the animal but used in their environment are not considered to be veterinary drugs but pesticides in the broader sense, even if they sometimes involve the same molecules. Their formulations (especially the excipients) are different and they are not regulated by the same legislation, nor do they require marketing authorisation as veterinary medicines.

In addition to the molecules used and their mode of action, it is important to chose formulations that will either facilitate product application, which is the case for spot-ons, palatable tablets and collars, or modify the pharmacokinetics to increase the duration of the product's activity. Development of parasiticidal drugs with both external and internal antiparasitic spectra is required to meet the demand for prevention of both ecto- and endo-parasitosis in domestic carnivores.

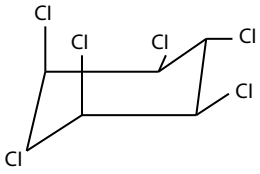
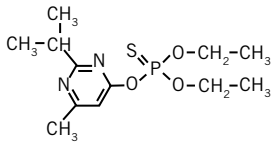
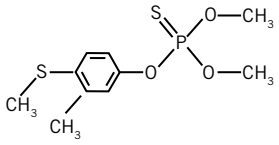
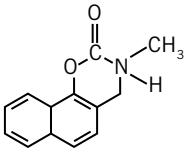
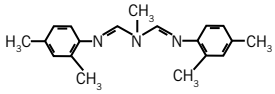
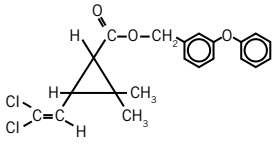
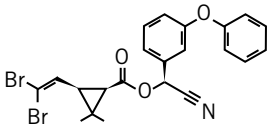
Chemical groups and molecules

Ectoparasiticides used in cats and dogs have evolved in terms of active ingredients, dosage forms and pharmacokinetics, and they mainly act on the arthropod nerve synapses and axons. Traditional chemical groups, especially organophosphates and carbamates, have been supplanted by new compounds. In addition to insecticides-acaricides, insect growth inhibitors have also emerged, and these can be separated into two categories: juvenile hormone analogues and chitin synthesis inhibitors.

Cyclodiene organochlorines

The cyclodiene organochlorine most commonly used in animals used to be **lindane**, which was isolated in 1912 and started to be used in animals in 1943, but it has now been banned in most countries. These molecules had a broad spectrum of activity against arthropods but were not free from toxicity; they are highly persistent in the environment, in milk and in meat, and may be retained in vertebrate fat. Organochlorines inhibit gamma aminobutyric acid (GABA) and/or stimulate the sodium channels located in the nerve cell membrane to open.

Table 1. Ectoparasiticides available for dogs or cats.

Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against ectoparasites
Insecticidal/acaricidal groups				
Cyclodiene organochlorines	Lindane *		Binding sodium channel. Stimulation of sodium entry into nerve cells. Arthropod hyperexcitation	Insects + ticks + mites
Organophosphates	Diazinon (= dympilate)		Acetylcholinesterase binding. Acetylcholine hyperactivity, nerve cell stimulation	Insects + ticks + mites
	Fenthion			Insects + ticks + mites
Carbamates	Carbaryl		Acetylcholinesterase binding. Acetylcholine hyperactivity, nerve cell stimulation	Fleas
Formamidines	Amitraz		Octopamine receptor binding (octopamine agonist) leading to adenylate cyclase and G protein activation	Ticks + mites
Pyrethroids	Permethrin		Sodium channel binding. Sodium entry, nerve cell hyperexcitation	Insects + ticks + mites (repellent)
	Deltamethrin			Ticks, and repellent to flying insects

* Not used for more than 20 years.

Table 1. Ectoparasiticides available for dogs or cats (continuation).

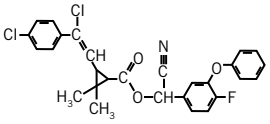
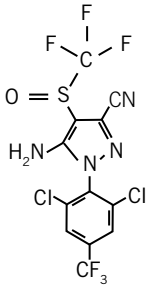
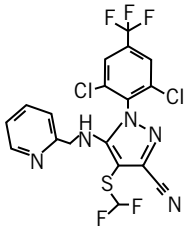
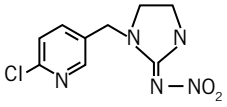
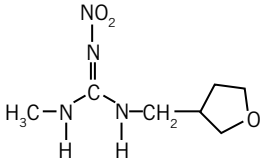
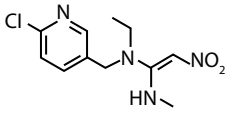
Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against ectoparasites
Insecticidal/acaricidal groups				
Pyrethroids	Flumethrin		Sodium channel binding. Sodium entry, nerve cell hyperexcitation	Ticks
Phenylpyrazoles	Fipronil		Binding to both GABA and glutamate receptors, chloride ion channel stimulation, blocking the entry of chloride ions, nerve cell stimulation	Insects + ticks
	Pyriprole			Insects + ticks
Chloronicotinyl nitroguanidines, neonicotinoids	Imidacloprid		Nicotinic acetylcholine receptor agonist, postsynaptic neuron stimulation	Insects
	Dinotefuran			Insects
	Nitenpyram			Insects

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Table 1. Ectoparasiticides available for dogs or cats (continuation).

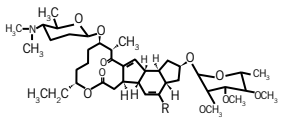
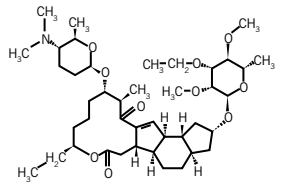
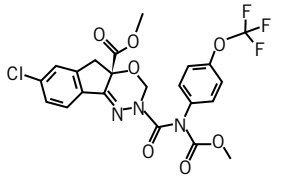
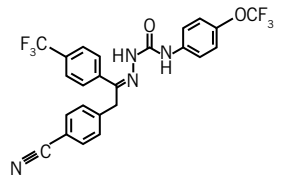
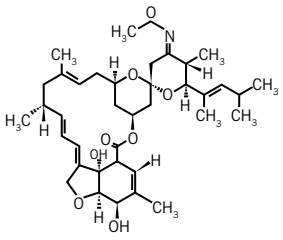
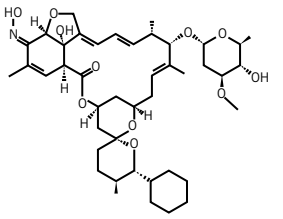
Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against ectoparasites
Insecticidal/acaricidal groups				
Spinosyns, macrocyclic lactones (Insects [some tick efficacy])	Spinosad (spinosyn A, spinosyn D)	 Spinosyn A, R=H Spinosyn D, R=CH_3	Nicotinic acetylcholine receptor and postsynaptic neuron stimulation	Insects
	Spinetoram			Insects
Oxadiazines	Indoxacarb		Voltage-dependent sodium channel blocker	Insects
Semicarbazones	Metaflumizone		Sodium channel antagonist, resulting in paralysis and death	Insects
Avermectins/ milbemycins (macrocyclic lactones)	Moxidectin		Glutamate receptor binding, chloride ion channel stimulation. Entry of chloride ions Inihibition of nerve cell activity	Insects + mites + nematodes
	Selamectin			Insects + mites + nematodes

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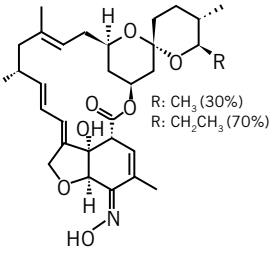
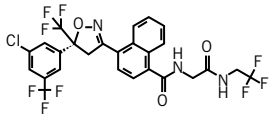
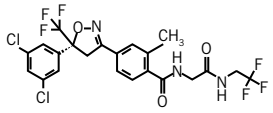
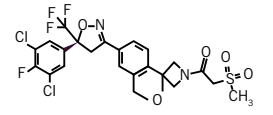
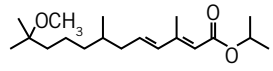
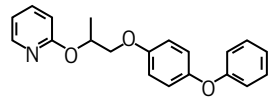
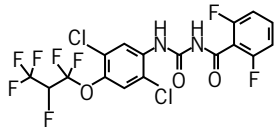
Table 1. Ectoparasiticides available for dogs or cats (continuation).

Chemical group	Scientific name	Molecule	Mode of action	Spectrum of activity against ectoparasites
Insecticidal/acaricidal groups				
Avermectins/ milbemycins (macrocyclic lactones)	Milbemycin oxime		Glutamate receptor binding, chloride ion channel stimulation. Entry of chloride ions. Inhibition of nerve cell activity	*Demodex* + *Sarcoptes scabiei*
Isoxazolines	Afoxolaner		Non-competitive GABA and glutamate receptor antagonists. Bind to chloride channels in arthropod nerve and muscle cells	Fleas + ticks + mites
	Fluralaner			Fleas + ticks + mites
	Sarolaner			Fleas + ticks + mites
IGR (insect growth regulator)				
Juvenile hormone analogues	Methoprene and s-methoprene		Reduce prolificacy, egg hatching, last moult from larval to pupal stage	Fleas
	Pyriproxyfen			Fleas
Organofluorines-benzoylureas	Lufenuron		Chitin synthetase inhibitor Inhibits egg hatching, induces mortality during moulting	Fleas

Organophosphates

Organophosphates exert an anti-cholinesterase effect which results in acetylcholine accumulation in the synapses. This neurotransmitter has a postsynaptic stimulating action and arthropods become hyperactive prior to death.

Their spectrum of activity is broad, and includes insects and acari. Organophosphates have been used for many years (since the 1950s) so cases of chemoresistance are not uncommon and have been reported all over the world. These cases involve flies, crawling insects, ticks and fleas.

Organophosphates have short residual activity (only a few days) apart from in certain formulations, such as collars. They are hydrolysed quite rapidly in the environment and are persistent for a few weeks on inert surfaces but far less on organic surfaces. They can be toxic (parasympathomimetic) in mammals and any overdose or accidental ingestion, of a collar for instance, must be avoided. Cats are much more sensitive than dogs.

The molecules belonging to this group, and their formulations, are numerous, as solutions, sprays, powders, spot-ons and collars are available on the veterinary health market. The molecules most widely used in pets are: **coumaphos**, **cythioate**, **diazinon** (also called **dimpylate**), **dichlorvos**, **fenitrothion**, and **fenthion**.

Carbamates

Carbamates, esters of carbamic acid and classified as methyl or dimethyl carbamates, also inhibit acetylcholinesterase. They are not long-acting (2 to 4 days), except in collars, and are neither retained in animal tissue nor in the environment. They are not very toxic as they are hydrolysed rapidly. Carbamates are mainly insecticidal and they are mainly used in veterinary medicine in the form of collars or powders. The most common are **bendiocarb**, **carbaryl**, and **propoxur**.

Both organophosphates and carbamates were mainly used in the field to prevent flea infestation (with *Ctenocephalides felis* and *C. canis*).

Amitraz

Amitraz is a formamidine that is selective towards arthropods and has been used since the late 1960s to control ticks. Amitraz does not act directly on nerve conduction but alters arthropod behaviour, which has been studied in ticks. It interferes with the arthropods' octopaminergic system, which is similar to the adrenergic system in mammals, by binding to the octopamine receptors, stimulating monoamine oxidases (adenylate cyclase activity) and G proteins. This induces the synthesis of cAMP and cGMP which have various intracellular activities. At a sublethal dose, attached ticks will fall off their host, and those that infest the host do not attach or feed. Reproduction is impaired, prolificacy is reduced and most of the eggs do not hatch.

Amitraz is used on dogs to control ticks of the main genera infesting dogs (*Rhipicephalus*, *Dermacentor*, *Ixodes*, *Haemaphysalis* and *Amblyomma*) or to treat mite infestations, such as demodicosis (*Demodex canis*), sarcoptic mange (*Sarcoptes scabiei* var. *canis*) or cheyletiellosis (*Cheyletiella yasguri*).

Amitraz may interact with the adrenergic system in carnivores. Intoxication can be caused by ingesting, chewing or sucking amitraz-impregnated collars, or by licking spot-ons or solutions. Intoxication has an α2-agonist effect which induces hyperglycaemia, causes a neurosedative effect (lethargy and ataxia) and, more rarely, leads to cardiovascular disorders (bradycardia, hypotension). Specific treatment for poisoning is possible with atipamezole, an α2-antagonist and/or yohimbine. Cats are sensitive to amitraz, probably because they ingest it when grooming.

Nowadays, amitraz is used in dogs in the form of collars to prevent tick infestation, and lotions to treat canine demodicosis.

Natural pyrethrins

Natural pyrethrins are extracted from the flower heads of pyrethrum (a chrysanthemum species, *Chrysanthemum cinerariifolium* or *Dalmatian pyrethrum*). These natural molecules are chrysanthemic acid and pyrethric acid esters. Modification of chrysanthemic acid led to the production of the first synthetic pyrethrins called **pyrethroids** in the 1950s: allethrin, bioallethrin, tetramethrin, phenothrin, resmethrin, bioresmethrin, and kadethrin. This first generation included molecules with low photostability, so their use as insecticidal sprays, mainly applied in the home, was limited.

A second generation of photostable pyrethrins emerged in the 1970s. These have residual activity of several weeks on the skin and in the environment. The main second-generation pyrethroids include **permethrin, cypermethrin, fenvalerate, deltamethrin**, and **flumethrin**.

They act on contact with insects and mites, and their mode of action is very similar to that of organochlorines: opening sodium channel and depolarising nerve cell membranes. This rapid effect on the insect's cerebral ganglia results in sudden shock, known as the "knock-down" effect, where insect stop moving and appears to be dead. This knock-down can be reversible and the insect may wake up after a few seconds and enter a second phase, which involves hyperexcitation due to the effect on the peripheral nerves, with rapid, brief and inconsistent movements, which can lead to death. The spectrum of activity depends on the molecule: permethrin and deltamethrin are both insecticidal and acaricidal, whereas flumethrin is mainly acaricidal. These molecules are volatile and their presence around treated animals explains their repellent effect on flying insects (mosquitoes and sand flies) and even ticks. This repellency is one of the main benefits of the pyrethroids developed recently for use in dogs and it varies according to the molecule, formulation and conditions:

- Animal living in a small or large interiors.
- Animal living outdoors.
- Wind and humidity. Activity is greater and longer-lasting with the use of a collar.

Pyrethroids are not very toxic to mammals but are highly toxic to poikilothermic vertebrates (fish). Pyrethroid metabolism includes an oxidative phase (through hepatic cytochrome P450-dependent mixed-function oxidases), followed by a conjugation phase. Cats are physiologically deficient in glucuronoconjugation enzymes, which explains their hypersensitivity to pyrethroids, especially permethrin.

Pyrethroids are applied to dogs in the form of sprays, shampoos, lotions, collars or spot-ons. They are also used in the environment, sometimes combined with insect growth regulators, in the form of sprays, diffusers, and solutions.

Pyrethroids are mainly used to prevent flea and tick infestation, but also to repel flying insects, especially sand flies (*Phlebotomus*), mosquitoes (*Aedes* and *Culex*) and biting flies (*Stomoxys* and *Haematobia*).

They may be combined with other molecules to increase efficacy (e.g., permethrin + fipronil; permethrin + dinotefuran).

Neonicotinoids

Neonicotinoids (or chloronicotinyl guanidines) act like agonists on postsynaptic nicotinic acetylcholine receptors, mainly in motoneurons, depolarising nerve membranes and causing spastic paralysis in insects. They were developed following observation of the insecticidal effect of nicotine and they target insect-specific receptors, which explains their wide safety margin in mammals.

Imidacloprid is used on both dogs and cats in spot-on formulations. Residual activity on the animal's coat lasts about a month and it acts through contact. Fleas and lice die rapidly, most fleas dying within 24 hours of infestation. Imidacloprid is now combined with moxidectin, ivermectin, permethrin and/or pyriproxyfen to broaden its spectrum of activity.

Nitenpyram has the same mode of action, but it is a systemic insecticide rather than acting on contact. It is administered to carnivores orally, in tablet form and is rapidly absorbed, with peak plasma levels occurring 30 minutes to 2 hours after administration. It is excreted in the urine (with a half-life of 4 hours in dogs and 8 hours in cats) and disappears within 2 days. Fleas ingest nitenpyram while feeding on blood and they die in the following 15 to 30 minutes. It is therefore a short-acting, systemic insecticide with a rapid effect.

Dinotefuran acts on contact, like imidacloprid, has similar pharmacological properties, so is used in the form of spot-ons with long-lasting effects. In dogs, its spectrum is broadened to include ticks and immature flea stages by combination with permethrin and pyriproxyfen.

Fipronil

Fipronil belongs to the phenylpyrazole group and it was introduced to veterinary medicine in the mid-1990s in the form of an alcohol spray for use on dogs and cats. It binds to GABA and glutamate receptors, which inhibits chloride ion channel opening and consequently leads to neuronal hyperactivity. GABA and glutamate are neurotransmitters that inhibit muscle activity in insects and mites. Their attachment to specific receptors opens neuronal chloride channels (in motoneurons or cerebral ganglion neurons in insects), inhibiting depolarisation. Glutamate receptors are specific to arthropods, resulting in a wide safety margin. Fipronil's spectrum of activity includes insects (fleas and lice) as well as acari (ticks, *Sarcoptes* and *Cheyletiella*) and it is lipophilic and photostable. Whether in a spot-on formulation or a spray, the effect on cats or dogs lasts from 15 days (ticks in cats) up to 2 months (fleas in dogs). The molecule remains active on animals which may get wet in the rain or be washed in shampoo, due to its lipophilicity.

It acts on contact with fleas, which die within approximately 24 hours, and ticks which die within 48 hours. Fipronil may be combined with S-methoprene to be active against immature flea stages. Synergistic combination with permethrin adds repellent effect to ticks and flying insects and sustained speed of kill against fleas and ticks.

Pyriprole, which is similar to fipronil, is lipophilic, so treatment and prevention of flea and tick infestations on dogs is by a spot-on solution.

Metaflumizone

Metaflumizone belongs to the **semicarbazone** group. It is a contact insecticide acting as sodium channel antagonist. Binding to its receptor blocks sodium entry, inhibiting nerve activity so insects are paralysed and die. This insecticide was used in some countries as a spot-on in cats and dogs.

Indoxacarb

Indoxacarb belongs to the oxadiazine group. It is an insecticide-only molecule acting mainly through ingestion by the insect. It induces a voltage-dependent sodium channel blockade by binding to a specific receptor, inhibiting nerve activity and causing lethal paralysis. Indoxacarb is used in the spot-on form to control fleas in dogs and cats. A combination with permethrin has been developed to eliminate ticks on dogs. Interestingly, indoxacarb must be bioactivated by enzymes in the insect in order to become active.

Spinosyns

Spinosyns are isolated from the bacterial culture of actinomycete species, such as *Saccharopolyspora spinosa*. The two main molecules are spinosyns A and D, but many other compounds are derived from this fermentation, and semi-synthetic modifications may change insecticidal and pharmacokinetic properties.

Spinosyns have a unique mode of action as they bind to and stimulate nicotinic acetylcholine receptors, which stimulates postsynaptic neurons. Spinosyns are most active as insecticides and have little effect on acarian. They can affect insects on contact or after ingestion so they may be used in topical formulations which have combined systemic activity (after absorption) and contact effects, or directly, in oral formulations such as tablets. In mammals, they are stored in fat tissues that act as reservoirs, which explains their possible persistence. As for any systemic drug, the rate of absorption, followed by storage and elimination, may vary between animals.

Spinosad, a mixture of spinosyns A and D, is used in the form of palatable tablets in dogs and cats. It has anti-flea effects, which begin 30 minutes after the tablet is ingested, and the curative effect against existing flea infestations reaches maximum efficacy within 4 hours. Anti-flea effects then persist for approximately 1 month against new infestations, and are effective within 48 hours. Combination with milbemycin oxime extends the spectrum of activity to include nematodes in dogs.

Spinetoram is the result of semi-synthetic modifications of spinosyn J and it has a longer half-life than spinosad. It was developed as a spot-on formulation for cats, with both systemic and contact activity and it is effective against fleas for a month.

Macrocyclic lactones

Macrocyclic lactones in the avermectin/milbemycin group constitute a generation of broad-spectrum antiparasitic drugs which were introduced in the 1980s to treat livestock. They are derived from fermentation of *Streptomyces* actinomycete bacteria and their active ingredients are produced by semi-synthesis. These molecules are considered to be endectocides, as their spectrum of activity includes internal nematodes, but also some external arthropod parasites, such as lice, maggots, mange mites, *Demodex* spp. and fleas.

Avermectins/milbemycins have GABA-mimetic effects as they bind to glutamate-gated chloride channels. The pharyngeal ganglia in nematodes are the main nerve ganglia

affected, leading to cessation of feeding and death. Avermectins/milbemycins have a sublethal effect on female nematodes and arthropods, stopping egg-laying. Avermectins/milbemycins are absorbed transcutaneously after spot-on application, then they circulate in the plasma and are stored in fat tissues. They are gradually eliminated, mainly in the faeces.

Ivermectin was widely used off-label to treat sarcoptic mange and ear mange in carnivores, as well as canine demodicosis.

Nowadays, several avermectins/milbemycins are marketed for use in domestic carnivores:

- **Milbemycin oxime** is used mainly as a broad-spectrum anthelmintic, but may be indicated in dogs with demodicosis. It is administered orally.
- **In addition to its nematicidal action, selamectin** is used as a spot-on in dogs and cats to treat and/or prevent certain ectoparasitoses (pulicosis, canine sarcoptic mange and ear mange).
- **Moxidectin** is available as a spot-on treatment in combination with imidacloprid and is indicated for very similar uses: nematicidal spectrum plus insecticidal/acaricidal spectrum.

Isoxazolines

Isoxazolines belong to a new chemical class discovered in the 2000s. They have very recently been introduced as veterinary products against fleas and ticks in dogs, but they are also effective against numerous other arthropods. Isoxazolines are all isoxazole derivatives and they are non-competitive GABA and glutamate receptor antagonists, much more selective for GABA receptors in insects or ticks than for those in mammals, including humans. They bind to a unique site on GABA-gated chloride channels in nerve and muscle cells, blocking pre- and postsynaptic transfer of chloride ions across cell membranes. This induces hyperexcitation due to uncontrolled activity in the central nervous system of the arthropod and causes its death.

Afoxolaner, fluralaner, sarolaner, and **lotilaner** are only used in veterinary medicine. They are characterised by high permeability and low aqueous solubility. Following oral administration, they are quickly absorbed and are highly bioavailable. They are highly bound to plasma proteins and metabolised slowly by P450 enzymes in the hepatocytes.

New antiparasitic drugs in this chemical class are likely to appear in the future. They may be combined with other molecules like milbemycin or selamectin to add nematodicidal activity. Other formulations than oral are possible.

Other insecticides

Use of insecticides that do not act on nerve conduction is very limited. Some oxidative phosphorylation inhibitors used as anthelmintics, especially flukicides, may be effective against haematophagous arthropods. This is the case for closantel and nitroxinil which can be used in carnivores to treat parasitoses such as linguatulosis or some myiases, such as *Cordylobia anthropophaga*.

Piperonyl butoxide, an oxidase inhibitor, is a synergist for the pyrethroids found in some environmental insecticidal/acaricidal formulations but is rarely used in veterinary medicine.

Organic silicones or polysiloxanes, especially dimethicone, are used for lice in human medicine. They asphyxiate the arthropod, blocking its respiratory openings and covering its entire cuticle.

Insect growth regulators

Insect growth regulators (IGRs) are molecules that interfere with hormones or enzymes. They inhibit reproduction in adult insects and block organogenesis in immature stages. Their use in veterinary medicine began 20 years ago, to control fleas in livestock and domestic carnivores. IGRs used in dogs and cats may be classified into two groups: juvenile hormone analogues and chitin synthesis inhibitors.

- **Juvenile hormone, or neotenin, analogues** act on contact or on ingestion. They enter flea eggs via adult fleas that come into contact with the active ingredients, or directly through the cuticle of the eggs. The first mechanism inhibits egg hatching and these neotenin analogs can also prevent the last stage 3 larvae from moulting into pupae. These analogs are either used in the environment, in the form of sprays or diffusers and often combined with an insecticide, or applied directly to the animal.

  Juvenile hormone analogues include methoprene and **S-methoprene** (active isomer), **pyriproxyfen** and **fenoxycarb**. Fenoxycarb is only used in the environment. In most cases, these analogues are used in animals in combination with an insecticide/acaricide, such as fipronil, permethrin, dinotefuran or imidacloprid.

  These molecules are lipophilic and persist for several weeks, which allows residual IGR effects to last for 1 to

3 months, depending on the formulation and the study concerned. Fleas that do not die are less prolific, and the hatching and development rates are reduced, and there will eventually be no further new flea generations.

- **Chitin synthesis inhibitors** affect female insect fecundity and prolificacy and inhibit egg hatching and larval moulting. In veterinary medicine, these molecules are used in the environment (flufenoxuron) or directly on the animal (lufenuron). **Lufenuron** acts systemically. It is either administered by subcutaneous injection or orally and is stored in fat tissues or fixed to plasma proteins.

Use of insecticides/acaricides to treat and prevent both ectoparasite infestation and arthropod-borne pathogen transmission

The ectoparasites of pets can be divided into two groups: ones that induce disease, are diagnosed and should be treated; and ones that represent a continuous nuisance and a threat as pathogen vectors.

Many mites, such as *Demodex*, *Otodectes*, *Notoedres*, *Sarcoptes* and *Cheyletiella* species are found in the first group. There are no preventive measures available against these mites and dogs and cats are treated after a veterinary diagnosis. There is no need for long-lasting protection against these parasites, so molecules/formulations that include them in their spectrum of activity are used.

Fleas, sandflies, mosquitoes and ticks are in the second group. They may induce clinical signs like itching, hair loss or flea allergy dermatitis, and they represent a major threat as vectors. Control measures for this group are curative (killing existing infestations) and preventive (killing new infestations as quickly as possible). Long-acting formulations are required for this purpose.

Regular protection will indirectly reduce the risk of arthropod-borne diseases, as well as having an external antiparasitic effect. This has been demonstrated in experiments using infected ticks and demonstrating the absence of transmitted diseases in dogs, or reduced pathogen transmission. Preventive efficacy has been demonstrated against *Borrelia burgdorferi sensu stricto* and *Anaplasma phagocytophilum* transmission by *Ixodes scapularis*, *Babesia canis* transmission by *Dermacentor reticulatus,* and *Ehrlichia canis* transmission by *Rhipicephalus sanguineus*. In those studies, dogs were treated and then infected by ticks carrying the pathogens. The number of ticks on the dogs was counted at different time points but, more interestingly, the dogs were clinically followed-up as well as serologically tested, biopsied, PCRed and blood sampled for culture for several weeks. Untreated dogs served as controls to confirm pathogen infection.

Another way to demonstrate the reduction in pathogen transmission is by field surveys where the rate of infection in control dogs and regularly treated dogs living in enzootic areas are compared. This has been used to assess the reduced risk of canine monocytic ehrlichiosis, canine leishmaniosis, or several pathogens at the same time.

Reasons for treatment failure

No cases of confirmed resistance to recent external antiparasitic drugs against ectoparasites in carnivores have been described. All follow-ups performed when failures were suspected highlighted many other reasons for the failure.

Failures in tick or flea control must first be attributed to ecological and biological factors. The high level of environmental tick infestation can often explain why infestations keep recurring in carnivores. Apart from *Rhipicephalus sanguineus*, which usually infests dogs, ticks are fairly non-specific and feed on many hosts: birds, rodents, insectivores, deer, wild boar, etc. The reservoir host is very important, and ticks are not subject to antiparasitic pressure. Acaricides used in carnivores have a certain speed of kill so it is still possible to see attached and engorging ticks on regularly treated dogs. Active ingredient release may have been irregular on the dog's body, or the concentration may have been reduced by frequent shampooing. The dog's weight might have been underestimated while choosing the dosage form. The product may not have been applied properly according to the guidelines: application sites, the coat being dry, application on skin and not on hair, or application of the entire treatment.

Similar observations apply to the choice of the product for cat and dog fleas and its method of use. There are many hosts, including all carnivores and even other mammals, whether domestic, stray or wild. Pupae are extremely resistant in the environment (4 to 6 months on average) and new adult fleas may therefore emerge and infest carnivores. A single flaw in protecting the animal means that the flea life cycle can restart. Flea susceptibility to insecticides varies according to physiology, which is why efficacy lasts for 1 to 3 months among the populations studied. It is unlikely that all dogs and cats living in the same area are treated regularly so fleas may easily appear as soon as preventive treatment is discontinued or at the end of its active period.

It is easier to question the efficacy of antiparasitic treatments than to seek the true cause and explain control measures in relation to flea and tick biology to pet owners.

Conclusion

External antiparasitic drugs have evolved in recent years with the development of new active ingredients and formulations. Older molecules are still being used, mainly due to their low cost. The number of external antiparasitic drugs available on the market has increased in the past 10 years, with some clear tendencies:

- Introduction of many generic products.
- Development of combination drugs to either cover a broader spectrum or improve properties such as speed of action, inhibition of attachment or repellency.
- Research into new insecticidal families with different modes of action and likely to be used in new formulations, such as oral insecticide formulations.
- Increased protection to reduce the risk of arthropod-borne pathogen transmission.

Vaccines against ectoparasitosis still do not exist and external antiparasitic drugs will probably remain the main therapeutic and preventive solution for many years to come.

Dosage forms used in domestic carnivores

Improvements in external and internal antiparasitic drugs are not only achieved through research into new active ingredients but the dosage form and innovative pharmacokinetic properties are also undoubtedly part of the success of these drugs. There are several objectives:

- Ease of administration to pets with minimal handling.
- Long-term efficacy to avoid repeat treatments and to evolve from the concept of cure to that of prevention.
- A broad spectrum of activity.
- A high level of safety for both animal and owner.

The parasiticidal properties of all formulations must be assessed by controlled experimental and field trials. Some guidelines are defined by drug agencies: the European Medicines Agency in Europe, and the Environmental and Pesticide Agency, or Food and Drug Administration in the United States, and occasionally by scientific associations, such as the World Association for the Advancement of Veterinary Parasitology.

Studies help to define the spectrum of activity, the speed of action against existing infestation (curative effect), or against reinfestation (preventive effect) and the duration (sustained effect). They are also useful to assess effects on immature stages (eggs, larvae), prolificacy in females or inhibition of reproduction. These studies, which have many different designs, are first conducted to licence the product, but many are carried out after the product launch, to refine some points, such as the speed of kill for existing infestation, the sustained speed of kill for new infestation, inhibition of egg production and new generation emergence and, of course, to compare the product with its competitors. The repellent effect, prevention of attachment, and inhibition of blood feeding in haematophagous arthropods are also assessed.

Powders, aerosols, shampoos and lotions are generally short-acting (less than a week) but spot-ons are designed for long-lasting effects, at least for fleas and ticks. They represent a large part of the external antiparasitic drugs available for domestic carnivores. They are based on the pharmacological properties of active ingredients and excipients which enables transdermal delivery followed by plasma distribution for some, and diffusion across the entire skin due to the lipophilic properties of others. Active ingredients remain in the sebum and the sebaceous glands for several weeks. Studies involving radiolabelling of active ingredients using hair clippings and skin samples (biopsies) help to demonstrate the cutaneous pharmacokinetics of these spot-ons. They must be applied to dry skin for optimal effectiveness, and it is therefore best not to wash the animal just before application, which enables the skin's lipid film to rebuild. Regular shampooing will remove the active ingredients and reduce their persistence.

Some active ingredients used in spot-on formulations act on contact with the arthropod; others are systemic and/or mixed in their mode of action, with transcutaneous penetration and plasma circulation. The molecules formulated as tablets act systemically and may have a short-term effect or a persistent efficacy. Oral palatable formulations have recently taken a large part of dog ectoparasiticides. Collars are made of plastic polymers, the matrix of which is impregnated with the insecticide/acaricide. Rubbing of the collar against the skin releases the active ingredients continuously and gradually.

Study design to assess the efficacy of external antiparasitic drugs

The effect of any insecticidal/acaricidal product is assessed in relation to the time post infestation. Performance is evaluated either in the crate/cage or on the animal itself, according to the study design.

A control group is always required to be sure that the infestation process is normal and the comparisons and calculations are always made between treated and control groups.

Arthropod infestation may be achieved by directly placing the arthropods on the animal (the classic method for fleas and ticks) or by placing them in a crate/cage/box/under a net where a sedated animal will be placed later (the classic method for mosquitoes, phlebotomes, flies and some ticks). Infestation is usually by a minimum number of arthropods: 50 ticks (25 males and 25 females) or 100 fleas, and it may be repeated (usually weekly).

Treatments may be applied before (usually 24–48 hours) infestation (to assess any preventive effect) or after (to assess any curative effect). The arthropods are counted on the animal by thumb counting (they remain on their host, which allows successive counts on the same host) or removal counts.

When dealing with fleas, flea eggs can be collected on the ground 12–36 hours after infestation in order to assess the impact of the treatment on female prolificacy, egg viability and the rate of development into new fleas.

Effect measured after experimental infestation. The arthropod can be deposited in a crate/under a net or directly on the animal			Repellency *sensu stricto* (with early expellency). * Applicable to flying insects and ticks	Prevention of attachment. Applicable to ticks only	Insecticidal/acaricidal "killing" effect. Applicable to all ectoparasites
			Time post infestation		
			0–4 hours	4–24 hours	0–48 hours
In the crate or cage (if applicable)	Arthropod status	Alive in the crate/net	Repelled/expelled		Arthropod can be collected and placed in an insectarium. A survival evaluation can be then performed on the repelled arthropod after 24/48 hours
		Dead in the crate/net	Repelled/expelled		
On the dog or cat		Alive free on the animal	Not repelled/expelled	Anti-attachment effect considered	Killing effect not demonstrated
		Dead free on the animal	Not repelled/expelled	Anti-attachment effect considered	Killing effect demonstrated. Depending on the timing of the counts, speed of kill can be determined
		Dead attached to the dog (applicable to ticks only)		No anti-attachment effect	Killing effect demonstrated
		Alive attached to the dog (applicable to ticks only)		No anti-attachment effect	No killing effect demonstrated

*Repellency: the arthropod does not touch/infest the host and remains separate. Expellency: the arthropod does touch/infest the host but falls off quickly due to irritative or behavioural effects after contact. Both are repellency *sensu lato*.

APPENDICES

General definitions

Parasites, predators, commensals and symbionts

Parasite	• Parasites are living organisms (animal or plant) that develop at the expense of another living organism (the host), sometimes causing its death. • A parasite is therefore defined by its relationship with other living beings, and must be distinguished from animals or plants with different relationships, such as predators, commensals, saprophytes, saprobes, symbionts and parasitoids.
Predator	• Predators are living beings that develop by destroying other living beings, their prey.
Commensal	• A commensal is an organism that depends on another organism for its development, but the relationship is not harmful to the host as it is in parasitism: it is mutually beneficial. • For example, certain protozoa (unicellular organisms) live commensally in the digestive tracts of herbivores, where conditions are suitable for their nutrition and reproduction (food supply, temperature, pH, etc.). In return, they allow cellulose to be digested and ensure that water-soluble vitamins, especially vitamin B, can be produced. Some other organisms also perform this function. • Commensal *Trichomonas* can be observed in the digestive and genital tracts of carnivores.
Symbiont	• When the positive relationship between the commensal and host becomes essential for development of the host and survival of the parasite this is known as symbiosis, and the two organisms are called symbionts. • For example, some ciliate protozoa in termite digestive tracts are symbionts. These protozoa themselves harbour symbiotic bacteria, and allow the termites to digest wood. • Some fungi associate with algae to create lichens.
Saprophyte/Saprobe	• When an organism lives off another without negative (parasitic) or positive (symbiotic) effects, it is called a saprophyte. • Some authors restrict the term "saprophyte" to plants, using the word "saprozoite" for animals. • The single term "saprobe" has been proposed and is particularly suitable for fungi, which are currently considered to belong to a kingdom between animals and plants.
Hyperparasite	• There is one other relationship, hyperparasitism, which is linked to parasitoid organisms. These feed at the expense of their host and inevitably cause its death. Numerous arthropod larvae exhibit this parasitoid behaviour. • For example, *Hymenoptera* larvae of the Ichneumonidae family (adults of which resemble wasps) develop on or in certain arthropods (caterpillars, ticks) and cause the death of the host during transformation of the larvae into adults by pupation.

Parasitic infections and infestations

There is debate over what should be called "infection" and what should be referred to as "infestation". For some parasitologists, they are just synonyms but, for others, external parasites cause infestation and internal parasites induce infection. We prefer a zoological and biological approach: infestations are caused by metazoa, while infections are caused by bacteria, viruses, protozoa and fungi.	
Parasitic infections	• The term "infection" suggests multiplication of the pathogenic agent in its host. "Infection" used to refer to viral or bacterial origins, but it can equally refer to parasitic infection. • Numerous protozoa, such as the parasitic coccidia of bird and mammalian gastrointestinal tracts, actively multiply in their host. *Babesia* is a piroplasm or protozoan belonging to the same phylum as the Apicomplexa, which multiply in red blood cells, causing severe anaemia. • We use "infection" for viruses, bacteria, protozoa and fungi.
Parasitic infestations	• The term "infestation" is specific to complex parasites that penetrate, and then develop in or on, their host. This is the general case for helminths and arthropods. • We use "infestation" for all metazoa (helminths and arthropods).

Different types of parasitism

Obligate parasitism	• Development in a host is essential. This is the most common relationship and includes *Dirofilaria immitis* and numerous other helminths, lice, fleas, ticks, etc.
Facultative (or occasional) parasitism	• Development in a host is not essential. The "parasite" may survive and reproduce in the external environment. • This is the case in the majority of fungi, such as *Aspergillus*, which is present in the soil and damp housing conditions. It is also the case in free-living nematodes and some mites (e.g., *Cheyletiella*).
Accidental parasitism	• This is when non-parasitic agents accidentally infect a host and may survive there for some time, or when parasitic agents mistakenly infest an unusual host. • The first case is rare, and can occur after ingestion of the larvae or eggs of the house fly, *Musca domestica*, or after eggs are deposited on a cutaneous wound. The larvae survive, developing at the expense of the host's tissues, causing accidental myiasis. • Parasites of wild carnivores may accidentally infest dogs or cats. This is the case with *Taenia crassiceps, Crenosoma vulpis, Dioctophyme renale* and numerous other helminths and arthropods.
Opportunistic parasitism	• A non-parasitic or commensal organism may become parasitic and pathogenic as a result of increased receptivity by the host. This is particularly common when hosts are immunosuppressed (e.g., by AIDS, or chemotherapy for transplants or cancer). Immunosuppression in animals may be linked to certain treatments (high-dose corticoid treatment), congenital disorders, breeding conditions (housing, nutrition, etc.) or age. • Most fungi are opportunistic parasites, as are some protozoa, such as the digestive tract coccidia belonging to the species *Cryptosporidium.*

Biological characteristics of parasites and parasitism

Permanent parasites	• Permanent parasites live in a host through all stages of their development. They can only survive in the external environment for a short time, from a few hours to a few days. They may also survive and develop in an intermediate host, in which case they are permanent parasites of both their definitive and intermediate hosts. • Permanent arthropod parasites include the mite that causes scabies, and lice (Phtiraptera). • The Taeniidae, cestodes which cause taeniosis in humans or carnivores in their adult state, and cysticercosis, coenurosis or echinococcosis in various mammals including humans in their larval state, are also permanent parasites. • All stages of Filariae are permanent parasites, and *Babesia* are permanent parasites of either mammals or of tick vectors.
Intermittent parasites	• Intermittent parasites combine phases of parasitism and free-living at some time, usually during the adult stage. • This applies most to haematophagous arthropods, such as mosquitoes, horseflies and some fleas (though not *Ctenocephalides felis).*
Temporary parasites	• Temporary parasites have extensive parasitic and free-living phases. • Ticks (Acari: Ixodoidea) have a parasitic phase, during which they take a blood meal, at each life stage. This phase may last between 1 and 3 weeks and is followed by a free-living phase during which the tick leaves its host and undergoes a moult, or lays, in the case of a fertile female. • Adult stages of the Strongylida (Strongylida nematodes, hookworms in carnivores), parasites which live in the small intestine, have an average lifespan of 4 months. The females lay eggs, which are released into the external environment (pasture) in the faeces. These larvated eggs then mature on the pasture and hatch to release a first stage larva. This then moults into L2, then L3, which is the infective stage. This final stage larva can wait weeks or months to be ingested by the final host.

Major parasitoses of dogs and cats

Major internal parasitoses

	Dogs	Cats
Intestinal helminthoses	• Ascaridoses (*Toxocara canis, Toxascaris leonina*) • Hookworm infestation (*Uncinaria stenocephala, Ancylostoma caninum*) • Trichuriosis (*Trichuris vulpis*) • Strongyloidosis (*Strongyloides stercoralis*) • Teniosis (*Taenia* spp., *Dipylidium caninum, Mesocestoides, Diphyllobothrium, Spirometra, Echinococcus* spp.)	• Ascaridoses (*Toxocara cati, Toxascaris leonina*) • Hookworms (*Uncinaria stenocephala, Ancylostoma tubaeforme*) • Ollulanosis (*Ollulanus tricuspis*) • Teniosis (*Taenia taeniaeformis, Dipylidium caninum, Mesocestoides, Diphyllobothrium* and *Spirometra species, Echinococcus multilocularis*)
Intestinal protozooses	• Giardiosis (*Giardia duodenalis*) • Coccidiosis (*Isospora*, *Neospora* species)	• Giardiosis (*Giardia duodenalis*) • Coccidiosis (*Isospora*, *Toxoplasma*, *Hammondia*, *Besnoitia* species)
Respiratory parasitoses	• Respiratory strongyloses (*Oslerus osleri*, *Crenosoma vulpis*) • Capillariosis	• Respiratory strongyloses (*Aelurostrongylus abstrusus*, *Troglostrongylus*) • Capillariosis
Blood parasitoses	• Babesiosis (*Babesia canis*) • Hepatozoonosis	• Babesiosis and cytauxzoonosis (*Cytauxzoon felis*)
Cardiovascular parasitoses	• Dirofilariosis (*Dirofilaria immitis*) • Angiostrongylosis (*Angiostrongylus vasorum*)	• Dirofilariosis (*Dirofilaria immitis*) • Aelurostrongylosis (*Aelurostrongylus abstrusus*)
General parasitosis	• Leishmaniosis (*Leishmania infantum*)	

Major arthropod parasitoses

Ectoparasitosis	Arthropod	Frequency (-/+ to +++)
Fleas and lice		
Pulicosis	• *Ctenocephalides felis felis* • *Ctenocephalides canis* • *Archeopsylla erinacei* • *Pulex irritans*	• +++ • + • +/- • +/-
Lice infestation	• *Trichodectes canis* (dog) • *Linognathus setosus* (dog) • *Felicola subrostratus* (cat)	• +/- • +/- • +/-
Ticks		
Tick infestation	• *Dermacentor* spp. • *Rhipicephalus* spp. • *Ixodes* spp. • *Amblyomma* spp. • *Haemaphysalis* spp.	• ++ • ++ • ++ • ++ • ++
Mites		
Otoacariosis (ear mange)	• *Otodectes cynotis*	• ++ (mostly in kittens)
Sarcoptic mange (dog)	• *Sarcoptes scabiei* var. *canis*	• +
Notoedric mange (cat)	• *Notoedres cati*	• +/-
Canine demodicosis	• *Demodex canis*	• + (frequent between 3 months and 2 years old; genetic predisposition)
Feline demodicosis	• *Demodex cati*	• +/- (rare; often in the ears)
Cheyletiellosis	• *Cheyletiella yasguri* (dog) • *Cheyletiella blakei* (cat)	• + (young carnivores, mainly in or from kennels) • +
Trombiculosis (chiggers)	• *Trombicula autumnalis*	• + (frequent in summer and autumn on carnivores with access to the outdoors)

Major intestinal helminths

Parasite	Epidemiological data
Dog	
Spirocerca lupi	• Parasite of oesophagus and stomach. • Found in tropical areas. • Transmitted through intermediate hosts (arthropods, such as beetles).
Ancylostoma caninum	• Common hookworm in warm countries.
Uncinaria stenocephala	• Main hookworm infesting dogs in northern Europe. • Infestation through infective L3 on grass.
Toxocara canis	• Common roundworm in dogs. • Infestation *in utero*, through suckling milk or ingesting embryonated eggs. • Very common in breeding kennels.
Toxascaris leonina	• Rare roundworm, found in young and adult dogs and cats, more common in rural areas. • Infestation by ingesting embryonated eggs or paratenic hosts (such as rats or mice).
Strongyloides stercoralis	• Parasite of small intestine, rare.
Dipylidium caninum	• Common tapeworm found in many pets infested by intermediate hosts, fleas or lice.
Mesocestoides spp.	• Rare tapeworm. • Infestation after ingesting second intermediate hosts (rodents or birds).
Taenia spp. (numerous species: *T. hydatigena, T. pisiformis, T. serialis, T. multiceps* and *T. ovis*)	• Tapeworms found in rural areas where dogs may ingest larvae in ruminant or rabbit offal.
Echinococcus spp. (*E. granulosus* and *E. multilocularis*)	• Rare tapeworms, but dangerous as dogs shed oviferous segments and eggs in their faeces which can directly infest humans. • *Echinococcus granulosus* is linked to sheep farming in Europe. • *Echinococcus multilocularis* is found in European forests where foxes are definitive hosts and Microtidae (voles) are the intermediate hosts.
Diphyllobothrium latum	• Lake tapeworm, found in large European lakes. • Infestation through ingesting infested raw fish.
Trichuris vulpis	• Whipworm, common in dog kennels. • Common on adult dogsboth in rural and urban areas. • Infestation through ingesting highly resistant embryonated eggs.
Opisthorchis felineus, Clonorchis sinensis (liver flukes)	• Possibility of dog infestation in Eastern and Central Europe, and most of Asia, through ingestion of infested fish.
Cat	
Spirura rytipleurites	• Rare parasite of the stomach. • Infestation after ingesting infested arthropods (beetles).
Ollulanus tricuspis	• Rare parasite of the stomach. • Infestation by ingesting infesting L3 larvae. • Infective larvae directly expelled in the vomit of infested cats.
Ancylostoma tubaeforme	• Main hookworm of cats.
Uncinaria stenocephala	• Rare hookworm in cats.
Toxocara cati	• Very common parasite of kittens and young cats (under a year old). • Infestation through suckling milk, ingesting embryonated eggs from the environment or ingesting infested paratenic hosts (rodents).
Toxascaris leonina	• Roundworm found in adult cats, mainly in rural areas. • Infestation through ingesting paratenic hosts (rodents).
Dipylidium caninum	• Common tapeworm in cats. • Infestation through ingesting intermediate hosts (fleas or lice).
Mesocestoides sp.	• Rare tapeworm.
Taenia taeniaeformis	• Tapeworm found in hunting cats, following ingestion of infested mice or rats.
Echinococcus multilocularis	• Infestation possible in cats, but extremely rare (cats can not be a source of *Echinococcus granulosus*).
Diphyllobothrium latum	• Rare lake tapeworm found in cats ingesting raw fish.
Opisthorchis felineus, Clonorchis sinensis (liver flukes)	• Infestation occurs in Central and Eastern Europe, and most of Asia.

Biological characteristics of the major gastrointestinal parasitoses of dogs and cats

Parasitosis	Source	Infective elements
Teniosis due to *Dypilidium caninum*	Fleas (and sometimes lice).	Cysticercoid larvae.
Taeniosis due to *Taenia hydatigena*	Sheep and cattle offal.	*Cysticercus tenuicollis:* larval stage looks like a fluid-filled balloon, located in the liver or peritoneum.
Taeniosis due to *Taenia pisiformis*	Rabbit offal.	*Cysticercus pisiformis:* metacestode looks like a bunch of grapes, located in the liver and peritoneum.
Taeniosis due to *Echinococcus granulosus*	Sheep, cattle and pig offal.	Hydatid larvae often located in the liver and lungs.
Taeniosis due to *Echinococcus multilocularis*	Voles.	Alveolar larvae located in microtid livers.
Teniosis due to *Diphyllobothrium latum*	Fresh water fish.	Plerocercoïd larvae.
Strongyloidosis due to *Strongyloides stercoralis*	Soil.	• Transcutaneous penetration by L3 larvae. • Free larvae and adult stages possible under ideal conditions (high temperature and humidity).
Ancylostomosis	• Soil. • Paratenic hosts (rodents). • Lactating females.	• L3 larvae. • Ingestion and transcutaneous penetration possible. • Transmission from female to offspring through suckling.
Toxocarosis	Soil.	Eggs containing L2 larvae.
	Lactating females.	Transmission from female to offspring *in vivo* (dog) or through suckling milk (dog and cat).
Trichuriosis	Soil.	Highly resistant eggs containing L3 larvae.
Coccidiosis due to *Isospora*	• Soil. • Various kennel materials. • Drinking water.	Sporulated oocysts either from clinically infected animals or healthy carriers.
Coccidiosis due to *Sarcocystis*	Sheep, goat, cattle, pig and horse meat and offal.	Muscle cysts.
Giardiosis due to *Giardia duodenalis*	• Healthy human, dog and cat carriers. • Environment: cages, bedding, drinking water.	Infective cysts excreted in the faeces of clinically infected animals or healthy carriers.

Main clinical characteristics of the major gastrointestinal parasitoses of dogs and cats

Parasitosis	Intestinal signs	Skin signs	General signs	Respiratory signs
Teniosis due to *Dipylidium caninum*	Loss of appetite, soft faeces or diarrhoea, excretion of segments.	Pruritus of the skin and anus.	Rare: weight loss or nervous signs with epileptic convulsions.	
Ancylostomosis	• Chronic enteritis with diarrhoea. • Faeces sometime dark due to digested blood.	Erythematous dermatitis with papules, following transcutaneous passage of L3 larvae.	• Anaemia, epistaxis, weight loss. • Signs more severe with *Ancylostoma* than *Uncinaria*.	Nasal discharge, cough, changes to the voice, loss of sense of smell all possible due to tracheal migration of hookworm larvae.
Toxocarosis	Alternating diarrhoea and constipation, abdominal bloating, vomiting roundworms.		Puppy/kitten growth slows, irregular appetite, weight loss, dull coat, osteoarticular pain, nervous signs with convulsions (if hypoglycaemic).	Cough caused by migration of ascarid larvae (ascarid pneumonia).
Trichuriosis	Diarrhoea, which can be haemorrhagic in cases of heavy infestation.		Anaemia and cachexia.	
Coccidiosis	Subacute to acute enteritis, possibility of abundant diarrhoea, sometimes haemorrhagic.		Dehydration and weight loss.	
Giardiosis and trichomonosis (cat)	Chronic enteritis with reduced intestinal absorption, foul-smelling diarrhoea with a fatty appearance (steatorrhoea), often yellow.		Weight loss, abdominal pain, excessive thirst.	

Arthropods and vector-borne diseases

Arthropods and the pathogens they transmit in humans or animals

Scientific name of arthropod	Common name of arthropod	Type of pathogen transmitted
Diptera		
Nematocera	**Mosquitoes**	
Anopheles	Mosquitoes	Viruses, *Plasmodium,* filarial worms
Culex	Mosquitoes	Viruses, *Plasmodium,* filarial worms
Aedes	Mosquitoes	Viruses, *Plasmodium,* filarial worms
Culicoides	Midges	Viruses, filarial worms
Phlebotomus	Sandflies = phlebotomes	*Leishmania*, viruses, bacteria
Lutzomyia	Sandflies	*Leishmania*, viruses, bacteria
Simulium	Black flies	Viruses, filarial worms
Brachycera		
Muscidae, Muscinae or Stomoxynae	Flies	Bacteria
Tabanidae	Tabanids	Bacteria, *Trypanosoma*
Muscidae, Glossininae	Tsetse flies	*Trypanosoma*
Hemiptera		
Reduviidae, Triatominae	Kissing bugs	*Trypanosoma cruzi*
Phtiraptera		
Pediculus	Lice	Bacteria
Siphonaptera		
Pulex, Ctenocephalides, Spilopsyllus, etc.	Fleas	Bacteria, filarial worms
Acari		
Ixodidae		
Ixodes, Rhipicephalus, Amblyomma, Hyalomma, Haemaphysalis, Dermacentor, etc.	Hard ticks	Viruses, bacteria, *Babesia*, *Theileria*, *Cytauxzoon*, *Hepatozoon*, filarial worms
Trombiculidae	Chiggers	Viruses, bacteria

Tick-borne diseases of cats and dogs

Disease agent	Tick vector	Geographical distribution
Viral tick-borne pathogens		
Tick borne encephalitis virus	*Ixodes ricinus*	Europe and Asia
Louping ill virus	*Ixodes ricinus*	England, Scotland, Ireland, Spain, Portugal, Bulgaria, Turkey, Japan
Bacterial tick-borne pathogens		
***Borrelia* spp.**		
Borrelia burgdorferi sensu stricto	*Ixodes scapularis*	USA, Canada
	Ixodes pacificus	USA (Pacific Coast)
	Ixodes ricinus, Ixodes persulcatus	Europe, Asia
Borrelia garinii	*Ixodes ricinus*	Europe, Asia
Borrelia afzelii	*Ixodes ricinus*	Europe, Asia
Borrelia japonica	?	Japan
***Ehrlichia* spp. / *Anaplasma* spp.**		
Anaplasma phagocytophilum	*Ixodes scapularis*	USA
	Ixodes pacificus	USA
	Ixodes ricinus	Europe
Anaplasma platys	*Rhipicephalus sanguineus*	USA, South America, Africa, Europe
Ehrlichia canis	*Rhipicephalus sanguineus*	Southern Europe, Middle East, Africa
	Dermacentor variabilis	Southeast Asia, Southern USA
Ehrlichia chaffeensis	*Amblyomma americanum*	Southeastern and South Central USA
Ehrlichia ewingii	*Amblyomma americanum*	Southeastern and South Central USA
***Rickettsia* spp.**		
Rickettsia rickettsii	*Dermacentor variabilis*	Southeastern and South Central USA
	Rhipicephalus sanguineus	Arizona, Mexico
	Amblyomma americanum	Southeastern and South Central USA
	Amblyomma cajennense	Central and South America
Rickettsia conorii	*Rhipicephalus sanguineus*	Southern Europe, North Africa, Southern Africa, Middle East, Indian subcontinent, Asia
Rickettsia parkeri	*Amblyomma maculatum*	USA, Central America
Protozoan tick-borne pathogens		
***Babesia* spp.**		
Babesia canis	*Dermacentor reticulatus*	Europe
Babesia vogeli	*Rhipicephalus sanguineus*	Tropical, subtropical and Mediterranean regions
Babesia rossi	*Haemaphysalis elliptica*	Sub-Saharan Africa (Nigeria, Sudan), South Africa
Babesia conradae	?	California
Babesia gibsoni	*Haemaphysalis longicornis*	Asia (Japan), sporadic worldwide
Babesia vulpes	*Ixodes hexagonus*	Portugal, Spain
***Hepatozoon* spp.**		
Hepatozoon canis	*Rhipicephalus sanguineus*	Southern Europe (Spain, France, Italy, Greece), Asia (Japan, Thailand, Philippines), India, Africa, Middle East and South America (Brazil), Southern USA
Hepatozoon americanum	*Amblyomma maculatum*	Southeastern and South Central USA
***Cytauxzoon* spp.**		
Cytauxzoon felis	*Amblyomma americanum*	North and South America
	Dermacentor variabilis	

Taxonomy of the main parasites of dogs and cats

Nematode taxonomy

The morphological and biological characteristics of Class, Order and Family are given. Genera of veterinary importance for dogs and cats are indicated in purple.

Phylum Nemathelminthes

Class Nematoda

Triploblastic pseudocoelomate worms, complete digestive tract and separate sexes. Males have two spicules.

Subclass Secernentea

Possess phasmids (chemoreceptor organs located at the posterior extremity of the worm).

Order Rhabditida

Oesophagus with a rhabditoid organ (bulb) assembles free and parasitic worms. The parasitic worms consist of parthenogenic females only.

- Family Strongyloididae: genus *Strongyloides*.
- **Family Rhabditidae:** genus *Pelodera*, *Rhabditis*.
- **Family Cephalobidae:** genus *Halicephalus*, *Cephalobus*.

Order Ascaridida

Buccal orifice with three lips.

- **Superfamily Ascaridoidea:**
  Three well-developed oral lips (one dorsal and two subventral).
  - Family Ascarididae: genus *Ascaris*, *Parascaris*, *Toxascaris*, *Baylisascaris*, *Lagochilascaris*.
  - Family Toxocaridae: genus *Toxocara*.
  - **Family Anisakidae:** genus *Anisakis*, *Pseudoterranova*, *Contracaecum*, *Hysterothylacium*.
- **Superfamily Subuluroidea:**
  Three oral lips, pre-cloacal sucker.
  - **Family Heterakidae:**
    - **Subfamily Heterakinae:** genus *Heterakis*.
    - **Subfamily Ascaridiinae:** genus *Ascaridia*.
  - **Family Subuluridae:** genus *Subulura*.
- **Superfamily Oxyuroidea:**
  Pharynx with a posterior bulb and valvules, some species have a buccal orifice with three lips. Females have thin posterior extremity (tail-like).
  - **Family Oxyuridae:** genus *Oxyuris*, *Enterobius*, *Passalurus*, *Skrjabinema*, *Syphacia*, *Aspiculuris*, *Probstmayria*.

Order Strongylida

Male have copulatory bursa and bursal rays. L1 and L2 larvae 1 free, L3 infective.

- **Superfamily Ancylostomatoidea:**
  Well-developed subglobular buccal capsule armed with teeth (*Ancylostoma*) or cutting plates (*Uncinaria*). Angle between head and rest of the body explains the name, based on the Greek "ankylos" which means hook.
  - Family Ancylostomatidae:
    - Subfamily Ancylostominae: genus *Ancylostoma*, *Agriostomum*.
    - Subfamily Necatorinae: genus *Necator*, *Uncinaria*, *Bunostomum*, *Gaigeria*.
    - **Subfamily Globocephalinae:** genus *Globocephalus*.

- **Superfamily Strongyloidea:**
  Unarmed buccal capsule, oral opening often surrounded by corona radiata.
  - **Family Strongylidae:**
    - **Subfamily Strongylinae:** genus *Strongylus*, *Triodontophorus*, *Oesophagodontus*, *Craterostomum*.
    - **Subfamily Oesophagostominae:** genus *Oesophagostomum*, *Chabertia*.
  - **Family Trichonematidae** (= Cyathostomidae):
    - **Subfamily Cyathostominae:** genus *Cyathostomum*, *Cyliclocyclus*, *Cylicostephanus*, *Cyliclodontophorus*, *Poteriostomum*, *Gyalocephalus*.
  - **Family Syngamidae:**
    - **Subfamily Syngaminae:** genus *Syngamus*, *Cyathostoma*, *Mammomonogamus*.
    - **Subfamily Stephanurinae:** genus *Stephanurus*.
- **Superfamily Trichostrongyloidea:**
  Reduced buccal capsule, corona radiata absent, copulatory bursa of male well developed.
  - **Family Amidostomidae:** genus *Amidostomum*, *Epomidiostomum*.
  - **Family Trichostrongylidae** (parasites of mammals):
    - **Subfamily Libyostrongylinae:** genus *Libyostrongylus*, *Obeliscoides*.
    - **Subfamily Graphidiinae:** genus *Graphidium*, *Hyostrongylus*.
    - **Subfamily Haemoncinae:** genus *Haemonchus*, *Asworthius*, *Mecistocirrus*.
    - **Subfamily Trichostrongylinae:** genus *Trichostrongylus*.
    - **Subfamily Cooperiinae:** genus *Cooperia*, *Paracooperia*, *Gazellostrongylus*.
    - **Subfamily Ostertagiinae:** genus *Marshallagia*, *Camelostrongylus*, *Ostertagia*, *Longistrongylus*, *Spiculopteragia*, *Teladorsagia*.
  - **Family Dictyocaulidae:** genus *Dictyocaulus*.
  - Family Molineidae:
    - **Subfamily Molineinae.**
    - Subfamily Ollulaninae: genus *Ollulanus*.
    - **Subfamily Nematodirinae:** genus *Nematodirus*, *Nematodirella*.
  - **Family Ornithostrongylidae:** genus *Ornithostrongylus*.
  - **Family Heligmosomidae:** genus *Heligmosomoides* (syn. *Nematospiroides*).
  - **Family Heligmonellidae:** genus *Nippostrongylus*.
- **Superfamily Metastrongyloidea:**
  Buccal capsule and copulatory bursa reduced or absent, bursal rays fused to varying degrees.
  - **Family Metastrongylidae:** genus *Metastrongylus*.
  - **Family Protostrongylidae:** genus *Protostrongylus*, *Muellerius*, *Cystocaulus*, *Spiculocaulus*, *Neostrongylus*, *Varestrongylus* (syn. *Bicaulus*), *Elaphostrongylus*, *Parelaphostrongylus*.
  - Family Filaroididae (syn. Angiostrongylidae): genus *Angiostrongylus*, *Aelurostrongylus*, *Perostrongylus*, *Gurltia*, *Parafilaroides*, *Filaroides* (syn. *Oslerus*).
  - Family Crenosomatidae: genus *Crenosoma*, *Troglostrongylus* (= *Bronchostrongylus*).
  - **Family Skrjabingylidae:** genus *Skrjabingylus*, *Metathelazia* (syn. *Vogeloides*, *Pneumospirura*).

Order Spirurida

Oesophagus divided into two parts: the anterior short and muscular, the posterior longer and glandular. Larval stages develop in arthropods.

Suborder Spiruroidea

Two lateral lips which can be further subdivided. Cylindrical buccal capsule. Vulva opens near the middle of the body in most cases.

- **Superfamily Gnathostomatoidea:**
  Large trilobed lips. Head bulb with tooth-like ridges.
  - **Family Gnathostomatidae:** genus *Gnathostoma*.
- **Superfamily Physalopteroidea:**
  Smooth collar-like head bulb.
  - **Family Physalopteridae:** genus *Physaloptera*.
- **Superfamily Habronematoidea:**
  Well developed lateral lips, often trilobed.
  - Family Spiruridae:
    - Subfamily Spirurinae: genus *Spirura*, *Protospirura*, *Spirocerca*.
    - **Subfamily Ascaropsinae:** genus *Ascarops*, *Physocephalus*, *Simondsia*.
    - **Subfamily Habroneminae:** genus *Habronema*, *Draschia*.
    - **Subfamily Hatertiinae:** genus *Hatertia*.
  - **Family Acuariidae:** genus *Cheilospirura* (syn. *Acuaria*), *Dispharynx* (syn. *Acuaria*), *Streptocara*, *Echinuria*.
  - **Family Tetrameridae:** genus *Tetrameres*.

- **Superfamily Thelazoidea:**
  No pseudolabia but possess mouth capsule. Parasite of conjunctival sac, lacrymal duct for the family Thelaziidae.
  - **Family Thelaziidae:** genus *Thelazia*, *Oxyspirura*.
  - **Family Gongylonematidae:** genus *Gongylonema*.
  - **Family Rictulariidae:** genus *Rictularia*.

Suborder Dracunculoidea

Reduced mouth capsule, atrophied vulva after fecundation.

- **Family Dracunculidae:** genus *Dracunculus*.

Suborder Filarioidea

Long thin worms. Mouth not surrounded by lips or by a mouth capsule. Vulva generally situated near the anterior extremity. Female often viviparous and expel fully developed larvae called microfilariae.

- **Family Filariidae:**
  - **Subfamily Filariinae:** genus *Parafilaria*, *Suifilaria*.
  - **Subfamily Stephanofilariinae:** genus *Stephanofilaria*.
- **Family Onchocercidae:**
  - **Subfamily Onchocercinae:** genus *Onchocerca*, *Acanthocheilonema* (syn. *Dipetalonema*), *Cercopithifilaria* (syn. *Dipetalonema*), *Mansonella* (syn. *Dipetalonema*), *Elaeophora*, *Brugia*, *Wuchereria*, *Litosomoides*, *Monanema*, *Molinema*
  - **Subfamily Dirofilariinae:** genus *Dirofilaria*, *Loa*, *Eulimdana* (syn. *Pelecitus*).
  - **Subfamily Setariinae:** genus *Setaria*.

Subclass Adenophorea

No phasmids.

Order Dioctophymatida

Muscular oesophagus, posterior extremity of the male forms a caudal sucker.

- **Family Dioctophymatidae:** genus *Dioctophyme*, *Hystrichis*, *Eustrongylides*.

Order Enoplida (syn. Trichinellida, Trichurida)

Reduced muscular tissue in the oesophagus, oesophageal glands form a single row of cells. Males have one or no spicules.

- **Family Trichuridae:** genus *Trichuris* (syn. *Trichocephalus*).
- **Family Capillariidae:** genus *Aonchotheca*, *Capillaria*, *Baruscapillaria*, *Eucoleus*, *Pearsonema*, *Calodium*.
- **Family Trichinellidae:** genus *Trichinella*.

Cestode and trematode taxonomy

Phylum Platyhelminthes

Flat worms: acoelomate and triploblastic metazoa. Triploblastic acoelomate

Class Cestoda

Tapeworms segmented in the adult stage. Hermaphrodite endoparasites. Long segmented body with no body cavity or alimentary canal. Divided into three parts: scolex, neck (producing segments), and strobila (chain of segments or proglottids).

Subclass Eucestoda

Order Pseudophyllidea

Unarmed scolex with two muscular grooves, called bothria, instead of suckers, oviferous segments with a uterine pore, the tocostome, from which the operculated eggs are laid.

- Family Diphyllobothriidae:
  - Subfamily Diphyllobothiinae: genus *Diphyllobothrium*, *Spirometra*.
  - **Subfamily Ligulinae**: genus *Ligula*.

Order Cyclophyllidea

True tapeworms. Rostrum often armed with one or several rows of hooks. Scolex with four muscular suckers. No uterine orifice; the oviferous segments containing eggs are expelled.

- Family Mesocestoididae: genus *Mesocestoides*.
- **Family Anoplocephalidae:**
  - **Subfamily Anoplocephalinae**: genus *Anoplocephala*, *Paranoplocephala*, *Aporina*, *Bertiella*, *Moniezia*, *Cittotaenia*.
  - **Subfamily Thysanosominae**: genus *Thysaniezia*, *Stilesia*, *Thysanosoma*.
- Family Dilepididae: genus *Amoebotaenia*, *Choanotaenia*, *Dipylidium*, *Joyeuxiella*, *Diplopylidium*.
- **Family Davaineidae**: genus *Davainea*, *Raillietina*, *Cotugnia*.
- Family Taeniidae: genus *Taenia* (subgenus *Taenia*, *Taeniarhynchus*, *Hydatigera*, *Multiceps*); genus *Echinococcus*.
- **Family Hymenolepididae**: genus *Hymenolepis*, *Drepanitotaenia*, *Fimbriaria*.

Class Digenea

Historically, trematodes were defined as unsegmented flatworms in the adult stage, endoparasitic, possessing suckers and an incomplete alimentary canal with a buccal orifice but no anus.

The trematodes were divided into two subclasses: the Monogenea, which are fish ectoparasites, and the Digenea, which are vertebrate endoparasites.

This class of trematodes does not exist anymore, and Monogena and Digenea are considered to be two distinct classes.

Their taxonomy is based on the morphological characteristics of the cercariae.

Superorder Anepitheliocystida

Order Strigeatida

Cercariae havedistally bifurcated tails and are called furcocercaria.

Suborder Strigeatoidea (Holostomes)

Two suckers and one specifically adhesive tribocytic organ.

- **Family Strigeidae**: genus *Parastrigea*, *Cotylurus*, *Apatemon*.
- **Family Diplostomidae**: genus *Alaria*, *Neodiplostomum*, *Posthodiplostomum*.

Suborder Schistosomatoidea (Schistosomes)

Trematodes with separate males and females.

- **Family Schistosomatidae:**
  - **Subfamily Schistosomatinae**: genus *Schistosoma*, *Orientobilharzia*, *Heterobilharzia*.
  - **Subfamily Bilharziellinae**: genus *Bilharziella*, *Trichobilharzia*.

Suborder Cyclocoeloidea (Monostomes)

No abdominal sucker (acetabulum).

- **Family Cyclocoeliidae**: genus *Cyclocoelum*, *Tracheophyllus*.

Suborder Brachylaemoidea (Distomes)

Ovary located between the testes.

- **Family Brachylaemiidae**: Genu *Brachylaemus*, *Postharmostomum*.

Order Echinostomatida

Cercaria with glandular cells, which develop into metacercaria. They encyst on plants, or in molluscs, arthropods or fish.

Suborder Notocotyloidea (Monostomes)

No ventral sucker.

- **Family Notocotylidae:** genus *Notocotylus*, *Catatropis*.

Suborder Paramphistomatoidea (Amphistomes)

Thick body, usually circular in transverse section. Ventral sucker well-developed and situated near the posterior extremity.

- **Family Paramphistomatidae:** genus *Paramphistomum*, *Cotylophoron*, *Calicophoron*, *Gigantocotyle*.
- **Family Gastrothylacidae:** genus *Gastrothylax*, *Carmyerius*, *Fischoederius*.
- **Family Gastrodiscidae:** genus *Gastrodiscus*, *Gastrodiscoides, Watsonius*, *Homalogaster*, *Pseudodiscus*.

Suborder Echinostomatoidea (Distomes)

Oral sucker surrounded by a "head-collar", which bears a single or double row of large spines.

- **Family Echinostomatidae:** genus *Echinostoma*, *Euparyphium*, *Echinoparyphium*, *Hypoderaeum*, *Echinochasmus, Hismasthla.*

Suborder Fascioloidea (Distomes)

Flat body, usually large. Oral and ventral suckers close together.

- **Family Fasciolidae:** genus *Fasciola*, *Fascioloides*, *Fasciolopsis*.

Superorder Epitheliocystida (Distomes)

Order Plagiorchiida (Distomes)

Suborder Plagiorchioidea (Distomes)

Usually ovoid and plump, concave ventrally and convex dorsally, with spiny teguments.

- **Family Prosthogonimidae:** genus *Prosthogonimus.*
- **Family Plagiorchiidae:** genus *Plagiorchis.*
- **Family Troglotrematidae:** genus *Collyriclum*, *Troglotrema*.
- **Family Paragonimidae:** genus *Paragonimus.*
- **Family Philophtalmiidae:** genus *Philophtalmus.*

Suborder Dicrocoelioidea (Distomes)

Ovary behind the testes.

- **Family Dicrocoeliidae:** genus *Dicrocoelium*, *Platynosomum*, *Eurytrema*.

Order Opistorchiida (Distomes)

Metacercaria usually encysted in fish.

Suborder Opistorchioidea (Distomes)

Testes situated at the end of the body.

- **Family Opistorchiidae:** genus *Opisthorchis*, *Clonorchis*, *Metorchis*, *Pseudamphistomum.*

Suborder Heterophyoidea (Distomes)

Very small, not usually more than 2 mm long. Genital pore surrounded by a genital sucker (gonotyl). Body covered with scale-like spines, less dense posteriorly.

- **Family Heterophyidae:** genus *Heterophyes*, *Cryptocotyle*, *Apophallus*, *Metagonimus.*
- **Family Nanophyetidae:** *Nanophyetus salmincola.*

Simplified classification of Class Trematoda

The complex Digenea classification is usually simplified for veterinary use. This classification is based on the morphology of the adult stages, not the cercariae.

- **Order Distomes:** Family Dicrocoeliidae, Brachylaemiidae, Heterophyidae, Echinostomatidae, Opisthorchiidae, Fasciolidae, Troglotrematidae, Prosthogonimidae, Philophtalmidae.
- **Order Amphistomes:** Family Paramphistomatidae, Gastrothylacidae, Gastrodiscidae.
- **Order Holostomes:** Family Strigeidae, Diplostomidae.
- **Order Monostomes:** Family Cyclocoelidae, Notocotylidae.
- **Order Schistosomes:** Family Schistosomatidae.

Simplified taxonomy of the main protozoan parasites of domestic animals

The Protozoa are now considered to be a subkingdom of the kingdom Protista, although in the classical system they were placed in the kingdom Animalia. More than 50,000 species have been described, most of which are free-living organisms, and protozoa can be found in almost every habitat possible.

Protozoan classification has been, and remains, a problematic area of taxonomy. DNA sequences are used as the basis for classification when available, but this material is unavailable for the majority of the protozoa described. Protozoa have been, and still are, mostly classified on the basis of their morphology and the species of their parasite hosts. They have traditionally been divided on the basis of their means of locomotion.

The following classification is a simplified adaptation from the ITIS (Integrated Taxonomic Information System) and the fourth edition of Veterinary Parasitology (Taylor et al., 2015).

Phylum Amoebozoa

Class Archamoebae

Order Amoebida

- Family Entamoebidae: genus *Entamoeba*, *Idamoeba*, *Endolimax*.
- Family Acanthamoebidae: genus *Acanthamoeba*.

Phylum Apicomplexa

Class Conoidasida

Order Eucoccidiorida

Suborder Eimeriorina

- Family Eimeriidae: genus *Eimeria, Isospora, Cyclospora*.
- Family Cryptosporidiidae: genus *Cryptosporidium*.
- Family Sarcocystidae: genus *Besnoitia, Hammondia, Sarcocystis, Neospora, Toxoplasma*.

Suborder Adeleorina

- Family Haemogregarinidae: genus *Haemogregarina*.
- Family Hepatozoidae: genus *Hepatozoon*.

Class Aconoidasida

Order Haemosporida

- Family Plasmodiidae: genus *Plasmodium, Leucocytozoon, Hepatocystis, Haemoproteus*.

Order Piroplasmida

- Family Babesiidae: genus *Babesia*.
- Family Theileriidae: genus *Theileria, Cytauxzoon*.

Phylum Euglenozoa

Class Kinetoplasta

Order Trypanosomatida

- Family Trypanosomatidae: genus *Leishmania, Trypanosoma*.

Phylum Fornicata

Class Trepamonadea

Order Diplomonadida

- Family Enteromonadidae: genus *Enteromonas*.
- Family Hexamitidae: genus *Spironucleus*.

Class Metamonada

Order Giardiida

- Family Giardiidae: genus *Giardia*.

Phylum Parabasalia

Class Trichomonadea

Order Trichomonodida

- Family Trichomonodidae: genus *Tritrichomonas, Trichomonas*.
- Family Dientamoebidae: genus *Histomonas*.
- Family Monocercomonadidae: genus *Monocercomonas*.

Phylum Percolozoa

Class Heterolobosea

Order Schizopyrenida

- Family Vahlkampfiidae: genus *Naegleria*.

Phylum Preaxostyla

Phylum Ciliophora

Class Litostomatea

Order Trichostomatida

- Family Balantiidae: genus *Balantidium*.

Parasites listed in purple are the main protozoa found in dogs and cats.

Taxonomy of arthropods of veterinary importance

Phylum Arthropoda

Definition:

- Segmented body.
- Jointed external skeleton (exoskeleton).
- Paired jointed appendages on each segment.
- Dorsal brain.
- Ventral nerve cord.
- Open circulatory system.
- Dorsal heart.
- Moults.

Subphylum Chelicerata

Chelicerae, no antennae.

Class Merostomata

Horseshoe crabs.

Class Arachnida

- Aerial respiratory system.
- Body divided in prosoma and opisthosoma.
- Adults have four pair of legs.

Subclass Aranae

Subclass Scorpiones

Subclass Pseudoscorpiones

Subclass Acari

Opisthosoma unsegmented and often merged with the prosoma, forming the idiosoma.

Order Astigmata

No visible respiratory orifice.

- **Family Sarcoptidae:** *Sarcoptes scabiei, Notoedres cati, Trixacarus caviae, Cnemidocoptes* spp.
- **Family Psoroptidae:**
  - Subfamily Chorioptinae: *Chorioptes bovis, Otodectes cynotis.*
  - Subfamily Psoroptinae: *Psoroptes ovis*.
- **Family Analgesidae, Dermoglyphidae, Listrophoridae** (*Listrophorus*, *Myocoptes*), **Acaridae** (*Acarus*), **Pyroglyphidae** (*Dermatophagoides*).

Order Trombidida

Anterior respiratory stigmata.

- **Family Trombiculidae:** *Trombicula autumnalis.*
- **Family Cheyletidae:** *Cheyletiella yasguri, Cheyletiella blakei, Cheyletiella parasitivorax*.
- **Family Myobiidae:** *Myobia musculi.*
- **Family Demodecidae:** *Demodex canis, Demodex cati, Demodex gatoi, Demodex folliculorum*.

Order Mesostigmata

Respiratory stigmata located btween 2nd and 3rd pair of legs.
Dorsal scutum.

- **Family Dermanyssidae:** *Dermanyssus gallinae, Ornithonyssus, Ophionyssus, Pneumonyssoides caninum.*
- **Family Argasidae:** *Argas*, *Ornithodoros.*
  Soft ticks: no dorsal scutum.

Order Ixodida (hard ticks)

Respiratory stigmata in posterior position, associated with peritremes.

- **Family Ixodidae:** *Amblyomma, Hyalomma, Dermacentor, Haemaphysalis, Ixodes, Rhipicephalus* (including *Boophilus*).
  Hard ticks: dorsal scutum.

Subphylum Mandibulata

- Mouth part with mandibles.
- Antennae.
- Adults have three pairs of legs.

Class Myriapoda

Subclass Chilopoda

- One pair of legs per segment.
- One pair of antennae.
- Jaws.
- Two pairs of maxillae.
- Carnivorous.

Subclass Diplopoda

- Two pairs of legs per segment.
- Chewing mouthparts.
- Detritivorous: eat decaying organic matter.

Class Crustacea

- Chitin exoskeleton, some reinforced with calcium (crawfish).
- Periodic moulting. Free-swimming larva (nauplius) has an unsegmented body and three pairs of appendages.
- Two or three body segments: head, thorax or cephalothorax, and abdomen.
- Carapace/shield.
- Two pairs of antennae.
- One median and two lateral eyes.
- Three pairs of biting mouthparts: mandibles and two sets of maxillae.
- First pair of thoracic appendages often modified into pincers.
- Breathe through gills.
- Sexual reproduction.

Class Insecta

- Body in three parts: head, thorax and abdomen.
- Three pairs of mouthparts.
- One pair of antennae.
- Compound and simple eyes.
- Adult has three pairs of legs.

Orders: Coleoptera, Hymenoptera and Dictyoptera.

Order Diptera

One pair of wings.

Suborder Nematocera

Thin body. Mosquito-like. Thin antennae with >6 segments.

- **Family Culicidae:** *Culex, Aedes, Anopheles.*
- **Family Ceratopogonidae:** *Culicoides.*
- **Family Psychodidae:** *Phlebotomus, Lutzomyia.*
- **Family Simuliidae:** *Simulium.*

Suborder Brachycera

- **Family Tabanidae:** *Tabanus, Haematopota, Chrysops.*
- **Family Hippoboscidae:** *Lipoptena, Melophagus, Hippobosca.*
- **Family Gasterophilidae:** *Gasterophilus.*
- **Family Oestridae:** *Oestrus, Cephalopina, Cephenemyia, Hypoderma.*
- **Family Muscidae:**
  - **Subfamily Muscinae:** *Musca.*
  - **Subfamily Stomoxyinae:** *Stomoxys, Haematobia.*
  - **Subfamily Glossininae:** *Glossina.*
- **Family Calliphoridae:** *Calliphora, Lucilia, Cochliomyia hominivorax, Cordylobia anthropophagi, Chrysomya.*
- **Family Sarcophagidae:** *Sarcophaga, Wohlfahrtia.*

Order Siphonaptera (fleas)

- Wingless.
- Laterally flattened body.
- Complex taxonomy with many families and genera.
- **Superfamily Pulicoidea:**
  - No ctenidium: *Pulex*, *Xenopsylla*, *Ceratophyllus*.
  - One ctenidium: *Spilopsyllus*.
  - Two ctenidia: *Ctenocephalides.*
- **Superfamily Sarcopsylloidea:**
  Tunga penetrans, Echidnophaga.

Order Phtiraptera (lice)

- Wingless insects.
- Ventro-dorsally flattened body.

Suborder Anoplura

Head thinner than thorax. Antennae in five segments.

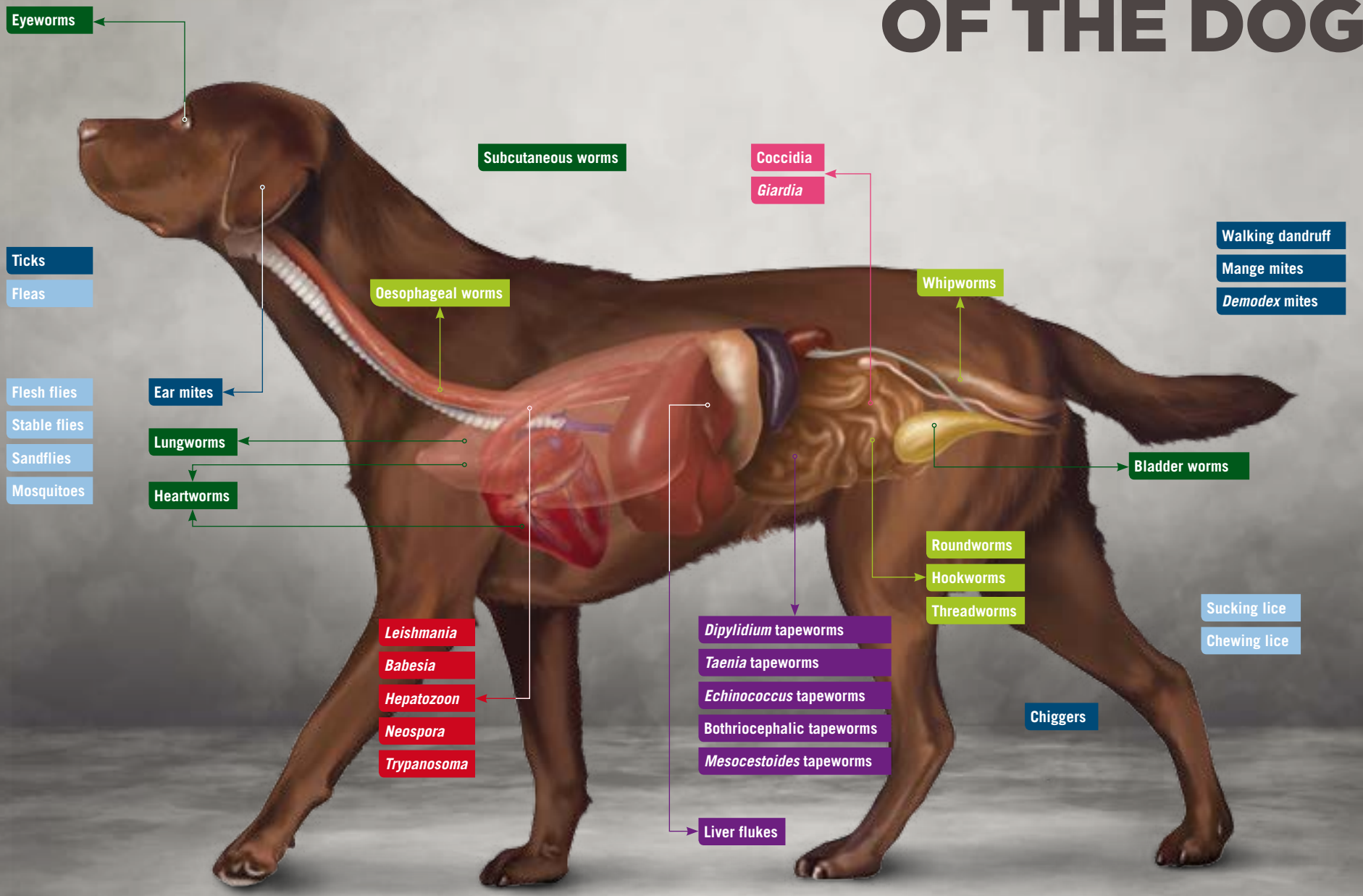
- **Family Pediculidae:** *Pediculus humanus* (var. *capiti* and var. *corporis*), *Phtirius pubis*.
- **Family Haematopinidae:** *Haematopinus*, *Linognathus*, *Solenopotes*.

Suborder Mallophaga

Head wider than thorax.

- **Family Trichodectidae:** *Trichodectes canis*, *Felicola subrostratus*, *Bovicola*.
- **Family Gyropidae:** *Gyropus*, *Gliricolla*.
- **Family Boopidae:** *Heterodoxus spiniger*.
- **Family Menoponidae:** *Menopon*.
- **Family Philopteridae:** *Goniodes*, *Lipeurus*, *Columbicola*.

MAIN PARASITES OF THE DOG



DOG ENDOPARASITES

Gastrointestinal nematodes

Roundworms
Toxocara canis,
Toxascaris leonina
Small intestine

Whipworms
Trichuris vulpis
Large intestine

Hookworms
Ancylostoma caninum,
A. braziliense,
A. ceylanicum,
Uncinaria stenocephala
Small intestine

Oesophageal worms
Spirocerca lupi
Oesophagus

Threadworms
Strongyloides stercoralis
Small intestine

Non-gastrointestinal nematodes

Heartworms
Dirofilaria immitis
Lungs, heart

Subcutaneous worms
Dirofilaria repens
Subcutaneous tissue

Lungworms
Angiostrongylus vasorum,
Oslerus osleri,
Crenosoma vulpis,
Capillaria aerophila
Lungs

Eyeworms
Thelazia callipaeda
Eyes

Bladder worms
Capillaria plica
Urinary tract

Platyhelminthes (cestodes and trematodes)

***Dipylidium* tapeworms**
Dipylidium caninum
Small intestine

***Taenia* tapeworms**
Taenia spp.
Small intestine

***Echinococcus* tapeworms**
Echinococcus spp.
Small intestine

Bothriocephalic tapeworms
Diphyllobothrium latum
Small intestine

***Mesocestoides* tapeworms**
Mesocestoides spp.
Small intestine

Liver flukes
Opisthorchis spp.,
Clonorchis sinensis
Liver

Gastrointestinal protozoa

Coccidia
Cystoisospora canis
C. ohioensis
C. neorivolta
Hammondia heydorni
Sarcocystis spp.
Cryptosporidium parvum
Small intestine

Giardia
Giardia duodenalis
Small intestine

Non-gastrointestinal protozoa

Leishmania
Leishmania infantum
Macrophages

Babesia
Babesia spp.,
Theileria spp.
Red blood cells

Hepatozoon
Hepatozoon canis,
H. americanum
Neutrophils and monocytes

Neospora
Neospora caninum
Muscle cells

Trypanosoma
Trypanosoma spp.
Macrophages and muscle cells

The sizes of the illustrations are not representative of actual parasite size.

DOG ECTOPARASITES

Insects

Fleas
Ctenocephalides felis,
C. canis
Skin and fur

Sucking lice
Linognathus setosus
Blood sucking

Chewing lice
Trichodectes canis
Hair

Flesh flies
Calliphora spp.
Hair and wounds

Stable flies
Stomoxys calcitrans
Blood sucking

Sandflies
Phlebotomus spp.
Blood sucking

Mosquitoes
Aedes spp.,
Culex spp.
Blood sucking

Acarids

Ticks
Ixodes spp., *Haemaphysalis* spp.,
Rhipicephalus sanguineus,
Dermacentor spp.
Entire body

Ear mites
Otodectes cynotis
Ears

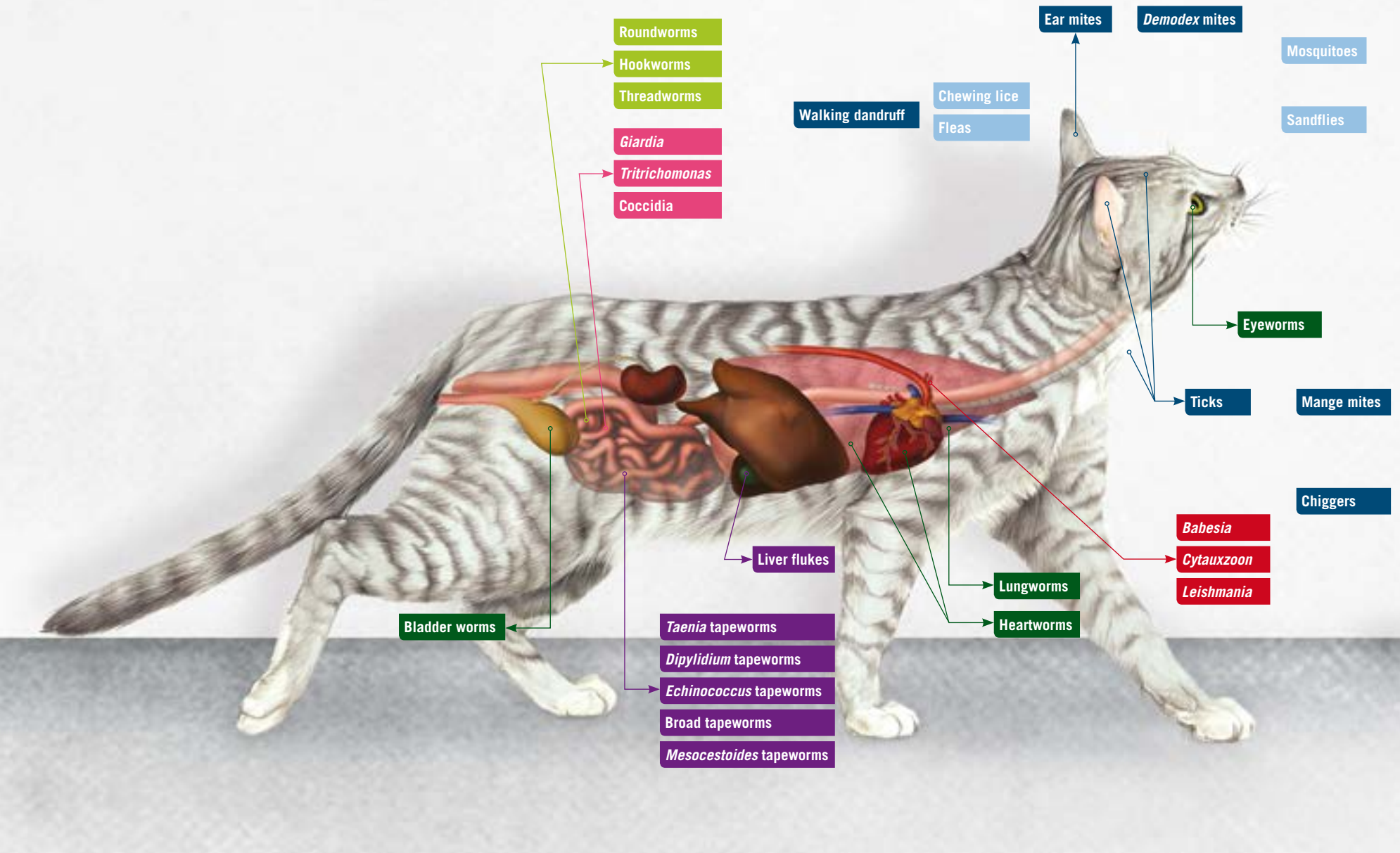
***Demodex* mites**
Demodex canis
Entire body, especially face and legs

Mange mites
Sarcoptes scabiei
Ears and abdomen

Walking dandruff
Cheyletiella yasguri
Back and upper body

Chiggers
Trombicula autumnalis
Ears and abdomen

MAIN PARASITES OF THE CAT



CAT ENDOPARASITES

Gastrointestinal nematodes

Roundworms
Toxocara cati,
Toxascaris leonina
Small intestine

Hookworms
Ancylostoma tubaeforme,
A. caninum, A. braziliense
Small intestine

Threadworms
Strongyloides stercoralis
Small intestine

Non-gastrointestinal nematodes

Heartworms
Dirofilaria immitis
Lungs, heart

Lungworms
Aelurostrongylus abstrusus
Lungs

Eyeworms
Thelazia callipaeda
Eyes

Bladder worms
Capillaria plica
Urinary tract

Platyhelminthes (cestodes and trematodes)

***Taenia* tapeworms**
Taenia taeniaeformis
Small intestine

Broad tapeworms
Diphyllobothrium latum
Small intestine

***Dipylidium* tapeworms**
Dipylidium caninum
Small intestine

***Mesocestoides* tapeworms**
Mesocestoides spp.
Small intestine

***Echinococcus* tapeworms**
Echinococcus multilocularis
Small intestine

Liver flukes
Opisthorchis spp.
Gallbladder

Gastrointestinal protozoans

Giardia
Giardia duodenalis
Small intestine

Tritrichomonas
Tritrichomonas foetus
Small intestine

Coccidia
Cystoisospora felis
Hammondia hammondi
Besnoitia besnoiti
Sarcocystis spp.
Cryptosporidium parvum
Small intestine

Toxoplasma gondii
Small intestine, various tissues and organs

Blood protozoa

Babesia
Babesia spp.
Red blood cells

Cytauxzoon
Cytauxzoon felis
Red blood cells

Leishmania
Leishmania infantum
Macrophages

The sizes of the illustrations are not representative of actual parasite size.

CAT ECTOPARASITES

Insects

Fleas
Ctenocephalides felis
Entire body

Chewing lice
Felicola subrostratus
Face, ears, back

Sandflies
Phlebotomus spp.
Exposed skin

Mosquitoes
Aedes spp.
Exposed skin

Acarids

Ticks
Ixodes spp.,
Haemaphysalis spp.,
Rhipicephalus spp.
Head, neck, ears

Ear mites
Otodectes cynotis
Ears

***Demodex* mites**
Demodex cati
Head, neck

Mange mites
Notoedres cati
Entire body

Walking dandruff
Cheyletiella blakei
Back and upper body

Chiggers
Trombicula autumnalis
Head, neck, feet

ALPHABETICAL INDEX

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The objective of this book is educational, and it is aimed at students and veterinarians.

It is impossible to propose an exhaustive list of references: selecting particular articles would lead to many omissions, and listing pages of references would not be helpful.

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Textbook of Clinical Parasitology in dogs and cats focuses on the main parasitoses of dogs and cats in Europe (helminthology, protozoology and entomology/acarology). Essential information has been collated for rapid access, so taxonomy, morphology and biology have not been extensively developed. The authors, renowned experts with extensive experience in this field, have emphasised the use of appropriate diagnostic methods and their importance in daily clinical practice. Treatment and prevention are also discussed, and any zoonotic risk linked to these parasitoses are highlighted. Numerous graphic resources (images, illustrations, tables) have been included to complement the information provided and make the contents more comprehensible and accessible to the reader.

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