

PRACTICAL GUIDE

Infectious diseases in dogs

Rafael Ruiz de Gopegui Fernández



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Plaza Antonio Beltrán Martínez nº 1, planta 8 - letra I
(Centro empresarial El Trovador)
50002 Zaragoza - Spain

First printing: November 2016

This book has been published originally in Spanish under the title:

Enfermedades infecciosas caninas

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ISBN Spanish edition: 978-84-16315-90-1

Translation:

Owen Howard

Illustrator:

Jacob Gragera Artal

ISBN: 978-84-16818-23-5

eISBN: 978-84-16818-36-5

DL: Z 1337-2016

Design, layout and printing:

Servet editorial - Grupo Asís Biomedica, SL

www.grupoasis.com

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Warning:

Veterinary science is constantly evolving, as are pharmacology and the other sciences. Inevitably, it is therefore the responsibility of the veterinary surgeon to determine and verify the dosage, the method of administration, the duration of treatment and any possible contraindications to the treatments given to each individual patient, based on his or her professional experience. Neither the publisher nor the author can be held liable for any damage or harm caused to people, animals or properties resulting from the correct or incorrect application of the information contained in this book.



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SERVET

For Yvonne, for your invaluable help and support.

Acknowledgments

To Yvonne Espada, Rosa Novellas, Ignacio Mesa, Rui Ferreira, Juan Rejas, Juan Ruiz, Xavier Julve, and Pedro Baringo.

Verus amicus amici nunquam obliviscitur.

The author

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Preface

When I was first asked to author this book I was delighted to accept the challenge of writing a text on infectious diseases in dogs from a clinical perspective. The first objective was to create an index consisting of the most relevant diseases, based on frequency and clinical significance, as well as an appendix on prophylaxis. This required finding motivated collaborators with expertise in the relevant fields. I put the proposal to Laia Solano (PhD in veterinary and clinical pathology) and internal medicine residents Rebeca Movilla and Jorge Castro. Between the four of us we were able to create a coherent index that prioritised what we consider to be the most important infectious diseases of dogs caused by viruses, bacteria, protozoa, and fungi. With this done, the next step was obvious: start writing. In this collaboration we sought to establish a synergy between the knowledge and clinical experience of the authors and their research experience in infectious diseases. At all times we have attempted to thoroughly review the diseases in question, always in the context of the relevant published scientific data. Ensuring that the text included the most up-to-date information required rewriting and modification of some of the chapters, particularly the appendix on vaccinations, which was almost completely rewritten for the final version.

Obtaining the best graphical material presented some difficulties; sufficiently high resolution versions of all the images one would like to include in a text are not always easy to come by. Fortunately, friends and colleagues have provided us with appropriately referenced images that accurately complement the text.

I would like to make special mention of the assistance provided by Professor Yvonne Espada, my wife, who corrected the mistakes in my descriptions of diagnostic imaging and provided the necessary images.

Knowing that my initial errors have been corrected in Tatiana Blasco's excellent revision of the text has also given me great peace of mind.

While only the most up-to-date information is contained in this text, new data is being continually published. Given the highly dynamic nature of internal medicine, it is thus up to each of us working in this field to ensure that our knowledge is continually updated.

Rafael Ruiz de Gopegui

Barcelona, April 2016

Introduction

This practical guide to infectious diseases in dogs is designed as a useful handbook for veterinary clinical activity, presenting up-to-date information on the relevant concepts, diagnostic approaches, treatments, and vaccine recommendations, based on published scientific evidence. Evidence-based medicine should not be an abstract concept, but rather should form the basis of our routine clinical activity.

The chapters are ordered by disease type: viral, bacterial, protozoan, and fungal infections.

The structure is traditional: definition, aetiology, incidence, pathogenesis, clinical presentation, diagnosis, treatment, and prevention. Our aim is to highlight the most useful and important concepts that guide clinical decision-making when establishing diagnosis and treatment.

Because those working in our profession require knowledge on how to treat canine diseases (in this case) and protect human health, we applied two basic premises in deciding which diseases to include and exclude: incidence and zoonotic risk.

Nonetheless, it is impossible to write a book without some influence of the authors' clinical experience in veterinary internal medicine. While this runs the risk of introducing subjective opinions, this is more than outweighed by the benefits of real-life research experience and critical review of the existing literature.

We hope that you find this guide useful and that perusing its pages is both an enjoyable and rewarding experience.

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**Recommendations and consensus for
vaccination of dogs**

Rabies

Rabies

Definition

Rabies is a zoonosis that has been recognised for millennia (Homer describes the transmission of rabies by dog bite).

It is considered the most serious of all zoonoses, with a mortality rate of over 99.9 %. Canine rabies accounts for 99 % of cases.

In Europe and America canine rabies has been controlled through vaccination campaigns, with a significant impact on human health. Excellent results have also been achieved using this approach in other territories including the island of Bohol (the Philippines), Zululand (South Africa), and Tanzania. However, in recent years outbreaks of vulpine rabies have occurred in Montenegro, Kosovo, Macedonia, Albania, and Greece.

Aetiology and incidence

The rabies virus belongs to the genus *Lyssavirus* , family Rhabdoviridae, order Mononegavirales. Within the genus *Lyssavirus* 12 species have been identified. The genus is divided into two phylogroups, of which the rabies virus belongs to phylogroup 1. It is a single-stranded, enveloped RNA virus, consisting of a nucleocapsid protein (N), a phosphoprotein (P), a glycoprotein (G), and an RNA-dependent polymerase (L). This virus, of which seven serotypes have been identified, is distributed worldwide. Serotype 1 is the classic virus that infects dogs.

Epidemiology

Although most *Lyssavirus* species infect bats, the role of carnivores as reservoirs is central to the epidemiology of these viruses. Rabies is a globally distributed zoonosis, but has the most significant impact in developing countries. According to WHO estimates, rabies causes 74,000 human deaths annually in Asia and Africa. In developing countries, the dog is the main source of this zoonosis. By contrast, in developed countries of the northern hemisphere, this zoonosis usually originates in wildlife.

Wild reservoirs show the following distributions:

- North America: raccoon (*Procyon lotor*), skunk (*Mephitis mephitis*), grey fox (*Urocyon cinereoargenteus*) (Figs. 1 –3).
- Eurasia and North America: red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), Arctic fox (*Alopex lagopus*) (Figs. 4 –6).
- America (Caribbean) and South Africa: mongooses (family Herpestidae) (Fig. 7).
- Worldwide: haematophagous bats, insectivores, and frugivores (Figs. 8 and 9).



Figure 1. Raccoons (Styve Reineck, Shutterstock.com).



Figure 2. Skunks (Bildagentur Zoonar GmbH, Shutterstock.com).



Figure 3. Grey foxes (Holly Kuchera, Shutterstock.com).



Figure 4. Red fox (davemhuntphotography, Shutterstock.com).



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Figure 6. Arctic fox (Erni, Shutterstock.com).



Figure 7. Mongoose (Vladimir Wrangel, Shutterstock.com).



Figure 8. Bats (Ethan Daniels, Shutterstock.com).



Figure 9. Flying foxes (EcoPrint, Shutterstock.com).

Pathogenesis

The infection is transmitted via the bite of an infected animal, which sheds the virus in saliva. In exceptional cases, infections can be transmitted by air, due to the excretion of large amounts of virus in poorly ventilated environments (e.g. rabies-infected bats in caves), through consumption of contaminated tissue, and possibly transplacentally.

Wildlife in endemic areas constitutes the greatest potential source of canine rabies, the effect of which can be controlled using appropriate vaccination programs.

Following infection, the incubation period ranges from 3 to 24 weeks, depending on the age of the animal, the innervation of the bite area, the distance from the bite to the central nervous system (CNS), and the amount of virus inoculated. Intramuscular viral inoculation allows the virus to replicate in tissue or directly access the neuromuscular junction. Once the virus reaches the peripheral nervous system it diffuses passively via the axon at a rate of 10 nm to 400 mm per day, until it reaches the CNS. Its final destination is the brain, specifically the limbic system, thalamic nucleus, reticular formation, vagal nucleus, and trigeminal nucleus.

Paralysis, which is progressive, is the result of lower motor neuron damage. The virus multiplies in the brain tissue and spreads via the peripheral nerves. The virus is widely disseminated, but given the mode of transmission, particularly affects the acini of the salivary glands, skeletal muscle, kidneys, pancreas, eyes, and the nerves around hair follicles. At this point, viral shedding is limited. Shedding begins between 1 and 5 days before neurological signs develop, and persists for up to 13 days, ending with the death of the animal.

The inflammatory response may promote dissemination of the virus within the CNS, resulting in a clinical picture of furious or paralytic rabies.

Clinical presentation

Development of the clinical picture occurs in several distinct stages (Box 1). However, some infected animals may show no clinical signs.

Prodromal stage

The prodromal stage lasts for 2 to 3 days, during which the dog presents with hyperthermia and behavioural changes, such as anxiety and irritability. This is accompanied by pruritus of the area of inoculation (bite), which may ulcerate, as well as mydriasis and corneal hyporeflexia.

Box 1. Clinical signs of rabies.

- » Aggression
- » Ataxia
- » Irritability
- » Anorexia
- » Lethargy
- » Sialorrhoea
- » Dysphagia
- » Lameness
- » Limb paralysis
- » Mandibular paralysis
- » Dysphonia
- » Hyperaesthesia
- » Seizures
- » Fever

Furious rabies

The furious phase has a maximum duration of one week. The animal overreacts to visual and auditory stimuli. It becomes excitable, aggressive, and develops photophobia and hyperaesthesia. It attempts to hide in dark, quiet places. Ultimately it becomes disoriented, develops ataxia, convulsions, and paralysis, and dies.

Paralytic rabies

This manifests between 1 and 10 days after the first clinical signs (prodromal signs). Lower motor neuron paralysis begins at the site of inoculation. Animals show dysphonia and salivation. This is the point at which dysphagia can occur, and maximum precautions should be taken. The paralytic phase progresses within 2 to 4 days to coma, arrhythmia, and respiratory arrest.

Asymptomatic carriers

In endemic areas (e.g. Africa) dogs may carry the rabies virus and shed the virus in saliva without displaying clinical signs.

Diagnosis

Clinical diagnosis

Rabies should be suspected in animals that have been in an endemic area and present with lower motor neuron paralysis and/or severe behavioural changes. Confirmation or suspicion that the animal may have suffered an unprovoked attack is key.

Direct diagnosis

The reference diagnostic technique is direct immunofluorescence, which confirms the presence of rabies virus antigen (Fig. 10). It is performed *post mortem* on brain tissue samples. Virus antigen can also be detected in other tissues and in saliva (latex agglutination test), although false negatives can occur.

Other techniques have been developed for the detection of rabies virus in saliva or cerebrospinal fluid (CSF), such as polymerase chain reaction (PCR) and immunochromatography, which have very high sensitivity and specificity. However, the possibility of false negative results remains.

PCR (RT-PCR) can be performed using samples taken from the brain, spinal cord, saliva, CSF, and other tissues. This technique is very sensitive and specific and allows identification of genotypes and strains of rabies virus.

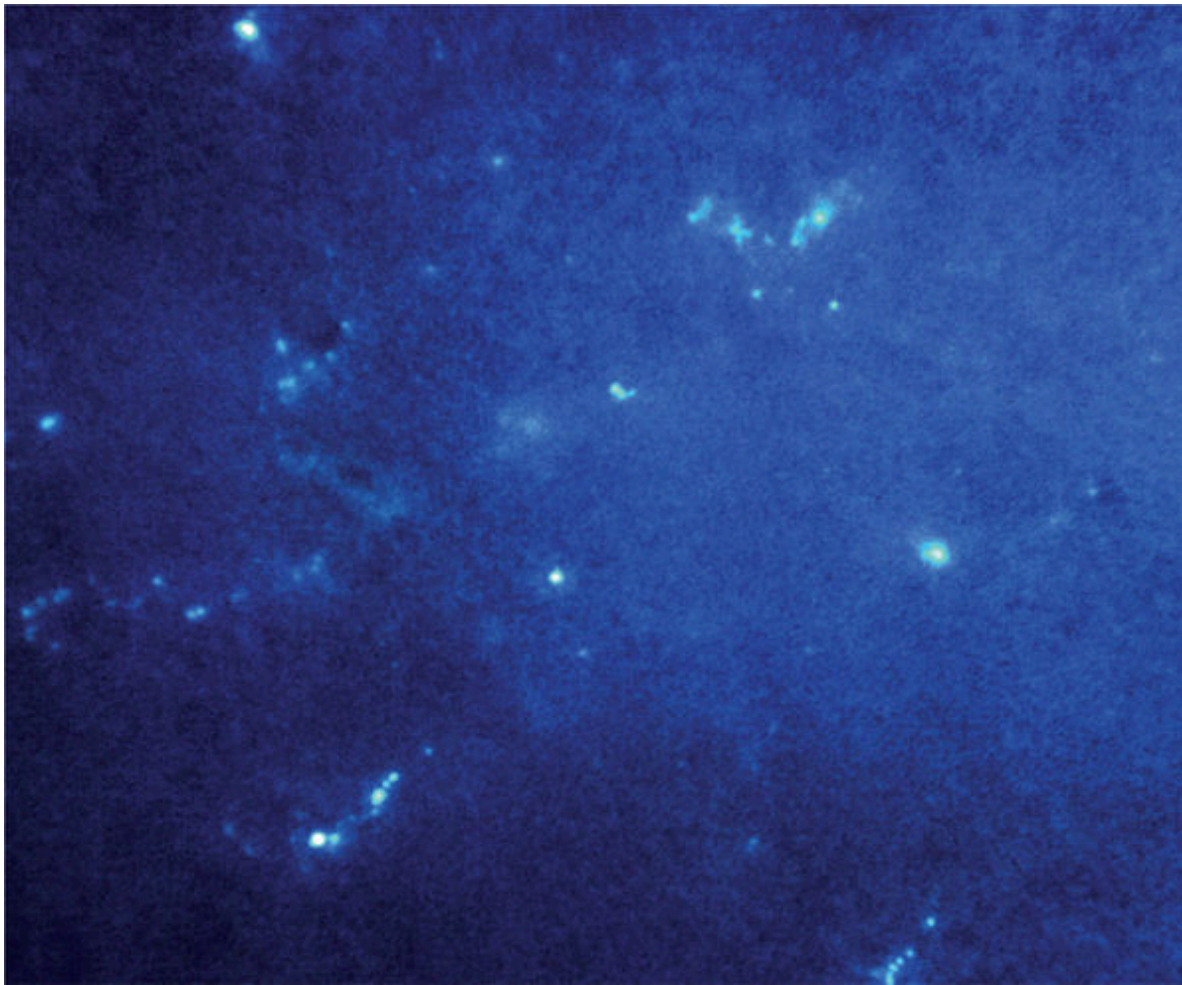


Figure 10. Direct immunofluorescence micrograph showing the presence of rabies virus antigen in the brain tissue of a fox (hippocampal region) (CDC / Dr. Hicklin).

Serological diagnosis

Serological tests are performed to evaluate vaccine immunity. Certain countries require quantification of vaccine antibodies in imported dogs. Various techniques can be used, including the rapid fluorescent focus inhibition test (RFFIT), enzyme-linked immunosorbent assay (ELISA), and fluorescent antibody virus neutralisation (FAVN). RFFIT is considered the reference technique, but FAVN is also acceptable for legal and importation purposes. ELISA is considered less precise, although the sensitivity of this technique can be increased with some modifications (i.e. by using staphylococcal protein A for antibody detection). A simplified immunochromatographic test with very high sensitivity and specificity has also been developed. Currently, FAVN is the most commonly used test. A

titre of 0.5 IU/ml is stipulated as a requirement for the importation of animals (in countries with obligatory FAVN testing).

In case of disease, it is possible to obtain a positive serological test result from serum or CSF samples. However, a negative titre neither rules out the disease nor permits distinction of viral from vaccine antibodies.

Definitive diagnosis requires detection of viral antigen by direct immunofluorescence in brain tissue samples.

Histopathological diagnosis

Both handling and necropsy of dogs with rabies entail a very high risk of contamination. When dealing with living animals destined for euthanasia, protective measures should be taken and the animal should be securely contained. For transport to a specialised laboratory, the skull or brain should be refrigerated but not frozen. It is also essential to adhere to the relevant regulations for the transport of biohazardous tissues.

The principal brain injury is moderate, nonsuppurative polioencephalomyelitis. This is accompanied by neural degeneration and neuronophagia, and even ganglioneuritis and necrotising encephalitis. Negri bodies are the typical lesion observed in classical rabies (Fig. 11). These are eosinophilic cytoplasmic inclusions in the neurons of the thalamus, hypothalamus, pons, cerebral cortex, and dorsal horns of the spinal cord. However, Negri bodies appear late in the course of the disease and are no longer used to establish a definitive diagnosis given their low sensitivity.

Nervous tissue from infected animals can be used to intracerebrally inoculate mice. The brain of the inoculated mouse is then analysed by direct immunofluorescence to identify the virus or used to perform a viral culture.

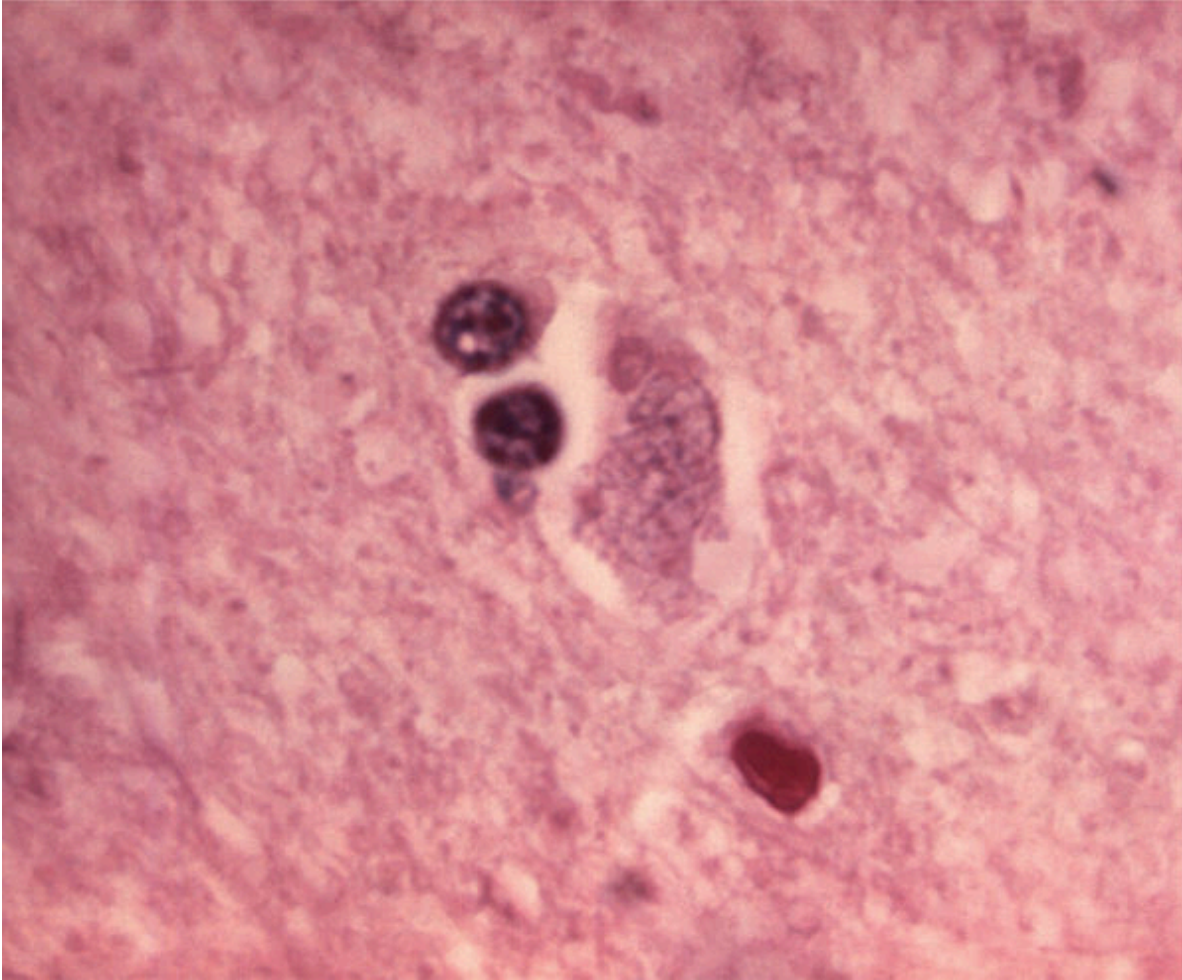


Figure 11. Histopathological image of encephalitis caused by rabies virus (HE staining). Negri bodies can be observed (CDC / Dr. Daniel P. Perl).

Treatment

Due to the zoonotic risk, any dog or cat with suspected rabies should be euthanised and diagnosis established *post mortem* . Animals that recover can shed the virus intermittently.

In the case of infected humans the course of action is to follow the Milwaukee protocol, which involves the induction of coma and the administration of antivirals. However this is a controversial approach, and new therapies combining hypothermia and antivirals, as well as other treatment strategies, have been proposed.

Prophylaxis

Active immunisation of domestic animals and wildlife is the cornerstone of rabies prevention. Annual vaccination of 70 % of dogs is considered sufficient to prevent enzootic rabies.

When administered parenterally attenuated vaccines (modified live virus) are associated with a risk of postvaccination encephalomyelitis or rabies, and are thus not recommended for use in dogs. However, these vaccines (initially containing the SAD B19 strain, currently containing SAG2 and V-RG) are used in oral baits to vaccinate wildlife.

Inactivated vaccines are obtained by inoculation of neonatal mice or, preferably, from cell culture. They require the addition of adjuvant to generate the necessary immunity.

Recombinant vaccines, which contain genetically modified viruses or viral vectors (V-RG), express a larger amount of glycoprotein to generate a stronger immune response.

Dogs should be vaccinated with an inactivated vaccine at 3 months of age, and revaccinated at 1 year, and again every 1 to 3 years depending on the vaccine used and the relevant regulations. These vaccines provide a high degree of protection, but are not 100 % effective. In endemic areas, primary vaccination against rabies is recommended in animals of less than 3 months of age to reduce the zoonotic risk. Postvaccination reactions, including fever, signs of anaphylaxis, vasculitis, or granuloma at the injection site may be observed. Postvaccination sarcomas are very rare in dogs.

Regulations relating to the exposure of vaccinated or unvaccinated dogs to the rabies virus differ between countries. Generally, unvaccinated dogs must be either confined or euthanised, while properly vaccinated dogs should be immediately revaccinated.

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Parvovirus infection

Parvovirus infection

Definition

Canine parvovirus is an infectious disease that presents with viral haemorrhagic gastroenteritis. This virus causes significant morbidity and mortality despite the availability of vaccines, and can lead to myocarditis in some cases.

Aetiology and incidence

Canine parvovirus (CPV) is a naked, single-stranded DNA virus, housed in a 25-nm capsid. It was first discovered in 1978. This resilient, highly contagious virus is a major cause of disease in young dogs. In the United States it is estimated that CPV affects over 1 million dogs each year, despite the availability of an effective vaccine.

Three variants of CPV-2, the aetiologic agent of canine parvovirus, have been described: CPV-2a, CPV-2b, and CPV-2c. The first strain detected (in the 1970s) was CPV-2, which was subsequently subclassified as CPV-2a or CPV-2b according to the corresponding mutation in the genes encoding the VP1/VP2 capsid proteins. In the first decade of the 21st century the CPV-2c variant, generated as a consequence of the Asp426Glu mutation, was described, although the Gly300Asp mutation had already been detected in 2000.

Currently, the three variants coexist, and their frequency varies according to geographical distribution; CPV-2b and CPV-2c are prevalent in North America while CPV-2a predominates in Asia and Oceania. Within Europe strain prevalence also varies by country.

The incidence of this disease is greatest in puppies of under 6 months of age and in dogs that have not been vaccinated for CPV.

CPV-2 is transmitted by direct contact between dogs or by contact with contaminated faeces. However, doubts persist as to the route of transmission in the case of neonatal parvovirus infections.

While the type of CPV does not appear to influence the severity of the disease or its prognosis, coinfection tends to exacerbate the clinical picture and worsen the prognosis. Coinfections with canine coronavirus type I and type II, canine morbillivirus, etc., have been recorded.

Epidemiology

In 1967 a diminutive canine virus (*canine minute virus*) that caused digestive and respiratory signs was identified. This was subsequently named CPV-1. Most CPV-1 infections are asymptomatic. From 1978 onwards symptomatic infections, caused by CPV-2, were described. The absence of natural immunity against CPV-2 led to a pandemic in the 1980s. Puppies are protected from CPV by maternal immunity during the first weeks of life (provided the mother has anti-CPV antibodies), but from 6 weeks to 6 months of age rely on vaccine immunity.

The following predisposing factors have been described: lack of vaccination, seasonality (summer), intestinal parasites, overcrowding, poor hygiene, stress, breed (see Box 1), and sex (male).

Pathogenesis

Transmission of infection occurs via the faecal-oral-nasal route or via fomites. Faecal shedding of the virus begins 3 days after infection and persists for 3 to 4 weeks after the onset of clinical signs. Following infection, CPV-2 replicates in rapidly dividing cells, especially cells of the lymphoid organs, myeloid progenitor cells in the bone marrow, and intestinal epithelial cells. Viral replication causes disruption of mitosis and cell death. After viraemia, CPV-2 can be found in the tongue, oral cavity, oesophagus, small intestine, bone marrow, and lymphatic tissues. Canine parvovirus, unlike other intestinal

viruses, replicates in the crypts of the small intestine and colonises the villi. It causes epithelial destruction and collapse of the villi. The clinical disease is characterised by severe vomiting, bloody diarrhoea, dehydration, lymphopaenia, and neutropaenia.

Effects of virus on the organism

After ingestion, the virus replicates in the lymphatic tissue of the throat, thymus, and mesenteric lymph nodes. It propagates to the bloodstream 1 to 5 days after the onset of infection. In the viraemic phase, the virus infects rapidly dividing cells, resulting in intestinal crypt necrosis and a decrease in the number of lymphocytes in the lymph nodes. Lesions of the intestinal mucosa cause haemorrhagic enteritis and promote secondary bacterial infection. This intestinal bacterial infection spreads to the blood and organ systems. Endotoxins (lipopolysaccharides) of Gram-negative bacteria pass into the peripheral circulation and induce systemic inflammatory response syndrome (SIRS), sepsis, and endotoxaemia.

Tissue tropism

The genus *Parvovirus* displays tropism towards tissues with high mitotic activity, such as the intestinal epithelium, bone marrow, and lymphatic tissues. Feline cerebellar Purkinje cells are also vulnerable to parvovirus infection during pregnancy. However, this kind of tropism has not been identified in the dog and has been ruled out by immunohistochemistry.

Box 1. Predisposed breeds and clinical signs in patients with parvovirus infection.

- » Purebred*
- » Breeds: Rottweiler, American pit bull terrier, Doberman pinscher, Labrador retriever, and German shepherd *
- » Vomiting*
- » Haemorrhagic diarrhoea
- » Anorexia
- » Lethargy/depression
- » Fever
- » Hypothermia

- » Dehydration
- » Hypotension

* Negative prognostic value.

Oxidative stress

- » Oxidative stress is a major problem resulting from the inflammatory response. It is caused by the production of free radicals in the cell when the capacity of antioxidant systems is exceeded. This scenario has been described in dogs with infections such as leishmaniasis, babesiosis, and ehrlichiosis. The production of free radicals results in lipid oxidation (peroxidation) and damages cell membranes. The ability of enzymes to exert their antioxidant activity is dependent upon adequate concentrations of microelements such as iron, cobalt, copper, zinc, and manganese.
- » Signs of erythrocyte oxidative stress identified in canine parvovirus infection include increases in lipid peroxidation and the activity of catalase and superoxide dismutase enzymes, and reduced levels of zinc.
- » CPV-induced myocarditis is thought to be caused by oxidative stress or apoptosis.

Clinical presentation

In dogs, clinical signs of enteritis caused by canine parvovirus include vomiting, profuse bloody diarrhoea, dehydration, signs of abdominal pain, fever, and shock. Laboratory findings often include leukopaenia and hypoproteinaemia, as well as electrolyte imbalance secondary to vomiting and diarrhoea (Boxes 1 and 2). Alterations in gastrointestinal motility predispose affected dogs to ileus and intussusception. Death may occur due to hypovolaemic shock, endotoxaemia and sepsis, or as a consequence of the systemic inflammatory response.

Neonatal parvovirus infection may follow a hyperacute course, resulting in sudden death. Clinical signs include vocalisation, gagging, shortness of breath, and sudden death. This is a multifocal lymphoplasmacytic myocarditic process that causes degeneration, necrosis, and the formation of intranuclear inclusion bodies. It occurs before 8 weeks of age. It may present with congestive heart failure, either left (pulmonary oedema) or right (pericardial effusion and liver congestion). This is a rare clinical manifestation that can be

a result of infection *in utero* or can affect neonates born to nonimmunised mothers.

Respiratory involvement can develop as a consequence of acute dyspnoea, aspiration pneumonia, or coinfection with *Morbillivirus* .

The evolution of the haemogram profile can provide a reliable prognosis in puppies with parvovirus infection (Table 1).

A count of less than 1,000 WBC/ μ l 24 to 48 hours after onset of the process is indicative of an unfavourable prognosis in terms of mortality.

Leukopaenia has been associated with atrophy of the thymus and lymph nodes and with relative bone marrow hyperplasia. High values in the total white blood cell count and the partial count of lymphocytes, monocytes, and eosinophils are indicative of a favourable prognosis.

In critical cases the disease may present with decreased levels of thyroxine (T_4) and increased cortisol levels, indicating a poor prognosis.

Acute phase proteins (APPs) are considered the most sensitive markers of inflammation. They are classified according to whether their levels increase or decrease in response to inflammation (Box 3).

The prognostic value of APPs in canine parvovirus infections has been investigated. Serum concentrations of C-reactive protein (CRP), ceruloplasmin, and haptoglobin increase in dogs with CPV, while serum albumin concentration decreases. Serum concentrations of CRP of over 92.4 mg/l predict mortality with a sensitivity of 91 %. In terms of lethality, the prognostic value of white blood cell concentration in peripheral blood has a sensitivity of 52 % and a specificity of 65 %, applying an optimum cutoff value of 3,020 cells/ μ l. One study reported a positive predictive value for leukocyte concentrations of over 4,500/ μ l in survivors. While serum CRP is associated with mortality in puppies with canine parvovirus infection, it is not an optimal predictor when used in isolation.

Box 2. Laboratory tests: altered parameters in parvovirus patients.

- » Anaemia
- » Leukopaenia*
- » Lymphopaenia*
- » Neutropaenia*
- » Thrombocytopaenia
- » Hypoglycaemia*
- » Hypoproteinaemia
- » Hypoalbuminaemia*
- » Hypoglobulinaemia
- » Hypocholesterolaemia*
- » Hypercoagulability (hyperfibrinogenaemia and decreased antithrombin III*)
- » Increased ALT and AP
- » Hypocarbica
- » CRP (increase)*
- » TNF (increase)*
- » Citrulline (decrease)*

* Negative prognostic value
 ALT: alanine aminotransferase
 AP: alkaline phosphatase
 CRP: C-reactive protein
 TNF: tumour necrosis factor

Box 3. Acute phase proteins.

Positive APP (increase)

- » Haptoglobin (Hp)
- » C-reactive protein (CRP)
- » α_1 -acid glycoprotein
- » Serum amyloid A protein

Negative APP (decrease)

- » Albumin
- » Transferrin

Table 1. Haemogram results in a case of parvovirus infection.

Haemogram	Results	Reference values
Erythrocytes ($\times 10^6 / \mu\text{ l}$)	4.37	5.5–8.5

Hematocrit (%)	27	37–55
Haemoglobin, g/dl	9.3	12–18
MCV (fl)	61.8	62–77
MCH (pg)	21.3	21.5–26.5
MCHC (g/dl)	34.4	33–37
Reticulocytes (%)	0.2	0.5–1
Normoblasts (%)	-	0–1
Leukocytes (cells/μl)	3,030	6,000–17,000
Lymphocytes (cells/μl)	1,606	1,000–4,800
Segmented neutrophils (cells/μl)	636	3,000–11,500
Band neutrophils (cells/μl)	0	0–300
Eosinophils (cells/μl)	0	100–1,500
Monocytes (cells/μl)	788	150–1,350
Platelets (cells/μl)	485,000	200,000–500,000

A simpler way of determining the degree of oxidative stress is to calculate total antioxidant capacity. Recently, laboratory tests have begun to include analyses of the levels of two enzymes that tend to be reduced in conditions of inflammation and oxidative stress; paraoxonase-1 and butyrylcholinesterase. One study of dogs diagnosed with parvovirus reported a decrease in paraoxonase-1 activity.

Citrulline synthesis occurs in enterocytes and hepatocytes. Citrulline of hepatic origin is integrated into the urea cycle, while intestinal citrulline is absorbed and distributed throughout the bloodstream. The concentration of citrulline is significantly lower in dogs with parvovirus infection than in healthy dogs, although this parameter has no prognostic value.

Imbalances in hydroelectrolytes (e.g. hyponatraemia and hypokalaemia) and in acid-base equilibriums are common in dogs with haemorrhagic gastroenteritis. However, no stable pattern has been identified, and these parameters must be determined in each patient. These are complex, multifactorial acid-base disturbances. Both hypochloraemic alkalosis and hyperchloraemic acidosis have been described in dogs with parvovirus infection (Table 2).

Table 3 shows a classification of the severity of canine parvovirus depending on the clinical picture.

Table 2. Biochemical analysis results in a case of parvovirus infection.

Serum biochemistry	Results	Reference values
Glucose, mg/dl	148.8	76–120
Total protein (g/dl)	4.04	5.5–7.3
Albumin (g/dl)	1.39	2.6–3.9
α_1 globulin (g/dl)	0.23	0.2–0.5
α_2 globulin (g/dl)	1.02	0.3–1.1
β globulin (g/dl)	1.33	1.3–2.5
γ globulin (g/dl)	0.08	0.5–1.2
Sodium (mmol/l)	139.9	141–152
Chlorine (mmol/l)	108.3	105–115
Potassium (mmol/l)	4.89	4.1–5.3

Table 3. Clinical severity score in canine parvovirus infection.

Score	Fæces	Depression	Dehydration	Leukocytes ($\times 10^3/\mu$
0	Normal	Normal	Normal	Normal
1	Pasty	Mild	Mild	4.5–5.5 or 12.5–15
2	Semiliquid	Moderate	Moderate	3.5–4.5 or 15–17
3	Liquid	Severe	Severe	<3.5 or >17.5

Diagnosis

Clinical diagnosis

The typical clinical picture includes acute haemorrhagic diarrhoea, vomiting, anorexia, depression, dehydration, and fever in unvaccinated puppies.

Diagnostic imaging

Abdominal radiography reveals no specific alterations. Findings suggestive of gastroenteritis, such as intestinal loops filled with gas, liquid, or both, may be observed.

Abdominal ultrasound reveals a fluid-filled atonic intestine (small and large) with irregular duodenal and jejunal mucosa (thin and hyperechoic or corrugated). Observable changes are indicative, but not pathognomonic. It is important to confirm or rule out intussusception (Fig. 1).

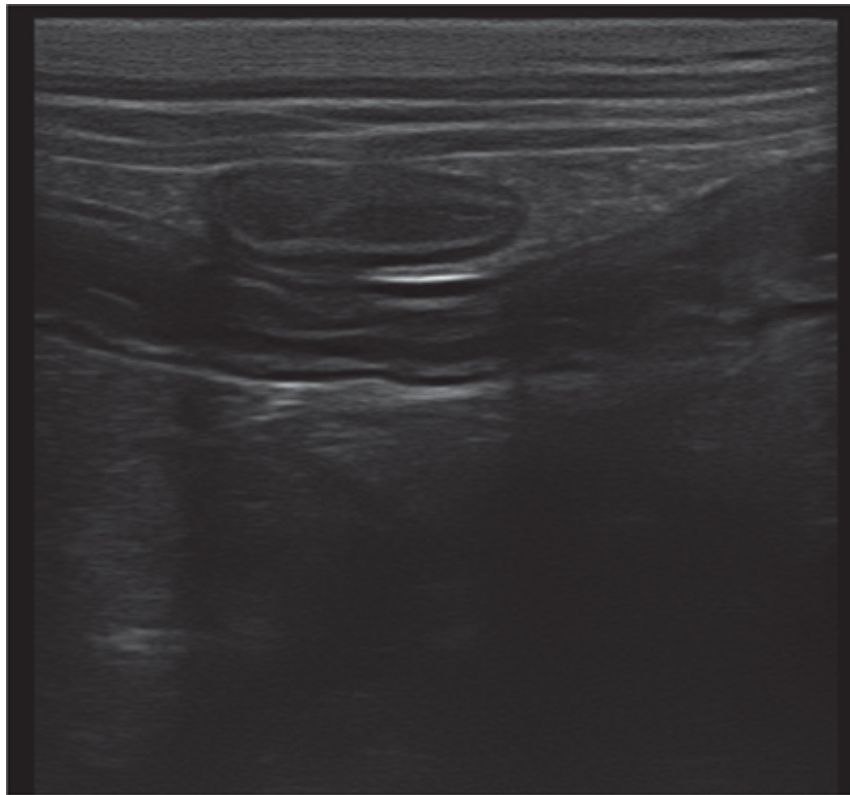


Figure 1. Abdominal ultrasound showing signs of enteritis in a case of parvovirus infection.

Laboratory diagnosis

A key sign in laboratory tests is leukopaenia with neutropaenia (in an animal that fulfils the criteria for clinical diagnosis).

Serological tests

Detection of early appearing anti-CPV antibodies (IgM) in an animal that fulfils the criteria for clinical diagnosis.

Direct diagnosis

The most widespread diagnostic test is ELISA, which detects the presence of CPV antigen (Ag) in faecal samples. Peak viral shedding in faeces occurs 4 to 7 days after infection. Current commercially available diagnostic tests provide a rapid means of establishing diagnosis and are widely used in veterinary practice. This is a test of high specificity, but variable sensitivity; depending on the study, sensitivities of 81.8 %, 56.2 %, and 18.4 % have been reported. As a result, the likelihood of a false negative diagnosis is high. While it has been speculated that ELISA is incapable of detecting the CPV-2c variant, this has been shown not to be the case, indicating that the CPV mutation is not the source of false negatives. However, the faecal viral load (i.e. the amount of CPV Ag) may determine whether this type of test produces a positive or negative result. Another question is what happens if there is a high titre of anti-CPV antibodies. It is possible that these antibodies end up binding to faecal Ag, hindering or preventing a positive reaction in the ELISA test. Dogs with a negative faecal ELISA (false negative) have been demonstrated to have a shorter prepatent period, lower body weight, a lower frequency of defecation, a lower faecal viral load, and a higher serum antibody titre. The causes of false negative results in faecal ELISAs are a lower antigenic load (viral) and a higher number of antibodies (which bind to the faecal antigen).

The faecal polymerase chain reaction (PCR) test appears to have the highest sensitivity and specificity for the diagnosis of CPV gastroenteritis. This greater sensitivity allows for earlier diagnosis.

Faecal electron microscopy (immuno-EM) has a very high specificity, but lower sensitivity than PCR.

Definitive diagnosis

Definitive diagnosis of parvovirus infection can be established at necropsy by analysing samples of the tongue, skin, lymph nodes, and intestine, and confirmed by direct immunofluorescence, PCR, immunohistochemistry, or electron microscopy. Confirmation of CPV myocarditis requires immunohistochemistry or PCR of myocardial tissue.

In cases of suspected CPV infection with a negative faecal ELISA, faecal PCR is recommended to establish a definitive diagnosis.

Treatment

General

This disease is almost always fatal if untreated, with a survival rate of 9 % (untreated). However, with treatment the survival rate ranges from 64 % to 95 %. These survival rates increase to 96 % to 100 % in reference centres, and range from 67 % to 75 % in nonspecialised care centres.

The objective of treatment for CPV enteritis is to control clinical signs of disease. Early enteral nutrition (EEN) is associated with a shorter recovery time and decreased morbidity.

Dogs with parvovirus infection should be treated in the same way as those with Gram-negative sepsis. Treatment should include appropriate fluid therapy, with crystalloids or colloids (hydroxyethyl starch), antiemetics, plasma, antibiotics against Gram-negative bacteria, and immune modulators to improve the immune response.

The initial lesion is a consequence of the viral infection, but the destruction of the intestinal crypt cells results in loss of the protective intestinal barrier. It has been shown that the clinical signs associated with parvovirus infection are caused by bacteraemia resulting from the translocation of bacteria from the intestinal lumen following epithelial damage. Patients develop Gram-negative sepsis and immunosuppression due to the destruction of white blood cells.

Experimental studies in parvovirus-infected dogs have confirmed that the administration of benzylpenicillin (penicillin G), procaine (20,000 IU/kg), and dihydrostreptomycin (20 mg/kg) can improve patient survival by 90 %.

Studies have also investigated the use of plasma from recovered dogs (i.e. a source of passive immunity) as a means of improving immune function, as well as recombinant human granulocyte stimulating factor, recombinant feline interferon ω , and oseltamivir.

Principal treatment regimen

Fluid therapy is essential in these patients. The aim is to resolve dehydration in 4 to 6 hours, maintaining fluid requirements and replacing fluid lost due to vomiting and diarrhoea. A crystalloid replacement solution or lactated Ringer's solution should be administered to resolve hypokalaemia and hypoglycaemia, which are usually present (Figs. 2–4).

For example, to every litre of fluid administered, 16 mEq KCl and 100 ml of 50 % dextrose can be added.

Colloids should be selected based on the severity of the patient's status (hypotension, shock, hypoalbuminaemia, leukopaenia, etc.), and include plasma (fresh frozen) (Fig. 5), hydroxyethyl starch, and albumin (human).

Antiemetics minimise electrolyte loss, facilitate oral nutrition, and improve the animal's attitude. The side effects of antiemetics are related to their mechanism of action. Those which act at the central level can cause anxiety, depression, or sedation, while those that act as antagonists of α adrenergic receptors may cause hypotension, complicating the situation in hypovolaemic animals. Finally, prokinetic drugs may increase the risk of intussusception or intestinal rupture.

Continuous infusion of metoclopramide (1–2 mg/kg/day) provides good results. An alternative option is intramuscular or subcutaneous prochlorperazine administration at doses of 0.1 mg/kg to 0.5 mg/kg, every 6 to 8 hours. Neither of these drugs can completely control vomiting in dogs with parvovirus enteritis. Animals that receive antiemetics generally require longer hospital stays. If vomiting does not subside, treatment with serotonin antagonists should be instituted (e.g. intravenous or subcutaneous ondansetron, 0.1–1 mg/kg every 8–12 hours). Persistent vomiting is considered a negative prognostic factor in canine parvovirus infection.

Nutrition is an important aspect of any treatment, especially in puppies. Parenteral administration requires placement of a catheter. Placement of a nasoesophageal tube resolves the clinical signs of parvovirus infection much faster than conventional therapies, in which nothing is administered until 12 hours after vomiting has resolved and the animal feeds voluntarily.

Neutropaenia is common in dogs with parvovirus infection. As a result, bacteria of the intestinal tract (most commonly *Escherichia coli* and *Clostridium perfringens*) can reach the bloodstream and cause septicaemia. Penicillins and aminoglycosides have the broadest antibacterial spectrum. The combination of ampicillin and amikacin (or gentamicin) is recommended. To avoid nephrotoxicity it is important to first ensure that the animal is well hydrated.

For example, ampicillin, 20 mg/kg IV every 8 hours + amikacin, 20 mg/kg IV, IM, or SC every 24 hours (with hydration).





Figures 2–4. Puppies with parvovirus infection.



Figure 5. Fresh frozen plasma.

Interferon

Recombinant feline interferon ω should be administered at a dose of 2.5 million units (MU)/kg every 24 hours for 3 days. Puppies treated with interferon show better recovery. A greater decrease in mortality is observed in unvaccinated puppies. The risk of mortality is higher in those previously vaccinated against parvovirus as compared with unvaccinated animals. It is likely that in these animals residual or reactivated immunity attenuates the therapeutic benefits of interferon treatment.

Plasma

Passive immunotherapy is effective for the treatment of various diseases, including tetanus and *Clostridium difficile* infection. In the case of parvovirus enteritis, one treatment option is hyperimmune plasma: infused antibodies neutralise free virus in the plasma, and prevent viral spread by blocking entry

into target cells and suppressing the release of new infectious virions from infected cells.

In a study of experimental CPV infection, plasma administration was shown to improve survival and reduce vomiting and diarrhoea in dogs. A prospective, randomised, double-blind, placebo-controlled clinical trial studied the effectiveness of a single 12-ml dose of CPV-immune canine plasma for the treatment of naturally-acquired CPV enteritis. The results revealed no statistically significant differences in neutrophil or monocyte counts, the magnitude of viraemia, body weight variation, the number of days of hospitalisation, or treatment costs between the two groups.

Oseltamivir

Oseltamivir is a neuraminidase (NA) inhibitor designed to treat human influenza. It has recently been proven effective for the treatment of avian influenza. This drug inhibits viral NA and prevents cleavage of sialic acid residues. This cleavage is required to release newly formed virions from the host cell and for aggregation of viral particles. The release and aggregation of virions are mechanisms necessary for the propagation and spread of the virus in the host. Viral NA cleaves the sialic acid residues of the mucin to penetrate this protective layer and infect the respiratory epithelium.

Unlike influenza virus, CPV is not dependent on NA for efficient replication. Therefore, any beneficial effects are not due to direct antiviral action. Studies in humans have demonstrated a significant decrease in the development of bacterial infections secondary to influenza. This effect may be due to reduced bacterial penetration of the mucin layer of respiratory epithelial cells, a process dependent on the action of NA. It has been postulated that oseltamivir may also inhibit penetration by similar bacteria of the mucin layer of the intestinal epithelial cells. This inhibitory effect would decrease bacterial translocation and the consequences thereof; endotoxaemia, sepsis, systemic inflammatory response, and multiple organ failure. However, based on the current literature the role of oseltamivir in treating parvovirus enteritis remains speculative.

Granulocyte colony-stimulating factor (G-CSF)

G-CSF has not been proven to effectively treat canine parvovirus neutropaenia. In fact, G-CSF levels increase during the neutropaenic phase and naturally decline upon resolution of leukopaenia.

Early enteral nutrition is the only treatment that improves patient outcomes.

Prevention

The first step is maternal immune protection, which provides the newborn with passive immunity lasting about 10 days.

The most effective vaccine immunity is obtained using modified live vaccines (attenuated virus). It is generally recommended that primary vaccination be performed at 8 to 9 weeks of age, with revaccination every 3 to 4 weeks up to the age of 14 to 16 weeks. Subsequently, revaccination is recommended at 1 year of age, and again every 3 years.

Hygiene and disinfection is essential in breeding facilities. Common bleach (NaClO) inactivates parvovirus following prolonged exposure (1 hour). The virus displays considerable environmental resistance.

Animals that recover maintain high antibody titres for more than 16 months.

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Distemper

Distemper

Definition

Canine distemper is a highly contagious infectious disease. Infection of unimmunised dogs with canine distemper virus (CDV) causes severe immunosuppression with multisystemic involvement, usually culminating in viral spread throughout the central nervous system (CNS). This is followed by multifocal leukoencephalopathy with progressive demyelination. Although an effective vaccination protocol has been developed for CDV, this virus remains an important pathogen of dogs.

Aetiology and incidence

Canine distemper virus (CDV) belongs to the genus *Morbillivirus*, family Paramyxoviridae, order Monnegavirales. It is a negative-sense, single-stranded, enveloped RNA virus, which causes usually lethal infections in various species of the order Carnivora. It consists of a ribonucleoprotein (RNP) complex composed of a nucleocapsid, phosphoprotein, a large polymerase protein, and RNA. Matrix protein forms the link between the RNP and the envelope, which is derived from the membrane of the infected eukaryotic cell. The envelope contains viral glycoproteins, haemagglutinin (H), and fusion proteins (F). H and F proteins regulate entry of the virus into the eukaryotic host cell. In addition, the envelope contains two nonstructural proteins: C and V.

Epidemiology

CDV was previously thought to infect species of the family Canidae. However, outbreaks have also been detected in the following families:

Felidae, Hyaenidae, Mustelidae, Procyonidae, Ursidae, Viverridae, Ailuridae, and Mephitidae.

Like other enveloped viruses, CDV is rapidly inactivated in the environment. Accordingly, infection usually requires direct contact.

CDV shows seasonal incidence, increasing during colder seasons, with temperatures of less than 4 °C representing ideal conditions. Susceptibility of puppies is greatest between 3 and 6 months of age, a period coinciding with the reduction of passive immunity prior to acquiring vaccine immunity.

Infected animals are thought to begin shedding the virus (prepatent period) around 7 days postinfection, and do so up to an estimated maximum of 90 days. Shedding begins when viral replication reaches the epithelia.

The incidence of CDV is higher in shelters (outbreaks) and in free-living dogs. Moreover, dolichocephalic dogs or breeds are more susceptible than brachycephalic counterparts, both in terms of incidence and poor prognosis.

The most commonly isolated CDV field strains, which display distinct H protein variations, are America-1, Asia-1, Asia-2, European, and Arctic.

Pathogenesis

The incubation period can vary from 1 to 4 weeks and depends on the viral strain, the age of the animal at the moment of infection, and its immunity status. The disease can vary from virtually asymptomatic to severe, with 50 % mortality.

Route of infection and propagation

Infected animals shed the virus primarily in oronasal secretions, faeces, and urine, although the role of fomites should also be borne in mind. Vertical (transplacental) transmission is possible, and can cause miscarriage or

immunosuppression in pups, which show neurological signs in the first 4 to 6 weeks of life.

Shed virus enters the host through inhalation of airborne particles. Subsequently, the virus replicates in the lymph tissue of the airways. The first targets, 24 hours postinfection, are macrophages and monocytes of the respiratory epithelium and tonsils. Viral replication occurs 2 to 4 days postinfection, after which the virus spreads via the lymphatic vessels and blood to the hematopoietic tissues during the first viraemic phase, 3 to 6 days postinfection. This gives rise to generalised infection of the mononuclear phagocyte system and lymph tissues, i.e. spleen, thymus, lymph nodes, bone marrow, gastric mucosa-associated lymphoid tissue, urothelium, lymphocytes and macrophages of the lamina propria of the gastrointestinal tract, and the Kupffer cells of the liver.

The second viraemic phase follows several days later (8–9 days postinfection), with high fever and infection of the parenchyma of all tissues. This is the point at which the virus reaches the cerebrospinal fluid (CSF) and CNS. CDV can thus be found in the cells of the urinary tract, lymphatic tissue, CNS, and the respiratory, gastrointestinal, endocrine, and vascular systems (in keratinocytes, fibroblasts, platelets, lymphocytes, bronchial epithelium, endothelium, and neuroectodermal cells). The virus spreads to the glial cells and neurons from the olfactory bulb, as well as haematogenously through the choroid plexus and the cerebral vasculature.

In some cases, the animal can recover thanks to an immune response that increases production of specific neutralising antibodies. However, CDV can persist in the uvea, CNS, lymphatic organs, and footpads. Viral persistence in the CNS can result in subsequent development of the nervous form of canine distemper. Dogs with nervous signs present with acute demyelination and usually die 2 to 4 weeks postinfection, although some can recover, with persistent myoclonus.

Immune response

Anti-CDV (IgM) antibodies are produced during the first two weeks of infection. The humoral immune response correlates with the final course

of the disease. Antiviral protection requires viral antibodies directed against the viral nucleoprotein (N) and the envelope protein, as well as the development of T lymphocyte-mediated cellular immunity. Cytotoxic T cells play a key role in removing the virus from infected cells and those in which viral antigens have been internalised.

The course of the infection depends on the immune response, with three possible scenarios:

- 1. Effective immune response:** if the first response neutralises the virus in the extracellular tissue (cytotoxic cellular response), CDV disappears by 14 days postinfection.
- 2. Intermediate immune response:** if the response is of intermediate effectiveness, as occurs in cases of delayed or incomplete immune responses, some clinical signs disappear after 2 weeks, but the virus persists in the footpads, uvea, and nervous system. This ineffective or late humoral immune response allows the development of persistent neurological disease, as well as hyperkeratosis of the footpads.
- 3. Ineffective immune response:** if the response is ineffective, the virus persists, giving rise to an acute disease course.

Immunosuppression

The acute phase of distemper is characterised by a transient decrease in CD4⁺ helper T lymphocytes, CD8⁺ cytotoxic T lymphocytes, and CD21⁺ B lymphocytes in peripheral blood. Interleukin (IL-1) is inhibited and prostaglandin E₂ release increased. The N protein of CDV exerts immunomodulatory activity, suppressing B cells, attenuating the function of T helper cells, and decreasing IgM and IgG expression. This N protein also modulates antigen presentation by dendritic cells and decreases the production of IL-12. The viral V protein is an interferon antagonist and inhibits the cytokine response.

Clinical presentation

In dogs, the clinical signs of canine distemper are gastrointestinal and respiratory, followed by CNS involvement (Boxes 1 and 2). The most common neurological sign is the development of myoclonus. The absence of this clinical presentation significantly complicates diagnosis.

Up to 50 % of cases may involve subclinical infection. Mild to moderate cases, which present with fever, depression, anorexia and upper respiratory tract involvement are also observed. These cases may result in keratoconjunctivitis sicca and permanent anosmia.

Systemic signs

Transient fever is observed 3 to 6 days postinfection, and is accompanied by anorexia, moderate depression, oculonasal discharge, and tonsillitis. Lymphopaenia and leukopaenia may be observed during this first febrile viraemic phase.

Initial clinical signs are followed by a more pronounced clinical manifestation that varies depending on the system predominantly affected. Biphasic fever is a characteristic symptom. The second fever peak occurs 10 days postinfection. In the acute phase, the virus is found in all bodily secretions and excretions. This phase is accompanied by skin rash, serous nasal discharge, ocular discharge, conjunctivitis, anorexia, vomiting, an initially dry cough that becomes productive, and both dehydration and emaciation (Fig. 1). Gastrointestinal and respiratory signs are complicated by secondary bacterial infections and neurological alterations. Nervous signs may be observed from 20 days postinfection.



Figure 1. Puppy with fever, depression, and cachexia due to distemper.

Neurological signs

These usually develop 1 to 3 weeks after the resolution of systemic signs, although both signs can overlap. A gap of several months between systemic and neurological signs is unusual.

Neurological signs are progressive and even recurrent, and depend on the areas of the nervous system affected by the virus. The following neurological signs can be observed: hyperaesthesia, cervical or paraspinal rigidity, myoclonus, nystagmus, circling behaviour, head tilt, ataxia, seizures, dementia, postural alterations, and tetraparesis or quadriplegia.

Distemper leukoencephalitis

- » CDV penetrates the CNS via the bloodstream, primarily through the choroid plexus, and, less commonly, via neural pathways including the nasal epithelium, cribriform plate, olfactory nerve, and olfactory bulb. This results in subsequent development of acute, subacute, or chronic encephalitis. The course of the disease depends on the inflammatory response and antigen expression.
- » Inclusion body polyoencephalitis may be observed either postvaccination or in dogs that exclusively present neurological signs.

» One component of the clinical picture is demyelinating leukoencephalomyelitis (DL). Recent years have seen considerable advances in the understanding of the pathogenesis of this disease, specifically axon-myelin-glia interactions and the endogenous mechanisms of regeneration and astroglial plasticity. DL caused by distemper is characterised by demyelinating lesions, with varying degrees of mononuclear infiltration, accompanied by dysregulation of cytokines, matrix metalloproteinases (MMP), and MMP inhibitors. The initial axonal injury represents the beginning of a progressive lesion, which precedes demyelination.

Axonopathy may trigger disruption of myelin-axon-glia interactions. Studies have established that demyelination is secondary to the axonopathy. This observation contradicts the established dogma, which holds that demyelination constitutes the primary event.

» Another unexpected finding is the expression of p75 neurotrophin receptor (NTR); bipolar cells positive for NTR are detected in distemper-induced DL. p75 NTR is a prototypical marker of immature Schwann cells. While the hypothesis remains unproven, NTR expression may constitute an endogenous remyelination and regeneration mechanism.

Respiratory signs

The respiratory phase is characterised by serous or mucopurulent nasal discharge (rhinitis), interstitial pneumonia, and necrotising bronchiolitis, which is often complicated by suppurative bronchopneumonia caused by secondary bacterial infections.

Digestive signs

Enteral infection causes catarrhal enteritis with a decrease in Peyer's patches, and presents with anorexia, vomiting, diarrhoea (which may become haemorrhagic), and tenesmus. These alterations can lead to dehydration and emaciation.

Dermatological signs

The vesicular and pustular dermatitis that affects dogs with distemper is known as distemper dermatitis. It affects the thighs, the ventral abdomen, and the inner aspect of the pinna, and is characterised by hyperkeratosis, parakeratosis, vesicles, pustules, and the presence of giant multinucleated syncytial cells.

Other signs

In animals with subclinical or subacute infection other signs such as nasal hyperkeratosis, hyperkeratosis of the footpads, and dental enamel hypoplasia may be observed. Hardening of the footpads is a rare cutaneous manifestation of distemper characterised by hyperkeratosis. The nasal plane also tends to be affected. Neonatal infections affect the development of permanent teeth, as CDV infects ameloblasts, resulting in enamel hypoplasia, partial eruption, or oligodontia. In certain cases this manifestation presents with cardiomyopathy.

Infection of the osteoclasts of the physis of long bones is known as metaphyseal osteosclerosis and presents with growth retardation (persistence of the primary spongiosa accompanied by bone modelling defects).

CDV antigen may be detected in the synovial fluid of some animals with rheumatoid arthritis.

Dogs with nervous signs present acute demyelination and usually die within 2 to 4 weeks postinfection, although some recover and are left with persistent myoclonus that can last for 40 to 50 days or persist indefinitely.

Box 1. Clinical signs in patients with distemper.

- » Dolichocephalic breed*
- » Oculonasal secretion
- » Dry/productive cough
- » Dyspnoea
- » Vomiting
- » Diarrhoea
- » Tenesmus
- » Anorexia
- » Lethargy/depression
- » Fever
- » Dehydration
- » Wasting

- » Osteosclerosis
- » Enamel hypoplasia
- » Oligodontia
- » Conjunctivitis
- » Uveitis
- » Chorioretinitis
- » Blindness
- » Exanthema
- » Hyperkeratosis
- » Seizures
- » Hyperaesthesia
- » Paresis or paralysis
- » Ataxia
- » Circling behaviour
- » Myoclonus

* Negative prognostic value.

Diagnosis

Clinical diagnosis

The typical clinical picture consists of respiratory signs, with or without digestive signs, and neurological signs (myoclonus) in unvaccinated puppies. Accordingly, distemper should be suspected in puppies that present with fever and multisystemic clinical signs.

Diagnostic imaging

Thoracic radiographs usually reveal an interstitial pattern that evolves to an alveolar pattern when secondary bacterial pneumonia is established (Fig. 2).

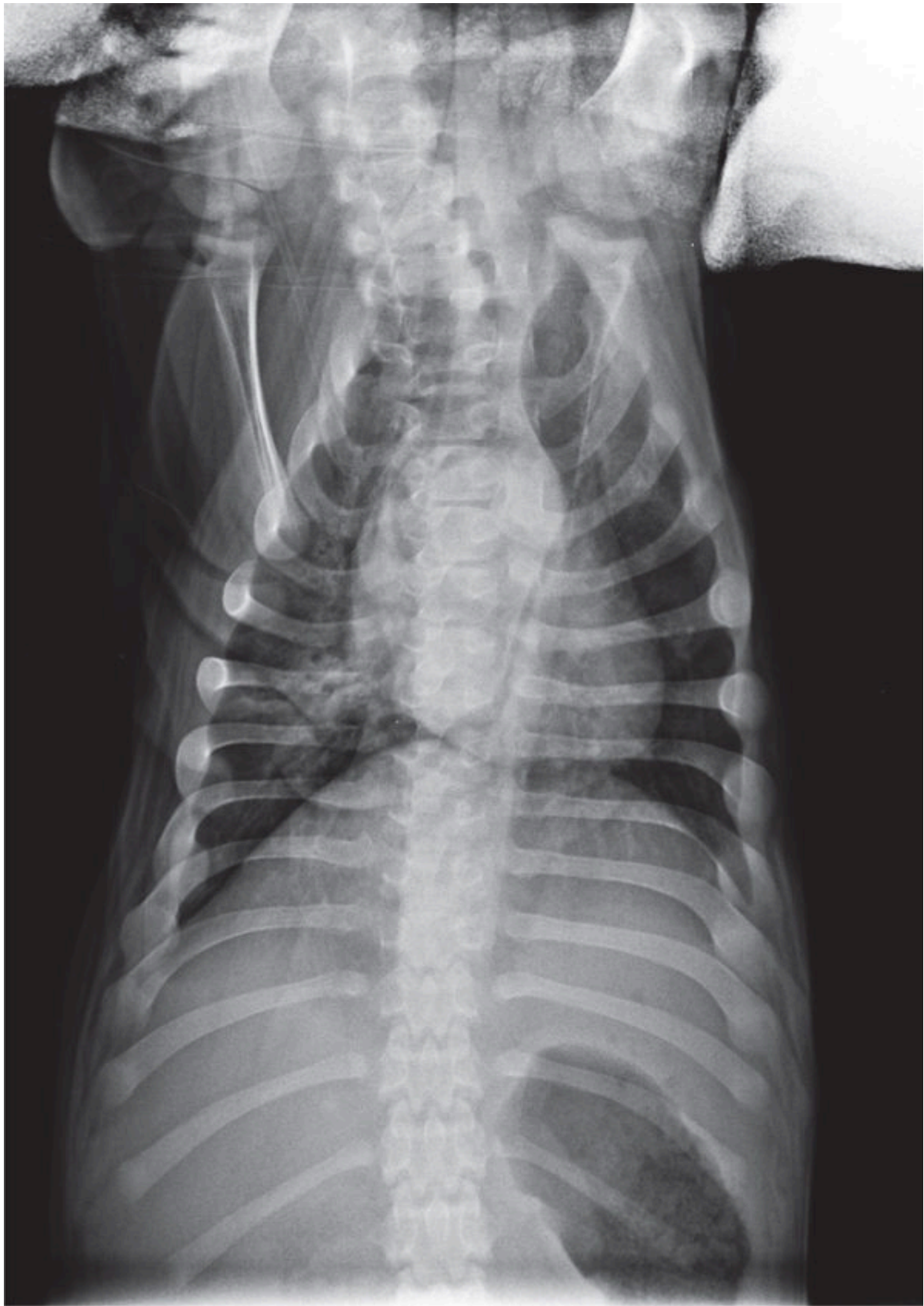


Figure 2. Simple thoracic radiograph in a case of pneumonia due to distemper.

Laboratory diagnosis

The most characteristic sign in laboratory analyses is lymphopaenia during the viraemic phase. Neutrophilic leukocytosis may be observed with progression of the disease. This is accompanied by anaemia and thrombocytopaenia. The presence of CDV inclusion bodies in lymphocytes, neutrophils, monocytes, and erythrocytes has been described.

Box 2. Laboratory tests: alterations in distemper patients.

Blood tests

- » Anaemia
- » Lymphopaenia
- » Neutropaenia
- » Neutrophilia with left shift
- » Thrombocytopaenia
- » Hypoalbuminaemia
- » Hyperglobulinaemia*

Cerebrospinal fluid analysis

- » Lymphocytic pleocytosis
- » Mixed pleocytosis
- » Presence of inclusion bodies
- » Presence of anti-CDV antibodies
- » Increased protein concentration

* Neonatal infection.

Serum biochemical analyses reveal hypoalbuminaemia and hyperglobulinaemia (Box 2).

Table 1 shows the haemogram results of a puppy with distemper and parvovirus infection.

Table 1. Haemogram results for a puppy with distemper and parvovirus infection.

Haemogram	Results	Reference values
Erythrocytes ($\times 10^6$ / μ l)	5.33	5.5-8.5
Hematocrit (%)	35.2	37-55
Haemoglobin (g/dl)	11.9	12-18
MCV (fl)	66.7	62-77
MCH (pg)	22.3	21.5-26.5
MCHC (g/dl)	35.3	33-37
Reticulocytes (cells/ μ l)	9,700	-
Normoblasts (%)	-	-
Leukocytes (cells/ μ l)	400	6,000-17,000
Lymphocytes (cells/ μ l)	250	1,000-4,800
Segmented neutrophils (cells/ μ l)	90	3,000-11,500
Band neutrophils (cells/ μ l)	0	0-300
Eosinophils (cells/ μ l)	10	100-1,250
Monocytes (cells/ μ l)	40	150-1,350
Platelets (cells/ μ l)	205,000	200,000-500,000

Serological tests

The benchmark test is the serum neutralisation test. Early detection of anti-CDV antibodies (IgG) in an animal that fulfils the clinical criteria is unreliable, as antibody titres can be high as a result of vaccination or infection. Moreover, the acute phase of the disease is associated with immunosuppression, which can result in low antibody titres. Vaccine-induced titres may persist for months. Anti-CDV IgM levels are a more accurate means of detecting the disease during the acute phase. However, these antibodies may be produced in response to vaccination (primary vaccination only).

Specific ELISA can detect IgM against the virus (specifically against the viral N protein), which can serve as a marker of recent infection and persists for at least 3 months postinfection. It is advisable to determine seroconversion in order to establish the diagnosis.

CDV ELISA can be used to determine the titre of neutralising antibodies in order to evaluate the vaccine protection status. A titre greater than or equal

to 1:16 or 1:20 is considered indicative of protection against CDV.

Specifically, a CSF:serum anti-CDV IgG ratio of >1 is indicative of CDV encephalitis.

[CSF anti-CDV IgG:serum anti-CDV IgG] $>1 \rightarrow$ CDV encephalitis

Direct diagnosis

The most widely used diagnostic test is direct immunofluorescence (DIF) to detect the presence of the virus in scrapings (conjunctival, nasal, or vaginal), leukocyte concentrate, bone marrow, CSF, or body fluids. This test is more useful during the first three weeks postinfection, after which the virus disappears from the epithelia. FID can thus produce false negative results in subacute and chronic cases.

RT-PCR of blood, urine, CSF, tonsil, or conjunctiva samples appears to show the highest sensitivity and specificity for diagnosis of distemper. This greater sensitivity also enables earlier diagnosis of the disease.

Other PCR techniques can produce false positives in recently vaccinated animals (3 weeks). This false positive will not occur in cases of vaccination using recombinant canarypox virus.

Post mortem diagnosis

Definitive diagnosis of canine distemper can be established upon necropsy by immunohistochemistry, using samples from the CNS, nasal mucosa, tonsils, spleen, lymph nodes, footpads, skin, stomach, duodenum, lung, or bladder, depending on where lesions are observed.

This technique is of limited sensitivity for monitoring the evolution of encephalomyelitis; because the immune response can result in elimination of the virus from demyelinating inflammatory lesions, false negative results are possible. For definitive *post mortem* diagnosis of canine distemper, the analysis of CNS samples can improve the sensitivity of RT-PCR while maintaining specificity.

To establish a definitive diagnosis, it is advisable to perform RT-PCR using samples of blood, secretions, or tissues taken from a suspected case.

Treatment

General

Canine distemper treatment is intended to control the associated clinical signs (Fig. 3). This involves antibiotic treatment and support therapy, including:

- Keeping the patient in a warm and secluded environment.
- Cleaning oculonasal secretions.
- Treating with expectorants or nebulisation, as well as massage with gentle, rapid pats (*coupage*) for pneumonia.
- Fluid therapy for dehydration, vomiting, and diarrhoea.
- Antibiotic treatment with ampicillin, cephalexin, or doxycycline.
- Antiepileptic medication: phenobarbital, diazepam, or levetiracetam.



Figure 3. Intensive treatment in a case of coinfection with parvovirus and distemper.

Botulinum toxin

Botulinum toxin is used in interventional neurology in human cases. It provides safe and effective treatment for symptomatic cases of strabismus, blepharospasm, hemifacial spasm, spastic torticollis, and myoclonus. Given that distemper-induced encephalitis characteristically presents with

myoclonus, the effects of the administration of botulinum toxin into the affected muscles have been investigated. Clinical use of this treatment is limited and transient adverse effects such as postinjection hyperthermia and weakness have been described. This treatment involves the administration of injections in the muscles affected by myoclonus, repeated periodically until clinical signs cease, or every 3 to 6 months (after which the effect of the administered toxin is lost). The dosage varies greatly, depending on the size of the muscle or muscle group to be treated, and there is no standard dose. Doses ranging from 0.3 IU/muscle up to 45 IU/muscle have been employed.

Ribavirin and interferon

Ribavirin (RBV), an analogue of the nucleoside 1-(β -D-ribofuranosyl)-1,2,4-triazole-3-carboxamide, exerts inhibitory effects on a wide variety of DNA and RNA viruses. RBV is a commercially available compound with known antiviral action against several members of the Paramyxoviridae family. The mechanism of intracellular viral inactivation involves the initial conversion of RBV to ribavirin-5'-monophosphate (RMP), a reaction catalysed by adenosine kinase. RMP is a potent inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH), and causes a decrease in the production of intracellular guanosine triphosphate (GTP). The resulting imbalance promotes the substitution of GTP with alternative nucleotides, increasing the frequency of viral mutation and the production of defective genomes. At the cellular level, RBV can reduce protein translation and RNA synthesis during viral replication, and can interfere with viral RNA capping. Despite poor selectivity and divergent outcomes, RBV has been used in several experimental protocols for the treatment of subacute sclerosing panencephalitis measles in humans.

Interferons (IFNs) are proinflammatory cytokines that are synthesised by the immune system in response to viral infections. Interferons enable development of a state of resistance against viruses and play an important role in modulating the adaptive immune system. An important element of the antiviral response is the production of type I interferons, especially interferons α and β (IFN- α/β). IFN- α displays antiviral, antiproliferative, and immunomodulatory effects, and promotes the production of CD8⁺ T

lymphocytes. Some members of the genus *Morbillivirus* inhibit interferon signalling pathways and block the action of IFN-I (α/β) and IFN-II (γ). The V, C, and N proteins of *Morbillivirus* viruses suppress transcriptional responses induced by IFN-I and IFN-II.

IFN- α and RBV show comparable antiviral efficacy against CDV in cell culture. In addition to the individual abilities of these two compounds to inhibit the intracellular stage of the viral life cycle, if used in combination their antiviral activity is also effective in the extracellular phase of the replicative cycle.

While these compounds exert powerful inhibitory effects on the intracellular stage of viral replication, their combined effects, which result from viral inhibition via intracellular and extracellular pathways, are poorly understood.

Alteration of the replicative cycle of CDV using a combination of RBV and IFN- α has the greatest therapeutic efficacy. These drugs, alone and combined, show considerable clinical promise.

Flavonoids

Phenolic compounds are plant metabolites with therapeutically useful pharmacological properties, owing to their antioxidant, anti-inflammatory, antimutagenic, antineoplastic, or antibacterial activity. Both flavonoids and phenolic acids display antiviral activity. Antiviral activity against CDV in cell culture has been demonstrated for several flavonoids and phenolic compounds, including quercetin, morin, rutin, and hesperidin (flavonoids), and the phenolic acids cinnamic acid, transcinnamic acid, and ferulic acid.

Fucoidans

Fucoidans are complex sulfated polysaccharides extracted from the edible brown alga *Cladosiphon okamuranus*. Fucoidans show antiangiogenic, anti-inflammatory, anticoagulant, and antiviral pharmacological properties. Analyses performed in cell culture have shown that fucoidan has antiviral properties, which are highly selective for CDV.

When establishing treatment for distemper it should be noted that the general prognosis ranges from guarded to unfavourable.

Prevention

Elimination of the virus requires an effective humoral and cellular immune response on the part of the infected animal. The first step is maternal immunity, which provides the newborn with passive immunity lasting about 10 days.

The most effective vaccine immunity is conferred using modified live vaccines (attenuated virus).

Occasionally, immunocompromised animals can develop postvaccination distemper. This is usually caused by natural infection, which develops before sufficient protection has been conferred, as opposed to reversion of the vaccine virus to a virulent strain. Vaccination of females with modified live virus during the puerperal period can cause systemic disease or encephalitis in puppies. The estimated incidence of postvaccination distemper is between 1:10,000 and 1:50,000, depending on the strain administered.

Several vaccine strains are available (Onderstepoort, Snyder Hill, and Rockborn), as well as a recombinant vaccine containing canarypox virus expressing CDV fusion protein and haemagglutinin. The recombinant virus is considered the safest for use during the puerperal period (and in other species) because the viral vector cannot replicate efficiently.

It is generally recommended that primary vaccination be performed at 6 to 8 weeks of age, and repeated every 3 to 4 weeks up to the age of 16 weeks. Subsequently, revaccination should be performed after one year and again every 3 years.

Several studies have examined vaccine efficacy: one such study estimated that an interval of approximately 30 days after vaccination is required for the development of neutralising protective antibodies, which then persist for over 2 years. However, more recent studies have proposed that protection (neutralising antibodies) is acquired within 2 weeks and persists for 9 years.

Hygiene and disinfection are essential preventive measures in breeding facilities, as is isolation of patients.

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Canine infectious tracheobronchitis

Canine infectious tracheobronchitis

Definition

Transmissible (or infectious) canine respiratory disease has many names, including kennel cough, canine infectious tracheobronchitis, and canine infectious respiratory disease complex. It is an acute infectious disease that affects the upper respiratory tract of the dog and can become complicated, resulting in BP or bronchopneumonia.

Aetiology and incidence

The aetiology of this disease is very complex, given the many agents implicated. The first descriptions of this disease date back to the 1960s, and identify the causative agent as canine adenovirus type 2 (CAV-2). However, with advances in diagnostic techniques, infections and coinfections with other viruses and bacteria have been identified.

Bacterial pneumonia (BP) is an inflammatory disease of the lower airways and lung parenchyma secondary to bacterial infection. BP is difficult to induce experimentally in healthy animals. Owing to the many predisposing factors involved, the pathogenesis of this disease is complex. The development of BP is dependent upon swallowing alterations (aspiration pneumonia), decreased ciliary function, or immune deficiency.

The role of canine respiratory viruses in the development of BP, and the possible connection between BP and canine infectious respiratory disease complex (CIRDC), have not been fully elucidated. CIRDC is an infectious respiratory disease of multifactorial aetiology, in which both viruses and pathogenic bacteria are implicated. The first viruses detected and identified in the 1960s were canine parainfluenza virus (CPiV) and canine adenovirus type 2 (CAV-2). Other viruses have since been implicated in the aetiology of this disease, including canine herpesvirus (CHV), whose role remains

controversial, canine respiratory coronavirus (CRCoV), canine influenza virus (CIV), and canine pneumovirus (CnPnV), the role of which remains unclear.

Bacterial pathogens that contribute to the aetiology of CIRDC include *Bordetella bronchiseptica* , *Mycoplasma cynos* , and *Streptococcus equi* subsp. *zooepidemicus*.

Canine distemper virus (CDV) is another important respiratory pathogen that causes severe systemic disease, and induces respiratory alterations.

Bacterial and viral coinfections of the respiratory tract are well documented in humans. Epidemiological data and laboratory studies support the conclusion that respiratory viruses predispose infected individuals to the development of secondary infections. The virus destroys the respiratory epithelium and facilitates bacterial adhesion. Moreover, viral infection regulates the expression of molecules that act as bacterial receptors. In addition, immunosuppression induced by the virus promotes infection. Concurrent or prior infections with viruses implicated in CIRDC are considered a possible aetiological factor in canine BP.

As described in humans, it is very likely that respiratory viruses are a major aetiological component of canine BP, although this has yet to be demonstrated.

Type 2 canine adenovirus (CAV-2)

CAV-2 is a naked DNA virus of the genus *Mastadenovirus* , family Adenoviridae. It replicates in the respiratory epithelium and tonsils of dogs, and causes acute laryngotracheitis. It is shed 1 to 2 weeks postinfection and displays long-lasting resistance both in the environment and on fomites. It is considered a major aetiological agent of CIRDC.

Type 1 canine herpesvirus (CHV-1)

Experimental infection with CHV-1 causes rhinitis, tracheobronchitis, keratitis, and conjunctivitis. Involvement of this virus is thought to exacerbate respiratory signs. The virus can remain latent and be shed during periods of stress, immune deficiency, or exposure to glucocorticoids. It

shows limited resistance in the environment, and its role as an aetiological agent in CIRDC is controversial.

Canine influenza virus (CIV)

This is an enveloped RNA virus that is classified according to the subtypes of the surface glycoproteins it expresses (haemagglutinin [H] and neuraminidase [N]). This virus was first isolated from greyhounds with haemorrhagic pneumonia (H3N8 CIV). The virus tends to propagate around 7 to 10 days postinfection, and displays little environmental resistance. Initially detected in the Americas, CIV has since been shown to be distributed worldwide. Its prevalence in the US is considered high. CIV is thought to induce moderate respiratory signs, unless bacterial complications ensue.

Canine parainfluenza virus (CPiV)

Enveloped RNA virus of the genus *Rubulavirus*, family Paramyxoviridae. This virus replicates in the upper respiratory epithelium of the dog, resulting in asymptomatic or mild infection, in the absence of coinfection. It is shed 8 to 10 days postinfection and has little environmental resistance. Given its incidence and contagiousness, it is a very important aetiological agent.

Canine respiratory coronavirus (CRCoV)

This is an enveloped RNA virus of the genus *Betacoronavirus*, family Coronaviridae, group II. Alone, this virus is thought to cause subclinical or mild respiratory infections. It is usually detected in dogs of less than 1 year of age, and displays limited environmental resistance. It is not considered a major aetiological agent of CIRDC.

Canine pneumovirus (CnPnV)

This enveloped RNA virus of the genus *Pneumovirus*, family Paramyxoviridae, was identified relatively recently (United States, 2010). While its pathogenicity has not been fully determined, this virus appears to be associated with CPiV and CIV infections.

Bocavirus

The genus *Bocavirus* belongs to the subfamily Parvovirinae, and consists of small, nonenveloped, single strand DNA viruses, with an icosahedral capsid. The genus is named for its two first members: bovine parvovirus (BPV) and canine minute virus (CMV). The aetiological involvement of Bocavirus in CIRDC remains unclear.

Bordetella bronchiseptica

B. bronchiseptica is a pleomorphic Gram-negative bacterium. It is distributed worldwide and affects diverse species of domestic animals. It infects the respiratory tract and is a major aetiological agent of CIRDC.

Mycoplasma cynos

This is the only *Mycoplasma* species linked to canine respiratory disease. It is found in the ciliated and nonciliated respiratory epithelia (including the lower airways). It has been isolated in several cases of BP, together with other aetiological agents. As such, its ability to induce pathological changes by itself is a source of debate.

Streptococcus equi subsp. *zooepidemicus*

This is a commensal bacterium of the upper respiratory tract of horses. It has been detected in dogs with respiratory infections that present with bloody nasal discharge and subsequent haematemesis. It manifests with fever and can be serious, and in some cases fatal. Its role as an aetiological agent of CIRDC is better demonstrated than that of other species that appear in BP cultures.

Epidemiology

CIRDC has a worldwide distribution. Incidence is highest in communities, e.g. refuges, shelters, and breeding facilities. Outbreaks also occur in dogs

that live with their owners and go to parks, contests, or events where they have contact with other dogs (Fig. 1). Clinically healthy dogs can carry and shed pathogens.

It is the most common respiratory disease of dogs, regardless of vaccination schedule, as vaccine immunity is incomplete. It affects dogs of all ages and does not confer natural immunity. These characteristics may be due to its multiple and complex aetiologies.

Three main aetiological agents of CIRDC have been identified: CAV-2, CPiV, and *B. bronchiseptica* . Other viruses and bacteria implicated in this condition are considered secondary agents, at least from the point of view of frequency of presentation in epidemiological studies. Coinfections may be less frequent than expected, or may go unnoticed in the event that the causative agent is undetectable at the moment of disease detection. The following data have been reported in three published epidemiological studies:

- A prospective study of 68 dogs with CIRDC performed in Japan in 2008 found that 10.3 % of dogs were positive for *B. bronchiseptica* , 7.4 % for CPiV, 5.9 % for CRCoV, 2.9 % for CAV-2, and 1.5 % for CDV. All dogs tested were negative for both CIV and *Bocavirus* .
- A prospective study conducted in the US in 2015 performed PCR analyses of ocular and oronasal samples taken from 503 asymptomatic dogs. A total of 240 asymptomatic dogs (47.7 %) were positive for at least one pathogen implicated in CIRDC, 12.7 % were positive for 2 pathogens, 3.8 % for 3 pathogens, 1.8 % for 4 pathogens, and 0.8 % for 5 pathogens. The most frequently detected pathogens were *Mycoplasma cynos* (29.2 % of cases), *B. bronchiseptica* (19.5 %), CAV-2 (12.5 %), CDV (7.4 %), and CPiV (3.2 %). Other pathogens detected included CIV (2 dogs), CHV-1 (4 dogs), CRCoV (9 dogs), and *S. zooepidemicus* (9 dogs). These findings indicate that new pathogens implicated in CIRDC are rare in asymptomatic dogs, although in general the percentage of dogs positive for CIRDC pathogens is high.
- A prospective study conducted in Germany in 2014 analysed healthy dogs and those with clinical signs of respiratory infection to determine the prevalence of CPiV, CAV-2, CIV, CRCoV, CHV-1, CDV, and *B. bronchiseptica*. Of the dogs that showed signs of respiratory disease 37.7 % were positive for CPiV, 9.8 % for CRCoV, and 78.7 % for *B.*

bronchiseptica . Coinfection was observed in 47.9 % of cases positive for *B. bronchiseptica* , 82.6 % of cases positive for CPiV, and 100 % of cases positive for CRCoV. Of the healthy dogs studied, 1.1 % were positive for CAV-2, 7.8 % for CPiV, and 45.6 % for *B. bronchiseptica* . The two microorganisms that were significantly more common in dogs with respiratory signs were *B. bronchiseptica* and CPiV.



Figure 1. Outbreaks of kennel cough can occur in any scenario involving contact with other dogs (Liga Gabrane, Shutterstock.com).

Pathogenesis

Transmission of infection occurs through direct contact and via aerosols and fomites. Objects and materials can become contaminated. These include clothing, drinkers, feeders, cages, and rooms.

The incubation period of the viral infection is less than two weeks, and can be as little as 2 to 3 days. Viral replication occurs in alveolar, nasal,

pharyngeal, laryngeal, tracheal, bronchial, and bronchiolar epithelia. The virus is predominantly localised in the larynx, trachea, and bronchi. Replication can also occur in tonsillar lymph tissue and in regional lymph nodes.

Shedding of the virus can begin before the appearance of clinical signs, but peaks during symptomatic disease. Differences in the duration of viral shedding are observed depending on the aetiologic agent involved.

Respiratory virus infections can be asymptomatic or complicated, and even fatal in rare cases. Symptomatic or complicated infections tend to be observed in immunosuppressed dogs and puppies, in which coinfection can exacerbate the clinical picture.

Bordetella bronchiseptica is transmitted via the airborne route, by direct contact, and via fomites. It is highly contagious. Following inhalation, the virus attaches to the cilia of the respiratory system, evades the immune system, and produces toxins that damage the respiratory epithelium. Adhesion of virulent strains of *B. bronchiseptica* to respiratory cilia is mediated by fimbrial adhesins (FIM), filamentous haemagglutinin (FHA), pertactin, and cell wall lipopolysaccharides. *Bordetella* colonisation factor A (BCFA) is another outer membrane protein that plays an important role in tracheal colonisation. The capsule of *B. bronchiseptica* protects against phagocytosis and destruction mediated by the complement system. The toxin adenylate cyclase, which is secreted by the bacteria, catalyses the conversion of ATP to cAMP, resulting in the accumulation of the latter in host cells and thereby inhibiting the migration and activation of phagocytes and T lymphocytes. Pertactin helps the microorganism avoid elimination.

The inflammatory response, combined with cellular dysfunction, increases the secretion of fluid and mucus. This limitation of the immune response facilitates coinfection with viruses and other bacteria.

The shedding of *B. bronchiseptica* begins 2 to 10 days postinfection, but can persist intermittently for one to several months (Fig. 2).

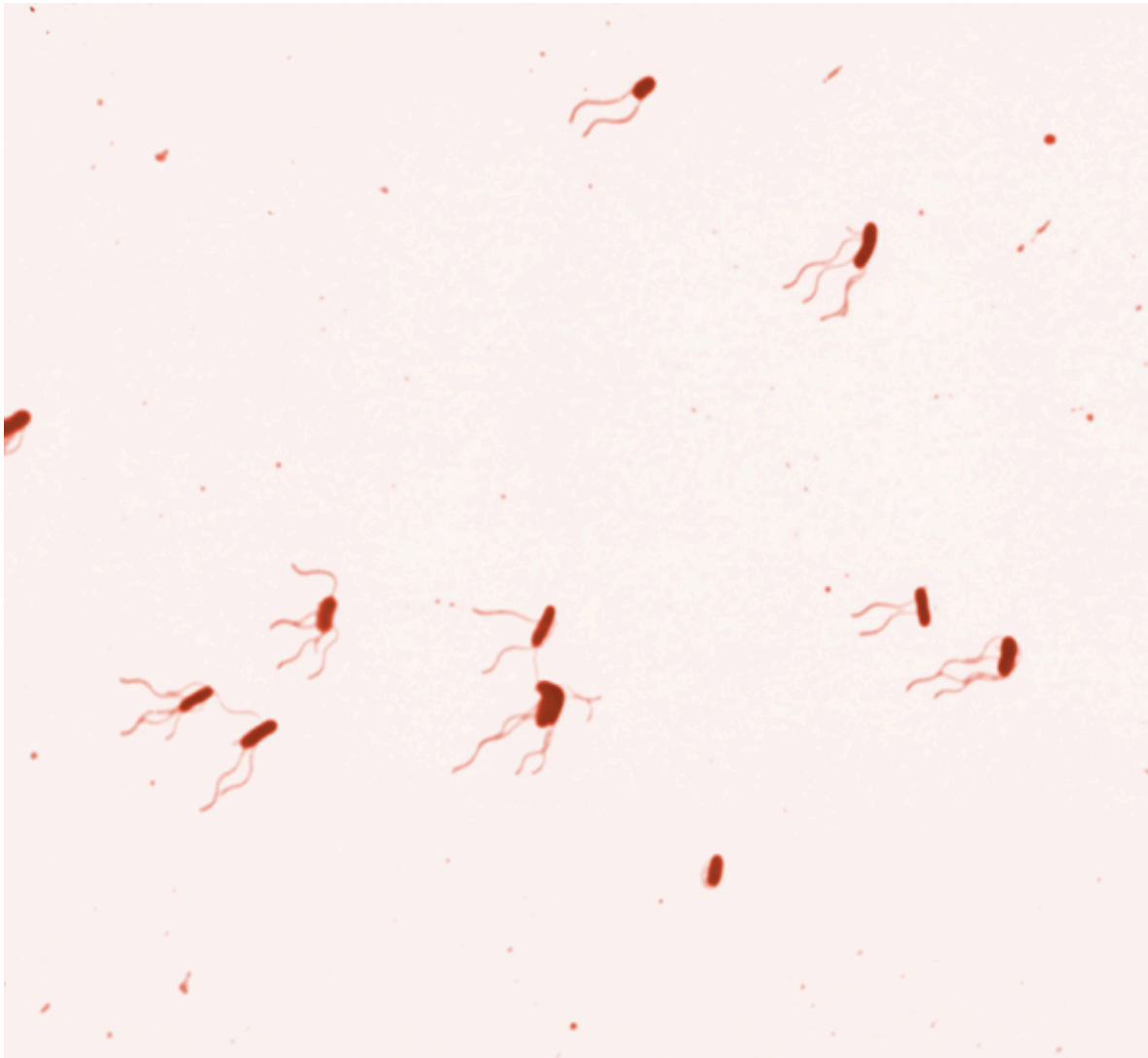


Figure 2. *Bordetella bronchiseptica* . Stained Leifson flagella (digitally coloured) (CDC / Dr. William A. Clark).

Clinical presentation

The clinical picture is milder in dogs that have been vaccinated (Box 1).

Box 1. Clinical signs of CIRDC with and without aetiological complication.

Clinical signs in patients with uncomplicated disease

- » Serous nasal discharge
- » Conjunctivitis
- » Fever
- » Dry/cavernous cough

Clinical signs in patients with complicated disease

- » Fever
- » Depression
- » Anorexia
- » Tachypnoea
- » Cough
- » Mucopurulent nasal/ocular discharge

The most common clinical presentation is self-limiting, in the absence of complication, and consists of a dry paroxysmal cough with hyperthermia, caused by the underlying viral infection. Affected dogs tend to be alert and active, and maintain their appetite. They may produce serous nasal discharge, accompanied by sneezing and a usually cavernous cough. A positive tracheal reflex is usually observed.

Moderate cases may present with a productive cough or reverse sneezing and dysphonia in cases involving laryngitis and tonsillitis.

The clinical signs associated with *B. bronchiseptica* infection vary in severity depending on the bacterial strain involved, host immunity, and the presence of coinfections. The incubation period ranges from 2 to 10 days, and clinical signs appear between 3 and 10 days after exposure. Tracheobronchitis and rhinitis may present with serous or mucopurulent nasal discharge, sneezing, and rales, with persistent, usually paroxysmal, coughing. In some dogs, tracheobronchitis presents with bronchial pneumonia, fever, productive cough, lethargy, and decreased appetite.

Complicated presentations are seen in immunosuppressed dogs and puppies, in which viral and bacterial coinfection occurs. The cough becomes productive and mucopurulent nasal discharge is observed. The infection progresses to bronchopneumonia or pneumonia, with systemic signs of fever, depression, and anorexia. Cases involving pneumonia and bronchopneumonia are generally associated with bacterial infection or coinfection. Dyspnoea and tachypnoea are characteristic, as are lung sounds (rales and stridor) upon auscultation.

The presence of keratitis is usually indicative of CHV-1 infection. In some cases CIV infection is associated with the development of pneumonia.

Diagnosis

Clinical diagnosis

In most cases diagnosis is established based on the clinical presentation. Diagnostic tests are not usually performed, except in complicated cases or outbreaks that occur in communities, in which case it may be appropriate to try to establish an aetiological diagnosis.

The diagnostic approach should take into account the emergence of an acute respiratory process, primarily involving signs of tracheobronchitis, where a possibility of infection exists. Diagnosis of CIRDC in other dogs in the immediate environment is a key indicator.

It is crucial to consider age and vaccination status in order to rule out the possibility of early stage distemper. In any case, the evolution of the process will facilitate confirmation of the presumptive diagnosis.

Diagnostic imaging

Thoracic radiographs generally reveal a diffuse bronchial or bronchointerstitial pattern, which evolves to an alveolar pattern if secondary BP is established (Fig. 3).

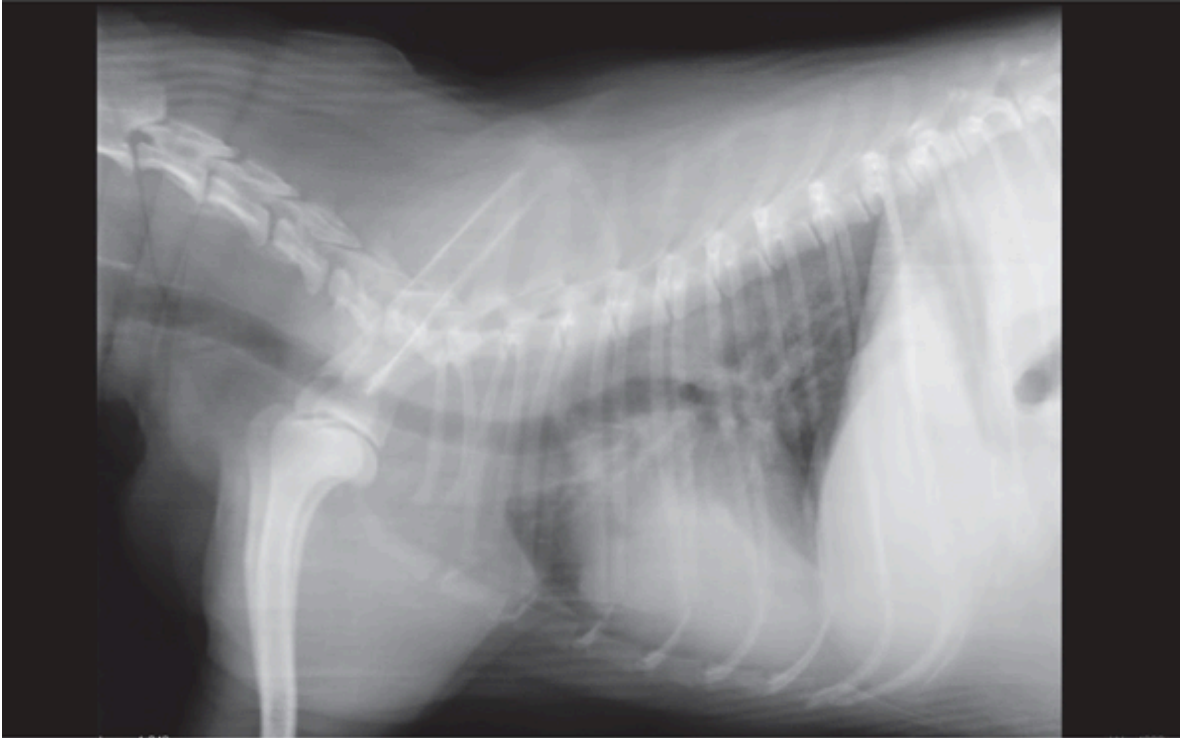


Figure 3. Thoracic radiograph showing a complication in a case of CIRDC with signs of pneumonia.

Laboratory diagnosis

No characteristic signs are observed in the haemogram, serum biochemistry, or urinalysis. Moderate neutrophilia may be observed. Neutrophilia with left shift and toxic changes in neutrophils may be observed in patients with bronchial pneumonia and BP. A haemogram should be performed in persistent cases or those involving more severe clinical signs, such as anorexia, depression, purulent discharge, dyspnoea, and high or persistent fever. Leukopaenia may develop in severe cases.

In cases involving complication with pneumonia or bronchopneumonia, it is advisable to perform a transtracheal or bronchoalveolar lavage for cytology, culture, and antibiogram (Figs. 4 and 5).



Figure 4. Transtracheal lavage procedure.

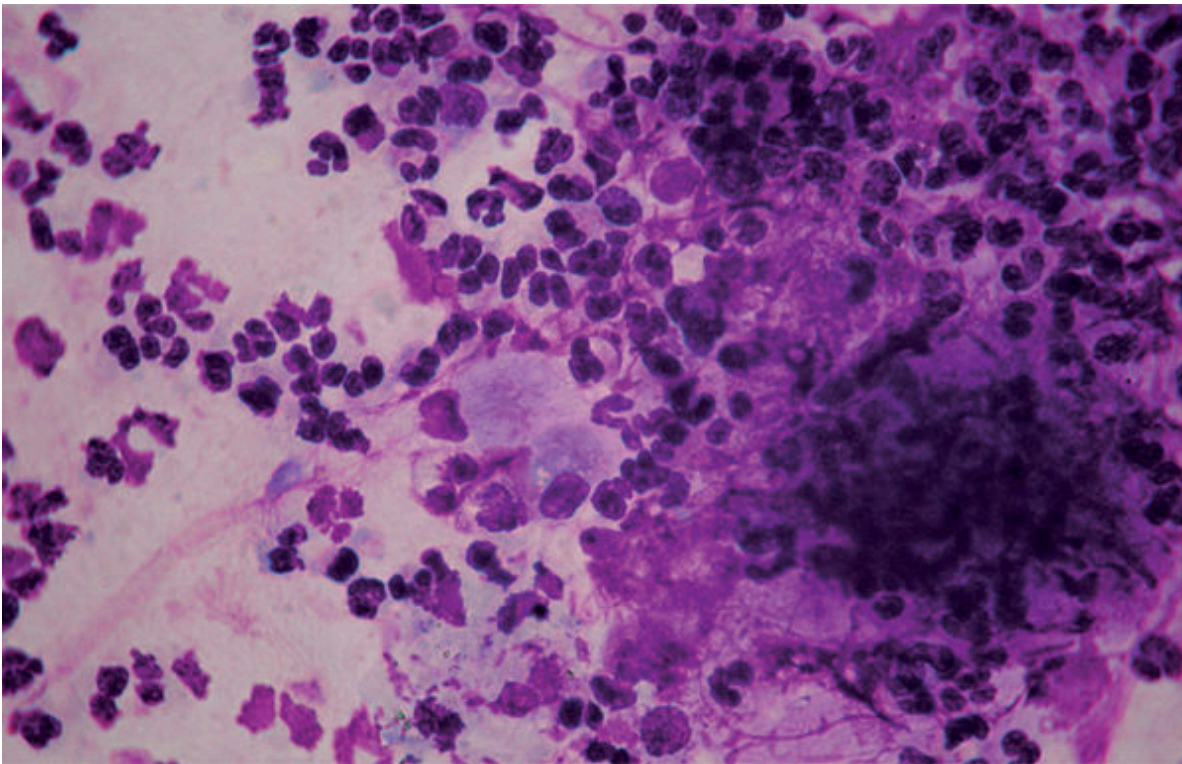


Figure 5. Transtracheal lavage cytology, with signs of purulent inflammation, mucus, and bacteria.

Serological tests

The benchmark test is the serum neutralisation test. It can be used to detect antibodies against CAV-2, CPiV, CIV, and CHV-1.

Exposure to CIV can be determined by ELISA, although the sensitivity and specificity of this technique are unknown.

ELISA for *B. bronchiseptica* is difficult to interpret, as a positive result can be observed following either virus exposure or vaccination, and does not necessarily indicate active infection.

Direct diagnosis

For isolation of the virus samples are obtained from the nasal, conjunctival, pharyngeal, and respiratory tract epithelium. However, this entails the use of complex and expensive techniques. Moreover, the use of attenuated virus vaccines can give rise to false negative and false positive results.

PCR techniques have been developed for some, but not all, viruses, and also suffer from potential false negatives, due to sample degradation and the detection of vaccine viruses. Aetiologic diagnosis of viral infection is justified for epidemiological studies and, where appropriate, can be applied to decide upon a vaccination strategy in communities or breeding facilities.

Isolation of *B. bronchiseptica* (like other bacteria) is achieved in aerobic bacterial culture, although false negative results are possible, particularly if antibiotics have been administered. A better approach is to obtain the sample by transtracheal or bronchoalveolar lavage, as sample collection by nasal or oropharyngeal swab complicates the interpretation of culture results due to the presence of commensal flora in these locations. PCR can also be employed.

Apart from epidemiological analyses or preventive strategies, the main reason to isolate *B. bronchiseptica* is to determine its sensitivity profile and antimicrobial resistance.

Post mortem diagnosis

Information pertaining to this type of diagnosis is primarily derived from cases of CIV-infected greyhounds that developed haemorrhagic pneumonia

prior to death.

Treatment

Therapeutic intervention is not required in most mild cases, which are generally self-limiting. Antitussives can be prescribed in cases involving a paroxysmal dry cough that persists for more than 1 or 2 weeks, but are not prescribed for productive coughs. It is often useful to replace the dog's collar with a harness to prevent stimulation and persistence of the cough.

Antibiotics (usually doxycycline) should be administered in cases that do not resolve and when bacterial infection is suspected or confirmed (Table 1). This will not help to eliminate the viral infection, but is commonly prescribed to treat or prevent opportunistic bacterial infections. Doxycycline has some advantages, including a broad antibacterial spectrum and a low incidence of resistance in *B. bronchiseptica* , but has the disadvantage of being a bacteriostatic agent, without bactericidal properties. Bacterial resistance is relatively frequent. As such it is advisable to perform bacterial culture and an antibiogram. In *B. bronchiseptica* infections resistance to antimicrobials such as amoxicillin and trimethoprim-sulfamethoxazole is relatively common.

Severe cases, involving bronchial pneumonia or BP, require supportive care with fluid therapy, massage with gentle, rapid pats (*coupage*), oxygen therapy, and nebulisation.

Table 1. Antimicrobials for oral administration.

Drug	Dose	Administration period
Doxycycline	2.5 mg/kg every 12 hours	10 days
Amoxicillin-clavulanic acid	12.5–25 mg/kg every 12 hours	10–14 days
Azithromycin	5 mg/kg every 24 hours	5–7 days

Enrofloxacin	5 mg/kg every 24 hours	10 days
Trimetoprim-sulfametoxazole	15 mg/kg every 12 hours	10–14 days

Prevention

Vaccination can reduce the prevalence of respiratory disease in dogs. Current vaccines do not protect against all aetiologic agents or confer complete immunity. However, it is possible that infection in vaccinated dogs results only in a mild clinical process.

Many vaccines contain a combination of viruses and bacteria, while others contain only one infectious agent. Vaccines are available for CAV-2, CHV-1, CIV, and CPiV. Vaccines against CAV-2 and CPiV are available for intranasal and parenteral administration. However, studies evaluating the benefits of one route of administration over another, in terms of the duration of immunity, interference with maternal immunity, and the speed or persistence of the immune response, have produced inconclusive results. It has been suggested that intranasal vaccine administration may result in less interference with maternal immunity and induction of an earlier but less persistent immune response. Moreover, the immune response obtained with a parenteral vaccine would be more prolonged and intense.

An inactivated CIV vaccine is used in endemic countries. This vaccine is first administered at 6 weeks of age, followed by 2 revaccinations every 3 weeks and subsequent annual revaccination.

Live attenuated vaccines for *B. bronchiseptica*, administered orally or intranasally, stimulate mucosal immunity (IgA) within three days without interfering with maternal immunity, and provide protection for one year. This type of vaccine cannot be administered in conjunction with antibiotic treatment and is more suitable for puppies that will live in communities. Inactivated vaccines are administered parenterally, and provide protection from one week after the second dose. Over the years, several studies have

examined the effectiveness of vaccines against *B. bronchiseptica* in order to understand the local and systemic immune response to this bacterium. However, many of these studies have inherent limitations due to the experimental designs employed. Many questions remain as to the duration of immunity conferred by these vaccines, as well as naturally-acquired immunity. As such, there are serious doubts about the adequacy and effectiveness of these vaccines.

Several vaccines against *B. bronchiseptica* , administered parenterally, intranasally, or orally have been tested to date. The composition of these vaccines is described below.

Despite the limitations of the aforementioned studies, findings suggest that intranasally and orally administered vaccines are more effective at rapidly generating mucosal immunity than parenterally administered forms, while vaccines containing several antigens appear to be more efficacious than those containing a single antigen. The immune response induced by an intranasal vaccine is thought to develop within 72 hours of administration. However, the duration of this immunity is likely to be shorter than that produced using a parenterally-administered vaccine. Vaccine protection can be of rapid onset and short duration, and is mediated by IgA. Revaccination using a parenterally-administered vaccine is likely a more effective means of generating long-lasting immunity.

Vaccines against *B. bronchiseptica* developed for parenteral administration

- » Heat-inactivated bacteria.
- » Formalin-inactivated bacteria to which an aluminium adjuvant is added.
- » Formalin-inactivated bacteria with carbopol as an adjuvant.
- » Bacteria inactivated with glutaraldehyde.
- » Vaccine with antigenic extract of *B. bronchiseptica*.

Vaccines against *B. bronchiseptica* developed for intranasal or oral administration

- » Intranasal attenuated vaccine.
- » Intranasal attenuated parainfluenza virus vaccine.
- » Vaccine containing attenuated parainfluenza virus and adenovirus type II for oral administration.
- » Inactivated bacteria for oral administration.

The recommendations of the WSAVA (World Small Animal Veterinary Association) do not include this group of vaccines within the essential vaccination program (core vaccinations). These guidelines recommend the use of intranasal over parenteral vaccines (CPiV and *B. bronchiseptica*), with a first dose at 3 weeks of age, another 3 to 4 weeks later, and subsequent annual revaccination. Vaccination against CPiV can also be achieved using multivalent, parenterally-administered vaccines. These vaccines require a primary vaccination series using various doses, followed by annual revaccination.

In conclusion, there remain many unresolved questions as regards the effectiveness of vaccines against *B. bronchiseptica*.

In communities, cleaning and disinfection, isolation of patients, ventilation, and control of new animals introduced into the group are key to preventing disease caused by this bacterium (Fig. 6).



Figure 6. In communities, it is essential to take all possible precautions to prevent the entry of CIRDC (Menna, Shutterstock.com).

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Leptospirosis

Leptospirosis

Definition

Leptospirosis is a disease of worldwide distribution that affects humans and most domestic and peridomestic mammals. Dogs can shed leptospires in urine. The disease is considered a zoonosis.

Aetiology and incidence

Leptospirosis is an infectious disease caused by spirochetes of the species *Leptospira interrogans (sensu lato)*. These are Gram-positive, filamentous, and flexible bacteria of 0.1–0.2 μm in width \times 6–12 μm in length (Fig. 1). The taxonomy and classification of these bacteria is highly complex, as until 1989 criteria based on culture and immunoreactivity were used. The reclassification currently underway is based on genomic criteria. The initial division groups these bacteria into two classes: *L. interrogans sensu lato* (pathogenic) and *L. biflexa sensu lato* (saprophytic). Subclasses are then established based on serovars, determined according to the immunoreactivity generated by bacterial membrane lipoproteins. Serovars are in turn subdivided into serogroups, based on crossreactivity in serological tests.

Leptospirosis is caused by multiple serotypes or serovars of spirochetes of the genus *Leptospira* . Of the more than 250 serovars identified for the *Leptospira interrogans* complex, most are of unknown pathogenicity. The following serogroups often affect canine species: *Canicola*, *Icterohaemorrhagiae*, *Pomona*, *Bratislava*, *Grippityphosa*, *Autumnalis*, *Batavia*, *Hardjo*, *Australis*, *Sejroe*, and *Zanoni* . The dog is thought to act as a reservoir only for serogroups *Canicola* and *Batavia*.

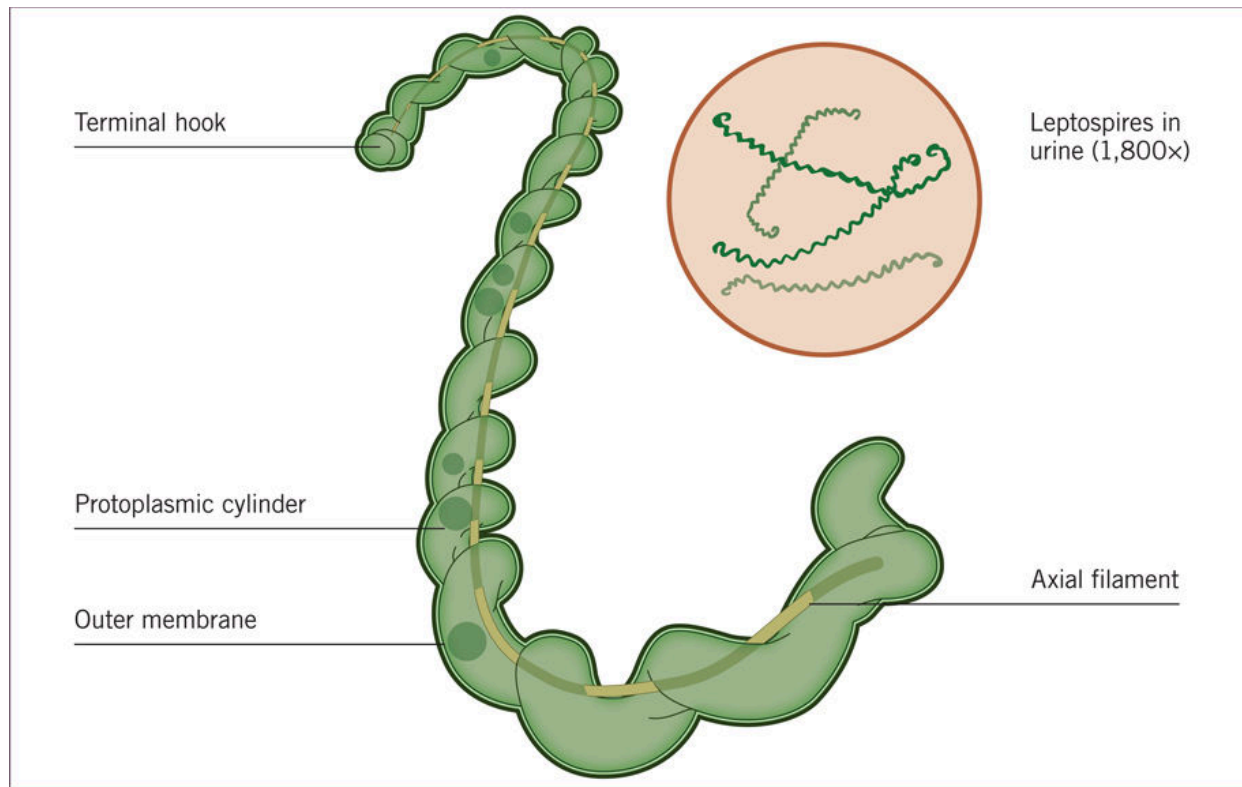


Figure 1. Ultrastructure of *Leptospira* .

Epidemiology

The bacteria can be transmitted directly between hosts through contact with urine, venereal or placental transmission, bites, or the ingestion of infected tissue. Depending on the serovar, shedding of the microorganism in urine is intermittent and persists for days or even months, as the bacteria colonises the renal tubules. It can even persist up to 4 years after the onset of infection. Indirect transmission is more frequent, and occurs via contact with contaminated water and the ingestion of contaminated food or tissue. This is the primary source of bacterial spreading. Optimal conditions of temperature (0 °C–25 °C) and humidity can lead to seasonal outbreaks.

Pathogenesis

Leptospire can penetrate the intact oral, nasal, genital, and ocular mucosae, as well as skin abrasions. They cause damage by replicating in the bloodstream and inducing inflammation. The endotoxic potential of their membrane lipopolysaccharides is considered low. The duration of leptospiraemia depends on the immune status of the affected dog.

Leptospirosis can range from subclinical to severe, with kidney, muscle, liver, vascular, spleen, neurological, genital, and ocular involvement. The cardiopulmonary and neurological involvement described in humans is not well defined in the dog. Leptospire replication initially damages the kidneys and liver, and is followed by bacterial invasion of additional organs, including the spleen, CNS, eyes, and genital tract. Genital tract involvement and miscarriages are somewhat anecdotal in dogs. Pulmonary involvement constitutes a serious complication that increases lethality. It should be borne in mind that dogs with leptospirosis can also present with vomiting and aspiration pneumonia. It is quite possible that respiratory involvement is due to the effect on lung tissue of leptospire toxins, which give rise to vasculitis and exudation. This results in interstitial pneumonia or, in the worst-case scenario, pulmonary haemorrhage (known as leptospiral pulmonary haemorrhagic syndrome). This is an emerging syndrome, the aetiology of which remains a topic of debate.

A recent study demonstrated the presence of deposits of IgG/IgM in the lung tissue of dogs with pulmonary leptospirosis, suggesting a link between the humoral immune response and the pathogenesis of pulmonary leptospirosis.

Disease progression

» Leptospirosis is relatively common in dogs and rare in cats, and was first described in dogs in the early 20th century. Initially, the most common varieties were *L. canicola*, *L. interrogans*, and *L. icterohaemorrhagiae*. Bacterins containing these serovars have been used to vaccinate the general dog population since the 1960s, and have contributed to a significant decrease in the prevalence of the disease in industrialised countries. However, a resurgence in the incidence of canine leptospirosis was observed in these same countries in the 1990s. The most commonly identified serogroups are *Pomona*, *Grippityphosa*, *Autumnalis*, *Hardjo*, *Bratislava*, and *Australis*. The geographical distributions of these serovars show considerable variability, and are the subject of several ongoing studies. The resulting data may help to improve preventive measures by allowing selection of the most appropriate vaccines for each geographic region.

Clinical presentation

While most *Leptospira* infections in dogs are subclinical, acute and (in rare cases) hyperacute forms may be observed. In general, the severity of the clinical course is greater in younger animals, large dog breeds, and dogs that live outdoors (although in the case of the latter, it is unclear whether severity or incidence is increased). The main clinical signs in **subacute infections** are nonspecific, and include loss of appetite, dehydration, weight loss, vomiting, diarrhoea, abdominal or lumbar pain, polyuria/polydipsia, tachypnoea, jaundice, lymphadenopathy, and pyrexia. It is important to note that this is accompanied by hepatic and renal dysfunction, with disorders of haemostasis.

Acute infections are characterised by a combination of the following signs: fever, muscle pain, tremor, vomiting, diarrhoea, intussusception (Fig. 2), dehydration, polyuria/polydipsia, prostration, paraspinal hyperaesthesia (of muscular origin), meningitis, nephritis, congestive mucosa, petechiae, ecchymosis, conjunctivitis, uveitis, rhinitis, tonsillitis, oliguria or anuria, cough, dyspnoea, and jaundice (Fig. 3). Some cases may involve both tissue oedema and acute disseminated intravascular coagulation (DIC), which presents with haematemesis, haematochezia, melaena, epistaxis, and purpura.

Because the microorganism replicates in renal tubular epithelial cells, colonisation of the kidney occurs in most infected individuals. The liver is the next most damaged organ during the period of leptospiroemia. Approximately 90 % of dogs show biochemical signs of lesions in the kidney, muscle, pancreas (Fig. 4), and liver. The consequences of liver disease due to leptospirosis include the development of cholestasis (Figs. 5 and 6), inflammation, and progression to chronic active hepatitis or chronic fibrosing hepatitis, which can lead to end-stage liver failure.

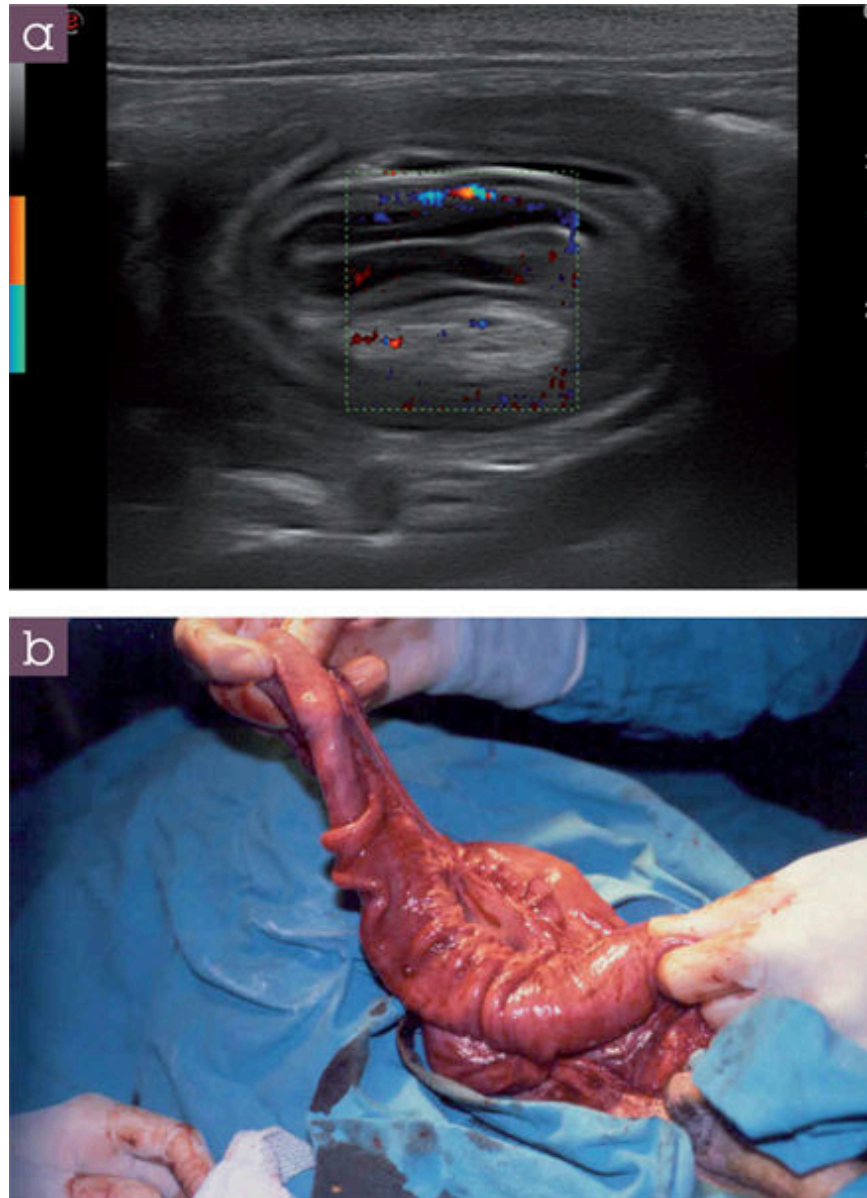


Figure 2. Abdominal ultrasound (a) and surgery (b) showing intussusception.



Figure 3. Jaundice of the oral mucosa of a dog who died due to leptospirosis.

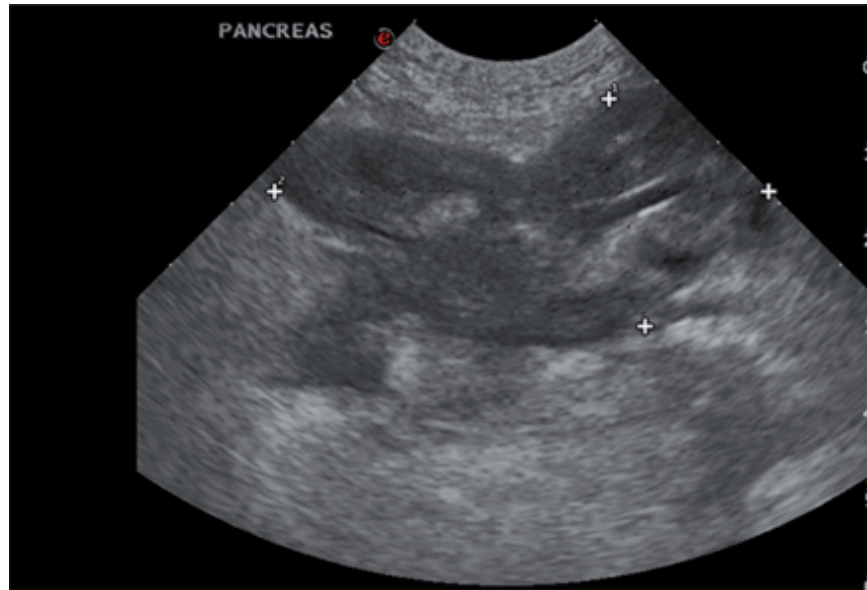


Figure 4. Abdominal ultrasound indicative of pancreatitis.



Figure 5. Abdominal ultrasound showing cholecystitis. A trilaminate structure is evident in the wall of the gallbladder.

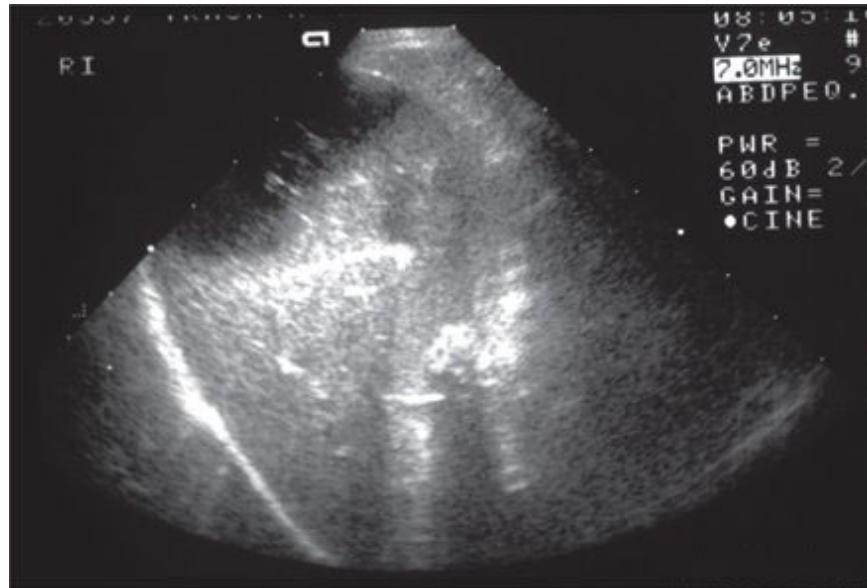


Figure 6. Gallstones in a case of leptospirosis.

The corresponding alterations in laboratory parameters are shown in Box 1 and Tables 1 to 4 .

The diagnostic protocol for animals with this clinical picture includes diagnostic imaging tests. A simple thoracic radiograph is important to determine whether the animal displays interstitial, nodular, or alveolar pulmonary patterns. Moreover, abdominal ultrasound can reveal nephromegaly, pyelectasis, increased echogenicity of the renal cortex, hepatomegaly, peritoneal effusion, pancreatitis, intussusception (rare), and lymphadenopathy.

In severe cases, electrocardiography may reveal ventricular arrhythmias, a consequence of myocarditis caused by *Leptospira* .

Box 1. Laboratory abnormalities in leptospirosis patients.

Blood test

- » Neutrophilic leukocytosis
- » Thrombocytopaenia
- » Alterations in haemostasis test
- » Hyponatraemia
- » Hypochloraemia

- » Hypokalaemia
- » Hyperphosphataemia
- » Hyperglycaemia
- » Increased levels of liver enzymes (ALT, AST, AP, LDH)
- » Hyperbilirubinaemia
- » Increased levels of bile acids
- » Increased amylase levels
- » Increased lipase levels
- » Azotaemia (urea and creatinine)
- » Increased creatine kinase (CK) levels
- » Increased C-reactive protein levels
- » Increased cardiac troponin I levels
- » Hypercholesterolaemia
- » Hyperglobulinaemia
- » Hypoalbuminaemia

Urinalysis

- » Isosthenuria or hyposthenuria
- » Glycosuria
- » Proteinuria
- » Bilirubinuria
- » Cylindruria
- » Haematuria
- » Increased urine protein/creatinine ratio

Table 1. Haemogram results in a case of leptospirosis.

Haemogram	Results	Reference values
Erythrocytes ($\times 10^6 / \mu\text{l}$)	5.47	5.5–8.5
Hematocrit (%)	33	37–55
Haemoglobin (g/dl)	12.2	12–18
MCV (fl)	59.6	62–77
MCH (pg)	22.3	21.5–26.5
MCHC (g/dl)	36.8	33–37
Reticulocytes (%)	0.3	0.5–1
Leukocytes (cells/ μl)	33,670	6,000–17,000
Lymphocytes (cells/ μl)	3,704	1,000–4,800

Band neutrophils (cells/ μ l)	337	0–300
Segmented neutrophils (cells/ μ l)	28,283	3,000–11,500
Eosinophils (cells/ μ l)	0	100–1,500
Monocytes (cells/ μ l)	1,347	150–1,350
Basophils (cells/ μ l)	0	0–200
Platelets (cells/ μ l)	645,000	200,000–500,000

Table 2. Biochemical analysis results in a case of leptospirosis.

Serum biochemistry	Results	Reference values
Glucose (mg/dl)	84.2	74–143
ALT (IU/l)	155	21–102
AP (IU/l)	1,357.35	20–156
GGT (IU/l)	40	1.2–6.4
Urea (mg/dl)	614	7.0–27
Creatinine (mg/dl)	11.83	0.5–1.5
Total protein (g/dl)	5.21	5.2–8.2
Total bilirubin (mg/dl)	15.26	0.1–0.5
Total cholesterol (mg/dl)	160	135–270
Calcium (mg/dl)	5.8	9–11.3
Phosphorus (mg/dl)	29.01	2.6–6.2
Sodium (mmol/l)	144.3	141–152
Chlorine (mmol/l)	94.1	105–115
Potassium (mmol/l)	6.99	4.1–5.35

Table 3. Protein alterations in a case of leptospirosis.

Proteinogram	Results	Reference values
Albumin (g/dl)	1.54	2.6–3.3
α_1 globulin (g/dl)	0.16	0.2–0.5
α_2 globulin (g/dl)	0.92	0.3–1.1
β globulin (g/dl)	1.81	0.9–1.6
γ globulin (g/dl)	0.78	0.3–0.8

Table 4. Urinalysis results in a case of leptospirosis.

Urinalysis	Results
Density (g/l)	1,020
pH	6.5
Glucose	Positive
Ketone bodies	Negative
Bilirubin (mg/dl)	+++
Proteins (mg/dl)	+
Leukocytes (cells/ μ l)	0
Erythrocytes (cells/ μ l)	+++

Diagnosis

Clinical diagnosis

It is important to pay close attention to the animal's vaccination history, habitat, clinical signs, and risk of exposure, as well as the time of year.

Diagnostic imaging

The diagnostic protocol should include simple thoracic radiographs and abdominal ultrasound, which is also useful for carrying out ultrasound-guided cystocentesis. In cases of pulmonary involvement, CT reveals lesions (peribronchovascular thickening, bronchial dilation, nodules, and consolidation) in all lung lobes, although the most severe lesions tend to be found in the caudodorsal lung fields.

Laboratory diagnosis

Laboratory diagnosis of the disease is very important, given that animal reservoirs have a high zoonotic potential.

In acute and subacute cases, various analyses including a haemogram, serum biochemistry, ionogram, haemostasis tests, and urinalysis will already have been carried out before reaching this point. Depending on the clinical picture, it is highly recommended to evaluate the acid-base balance, which can be done using venous blood samples in cases that present with nephropathy, but requires arterial blood samples in cases with severe respiratory involvement. Metabolic acidosis tends to occur as a result of kidney failure. Patients can display kidney, liver, respiratory, and haemorrhagic signs (alone or in combination).

The microscopic agglutination test (MAT) and polymerase chain reaction (PCR) are the two *in vivo* reference techniques.

Serological tests

The traditional reference technique is the microscopic agglutination test. This allows identification of *Leptospira* serogroups, but cannot distinguish between serovars. Laboratories can usually identify the following serogroups: *Autumnalis*, *Bratislava*, *Canicola*, *Grippotyphosa*, *Hardjo*, *Icterohaemorrhagiae*, and *Pomona*. Failure to identify a specific serogroup to which the aetiologic agent belongs may be indicative of a false negative result.

Other serological tests can also be used. Enzyme-linked immunoassay (ELISA) can detect anti-*Leptospira* IgG and IgM antibodies. IgG levels peak within the first month postinfection, while IgM levels increase one week postinfection, peaking the following week.

Latex agglutination tests allow determination of IgM levels early in the disease process (first two weeks) (Fig. 7). Indirect immunofluorescence (IIF) allows detection of IgG.

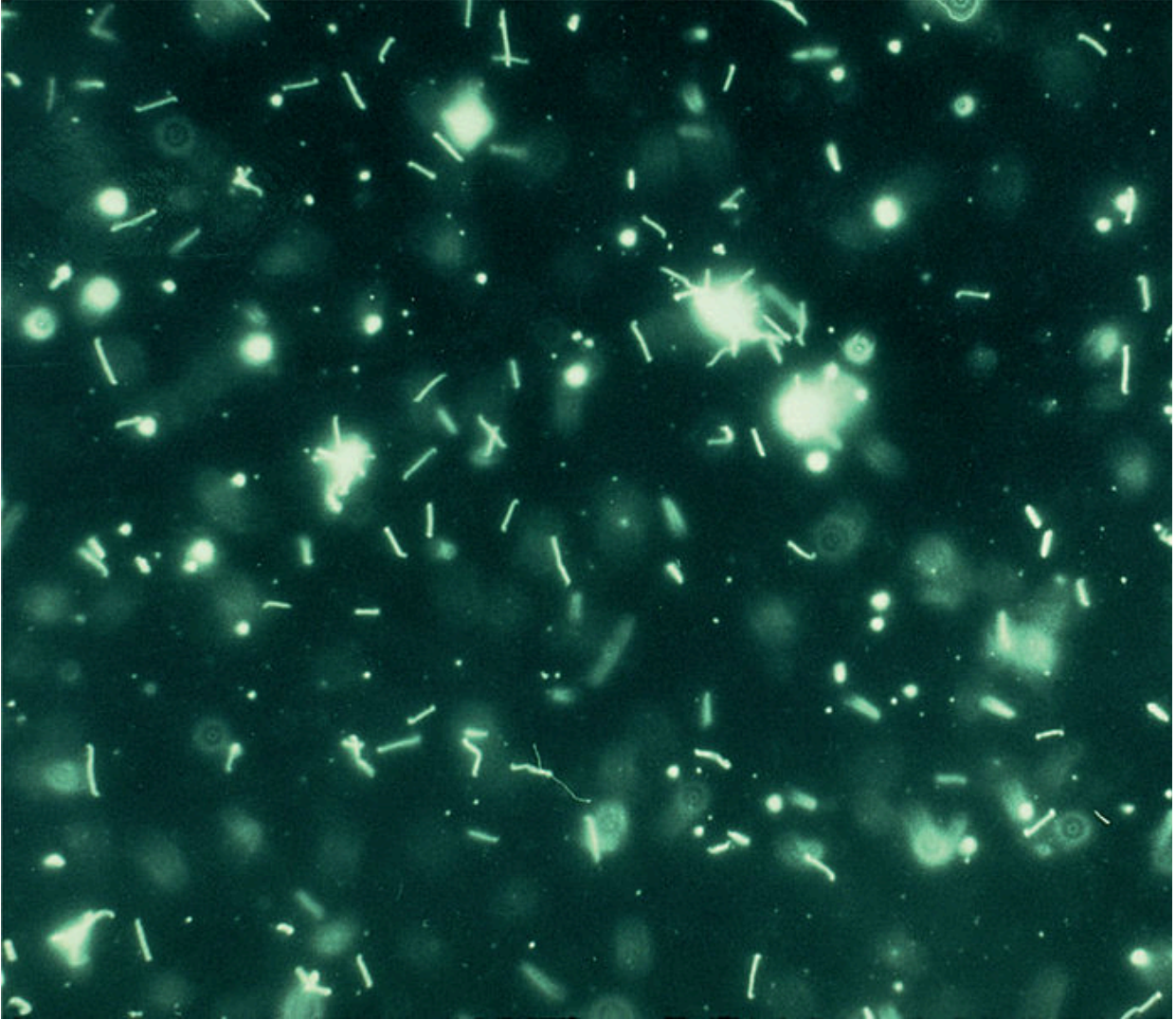


Figure 7. Darkfield microscopy image of an agglutination test with live *Leptospira* antigen (CDC / M Gatton).

Direct diagnosis

Leptospira can be cultured from samples of liver, kidney, urine, and other body fluids, providing a useful means of accurately diagnosing species identity using genetic techniques.

Observing leptospires in urine by dark field microscopy requires the presence of an enormous amount of bacteria, and is associated with a very high likelihood of false negative diagnosis.

While PCR is the ideal diagnostic techniques, the challenge lies in selecting the most appropriate sample type for analysis (blood, urine, cerebrospinal

fluid, aqueous humour). In cases of a suspected acute process, before onset of the disease, the sample of choice is blood and urine (both), which should always be collected before commencing any antibiotic therapy.

The immunoperoxidase technique, after necropsy, allows establishment of a definitive diagnosis. However, this technique may produce a negative result for certain serovars that are present in the sample but cannot be detected.

Presumptive and definitive diagnosis

A presumptive diagnosis can be established based on the animal's clinical history, habitat, clinical signs, laboratory test results, and histopathology. Sepsis caused by another infectious agent may constitute a possible differential diagnosis.

Definitive diagnosis of canine leptospirosis is established based on the sum of the following parameters: neutrophilic leukocytosis, azotaemia, hyperphosphataemia, hyperbilirubinaemia, increase in liver enzyme levels, proteinuria, isosthenuria, haematuria, and, as a necessary condition, a positive microscopic agglutination test ($>1:100$ or $1:800$) or positive PCR (blood, urine, or other fluid or tissue).

Detection of antibodies using the MAT is the most widely used diagnostic method.

However, the high prevalence of subclinical infections and the persistence of antibodies can complicate interpretation of the results. Moreover, because vaccines against *Leptospira* induce the production of antibodies, their

presence is not necessarily indicative of disease. For this reason, more sensitive and specific methods of direct diagnosis, such as PCR analysis of blood or urine, tend to be preferred.

The results of PCR analysis of urine samples from dogs with clinical signs of leptospirosis, after comparison with the results of conventional diagnostic methods, show 100 % sensitivity, 88.3 % specificity, a positive predictive value of 33 %, and a negative predictive value of 100 %. Furthermore, PCR allows diagnosis of positive cases before seroconversion, and is thus highly useful for early diagnosis of infection.

Serological tests cannot distinguish between vaccination and disease, although postvaccination titres are generally low (<1:400) and persist for less than three months. Titres >1:800 in dogs that have not been recently vaccinated are indicative of disease. In exceptional cases, some dogs develop titres of 1:1,600 and higher after vaccination. Accordingly, a positive titre threshold of 1:1,600 can be established to ensure greater specificity. Moreover, in infected dogs, antibody titres can increase 4 fold in 2 to 4 weeks (seroconversion), an alteration that can also be used to confirm the diagnosis.

In summary, evaluation of the immunisation history and results of the seroconversion test are the two keys necessary for reliable interpretation of the microscopic agglutination test.

Treatment

Leptosiraemia treatment is divided into two phases.

- The objective of the **first phase** is to prevent multiplication of the microorganism and complications of the infection, such as DIC, liver failure, and kidney failure. Stabilisation of these patients requires intensive

fluid therapy, transfusion, and infection control. Cases of oliguria and anuria require adaptation of fluid therapy protocols to treat osmotic diuresis, and although controversial, administration of dopamine or furosemide should be considered. It is important not to ignore digestive signs (e.g. vomiting and diarrhoea) that may occur. Antacid administration and even enteral nutrition may be required. In cases of severe dyspnoea ventilation can be employed. However, no effective alternatives are available in cases of severe pulmonary haemorrhage. Antimicrobial treatment is very important. Penicillin and its derivatives (penicillin G, ampicillin, and amoxicillin) are the antibiotics of choice, to be administered for three weeks starting at the moment of disease onset.

For example 20–30 mg/kg ampicillin, IV every 6–8 hours during hospitalisation.

- In the **second phase** of the disease, the goal of treatment is to eliminate the carrier state. In this case the choice of antibiotics changes. Administration of tetracyclines or macrolides for three or more weeks is recommended. Doxycycline is the drug of choice, followed by azithromycin and erythromycin.

For example: 5 mg/kg doxycycline orally every 12 hours.

Prevention

The first preventive measure is to avoid exposure to reservoirs and contaminated areas (Fig. 8). The second measure is to strengthen humoral immunity.

Inactivated vaccines contain the outer membrane proteins of leptospire, and should contain antigens from different serovars. Commercially available vaccines contain bacterins containing the following serogroups: *Canicola* , *Icterohaemorrhagiae* , *Grippotyphosa* , *Pomona* , and *Australis* . Commercial vaccines can contain up to four serogroups. Increasingly, it is

recommended to extend the protection range rather than limiting it to *Canicola* and *Icterohaemorrhagiae*. The ability to protect against *Bratislava*, *Sejroe*, and *Autumnalis* is limited, although immunity to the *Bratislava* serovar can be conferred by including the serogroup *Australis* in the vaccine.

Nonetheless, there is general agreement that vaccination can provide sufficient protection to reduce disease prevalence and severity. Another issue is the extent to which vaccination can prevent an asymptomatic carrier state and minimise zoonotic risk, as not all natural infections involve the same serogroups or confer the same degree of protection.

The duration of immunity conferred by vaccination is also a matter of debate, but is thought to exceed 12 months.

According to the criteria of the WSAVA, vaccination against leptospirosis is optional (i.e. vaccination is considered necessary only in geographical areas of risk). In conclusion, further epidemiological studies will be required to provide better insight into vaccination requirements, particularly for specific serogroups.



Figure 8. It is important to ensure that dogs do not drink water that may be contaminated (Gabriele Niepenberg, Shutterstock.com).

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Ehrlichiosis, anaplasmosis, and
rickettsiosis

Laia Solano Gallego

Ehrlichiosis, anaplasmosis, and rickettsiosis

Definition

Canine ehrlichiosis, anaplasmosis, and rickettsiosis are rickettsial diseases transmitted by ticks and caused by different species of the genera *Ehrlichia* , *Anaplasma* , and *Rickettsia* . These microorganisms can induce leukocyte and platelet abnormalities, infiltrate plasma cells in the parenchyma of various organs, induce immune complex deposition, and affect the vascular endothelium. They produce complex and variable clinical presentations that vary depending on the host's immune response and the rickettsial species involved.

Aetiology and transmission

Phylogenetic analysis of the *16S* gene sequence of the ribosomal RNA of *Ehrlichia* has led to a new classification scheme for this genus. Historically, all bacteria belonged to the genus *Ehrlichia* . Currently, four genera are described within the family Anaplasmataceae: *Anaplasma* , *Ehrlichia* , *Wolbachia* , and *Neorickettsia* (Dumler *et al.*, 2001). With the exception of those of the genus *Wolbachia* , all other microorganisms are pathogenic to mammals.

This chapter describes the species documented to date that infect dogs in Europe: *Ehrlichia canis* , *Anaplasma phagocytophilum* , and *Anaplasma platys* . The remaining *Ehrlichia* species (*E. chaffeensis*, *E. ewingii*, *E. muris* , and *E. ruminantium*) have not been described in dogs in Europe to date (Sainz *et al.* , 2015). Canine *Rickettsia conorii* infection is also described in this chapter.

Ehrlichia canis , *A. phagocytophilum* , *A. platys* , and *R. conorii* are obligate intracellular Gram-negative bacilli, which are transmitted mainly by ticks. *Ehrlichia* and *Anaplasma* form clusters of microorganisms called morulae.

Ehrlichia canis infects lymphocytes and monocytes, *A. phagocytophilum* mainly infects neutrophils and occasionally eosinophils, and *A. platys* infects platelets and megakaryocytes (De Tommasi *et al.* , 2014). *Rickettsia* invade mammalian endothelial cells, in which they subsequently multiply (Fournier and Raoult, 2009).

Ehrlichia canis is transmitted by the tick *Rhipicephalus sanguineus* . The life cycle of this tick consists of three stages: larva, nymph, and adult. Because transovarial transmission does not occur *R. sanguineus* is not a reservoir of *E. canis* . However, transstadial transmission occurs in both females and males. The most likely vector of *A. platys* is also *R. sanguineus* . However in Europe *A. phagocytophilum* is transmitted by ticks of the species *Ixodes ricinus* .

Rickettsia conorii , after infecting *R. sanguineus* , multiplies in the tick, and is found primarily in the salivary glands. Transmission of bacteria to vertebrate hosts occurs during the blood meal (Brouqui *et al.* , 2007; Parola *et al.*, 2005). *Rickettsia conorii* maintains its presence in ticks via transstadial and transovarial transmission (Socolovschi *et al.* , 2009). For this reason, ticks of all developmental stages constitute the main reservoir of *R. conorii* . The distribution of *R. conorii* is similar to that of the tick, and the seasonality of the disease mirrors that of *R. sanguineus* activity. In both humans and dogs, the disease is observed in summer (usually between June and September), when the density of *R. sanguineus* peaks (Parola *et al.* , 2005; Cascio and Iaria, 2006; Solano-Gallego *et al.* , 2015).

Transmission of these infections by blood transfusion has also been described. It is thus recommended to evaluate the possible presence of these agents in donor dogs.

Epidemiology

The geographical distribution of these microorganisms and the vector species involved in their transmission are described in Table 1 .

Prevalence of infection and disease

E. canis , *A. platys* , and *R. conorii* infections are endemic throughout the Mediterranean basin. Seroprevalence ranging from 0.2 % to 50 % has been reported for *E. canis* (Sainz *et al.* , 2015).

Few studies have used PCR to study the prevalence of *E. canis* infection, reporting prevalence rates ranging from 4 % to 22 %. The prevalence of *A. platys* infection, determined by PCR, ranges from 4 % to 70 % (Sainz *et al.* , 2015). *R. conorii* seroprevalence is generally very high, reaching 50 % to 60 % in some studies performed in Italy and Spain. However, prevalence rates as determined by PCR are much lower, and this species is only detected in dogs with a febrile presentation (Solano-Gallego *et al.* , 2015; Solano-Gallego *et al.* , 2006).

A. phagocytophilum infections are endemic in central and northern regions of Europe, including Germany and the Netherlands, and in northern Spain. The prevalence of this infection, as determined by PCR, ranges from 0.02 % to 6 %, with higher seroprevalence rates reported (1 % to 56 %) (Sainz *et al.* , 2015).

Few studies in the field of veterinary medicine have examined the seroconversion of these pathogens in dogs. A study conducted in Sicily (Italy) reported seroconversion rates for *R. conorii* , *E. canis* , and *A. phagocytophilum* of 20.7 %, 14.3 %, and 8.8 %, respectively (Solano-Gallego *et al.* , 2015). Furthermore, those authors found that the seroconversion rate for *R. conorii* in dogs with febrile illness was significantly higher than in dogs without febrile illness (Solano-Gallego *et al.* , 2015). These differences were not observed for the other two pathogens.

Risk factors

Ehrlichiosis can affect any breed. However, German shepherds, Dobermans, and Siberian huskies are especially prone to more severe clinical forms of *E. canis* infection, and therefore often have a worse prognosis (Harrus *et al.* ,

1997; Nyindo *et al.* , 1980). No age or sex predisposition has been described. Similarly, no breed, age, or sex predisposition has been described for *A. phagocytophilum* , *A. platys* , or *R. conorii* infections. Tick infestation is a common risk factor for these diseases.

The severity of these diseases can also be influenced by other factors, including immune status, coinfections with other vector-transmitted pathogens, and the presence of other concomitant diseases.

Pathogenesis

The pathogenesis of *Ehrlichia* and *Anaplasma* is complex and variable. These microorganisms can induce leukocyte and platelet abnormalities, plasma cell infiltration in the parenchyma of various organs, and immune complex deposition (uvea, renal glomerulus, etc.).

The course of infection depends on numerous factors, including the infecting species. *E. canis* infections are the best studied and known, and can be subdivided into three stages or phases (acute, subclinical, and chronic), although in clinical practice they are not easily distinguished. The duration of the acute phase is 1 to 4 weeks. Clinical signs are minimal or absent. Replication occurs in mononuclear cells. The microorganism spreads from the site of the tick bite to the lymph organs. Vascular endothelial cells are destroyed. The most important pathological findings are lymphoreticular hyperplasia and vasculitis. The acute phase can result in the appearance of clinical signs, culminating in spontaneous elimination of bacteria without the need for treatment, or progress to the subclinical phase, which can last from weeks to years, followed by the chronic phase. In the chronic phase, dogs often exhibit the typical clinical signs or pathological abnormalities associated with the disease. This is the phase during which clinical diagnosis is usually established (Figs. 1 and 2).

The incubation period for *A. phagocytophilum* infection is 1 to 2 weeks. The clinical signs or pathological abnormalities attributed to this infection occur in the acute phase. Persistent subclinical infections have not been described.

Species	Biogeographical distribution	Ticks involved	Clinical presentation	
			Main clinical signs	Laboratory abnormalities
<i>Ehrlichia canis</i>	Mediterranean basin and surrounding areas	<i>Rhipicephalus sanguineus</i>	<ul style="list-style-type: none"> ■ Nonspecific signs: fever, lethargy, anorexia, weight loss, lymphadenopathy, splenomegaly, pale mucous membranes, lameness, generalised pain, and muscle atrophy. ■ Haemorrhagic alterations: epistaxis, petechiae, ecchymosis, melaena, retinal haemorrhage, and haematuria. ■ Eye lesions and neurological signs. 	<ul style="list-style-type: none"> ■ Haematologic: thrombocytopaenia (common), variable anaemia (nonregenerative [frequent] or regenerative), leukopaenia (particularly neutropaenia), granular lymphocytosis (rare), and pancytopaenia (usually in severe cases). ■ Biochemical: hyperproteinaemia, hypoalbuminaemia, gammopathy (oligoclonal or polyclonal), elevated ALT and ALP, and renal azotaemia. ■ Urinalysis: proteinuria, decrease in urinary specific gravity. ■ Haemostatic parameters: platelet dysfunction (increased bleeding time), increased PT and PTT (rare). ■ Cerebrospinal fluid: elevated protein concentration and mononuclear, lymphocytic, or neutrophilic pleocytosis. ■ Bone marrow: cell line hyperplasia or hypoplasia (chronic forms: 15 %–20 % clinical cases) and plasma cell infiltration. ■ Synovial fluid: neutrophilic inflammation.
<i>Anaplasma phagocytophilum</i>	Central and northern Europe and northern Spain	<i>Ixodes ricinus</i>	Fever, lethargy, anorexia, weakness, and lameness.	<ul style="list-style-type: none"> ■ Haematologic: thrombocytopaenia, mild to moderate normocytic and normochromic nonregenerative anaemia, regenerative anaemia (rare), lymphopaenia, neutropaenia, and neutrophilia. ■ Biochemical: hyperglobulinaemia, hypoalbuminaemia, elevated ALP, mild hyperbilirubinaemia. ■ Synovial fluid: neutrophilic inflammation in synovial fluid.
<i>Anaplasma platys</i>	Mediterranean basin and surrounding areas	<i>Rhipicephalus sanguineus</i>	Fever, lethargy, anorexia, pale mucous membranes, and lymphadenomegaly.	<ul style="list-style-type: none"> ■ Haematologic: thrombocytopaenia, mild to moderate normocytic and normochromic nonregenerative anaemia. ■ Biochemical: hyperglobulinaemia and hypoalbuminaemia.
<i>Rickettsia conorii</i>	Mediterranean basin and surrounding areas	<i>Rhipicephalus sanguineus</i>	High fever.	<ul style="list-style-type: none"> ■ Haematologic: thrombocytopaenia, mild to moderate normocytic and normochromic nonregenerative anaemia, leukocytosis, and leukopaenia. ■ Biochemical: increased C-reactive protein, hypoalbuminaemia, decreased total iron, elevated ALP, and hyperglobulinaemia.

ALT, alanine aminotransferase; ALP, alkaline phosphatase; PT, prothrombin time; PTT, partial thromboplastin time.

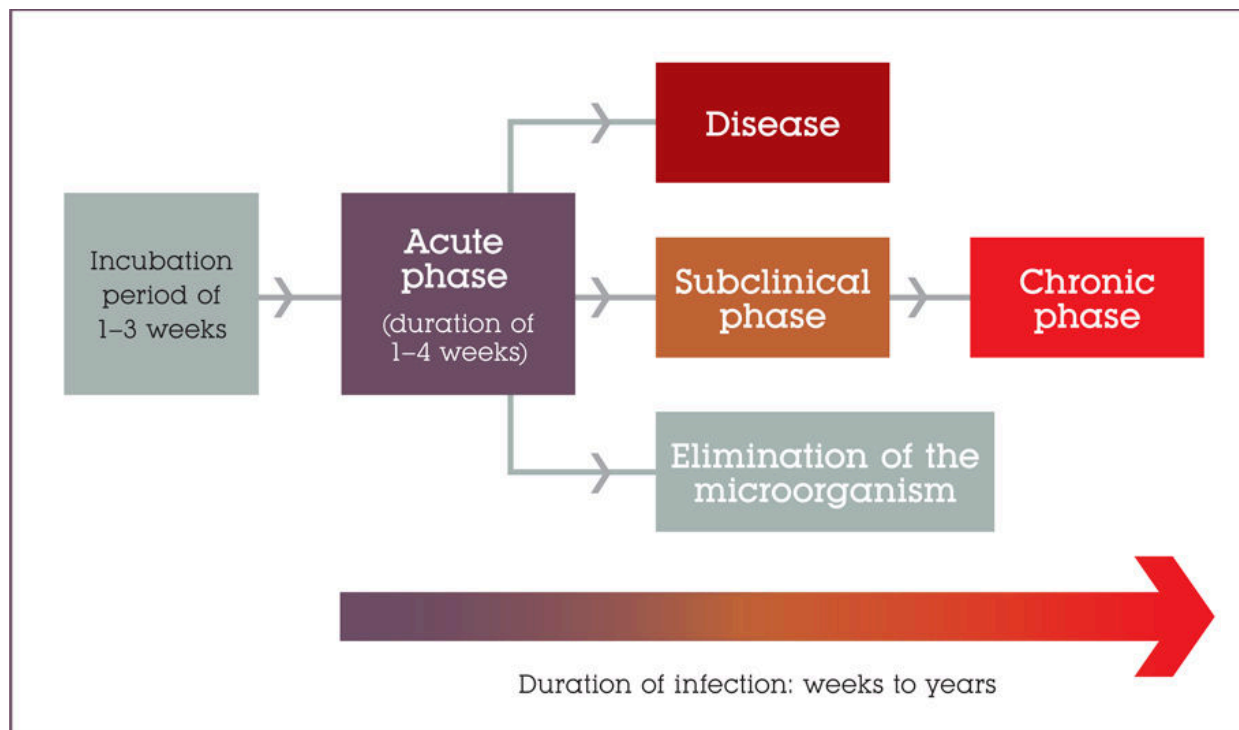


Figure 1. Stages of *E. canis* infection.

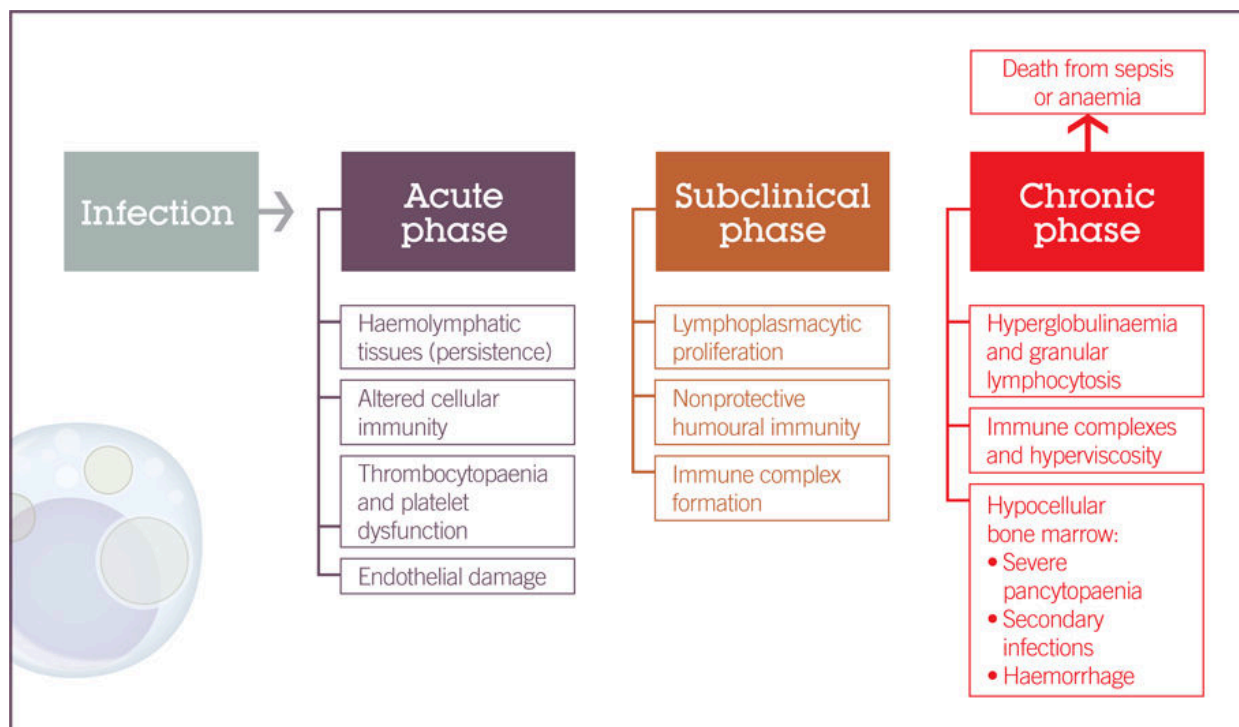


Figure 2. Pathogenesis of ehrlichiosis.

Clinical presentation

The clinical presentation can vary in severity depending on the causative species of *Ehrlichia* or *Anaplasma*, the immune response of the dog, and the presence of other debilitating diseases or concurrent infections with other tick- or flea-borne pathogens. In general, *E. canis* appears to produce a more severe clinical presentation than *A. phagocytophilum*, *A. platys*, or *R. conorii*. It is important to note that all of these infections can resolve spontaneously.

Table 1 shows the main clinical signs, and laboratory abnormalities for ehrlichiosis, anaplasmosis, and rickettsiosis.

Ehrlichia canis

The most common clinical signs in the acute phase are fever, conjunctival erythema, lymphadenopathy, and spontaneous haemorrhage (petechiae and ecchymosis). The most common laboratory abnormalities seen in the acute phase are thrombocytopaenia, leukopaenia, and in some cases monocytosis or leukocytosis. Morulae are rarely detected in blood smears.

In the subclinical stage, *E. canis* is found in the tissues. Affected dogs show no clinical signs and appear healthy, but may show laboratory abnormalities such as thrombocytopaenia, nonregenerative anaemia, leukopaenia, and hyperproteinaemia with hypergammaglobulinaemia. The subclinical phase can progress to pancytopaenia. This phase can last from weeks to years.

The chronic phase is that which is classically associated with disease. The clinical signs of chronic *E. canis* infection are highly variable and nonspecific, and include fever, lethargy, anorexia, weight loss, lymphadenopathy, splenomegaly, pale mucous membranes, peripheral oedema, lameness, generalised pain, muscle atrophy, and joint inflammation (polyarthritis). A tendency to bleed is observed, manifesting as epistaxis, petechiae, ecchymosis, melaena, retinal haemorrhage, or haematuria. Physical examination may reveal ocular lesions such as anterior uveitis and retinal diseases including chorioretinitis, papilloedema, retinal perivascular infiltrate, bullous retinal detachment, and acute blindness. Neurological signs (caused by inflammation or bleeding) may be observed. These include seizures, ataxia, paresis, vestibular disease, anisocoria, tremors, and hyperaesthesia.

The main laboratory abnormalities are thrombocytopaenia, mild normocytic and normochromic anaemia, hyperglobulinaemia with polyclonal gammopathy, and hypoalbuminaemia.

Anaplasma phagocytophilum

Anaplasmosis caused by *A. phagocytophilum* is an acute disease, which in dogs gives rise to less varied and potentially less serious clinical signs. In fact, some authors have suggested that self-limiting infections are common (Carrade *et al.* , 2009).

Clinical signs are nonspecific: fever, lethargy, anorexia, weakness, and lameness (polyarthritis). Less common clinical signs include diarrhoea, vomiting, and respiratory signs (cough and breathing difficulties). Haemorrhage is rarely observed in this infection, and the existence of central neurological signs is a matter of debate (Sainz *et al.* , 2015).

The following are the most commonly observed alterations in laboratory parameters, listed from mild to moderate: normocytic and normochromic nonregenerative anaemia, lymphopaenia, thrombocytopaenia, and hypoalbuminaemia.

Anaplasma platys

The most common clinical signs include fever, lethargy, anorexia, pale mucous membranes, and lymphadenomegaly. Other less common clinical signs include weight loss, petechiae, and nasal discharge.

The most commonly observed laboratory abnormalities are thrombocytopaenia, mild normocytic and normochromic nonregenerative anaemia, and hypoalbuminaemia.

Rickettsia conorii

The main clinical sign in dogs is fever, which can be quite high, even reaching 41 °C (Alexandre *et al.* , 2011; Solano-Gallego *et al.* , 2015; Solano-Gallego *et al.* , 2006). Other less frequently observed clinical signs include lameness, lethargy, anorexia, myalgia, lymphadenopathy, abdominal pain (kyphosis),

vomiting, and diarrhoea (Alexandre *et al.* , 2011; Solano-Gallego *et al.* , 2015; Solano-Gallego *et al.* , 2006).

The most common laboratory abnormalities are increased C-reactive protein, thrombocytopaenia, mild normocytic and normochromic nonregenerative anaemia, and hypoalbuminaemia (Solano-Gallego *et al.* , 2015).

Diagnosis

Parasitological and molecular diagnosis

Identification of the organism

Observation of these microorganisms by light microscopy using a blood smear or cytology depends on the species with which the dog is infected. For example, morulae of *E. canis* are rarely observed in blood smears, and more commonly detected in buffy coat smears (Mylonakis *et al.* , 2003). For this reason, direct detection of *E. canis* infection in blood smears is of low sensitivity, and should be followed by additional diagnostic tests such as serology or molecular techniques such as PCR. A higher percentage of morulae is observed (50 %) when a specialist examines cytological smears of lymph node aspirates (Mylonakis *et al.* , 2003; Mylonakis *et al.* , 2011). The detection of morulae in platelets during *A. platys* infection also appears to be of low sensitivity. By contrast, direct detection in dogs with *A. phagocytophilum* infections is higher, with neutrophilic morulae observed in 40 % to 60 % of clinical cases (Fig. 3) (Kohn *et al.* , 2008; Kohn *et al.* , 2011).

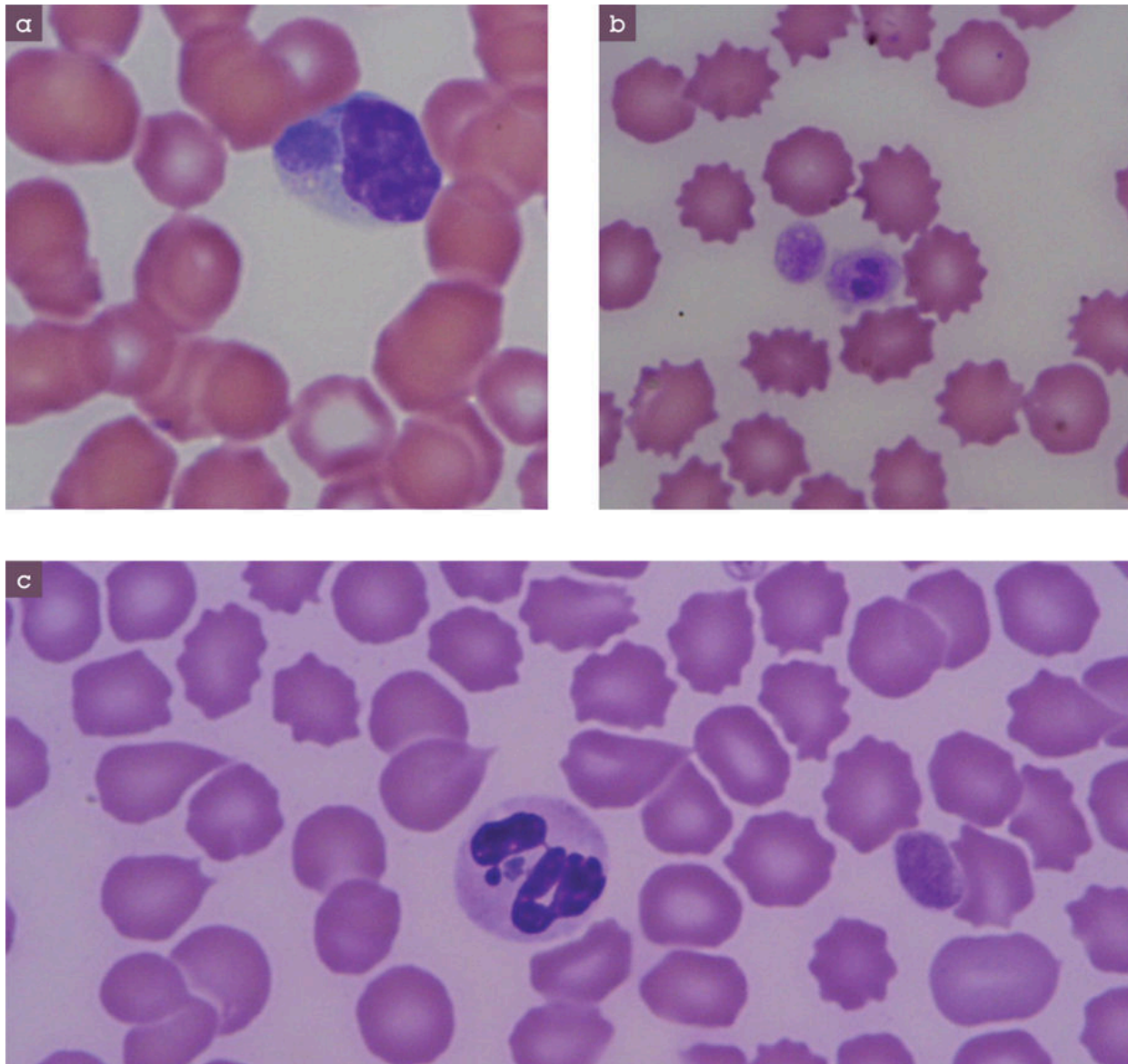


Figure 3. *E. canis* morula in the cytoplasm of a lymphocyte (100×) (a). *A. platys* morula in a platelet (100×) (b). *A. phagocytophilum* morula in the cytoplasm of a neutrophil (100×) (c).

Molecular tests

PCR is very useful for diagnosing infections caused by *E. canis* , *A. platys* , and *A. phagocytophilum* , as it is more sensitive than direct microscopic observation. Furthermore, this technique allows identification of the species infecting the dog, which is not possible by direct detection of the agent by microscopy or serology. The biological material of choice is blood, spleen, or bone marrow. PCR is used for both diagnosis and treatment monitoring.

It is important to bear in mind that false negatives can occur in these infections. This can be due to an absence of pathogens in the sample, owing either to intermittent bacteraemia, which is observed in many dogs, or prior administration of antibiotics such as doxycycline. Therefore, to improve the utility of PCR and obtain maximum information from this technique, it is important to evaluate the results together with the analysis of antibodies, clinical signs, and laboratory abnormalities (Sainz *et al.* , 2015).

DNA detection of *R. conorii* in blood is rare, likely due to the low abundance and transient presence of rickettsia in blood (Solano-Gallego *et al.* , 2015; Solano-Gallego *et al.* , 2006). Thus, while positive PCR is confirmatory for *Rickettsia* infection, a negative result does not rule out the presence of infection. In addition, molecular sequencing techniques allow the identification of the *Rickettsia* species and subspecies involved.

Serological diagnosis

The most commonly used serological methods are rapid tests, indirect immunofluorescence (IIF), and ELISA for *E. canis* and *A. phagocytophilum* infections. In general, rapid tests are very specific, but of low sensitivity, and cannot be used to determine levels of antibodies. Serology for *R. conorii* using IIF is the most widespread technique (Fig. 4).

To date, there are no specific commercial serological tests to detect antibodies against *A. platys* .

One problem that arises is that crossreactions can occur between different pathogens of the same genus. For example, strong crossreactions are observed between *A. phagocytophilum* and *A. platys* , and between *R. conorii* and *R. rickettsii* . This crossreactivity and the possibility of coinfection with different organisms can complicate interpretation of serological tests in certain geographical areas. In cases of possible vector-borne diseases, it is advisable to perform a complete panel of serological tests for the most common pathogens in a given geographical area.

Serological tests for the detection of all the pathogens described in this chapter can produce positive results, even in healthy dogs. Positive serology indicates that the dog has been exposed to the pathogen, but is not indicative of infection or disease. Similarly, negative serology does not rule out the

possibility that the dog is infected or ill. When levels of a given antibody are very high it is likely that the corresponding pathogen is the cause of the disease.

Seroconversion is very useful for the diagnosis of canine rickettsiosis, as this is an acute disease like *A. phagocytophilum* anaplasmosis or acute forms of *E. canis* or *A. platys* infection (Solano-Gallego *et al.* , 2015). It is important to determine the levels of antibodies during both the acute phase and the convalescent phase (2–4 weeks after the onset of the clinical presentation). A 2- to 4-fold increase in antibody titre compared to the initial serological titre, or transition from a seronegative to a seropositive state, confirms infection by these pathogens.

For better diagnosis of this type of disease we recommend using serology, including seroconversion analysis and PCR.

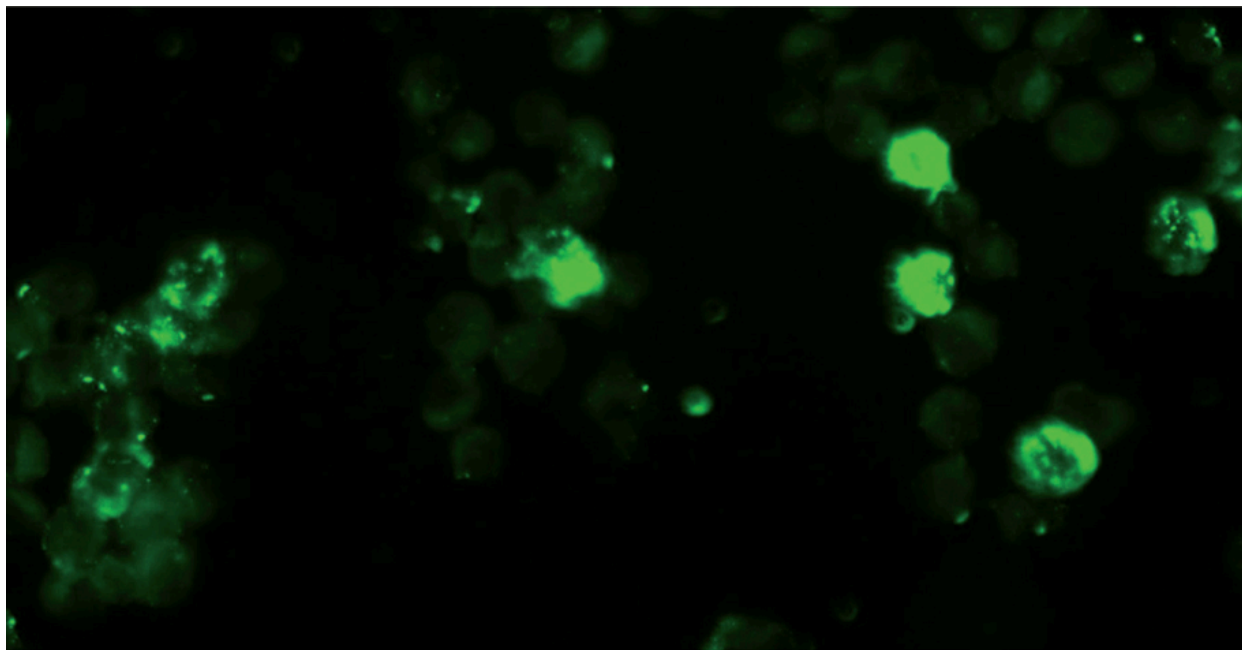


Figure 4. IgG reaction against *R. conorii* detected by indirect immunofluorescence (IIF) in a febrile and seropositive dog.

Treatment and prognosis

Doxycycline is the treatment of choice for all these diseases.

Doxycycline: 10 mg/kg orally every 24 hours, for 4–6 weeks.

The duration of treatment can be reduced (10–21 days) in cases of disease caused by *A. platys* , *A. phagocytophilum* , or *R. conorii* . If the species is unknown, longer duration therapy (4–6 weeks) is recommended.

Supportive treatment with intravenous fluids or blood transfusions are performed in patients for which they are deemed necessary.

In general, dogs with ehrlichiosis or anaplasmosis respond quickly once doxycycline treatment is instituted, and the prognosis is favourable. In the acute phase of *E. canis* infection, clinical improvement is observed within 72 hours. Antibody levels tend to decrease with time after treatment. The number of platelets normally increases 48 hours after initiation of treatment, reaching normal values within 14 days. In the chronic phase of *E. canis* infection, dogs can sometimes show an incomplete response, or no response at all. In particular, patients with severe pancytopenia caused by *E. canis* do not respond to treatment and have a poor prognosis.

Dogs infected with *R. conorii* respond rapidly to oral treatment with doxycycline, usually within 24 to 48 hours, and the prognosis is good (Solano-Gallego *et al.* , 2015; Solano-Gallego *et al.* , 2006).

Prevention

Prevention is primarily based on individual administration of topical acaricide treatments in order to reduce exposure to ticks and the transmission of pathogens to dogs (Dantas-Torres and Otranto, 2015).

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Canine bartonellosis

Laia Solano Gallego

Canine bartonellosis

Definition

Bartonellosis is a disease caused by bacteria of the genus *Bartonella* that can affect various mammalian hosts, including humans.

The clinical presentation in dogs is highly variable; subclinical infections predominate, although some dogs may develop infections ranging from mild to very severe (e.g. endocarditis).

Aetiology and transmission

Bartonella is a genus of Gram-negative bacteria that belongs to the phylum Proteobacteria, class Alphaproteobacteria, and is composed of numerous species. *Bartonella* species are well adapted to their specific mammalian host, in which they usually cause persistent intraerythrocytic bacteraemia, although they can also induce different clinical signs in domestic animals, such as dogs, and in humans (Breitschwerdt *et al.* , 2010). The first bacterium described was *Bartonella bacilliformis* , which was identified as the cause of disease in humans in 1905 (Barton, 1909). It was not until 1983 that *Bartonella henselae* , the causative bacteria of cat scratch disease, was identified (Wear *et al.* , 1983).

Since then, thanks to improvements in diagnostic tests that can detect different *Bartonella* species, these infections are recognised more frequently in dogs. The first *Bartonella* species described in the dog was *B. vinsonii* subsp. *berkhoffii* , as a cause of endocarditis (Breitschwerdt *et al.* , 1995). Table 1 shows an extensive list of *Bartonella* species that can infect dogs, which act as either the primary reservoir or as an accidental host. The *Bartonella* species most prevalent in dogs are *B. henselae* , *B. vinsonii* subsp. *berkhoffii* (main reservoir), *B. koehlerae* , *B. volans-like* , and *B. bovis* . The other species described to date are more sporadic (Sykes and Chomel, 2014).

The modes of transmission of most of the *Bartonella* species that infect dogs are unknown, although they are thought to be mediated by arthropods such as fleas, ticks, or lice (Table 1). For example, fleas mediate the transmission of *B. henselae* and *B. clarridgeiae* between cats, and serve as primary reservoirs (Breitschwerdt *et al.* , 2010).

Epidemiology

Table 1 describes reservoirs for the *Bartonella* species that most commonly infect dogs (Sykes and Chomel, 2014).

Canine bartonellosis (both subclinical infection and clinical cases) has mainly been studied and diagnosed by North American researchers and veterinary surgeons. Because it is difficult to diagnose *Bartonella* by routine blood culture, most epidemiological studies are based on the analysis of seroprevalence. In general, these infections are considered to be distributed worldwide. Seroprevalence against *B. vinsonii* subsp. *berkhoffii* ranges from 1 % to 47 %, and is higher in tropical and subtropical regions. Seroprevalence against *B. henselae* fluctuates between 1 % and 27 % (Solano-Gallego *et al.* , 2004; Solano-Gallego *et al.* , 2006). Molecular prevalence is usually lower than the corresponding seroprevalence in a given area (Sykes and Chomel, 2014).

However, there is little information available on *Bartonella* infection in dogs in Europe and the Mediterranean basin; few epidemiological studies have been performed, and few well documented cases have been diagnosed with infection caused by *Bartonella* species.

In Europe, documented clinical cases of
bartonellosis in dogs are extremely scarce (Drut *et al.* , 2014; Tabar *et al.* , 2011).

Few European studies have used molecular tests to study *Bartonella* infection. Some epidemiological studies of large numbers of dogs have reported zero infected animals, including studies conducted in Portugal (Maia *et al.* , 2015), Spain (Tabar *et al.* , 2009), and Finland (Pérez Vera *et al.* , 2014). However, other authors have documented the presence of *B. rochalimae* , *B. vinsonii* subsp. *berkhoffii* , and a new species of *Bartonella* (*Candidatus B. merieuxii* , HMD strain) in dogs from shelters in Italy and Greece (Diniz *et al.* , 2009) and in dogs with leishmaniasis in Greece (Mylonakis *et al.* , 2014).

Examples of risk factors identified in the south-eastern United States include tick exposure, living in rural environments, and stray or outdoor-living dogs (Pappalardo *et al.* , 1997) (Fig. 1). Other suggested risk factors include immunosuppression, malnutrition, and coinfections with other pathogens or debilitating diseases (Sykes and Chomel, 2014).



Figure 1. An outdoor lifestyle and the presence of ticks are risk factors for bartonellosis (Seeme, Shutterstock.com).

Pathogenesis

Bacteria of the genus *Bartonella* are highly adapted pathogens that can cause persistent intravascular infections in animal reservoirs, a strategy that allows them to evade the host's immune system and promotes zoonotic transmission via vectors. However, these bacteria can be located in other tissues such as endothelial cells (used primarily as a niche in nonreservoir animals) and

(CD34+) blast cells of the bone marrow. Infection of endothelial cells by *Bartonella* can occur more frequently in accidental hosts, and appears to be essential for the development of vasoproliferative disorders and endocarditis. *Bartonella* causes these vasoproliferative disorders by inducing cytokines, which promote the proliferation of endothelial cells while inhibiting their apoptosis. Virulence factors described in different *Bartonella* species include adhesins and the type IV secretion system (Eicher and Dehio, 2012; Pulliainen and Dehio, 2012).

Clinical presentation

The clinical manifestations of *Bartonella* infection are highly variable. Subclinical infections predominate, although some dogs can develop forms of the disease that range from mild to very severe (Table 1).

Table 1. *Bartonella* species that infect dogs, including the primary reservoir, primary vector, geographic distribution, and clinical manifestations. Adapted from Sykes and Chomel, 2014.

Species of <i>Bartonella</i>	Primary reservoir	Primary vector	Geographic distribution	Clinical manifestation
<i>B. henselae</i>	Domestic cats.	Faeces of the cat flea (<i>Ctenocephalides felis</i>).	North America, Europe (Spain, France, Greece, Italy), Israel, Gabon.	Subclinical infection (bacteraemia), fever of unknown origin, endocarditis, lymphadenitis, splenitis, pyogranulomatous or granulomatous panniculitis, splenic thrombosis, granulomatous hepatitis, hepatic peliosis, chronic erosive polyarthritis
<i>B. vinsonii</i> subsp. <i>berkhoffii</i>	Coyotes, dogs, foxes.	Unknown (Fleas, ticks?).	North America, Peru, Colombia, Brazil, Europe (Greece, Italy).	Subclinical infection (bacteraemia), endocarditis, bacillary angiomatosis, chronic erosive polyarthritis, anterior uveitis, and choroiditis.
<i>B. rochalimae</i>	Wild carnivores, dogs.	Fleas (<i>Pulex irritans</i>).	Peru, Colombia, Greece.	Subclinical infection (bacteraemia), endocarditis.
<i>B. clarridgeiae</i>	Domestic cats.	Cat flea (<i>Ctenocephalides felis</i>).	Gabon, Thailand.	Subclinical infection (bacteraemia), endocarditis.
<i>B. koehlerae</i>	Domestic cats.	Unknown.	Israel.	Endocarditis.
<i>B. quintana</i>	Humans.	Lice (<i>Pediculus humanus</i>).	Thailand, North America, New Zealand.	Subclinical infection (bacteraemia), endocarditis.
<i>B. washoensis</i>	California ground squirrel.	Unknown.	North America.	Mitral endocarditis.
<i>B. bovis</i>	Ruminants (cattle, deer).	Unknown.	North America.	Detected in some sick dogs. Undefined disease.
<i>B. elizabethae</i>	Rat.	Flea (<i>Xenopsylla cheopis</i>).	North America.	Subclinical infection, systemic clinical signs (decreased appetite, weight loss).
<i>B. grahamii</i>	Wild mouse.	Rodent fleas (<i>Ctenophthalmus nobilis</i>).	Thailand.	Subclinical infection (bacteraemia).
<i>B. taylorii</i>	Wild mouse.	Rodent fleas (<i>Ctenophthalmus nobilis</i>).	Thailand.	Subclinical infection (bacteraemia).
<i>B. vinsonii</i> subsp. <i>arupensis</i>	White-footed mouse.	Unknown.	Thailand.	Subclinical infection (bacteraemia).
<i>B. volans-like</i>	Flying squirrel.	Unknown.	North America.	Bacteraemia described in sick dogs. Undefined disease.
<i>Candidatus B. merieuxii</i> (HMD strain)	Dog, jackal.	Unknown.	Iraq, Greece, Sri Lanka.	Subclinical infection (bacteraemia), dogs with leishmaniasis.

Several different species of *Bartonella* can cause endocarditis in dogs (Fig. 2). The most commonly identified species is *B. vinsonii* subsp. *berkhoffii*, followed by *B. henselae*. However, other endocarditis-causing species have also been described, including *B. clarridgeiae*, *B. koehlerae*, *B. quintana*, and *B. rochalimae*. In dogs, *Bartonella* endocarditis generally affects the

aortic valve, although occasionally the mitral valve can be affected. Typically, these dogs are afebrile and present with congestive heart failure. The median survival time for this clinical condition is short. Complications of endocarditis include thromboembolic disease and neutrophilic polyarthrititis (Breitschwerdt *et al.* , 2010; Sykes and Chomel, 2014).

The presence of different *Bartonella* species or *Bartonella* DNA in dogs is associated with other clinical conditions. Macrophage or macrophage-neutrophil inflammation in the presence of *Bartonella* in different tissues has been documented in several clinical cases, mainly in North America. Pyogranulomatous lymphadenitis and splenitis (Friedenberg *et al.* , 2015), granulomatous hepatitis, granulomatous or pyogranulomatous systemic disease, and granulomatous and pyogranulomatous dermatitis and panniculitis, caused primarily by *B. henselae* , have all been described (Sykes and Chomel, 2014). Hepatic peliosis and bacillary angiomatosis (vasoproliferative diseases of the liver and skin) are particularly associated with *Bartonella* infection in humans, but are rarely observed in dogs (Breitschwerdt *et al.* , 2010).

Haematological and biochemical alterations in dogs with *Bartonella* endocarditis are nonspecific and usually mild, and include mild nonregenerative anaemia, leukocytosis with variable neutrophilia, and mild thrombocytopaenia. Biochemical analyses may reveal mild azotaemia with hypoalbuminaemia and, less frequently, hyperglobulinaemia.

Dogs with liver abnormalities associated with *Bartonella* can present a moderate increase in the activities of the enzymes ALT and alkaline phosphatase in serum (Sykes and Chomel, 2014). Monoclonal gammopathies caused by *Bartonella* in dogs have been sporadically documented (Tabar *et al.* , 2011).

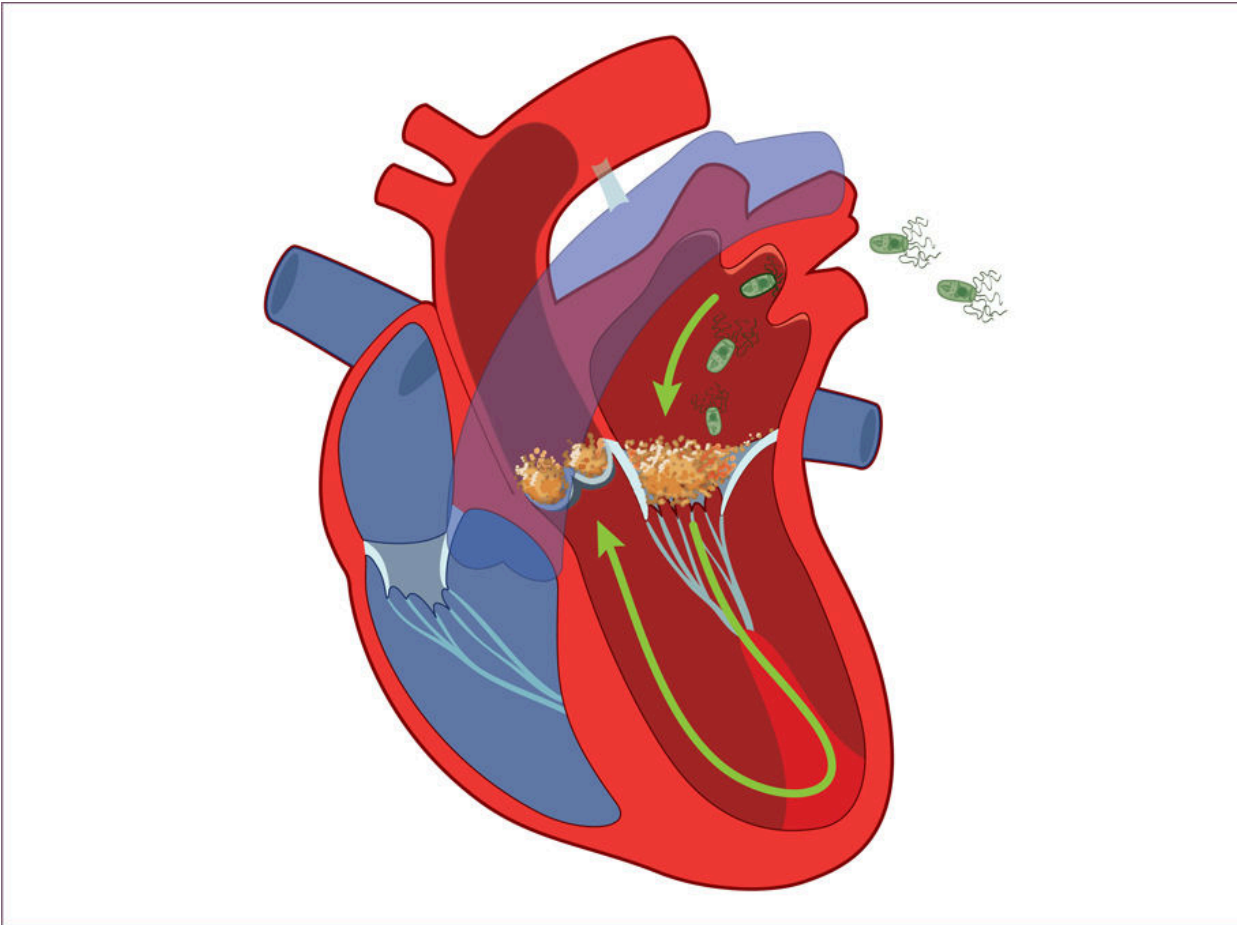


Figure 2. *Bartonella* causes endocarditis of the aortic valve and, less frequently, the mitral valve. The most common species are *B. vinsonii* subsp. *berkhoffii* and *B. henselae*.

Diagnosis

The most sensitive diagnostic test in dogs is the use of pre-enrichment cultures of *Bartonella*, such as the liquid medium BAPGM (Bartonella alpha-proteobacteria growth medium), followed by highly sensitive PCR analysis using primers that detect the 12S–23S internal transcribed spacer (ITS) region of the ribosomal DNA (Duncan *et al.*, 2007). By contrast, the use of conventional blood agar culture or direct PCR amplification of blood, biological fluid, or tissue samples offer much lower sensitivity than that of the aforementioned combined techniques (Breitschwerdt *et al.*, 2010).

Other techniques include specific histological stains, such as Warthin-Starry staining of suspicious inflammatory lesions, and immunofluorescence or

immunohistochemical analyses of tissue samples (Sykes and Chomel, 2014).

Serology is only positive in about half of all dogs infected with *B. henselae* or *B. vinsonii* subsp. *berkoffii*. The detection of antibodies can be indicative of either current infection or exposure to a microorganism. Although serology has limitations, such as crossreactions with other *Bartonella* species and poor sensitivity, it is an important diagnostic test (Breitschwerdt *et al.*, 2010).

The combined use of different diagnostic tests is recommended in suspected clinical cases of *Bartonella* infection (Breitschwerdt *et al.*, 2010).

Treatment and prognosis

In general, treatment with doxycycline, amoxicillin, enrofloxacin, or azithromycin is clinically effective, provided it is sustained for at least 4 to 6 weeks. However, antibiotic treatment usually does not result in elimination of the microorganism.

Oral administration of antibiotics of the macrolide family is recommended. Fluoroquinolones, alone or in combination with amoxicillin, have proven effective in the treatment of dogs with bartonellosis. Doxycycline is also effective in treating infections caused by some species of *Bartonella* in animals, although the results of one study of infected dogs suggest that high doses are necessary (10 mg/kg every 12 hours for 4–6 weeks) (Sykes and Chomel, 2014).

In cases of *Bartonella* endocarditis, supportive treatment is required for management of congestive heart failure (Sykes and Chomel, 2014).

The prognosis ranges from good to severe depending on the clinical picture presented by the patient. *Bartonella* endocarditis generally has a poor prognosis.

Prevention

Bartonella infections in dogs can be prevented by applying control measures that target the relevant vectors, especially fleas and ticks (Dantas-Torres and Otranto, 2016). Transfusions of blood from infected dogs should be avoided (Fig. 3), as this can result in the transmission of *Bartonella* (Becker, 2003; Kordick and Breitschwerdt, 1997; Magalhaes *et al.* , 2008; Reine, 2004; Velho, 2009). There is still no vaccine to prevent *Bartonella* infection in cats or dogs (Sykes and Chomel, 2014).



Figure 3. One possible mode of *Bartonella* transmission is via infusion of infected blood (Nicole Ciscato, Shutterstock.com).

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Canine mycoplasmosis

Jorge Castro López

Canine mycoplasmosis

Definition

Mycoplasmas are bacteria that lack a cell wall and can be found in mammals, birds, reptiles, insects, and plants. They measure between $0.3 \mu\text{m}$ and $0.8 \mu\text{m}$. Several of these species are pathogenic and can cause anaemia, arthritis, infertility, and respiratory diseases. The term “canine mycoplasma” is very broad, as several of the species that have been isolated in dogs can affect other animal species. Some authors have proposed that this term should be applied to mycoplasmas that are isolated exclusively in canids, or rarely in other species. Mycoplasmas are classified as haemotropic or nonhaemotropic.

Haemotropic mycoplasmas

Haemotropic mycoplasmas attach to and grow on the surface of erythrocytes. These species were previously classified as Rickettsia, as they belonged to the genera *Haemobartonella* and *Eperythrozoon*, but were reclassified as members of the genus *Mycoplasma* following sequencing of their ribosomal RNA (16S segment) (Box 1). However, species that have not been fully described are called *Candidatus* species. Haemotropic mycoplasmas possess both DNA and RNA and multiply by binary fission. They can be round, ringed, or stick-like, and can be found alone or forming chains on erythrocytes (Fig. 1).

Several species that infect dogs have been identified. The first such species to be described was *Mycoplasma haemocanis*, which is the largest species of this genus ($\sim 0.8 \mu\text{m}$) and possesses a genome similar to that of *Mycoplasma haemofelis*. *Candidatus M. haematoparvum*, which was described later, is a smaller species ($0.3 \mu\text{m}$) that resembles feline *M. haemominutum*. These haemoplasmas can participate in coinfections. Other species detected in dogs include *Candidatus M. haemominutum*, which more closely resembles the species that infects cats, *Candidatus M.*

turicensis, which is also very similar to the feline form, *Mycoplasma ovis* , and *Candidatus M. haemobos* .

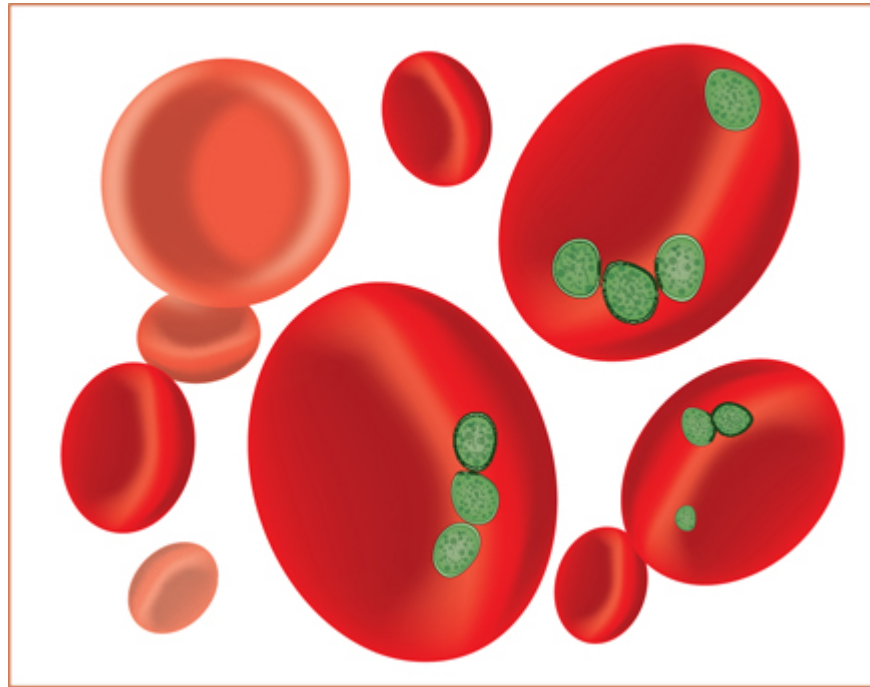


Figure 1. Representation of the appearance of haematropic mycoplasmas adhered to the surface of erythrocytes.

Transmission

It has been suggested that haematropic mycoplasmas in dogs may be transmitted by ticks. An experimental study demonstrated transmission of *M. haemocanis* by *Rhipicephalus sanguineus* . Moreover, transovarial and transstadial transmission of this *Mycoplasma* has been demonstrated in these ticks. Infection can also be transmitted iatrogenically, through blood transfusions or experimental oral administration of infected blood. Although vertical transmission has not yet been demonstrated, indirect transmission in the womb has been described.

Box 1. Haematropic mycoplasmas described in dogs (from most to least common).

- » *Mycoplasma haemocanis*
- » *Candidatus M. haematoparvum*

- » *Candidatus M. haemominutum*
- » *Candidatus M. turicensis*
- » *Mycoplasma ovis*
- » *Candidatus M. haemobos*

Pathogenesis

The mechanism by which haemoplasmas damage erythrocytes is unclear. It has been suggested that these bacteria can alter the shape of the erythrocyte and affect its deformability, thus interfering with microcirculation and altering tissue perfusion and oxygen delivery. This in turn would result in the sequestration or phagocytosis of infected erythrocytes. Another possible mechanism is the induction of immune-mediated haemolysis.

The prepatent period in splenectomised dogs is 1 to 2 weeks. Some dogs can develop progressive anaemia associated with constant bacteraemia. However, other dogs present with gradual anaemia caused by repeated episodes of bacteraemia.

The risk factors for infection and development of disease in dogs are listed in Box 2 .

Box 2. Risk factors for mycoplasma infection.

- » Living in communities.
- » Young or adult dogs and crosses.
- » Splenectomised or immunocompromised animals.
- » Presence of other diseases such as scabies or neoplasms.
- » Coinfections with other agents such as:
 - *Bartonella*.
 - *Leishmania infantum*.
 - *Babesia*.
 - *Anaplasma platys*.
 - *Ehrlichia canis*.
 - *Hepatozoon* spp.
- » Sepsis.
- » Blood transfusion.

Epidemiology

M. haemocanis infections have been described worldwide (United States, Canada, Brazil, Europe, Africa, and Australia). A recent US study reported a prevalence of *M. haemocanis* and *Candidatus M. haematoparvum* of 1.3 % (0.6 % and 0.8 %, respectively). An Australian study reported a higher prevalence of *M. haemocanis* (7.1 %), and prevalences of 1.7 % and 0.4 % for *Candidatus M. haematoparvum* and *Candidatus M. haemobos* , respectively. In Europe, a prevalence of 15.4 % has been reported in France (9.6 %, 3.3 %, and 2.6 % for *Candidatus M. haematoparvum* , *M. haemocanis* , and both haemoplasmas, respectively) and of 1.2 % in Switzerland (0.9 % for *M. haemocanis* and 0.3 % for *Candidatus M. haematoparvum*). Studies conducted in Brazil revealed a prevalence of between 0.48 % and 5.1 % for *M. haemocanis* , while another study reported a prevalence in Spain of 0.5 % to 14.3 % for *M. haemocanis* and 0.6 % to 2 % for *Candidatus M. haematoparvum* . In Italy, prevalences of 3.7 % and 5 % have been reported for *M. haemocanis* and *Candidatus M. haematoparvum* , respectively. In Portugal, the prevalence of *M. haemocanis* is 40 %, and no cases of *Candidatus M. haematoparvum* have been described.

Clinical presentation

Typically infection does not result in the development of disease, but rather a chronic asymptomatic infection. Clinical signs of *M. haemocanis* infection are most commonly observed in immunosuppressed animals or those that have undergone splenectomy.

Anaemia may be observed and can be mild, moderate, or severe. Only a handful of cases of severe haemolytic anaemia have been reported. In these dogs the most common clinical signs are apathy and pale mucous membranes (Fig. 2). The clinical manifestation of anaemia will depend on the speed with which it develops and its severity. Thrombocytopaenia may also be observed. Fever and anorexia are rare. Jaundice has not been reported in dogs with anaemia due to haemoplasmosis (Box 3).

The pathogenic potential of *Candidatus M. haematoparvum* is open to debate; only two cases of haemolytic anaemia caused by this bacterium have been reported.

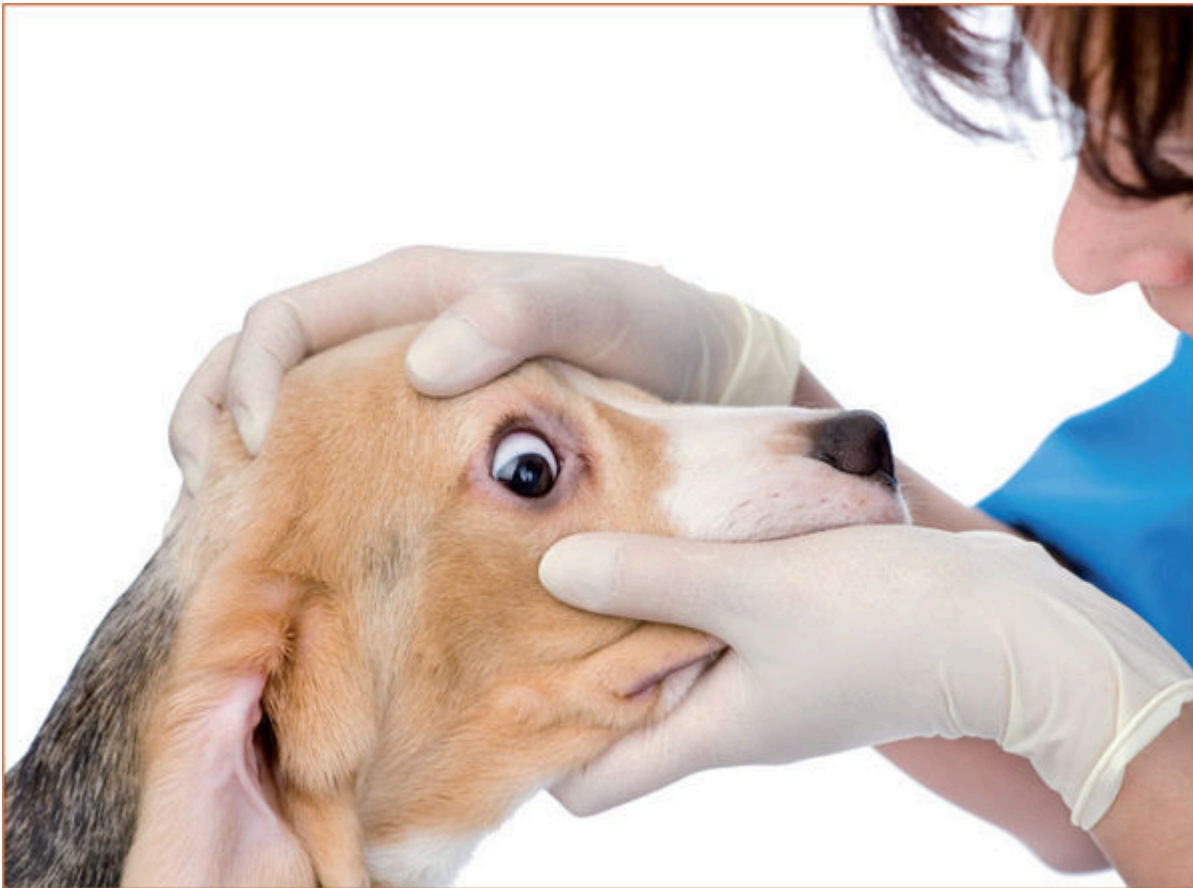


Figure 2. Examination of mucosal colour to detect possible anaemia (Ermolaev Alexander, Shutterstock.com).

Box 3. Summary of the clinical presentation of haemotropic mycoplasma infection.

- » Chronic infection without clinical signs (most common).
- » Splenectomised or immunocompromised dogs (develop disease).
- » *M. haemocanis* (produces clinical signs in cases in which disease develops).
- » *Candidatus M. haematoparvum* infection (rarely results in clinical signs).

- » Apathy and pale mucous membranes.
- » Fever and anorexia (uncommon).
- » Mild to severe anaemia.
- » Thrombocytopaenia (uncommon).
- » No jaundice.

Diagnosis

The haemogram can reveal anaemia, which in most cases is regenerative given the time elapsed between the onset of anaemia and the appearance of clinical signs, as well as reticulocytosis, polychromasia and anisocytosis, circulating nucleated erythrocytes, and Howell-Jolly bodies. Macrocytosis and spherocytes are rare. No abnormalities are observed in the leukogram. Most cases show no signs of hyperbilirubinaemia or haemoglobinaemia. In some cases the Coombs test may be positive. Dogs with a latent infection show no haemogram alterations.

M. haemocanis can be seen in blood smears
from dogs displaying clinical signs of anaemia
(Fig. 3).

This mycoplasma tends to form chains on the surface of erythrocytes. By contrast, *Candidatus M. haematoparvum* is smaller (0.3 µm), and is thus even more difficult to observe. Moreover, it can be solitary or form pairs, but does not form rings or chains.

The quantitative PCR assay developed to identify *M. haemocanis* and *Candidatus M. haematoparvum* is the test of choice and is sufficiently sensitive to detect subclinical and clinical infections.

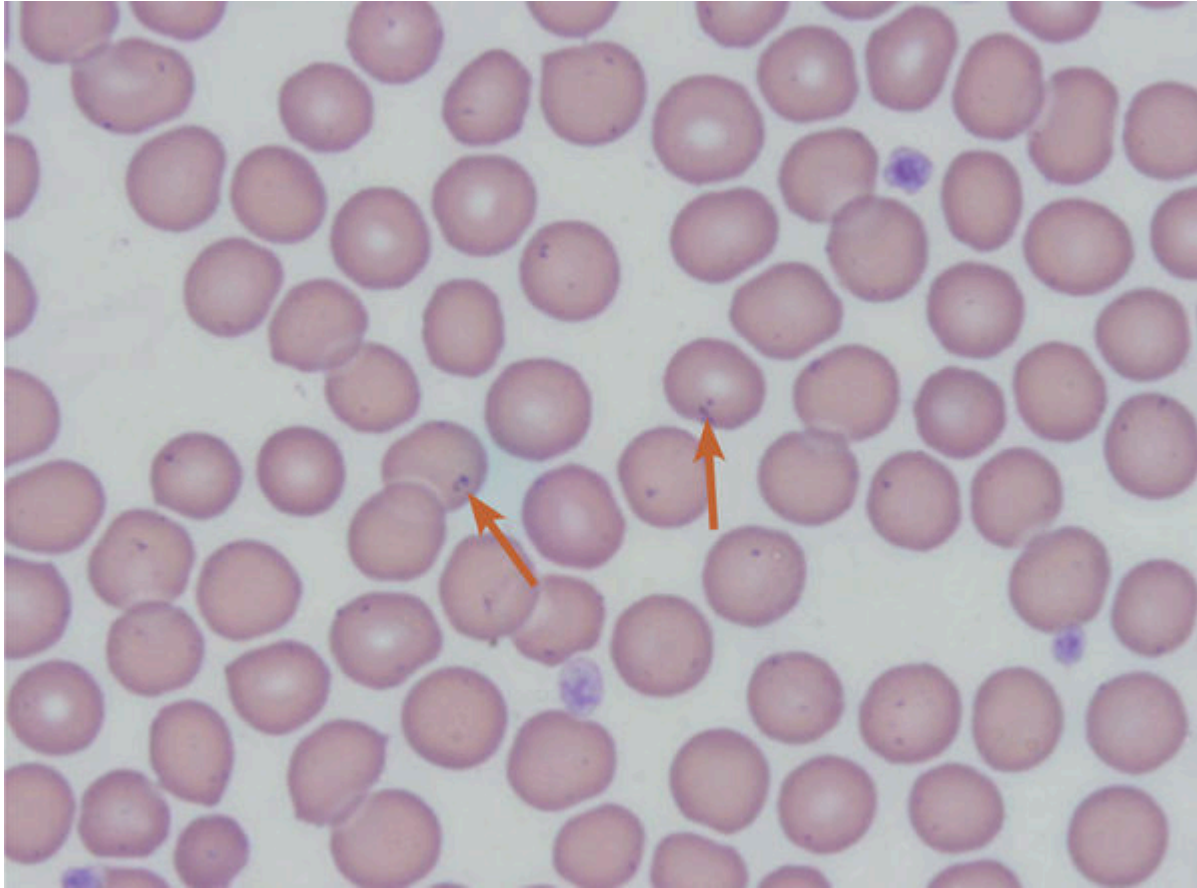


Figure 3. *Mycoplasma haemocanis* in a blood smear. Photo courtesy of Silvia Tasca, Laboratorio Veterinario Privado San Marco (Padua, Italy).

Treatment

Treatment with doxycycline is recommended for cases of infection with *M. haemocanis*. However, no clinical studies have confirmed its effectiveness for the treatment of canine haemoplasmosis, and even the minimum duration of treatment for *M. haemocanis* infection is unknown.

The administration of oral tetracyclines is effective for the treatment of *M. haemocanis* infection.

Tetracycline or oxytetracycline: 20 mg/kg every 8 hours, orally or intravenously, for 21 days.

Doxycycline: 10 mg/kg every 24 hours, orally, for 21 days.

Blood transfusions are only recommended when anaemia is very severe and clinical signs are evident.

Most studies of treatment approaches have been experimental, and performed in splenectomised dogs. A decrease in the number of DNA copies has been reported after treatment for 11 months, although this does not result in complete elimination of the infection, and may be indicative of a latent infection.

Quantitative PCR should be performed to monitor the effectiveness of treatment and bacteraemia, as blood smears are of lower sensitivity. Two cases that were monitored by quantitative PCR, with opposite responses to therapy, have been described:

- The first case presented with anaemia, and haemoplasma was readily detected by cytology. Oxytetracycline treatment was instituted. After 17 days mycoplasmas were no longer detectable by cytology and treatment was withdrawn at day 29. However, the anaemia recurred and the patient tested positive for *M. haemocanis* by PCR despite continued negative cytology.
- The other case was a splenectomised dog with anaemia secondary to *M. haemocanis* that was treated with doxycycline, resulting in the remission of clinical signs and levels of haemoplasma that were undetectable by quantitative PCR.

In most cases described in dogs glucocorticoids (prednisolone, 1–2 mg/kg every 24 hours) have been administered together with doxycycline to limit haemolysis and phagocytosis of erythrocytes. However, no scientific evidence supports the efficacy of this treatment approach, which theoretically could have a negative impact on infection. It is not even known whether this treatment could delay elimination of the organism.

To date, no effective treatment for *Candidatus M. haematoparvum* infection in dogs has been identified. The literature describes one case treated with doxycycline and another with ciprofloxacin (20 mg/kg every 24 hours for 7 days) and prednisolone (0.5 mg/kg every 12 hours for 3 days). Clinical signs improved after 3 days of treatment.

Prevention

It is recommended to remove haematophagous arthropods, which can transmit haemotropic mycoplasmosis. The presence of these bacteria should also be ruled out by PCR in blood donor dogs to prevent iatrogenic transmission to splenectomised recipients.

Nonhaemotropic mycoplasmas

These mycoplasmas have a very small genome, which allows them to live outside the cell, and have a limited metabolic capacity. As such, their metabolism is largely dependent on their enriched surrounding environment (e.g. respiratory or urogenital mucosa). The genera included in this group are *Mycoplasma*, *Ureaplasma*, and *Acholeplasma*, which form part of the normal mucosal flora of dogs. The pathogenicity of this group, which has been analysed in only a few studies, remains unclear. These mycoplasmas can cause infections of the respiratory and urogenital tract, musculoskeletal system, meninges, and conjunctiva.

Pathogenesis

These microorganisms can give rise to an increase in mucosal immunoglobulins to eliminate the infection, as occurs for example in the respiratory tract (increased IgA levels). They can also induce a T cell response, which increases the release of cytokines. These cytokines have an antibacterial function, but perpetuate inflammation. In addition, the metabolic products of mycoplasmas can act as superantigens, stimulating the onset of chronic immune-mediated diseases. Another mechanism by which some mycoplasmas exert their pathogenic effects is by causing intracellular infection, which in turn results in chronic recurrent infection.

Infections

The pathogenicity of mycoplasmas, which are part of the normal flora of different mucous membranes in the dog, remains unclear (Box 4).

Box 4. Diseases caused by nonhaemotropic mycoplasmas.

- » Respiratory infections.
- » Urinary infections.
- » Genital infections.
- » Polyarthritis.
- » Meningoencephalitis.

Respiratory infections

Mycoplasmas are part of the normal flora of the upper respiratory tract in dogs, and have even been isolated in the lungs. They are found in the trachea and lungs of 20 % to 25 % of healthy dogs. Available data indicate that mycoplasmas are isolated from 78 % of dogs of less than 1 year of age with lung disease. Furthermore, a retrospective study reported that *Mycoplasma* and *Bordetella bronchiseptica* bacteria were isolated more frequently in dogs with lower respiratory tract infection. However, another recent study has reported that there is a high probability of oropharyngeal contamination occurring when performing bronchoalveolar lavage, suggesting that positive *Mycoplasma* results should be interpreted with caution. As such, their pathogenic role remains unclear.

Mycoplasma infection of the lower respiratory tract has been associated with coinfections with bacteria (*Bordetella* and *Streptococcus*) and viruses (canine adenovirus type 2, canine distemper virus, and canine parainfluenza virus). It is also associated with predisposing anatomical abnormalities, such as tracheal collapse and lung parenchymal disease. Studies of this species have failed to demonstrate a clear association between the presence of *Mycoplasma* and disease, as the prevalence of these bacteria is similar in healthy and diseased animals. However, one study described a correlation between *M. cynos* infection and canine infectious respiratory disease. Experiments have shown that this *Mycoplasma* destroys the cilia and generates infiltration of neutrophils and macrophages.

Urinary infections

Mycoplasmas are also part of the normal flora of the distal urogenital tract. It has been proposed that the presence of tumours or bladder stones may

predispose to *Mycoplasma* infection. To diagnose these infections it is necessary to obtain a sample by cystocentesis as spontaneous urination can result in contamination of the sample in the urethra. No other bacteria should be detected in the sample. However, diseases that cause urine stasis, such as urethral obstruction due to stones, can result in contamination of the bladder by mycoplasma; in these cases a positive result may be observed despite obtaining the sample by cystocentesis. In dogs, ureaplasmas are thought to be involved in infections, as they are more resistant to urine-induced osmotic damage. One study reported isolation of *M. canis* in cultures from 4 % of dogs with urinary infection and azotaemia.

Genital infections

Mycoplasmas of the reproductive tract are considered opportunistic. These bacteria are found in the genital tracts of 30 % to 50 % of males and 23 % to 75 % of females.

M. canis has been isolated in bitches with endometritis and experimentally in males with urethritis and chronic epididymitis and females with endometritis and enlarged uterus. It has also been isolated from the prostate. In the case of endometritis, the condition is thought to constitute a coinfection. The role of mycoplasmas in infertility, and the significance of a positive result in semen or vaginal samples, remain unclear. Infertility in dogs may be associated with infections caused by the genus *Ureaplasma*. This view is supported by a study in which these organisms were more frequently isolated in infertile animals, although no clear conclusions can be drawn.

Other infections

The species most commonly isolated from the conjunctiva of dogs is *M. canis*. However, no direct association between infection with this species and conjunctivitis has been demonstrated.

Musculoskeletal infections have been described in dogs. These include the case of a greyhound with polyarthritis caused by *M. spumans* and another case of an immunosuppressed dog that presented with sepsis and acute polyarthritis caused by *M. edwardii*. The latter *Mycoplasma* has also been detected in a case of suppurative meningoencephalitis in a puppy.

Mycoplasmas form part of the normal flora of the colon in 30 % of dogs. One study failed to reproduce colitis following experimental inoculation of *Mycoplasma* into the colon of dogs.

Diagnosis

Cytological analyses of fistulas, mucous membranes, or body cavity effusions reveal nondegenerate neutrophils and an absence of other bacteria. Moreover, mycoplasmas cannot be observed by Gram staining. While they can be detected by electron microscopy, this technique is primarily used in the field of research. Mycoplasmas can also be cultured. This requires transport of samples for laboratory analysis in enriched media (Hayflick, Amies, or modified Stuart medium). Samples should be refrigerated and shipped with ice packs, and transportation should not take more than 3 days. If transport will take more than 3 days, samples should be frozen and transported on dry ice. Canine mycoplasmas can grow in special media prepared according to Hayflick's formula. Cultures should be incubated at 37 °C with 5 % carbon dioxide and subsequently in anaerobic conditions for 48 hours. The resulting colonies are fried-egg-shaped.

PCR enables the detection and differentiation of mycoplasma species. This technique detects the 16S-23S region of ribosomal RNA and is an easy test to perform. PCR can be conducted using different types of samples (liquid, smears, tissue), does not require special transportation conditions, and offers greater sensitivity than cytology. Furthermore, this technique can be performed using paraffin-embedded tissue samples.

It is recommended to always contact the reference laboratory to ascertain which sample is most representative of a given patient's clinical presentation, the most appropriate medium, and any specific transport requirements.

Treatment and prevention

Mycoplasmas are sensitive to macrolides, pleuromutilins, tetracyclines, lincosamides, chloramphenicol, fluoroquinolones, aminoglycosides, and nitrofurantoin (Table 1). Eradication of infection is dependent on a competent immune system.

Neither tetracyclines nor chloramphenicol can be used in pregnant animals. Erythromycin and lincomycin, which are safer but less effective, can be used. Treatment should last at least one week.

Currently, there are no vaccines available for use in dogs.

Table 1. Treatments for nonhaemotropic mycoplasmas.

Drug	Dose
Doxycycline	5–10 mg/kg PO every 12 hours
Tetracycline	22–30 mg/kg PO every 8 hours
Erythromycin	15–25 mg/kg PO every 12 hours
Azithromycin	5–10 mg/kg PO every 24 hours
Chloramphenicol	25–50 mg/kg PO every 8 hours
Clindamycin	5–11 mg/kg PO or IV every 12 hours
Enrofloxacin	5 mg/kg PO or IV every 24 hours

PO, oral; IV, intravenous.

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Canine toxoplasmosis and neosporosis

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Canine toxoplasmosis and neosporosis

Definition

Toxoplasmosis and neosporosis are diseases caused in dogs by the protozoa *Toxoplasma gondii* and *Neospora caninum*, respectively. Until recently these parasites were indistinguishable from one another. Canids play multiple roles in the epidemiology of these diseases, acting as intermediate hosts for *T. gondii* and as definitive hosts for *N. caninum*. Both microorganisms can give rise to similar clinical presentations in dogs, mainly affecting the respiratory, gastrointestinal, and neuromuscular systems with neuromuscular involvement predominating in the case of neosporosis.

Canine toxoplasmosis

Aetiology

Toxoplasma gondii is an obligate intracellular coccidia parasite (kingdom Protista, phylum Apicomplexa) capable of infecting all warm-blooded animal species. Felids, including the domestic cat, act as its definitive host. All other infected animals act as intermediate hosts (Fig. 1).

After ingestion of oocysts or tissue cysts by the definitive host, sporozoites and bradyzoites are released and penetrate the intestinal epithelium, where they reproduce asexually and sexually to form zygotes, which later transform into oocysts ($10 \times 12 \mu\text{m}$) and are shed into the environment in cat faeces. In the sporogonic phase, nonsporulated oocysts, in appropriate environmental conditions, transform into infective sporulated oocysts over the course of 1 to 5 days. The extraintestinal phase occurs following ingestion of the oocysts. These infective stages multiply in the vascular endothelium, fibroblasts, mononuclear cells, and segmented leukocytes. This results in the generation of tachyzoites, followed by bradyzoites, which

remain inside tissue cysts (15 μm –60 μm) in different organs, establishing the chronic phase of the disease (Fig. 2).

Three distinct *T. gondii* genotypes with varying degrees of virulence have been described: types I, II, and III. Types I and III have been detected in the brains of dogs with neurological dysfunction.

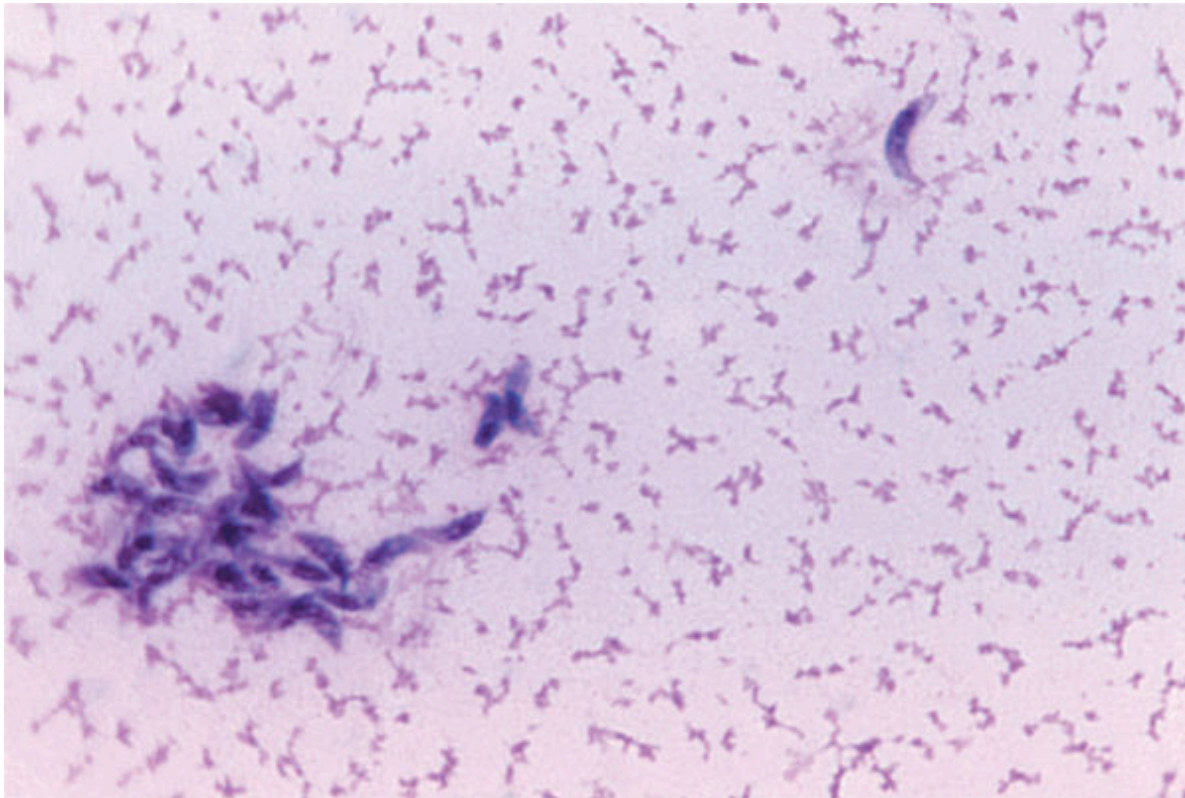


Figure 1. *Toxoplasma gondii* in an ascites smear from a mouse (CDC / Dr. LL. Moore, Jr.).

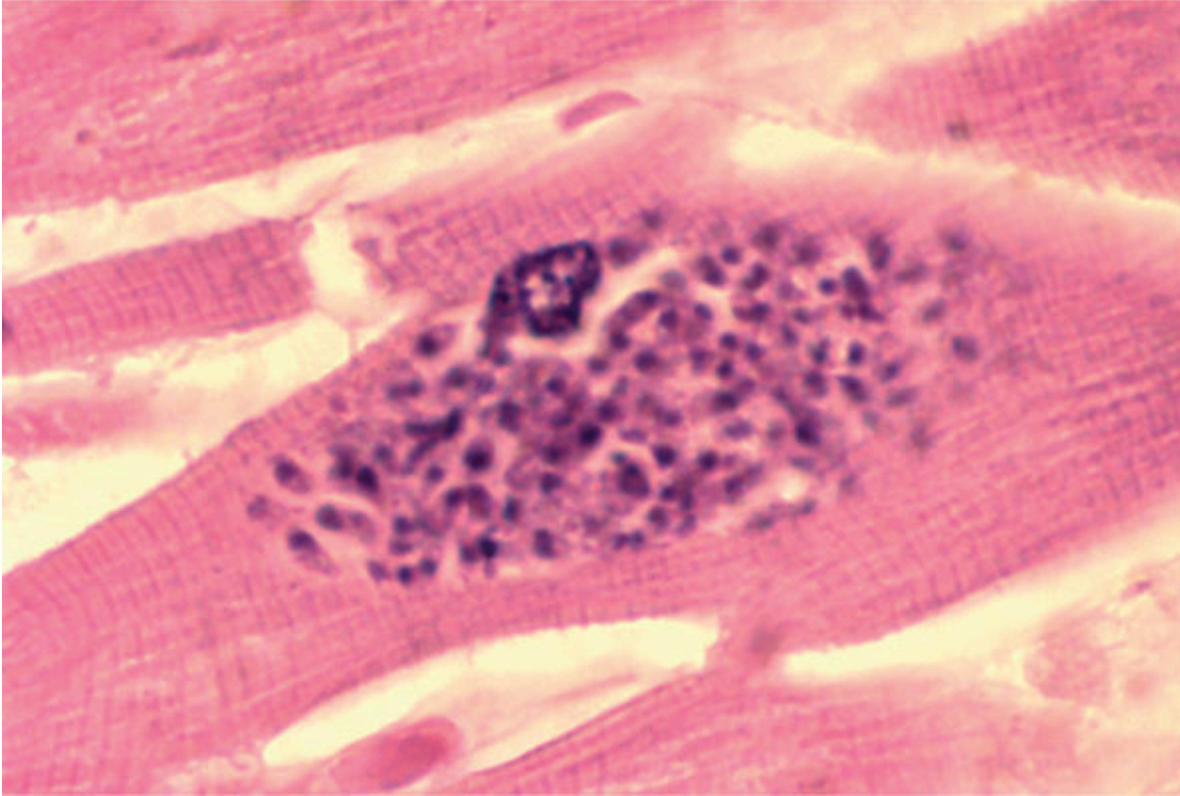


Figure 2. Myocardial toxoplasmosis in a case of AIDS. Tachyzoites of *Toxoplasma gondii* can be seen in a pseudocyst within a myocyte (CDC / Dr. Edwin P. Ewing, Jr.).

Epidemiology

T. gondii is most common in warm, moist environments. However, infections in humans and animals are widely distributed.

It is estimated that 60 % of the human population is seropositive for *T. gondii*.

While the level of seroprevalence in cats is between 30 % and 50 % in countries in central and southern Europe, the prevalence of canine toxoplasmosis in this region is poorly documented. A recent study conducted in Mallorca (Spain) revealed a seropositivity rate of 58.7 %, which was higher than expected. This may be due to the high seroprevalence in cats in

this area and the characteristics of the population studied. Results of a study performed in northeast Portugal indicate a prevalence of 38 %.

Box 1 shows the risk factors for canine toxoplasmosis.

Box 1. Risk factors for canine toxoplasmosis.

- » Feeding on raw or undercooked meat.
- » Dogs of over 1 year of age, with increased risk of exposure.
- » Animals that live outdoors with access to hunting or contact with wild animals.
- » Close contact with cats.
- » Pups of infected mothers.
- » Concurrent diseases.
- » Immunosuppressive drugs.

Dogs are primarily infected through the ingestion of sporulated oocysts. Vertical and transfusional transmission are less frequent. While *T. gondii* has been detected in *Ixodes ricinus*, the importance of this tick as a vector is unclear. Transmission in semen has been demonstrated experimentally.

Dogs can act as mechanical vectors of sporulated oocysts. Although these animals display seroconversion, enteroepithelial replication of the parasite does not occur and no associated clinical signs develop.

Key aspects of canine toxoplasmosis

1. Can cause serious illness in dogs.
2. Dogs can be used as sentinels of environmental contamination with oocysts of *T. gondii*.
3. Dog populations can constitute a direct source of infection for humans.

Pathogenesis

The pathogenesis of toxoplasmosis is attributed to the cytopathic effect of the parasite (i.e. cell necrosis caused by intracellular growth of the microorganism), as *T. gondii* does not produce toxins that can damage cells.

It is not known why some infected dogs develop clinical disease and others do not, although it is clear that adequate cellular immunity is essential to control infection. Macrophages, CD4+ and CD8+ T lymphocytes, natural killer cells, and cytokines are the main components of the innate immune response to the parasite. Antibodies play a minor role in the immune defence, but are essential for diagnosis of the disease.

The type and severity of toxoplasmosis depends on the extent and location of tissue damage. The ingestion of oocysts or tissue cysts results in the appearance of clinical signs due to necrosis of the intestine and associated lymphoid organs caused by tachyzoites. *T. gondii* spreads via the blood or lymph fluid to the extraintestinal organs, where it induces focal necrosis. The heart, liver, lungs, skeletal muscles, and eyes are common sites of initial replication. Approximately three weeks after infection tachyzoites can localise in tissue cysts in the form of bradyzoites. This phase is associated with a systemic, humoral and cellular, interferon γ -dependent immune response that inhibits parasitaemia. These tissue cysts can remain in the host for life, and can rupture and release bradyzoites during periods of immunosuppression, resulting in clinical recurrence of the disease.

Toxoplasmosis in dogs is often associated with distemper virus infection and other infections such as ehrlichiosis. Historically, the prevalence of canine toxoplasmosis has decreased with the routine use of vaccines against distemper virus.

Clinical presentation

In dogs, the main affected systems are the respiratory, intestinal, and neuromuscular systems. The neuromuscular form of the disease may have a longer course and is more common in adult animals. In young or immunosuppressed dogs, the disseminated form can result in a fulminant infection.

Systemic findings are varied. Jaundice may develop as a result of extensive hepatic necrosis. Myocardial involvement is usually subclinical, and occasionally presents with arrhythmias and heart failure. In immunocompromised animals toxoplasmosis can result in skin lesions, including pustular alopecic dermatitis and subcutaneous nodules.

Dogs with myositis can develop atrophy and muscle stiffness. Paresis can rapidly evolve to lower motor neuron paralysis. Dogs with polyradiculoneuritis should undergo serological analysis for antibodies against *T. gondii*.

Lesions of the eye due to canine toxoplasmosis are relatively rare, and are generally associated with the systemic form of the disease (Fig. 3).

The consequences of neonatal toxoplasmosis have been studied in experimental and natural infections.

Box 2 lists the clinical signs associated with different presentations of canine toxoplasmosis.

Box 2. Clinical signs of canine toxoplasmosis.

Generalised form

- » Nonspecific: anorexia, lethargy, fever, lymphadenopathy.
- » Respiratory: cough, dyspnoea.
- » Digestive: vomiting, diarrhoea, jaundice.
- » Cardiac: arrhythmias, congestive heart failure.

Neuromuscular form

- » Muscular: lameness, generalised hyperaesthesia, atrophy, stiffness.
- » Neurologic: seizures, cranial nerve deficits, tremors, ataxia, paresis, paralysis.

Ocular form

» Retinitis, anterior uveitis, iridocyclitis, optic neuritis, keratoconjunctivitis.

Neonatal form

» Weakness, foetal death.

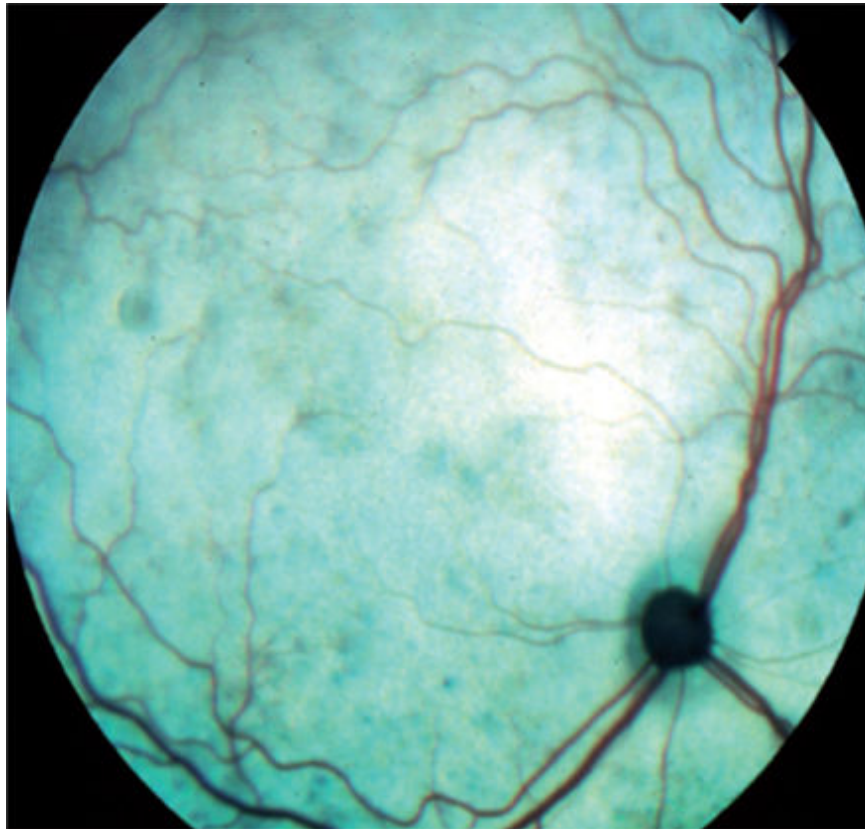


Figure 3. Active chorioretinitis in a confirmed case of canine toxoplasmosis. Image courtesy of Dr. Michael Davidson, Faculty of Veterinary Medicine, North Carolina State University (Raleigh, NC, USA).

Diagnosis

Laboratory tests

Haematological and biochemical alterations are usually nonspecific. The haemogram may reveal nonregenerative anaemia, neutrophilic leukocytosis, lymphocytosis, monocytosis, and eosinophilia. Biochemical abnormalities include marked increases in the liver transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP),

and indirect bilirubin due to hepatic necrosis. Increased creatine kinase (CK) levels are indicative of acute muscle necrosis. Dogs with pancreatitis may show an increase in pancreatic enzymes.

Marked proteinogram alterations have been demonstrated in dogs with toxoplasmosis, including decreased levels of albumin and α_1 , α_2 , and β globulins. Decreases in the levels of α_1 and α_2 globulins have been associated with liver damage, while decreased β globulin levels may be a consequence of reduced synthesis of this protein in the liver. Conversely, increases in γ globulin levels have been reported, possibly the result of the release of acute phase proteins in the liver in response to the inflammatory process. Increased levels of the liver aminotransferases ALT and AST have been correlated with hypoalbuminaemia and hyperglobulinaemia.

Cytological diagnosis

In the acute phase, tachyzoites can be detected by cytology. Giemsa staining reveals oval-shaped structures with little cytoplasmic staining. Tachyzoites are rarely found in the blood or cerebrospinal fluid (CSF), or in samples obtained by fine needle aspiration or transtracheal or bronchoalveolar lavage. They are more commonly observed in thoracic and abdominal effusions.

T. gondii can be detected by polymerase chain reaction (PCR), which enables identification and amplification of specific DNA sequences (B1 and P30) or the small subunit of ribosomal RNA (rRNA). The peroxidase-antiperoxidase test is used to detect antigens in tissues. Additional inflammatory changes can also be observed, predominantly in lymphocytes.

In dogs and cats with encephalitis or uveitis, increases in both protein and leukocyte concentrations may be observed in the aqueous humour or CSF. The cell population is usually mixed, consisting mainly of mononuclear cells and neutrophils. The microorganism is rarely observed in these fluids.

Diagnostic radiology

This is a complementary diagnostic technique. Thoracic radiographs may reveal the presence of pleural effusion, while abdominal radiographs may be

compatible with masses of the intestine or mesenteric lymph nodes, abdominal effusion, or pancreatitis.

Lesions of the central nervous system (CNS) can be evaluated by myelography, computed tomography (CT), or nuclear magnetic resonance (NMR). These techniques often reveal multifocal lesions, but also can detect solitary masses compatible with granulomas.

Serology and agent detection

Multiple serological tests have been used for the diagnosis of canine toxoplasmosis.

Once infected, animals harbour *T. gondii* cysts for life, resulting in long-lasting stimulation of a humoral immune response. In dogs the presence of IgM has been experimentally detected from 7 to 27 days after infection with *T. gondii* , while IgG can be detected for 1 to 2 months postinfection. Therefore, detection of IgM can be an indicator of either recent or active infection.

It should be noted that indirect immunofluorescence (IIF) for specific detection of IgM and IgG has been widely used in epidemiological studies. Comparison of different techniques, such as the agglutination test for the detection of IgG, indirect haemagglutination, latex agglutination, modified agglutination, and ELISA, has demonstrated comparable efficacies.

A presumptive diagnosis can be established based on clinical data together with laboratory results (Box 3).

Studies have investigated the utility of ELISA using recombinant surface antigen SAG1 (P30) for the detection of antibodies in naturally infected dogs. This antigen is secreted by tachyzoites and is thus specific for the clinical stage, and its detection is considered diagnostic of clinically active infections.

The presence of *T. gondii* can be confirmed by bioassays in animals or by inoculation into cell cultures. Laboratory mice are the most sensitive animals.

PCR can be used to determine the presence and genotype of the parasite in biological samples. The presence of uveitis potentially caused by *T. gondii* can be detected even before identifying antibodies in the aqueous humour, but a positive PCR result does not prove that the ocular signs are due to toxoplasmosis. A multiplex PCR has been developed to detect and distinguish between *Toxoplasma* and *Neospora* in CSF, skeletal muscle, and nervous tissue.

Box 3. Basic aspects of presumptive ante mortem diagnosis.

- » Increased IgM levels, indicative of an active or recent infection.
- » Increased IgG levels.
- » Exclusion of other possible causes of clinical signs.
- » Favourable clinical response to treatment of *T. gondii*.

Pathological examination

Histopathological lesions observed in canine toxoplasmosis are listed in Box 4. Immunoperoxidase staining and electron microscopy can confirm the presence of the parasite in tissue.

Box 4. Histopathological lesions.

- » Musculature:
 - Pallor
 - Necrosis
 - Fibrosis in chronic cases (more common in neosporosis)
- » CNS:
 - Necrosis
 - Discolouration
 - Gliosis
 - Vasculitis
 - Multifocal nonsuppurative meningoencephalitis
 - Encephalomalacia
 - Multifocal inflammatory infiltrate
 - Cerebellar atrophy
- » Lungs:
 - Fibrinous exudate
 - Necrosis
 - Inflammatory infiltrate
 - Hypoplasia of alveolar cells and epithelia
- » Other organs:

- Liver necrosis and mesenteric lymph nodes
- Ulcers in the digestive system

Treatment

The goal of treatment is to suppress the replication of *T. gondii*, as killing the parasite is not a completely effective approach.

Clindamycin is the drug of choice. Owing to its good intestinal absorption, oral and parenteral dosages are similar. It crosses the blood brain barrier and other vascular barriers.

Clinical signs usually start to subside 24 to 48 hours after initiation of therapy. Muscle atrophy and neurological signs may take weeks to resolve in animals with polymyositis. Due to the irreversibility of the damage caused by inflammation, neurological signs may not completely resolve. Chorioretinitis usually improves within a week. Usually, ophthalmologic signs require the administration of topical, oral, or injected glucocorticoid together with clindamycin. Potential side effects of clindamycin include anorexia, vomiting, and diarrhoea, possibly with gastrointestinal irritation, which disappears once therapy is discontinued.

There are other less effective therapeutic possibilities. The combination of pyrimethamine and fast-acting sulfonamides such as trimethoprim-sulfa, administered for 4 weeks, has a synergistic effect. Doxycycline treatment can also be considered if adverse reactions to clindamycin occur or in cases of coinfection with other parasites. Table 1 lists the various therapeutic options.

Table 1. Therapeutic options for *Toxoplasma gondii*.

Drug	Dose
Clindamycin	<ul style="list-style-type: none"> ■ 3–13 mg/kg PO or IM every 8 hours for 4 weeks. ■ 10–20 mg/kg PO or IM every 8 hours for 4 weeks.
Sulfonamides	20–30 mg/kg PO every 24 hours for 4 weeks.

Pyrimethamine (in combination with sulfonamides)	1 mg/kg PO every 24 hours for 4 weeks.
Trimethoprim-sulfadiazine	15 mg/kg PO every 12 hours for 4 weeks.
Doxycycline (for concomitant infections)	<ul style="list-style-type: none"> ■ 10 mg/kg PO every 24 hours for 4 weeks. ■ 5 mg/kg PO every 12 hours for 4 weeks.

Prevention

Preventive measures seek to reduce the incidence of feline infections and the consequent shedding of oocysts into the environment. It is recommended to cook meat well and even freeze it before consumption. Transfusions of unscreened blood should be avoided.

Canine neosporosis

Aetiology

Neospora caninum is a coccidia (kingdom Protista, phylum Apicomplexa) with a worldwide distribution. Domestic and wild dogs, coyotes, and foxes act as definitive hosts. These animals start to shed oocysts in their faeces beginning between 5 and 13 days postinfection and lasting for 27 days. The number of oocysts shed is greater in puppies than in adult animals and in dogs infected for the first time than in reinfected dogs. The oocysts sporulate 24 to 72 hours after shedding into the environment.

Herbivores such as cows, sheep, goats, horses, and deer act as intermediate hosts. They become infected after ingestion of sporulated oocysts or by vertical transmission. Once ingested the parasite can spread systemically, contaminate the placenta or milk and infect the foetus, resulting in infertility, miscarriages, and neonatal infection.

Dogs are primarily infected transplacentally. Another possible mode of transmission is through ingestion of contaminated raw meat or foetal

membranes derived from an intermediate host. This route of transmission does not result in faecal shedding of oocysts by infected dogs, but can potentially induce the development of antibodies against *N. caninum*.

Tachyzoites cause cell necrosis after multiplying rapidly inside the cells of the definitive host, and can potentially spread to various organs during the acute phase of the disease. The immunity of the infected individual restricts the chronic infection to muscle or neural tissue in the form of cysts (100 µm) encapsulating bradyzoites. The fragmentation of these cysts causes a granulomatous inflammatory tissue reaction.

Epidemiology

Natural infections in dogs have been detected worldwide. A study of domestic dogs in Catalonia, Spain, reported a prevalence of 12.2 %. Other epidemiological studies conducted in central and southern Europe have reported varying prevalences depending on the characteristics of the study population, with seropositivity rates of 6.4 % to 28.9 % in Italy; 22.7 % in France; and 7.9 % in Portugal. Seroprevalence in domestic dogs is greater than the incidence of disease, suggesting the presence of subclinical infections.

Risk factors for canine neosporosis are listed in Box 5 .

Box 5. Risk factors for canine neosporosis.

- » Ingestion of raw meat.
- » Dogs living in rural areas.
- » Hunting dogs.
- » Older animals, with increased risk of exposure.
- » Breed predisposition:
 - Pointer.
 - Labrador retriever.
 - Boxer.
 - Golden retriever.
 - Basset hound.
 - Greyhound.
- » Cubs of infected mothers.
- » Presence of concurrent illnesses.

Successive litters of chronically infected bitches can also be infected by transplacental or lactogenic transmission. A variable number of puppies may show clinical signs, while others may develop subclinical infection with reactivation.

Reactivation of the infection, which can cause cutaneous, muscular, or neurological conditions, has been linked to concurrent diseases and the administration of immunosuppressants, chemotherapeutic drugs, and modified live vaccines. Glucocorticoid administration may extend the period of oocyst shedding.

Infection of intermediate hosts is important for the maintenance of the disease in the wild, and has a subsequent economic impact on the livestock industry. A wildlife cycle involving wild canids and their prey has also been described. Birds can also act as intermediate hosts.

The zoonotic potential of *N. caninum* is unknown, although some serological evidence indicates that humans can be exposed to this microorganism.

Clinical presentation

Two possible disease presentations are described in clinically affected dogs: encephalomyelitis and myositis/polyradiculoneuritis. This type of myositis is the most commonly observed form in dogs and usually occurs in transplacentally infected animals of less than 6 months of age. Clinical signs begin to appear at 3 to 9 weeks of age.

In some cases, no serious intracranial manifestations develop and the animal can survive for months, but with paralysis-associated complications.

While infection can be spread by vertical transmission, miscarriages in dogs have not been documented.

The clinical presentation may vary depending on the dog's age (Box 6).

Box 6. Clinical signs of neosporosis.

Dogs of less than 6 months of age

- » Progressive ascending paralysis of the hindlimbs.
- » Gradual muscle atrophy, paralysis and rigidity (result of damage caused by the parasite in lower motor neurons and myositis).
- » Cervical weakness, dysphagia, megaesophagus, death.

Dogs of over 6 months of age

- » Chronic subclinical infection.
- » Encephalomyelitis, reflecting focal or multifocal involvement.
- » Less frequent: dermatitis, neuritis *cauda equina* , lower motor neuron dysfunction, necrotising cerebellitis, hepatitis, myocarditis, pneumonia, and peritonitis.

Diagnosis

Laboratory and radiological tests

Haematological and biochemical findings are nonspecific. Myositis induces elevations in CK and AST activity. In the case of liver disease, increases in ALP and ALT levels may be observed.

CSF analysis may reveal a slight increase in proteins and nucleated cells, mainly monocytes, lymphocytes, and neutrophils. Occasionally, tachyzoites can be detected in CSF.

Thoracic radiographs of affected animals can reveal interstitial and alveolar patterns. NMR findings are nonspecific but may show the presence of a diffuse inflammatory infiltrate in the muscle and CNS (Fig. 4).

Electromyography may show some alterations such as spontaneous activity, repeated discharges, reduced conduction velocity, and diminished evoked action potentials.

Neosporosis should be suspected in any dog with concurrent signs of CNS disease and myositis such as hyperaesthesia, swelling, or muscle atrophy, and increased activity of the muscle enzyme CK.

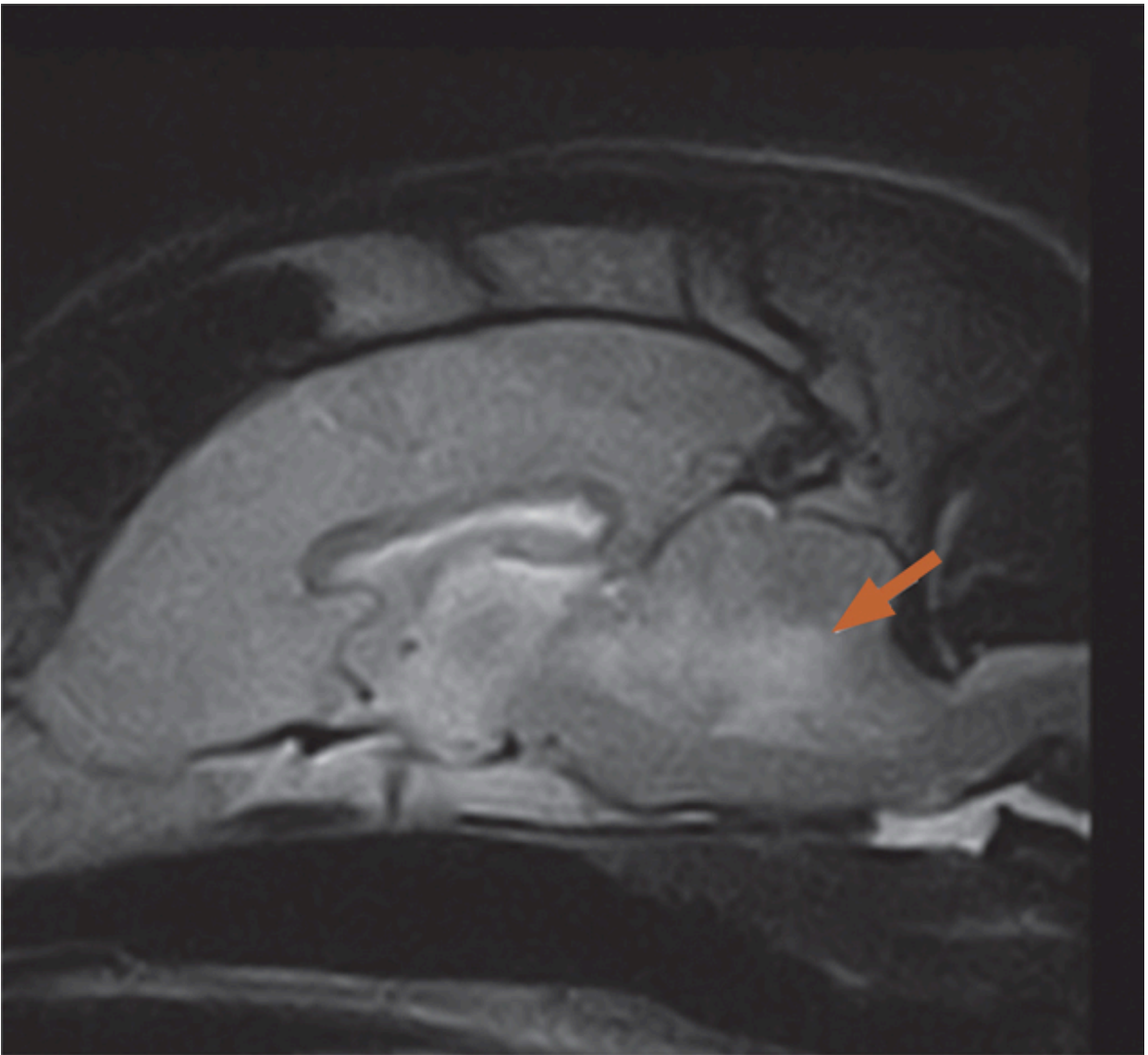


Figure 4. Cerebellitis in a confirmed case of canine neosporosis (T2 sagittal section). Image courtesy of Dr. Cristian de la Fuente, Diplomate ECVN, Clinical Veterinary Hospital, Autonomous University of Barcelona.

Serological tests

Given the difficulty in identifying *N. caninum* in tissues and CSF, presumptive diagnosis of neosporosis is based on clinical signs and serology.

IIF and ELISA for the detection of IgG and IgM, at 98 % sensitivity and 96 % specificity, are the most commonly used methods. Most dogs that shed oocysts do not show positive serology and thus cannot be identified. Positive titres may be observed in dogs that have previously been exposed but are asymptomatic, and in clinically ill dogs. No correlation between the magnitude of the increase in antibody levels and clinical signs has been reported. In most species IgG levels are increased 1 to 2 weeks after infection. The presence of maternal antibodies can lead to false positives. However, these antibody titres disappear by 32 days of age.

Although infection of dogs with *Neospora hughesi* is unlikely, crossreactions can occur in the presence of *N. hughesi* antigen. Crossreactions between *N. caninum*, *Babesia gibsoni*, *Toxoplasma gondii*, and *Hammondia heydorni* have been documented. ELISA with recombinant protein can be used for the diagnosis of acute *N. caninum* infections in dogs.

Organism detection

Visualisation of *N. caninum* oocysts in faeces is complex; they are rarely found and are very similar in morphology to those of *Hammondia heydorni*. The use of molecular techniques such as PCR, despite its low sensitivity, can facilitate diagnosis.

Tachyzoites can be detected in aspirates or smears of parasitised tissues or fluids, such as CSF. Monoclonal antibodies can be used for immunostaining of *T. gondii* to avoid crossreactions.

Tissue cysts are sporadically present and may not be detected. Tachyzoites can be visualised in biopsies of affected muscle tissue. These are very similar to those of *T. gondii* when viewed under an optical microscope, but can be differentiated by electron microscopy.

The microorganism can also be identified following isolation from cell cultures or immunocompromised mice.

Pathological examination

Histopathological examination may reveal meningoencephalomyelitis and myositis lesions in various muscles of the body. Tissue cysts are mainly found in the peripheral and central nervous tissue, while tachyzoites are found in many tissue types.

N. caninum induces more severe inflammation than *T. gondii*.

Confirmation of the aetiology requires serology, PCR, and immunohistochemical methods.

The histopathological lesions observed in canine neosporosis are listed in the box below.

Treatment

Although many dogs with neosporosis die, early treatment can result in resolution of the clinical disease.

In neonates, clinical improvement is unlikely in the presence of muscle stiffness or rapidly progressing paralysis. Puppies of over 16 weeks of age and adult dogs respond better to treatment. With early treatment, lower motor neuron paralysis caused by myositis is often reversible, as tissue scarring has not yet occurred. By contrast, 50% of cases in which affected dogs present with muscle stiffness do not respond to treatment.

Recommended drugs are similar to those used for the treatment of toxoplasmosis (Table 2).

Dermatitis and myositis respond well to treatment with clindamycin. Trimethoprim-sulfa and pyrimethamine are the antibiotics indicated for neurological diseases, as these compounds can adequately penetrate the CNS.

Table 2. Therapeutic options for *Neospora caninum* .

Drug	Dose
Trimethoprim-sulfadiazine	<ul style="list-style-type: none"> ■ 15–20 mg/kg PO every 12 hours for 4–8 weeks. ■ 10–15 mg/kg PO every 8 hours for 4–8 weeks.
Clindamycin	<ul style="list-style-type: none"> ■ 7.5–15 mg/kg PO or SC every 8 hours for 4–8 weeks. ■ 15–22 mg/kg PO or SC every 12 hours for 4–8 weeks.
Sulfonamides	15–30 mg/kg PO every 12 hours for 2–4 weeks.
Pyrimethamine (in combination with sulfonamides)	1 mg/kg PO every 24 hours for 2–4 weeks.

Prevention

No drugs or vaccines are currently available for the prevention of neosporosis. Similarly, there is no known therapy to prevent transmission from mother to pup. While oral toltrazuril does prevent this form of transmission in mice, further studies are required in dogs. Transmission from one generation to the next can be prevented by restricting breeding by bitches infected with *N. caninum*.

To reduce the likelihood of disease, all dogs of the same litter should be treated if one is diagnosed with neosporosis.

It is important to ensure that dogs do not ingest undercooked meat, especially beef. On farms, dogs should not have access to biological material derived from miscarriages, and should be prevented from defecating in areas to which livestock have access, particularly areas in which livestock feed.

The most effective treatment for the removal of oocysts from utensils and the environment is exposure to high temperatures for one minute with 10 % sodium hypochlorite.

Histopathologic findings

» Parasitic forms:

- In the thymus, liver, kidneys, stomach, adrenal glands, and skin in disseminated presentations.
- In the skeletal muscle, heart, brain, spinal cord, nerve roots, and retina in muscular and neurological presentations.

» CNS: discolouration, nonsuppurative encephalomyelitis, polyradiculoneuritis, ganglionitis, cerebellar necrosis.

» Musculature: necrosis, mineralisation, myositis, and myofibrosis.

» Other: phlebitis, dermatitis, nonsuppurative myocarditis, pneumonia, and hepatitis (usually subclinical).

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Canine leishmaniasis

Laia Solano Gallego

Canine leishmaniasis

Definition

Leishmaniasis is a parasitic zoonosis caused by *Leishmania infantum* and transmitted by the bite of the sandfly. The clinical manifestations of this infection are highly variable and include subclinical infections, mild self-limiting disease, and moderate to very severe disease.

Aetiology, biological cycle, and transmission

Aetiology

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania* (family Trypanosomatidae, class Kinetoplastida).

Human leishmaniasis is one of the most important globally distributed parasitic diseases after malaria and lymphatic filariasis. In humans, the term leishmaniasis describes a heterogeneous group of diseases caused by different species of the genus *Leishmania* that are transmitted by sandflies. Clinical manifestations are classically divided into three forms: cutaneous, mucocutaneous, and visceral leishmaniasis (Solano-Gallego and Villanueva-Saz, 2013b). In Europe, there are two cycles of endemic transmission:

1. Cutaneous and visceral leishmaniasis, caused by *Leishmania infantum* , which affects the Mediterranean region.
2. Anthroponotic cutaneous leishmaniasis, caused by *Leishmania tropica* , which causes sporadic outbreaks in Greece.

Research carried out in some parts of southern Europe suggests frequent exposure of humans to *L. infantum* . However, human susceptibility is low and asymptomatic infections are common in healthy populations (Michel *et al.* , 2011). In Europe cutaneous leishmaniasis accounts for the majority of

cases with a smaller number of visceral leishmaniasis cases (the mucocutaneous form is rarely described) (Solano-Gallego and Villanueva-Saz, 2013b).

The parasite *L. infantum* was first described in dogs in Tunisia in 1908 and is now endemic in more than 70 countries worldwide. Up to now, canine leishmaniasis (CanL) in Europe has been caused by *L. infantum* (Solano-Gallego and Villanueva-Saz, 2013b). However, infections caused by other species of *Leishmania*, such as *L. tropica* and *L. major*, have been sporadically reported in dogs in the Middle East (Baneth *et al.*, 2014) and North Africa (Lemrani *et al.*, 2002). Although both coincide in southern European countries, CanL is more prevalent and more widely distributed than human leishmaniasis, which can be hypoendemic (Solano-Gallego and Villanueva-Saz, 2013b). This chapter provides a detailed description of *L. infantum* infection in dogs in the Mediterranean basin.

Biological cycle

The life cycle of *L. infantum* comprises two main, morphologically distinct forms: the amastigote and promastigote. Amastigotes are round or oval, with a diameter of 2 µm to 6 µm, and parasitise cells of the mononuclear phagocyte system of vertebrate hosts. In addition to a basophilic nucleus, these forms contain a rod-shaped kinetoplast which is darker in colour when stained with Giemsa (Fig. 1). Promastigotes, which are found in the digestive tract of invertebrate hosts or vectors, are elongated extracellular microorganisms with a free flagellum. They measure 15 µm to 30 µm in length and 2 µm to 3 µm in width (Fig. 2) (Cardoso and Solano-Gallego, 2013).

After their inoculation via vectors into dogs and other vertebrates, infective metacyclic promastigotes are phagocytosed by macrophages. They transform into amastigotes and multiply by binary division, causing rupture of the host cells. The parasitic forms released are subsequently captured by other phagocytic cells. Repetition of this process leads to the spread of amastigotes and their presence in various tissues of susceptible animals, including the skin, spleen, bone marrow, and lymph nodes (Cardoso and Solano-Gallego, 2013).

Circulating parasitised cells can be ingested by other sandflies, in whose digestive tract they release amastigotes, which subsequently transform into

promastigotes. These multiply by longitudinal fission and achieve infectivity over a period of several days (metacyclogenesis). At this point, leishmania can be inoculated into another vertebrate host, completing the biological cycle of the parasite (Cardoso and Solano-Gallego, 2013).

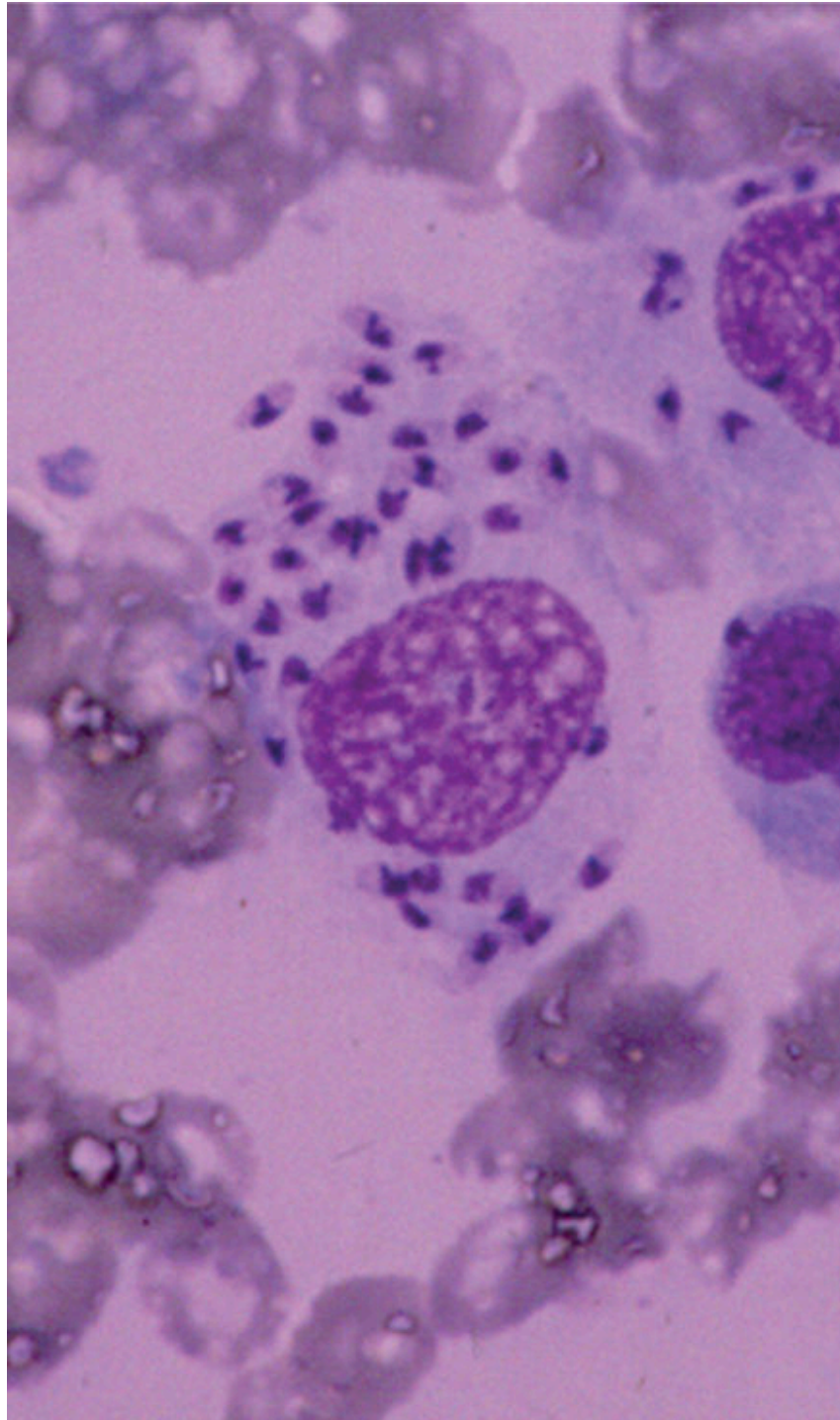


Figure 1. *Leishmania* amastigotes inside a macrophage (Giemsa stain, 1,000×). Image courtesy of Luis Cardoso, taken from the book *Leishmaniosis: una revisión actualizada* (Solano-Gallego *et al.* , Servet, 2013).

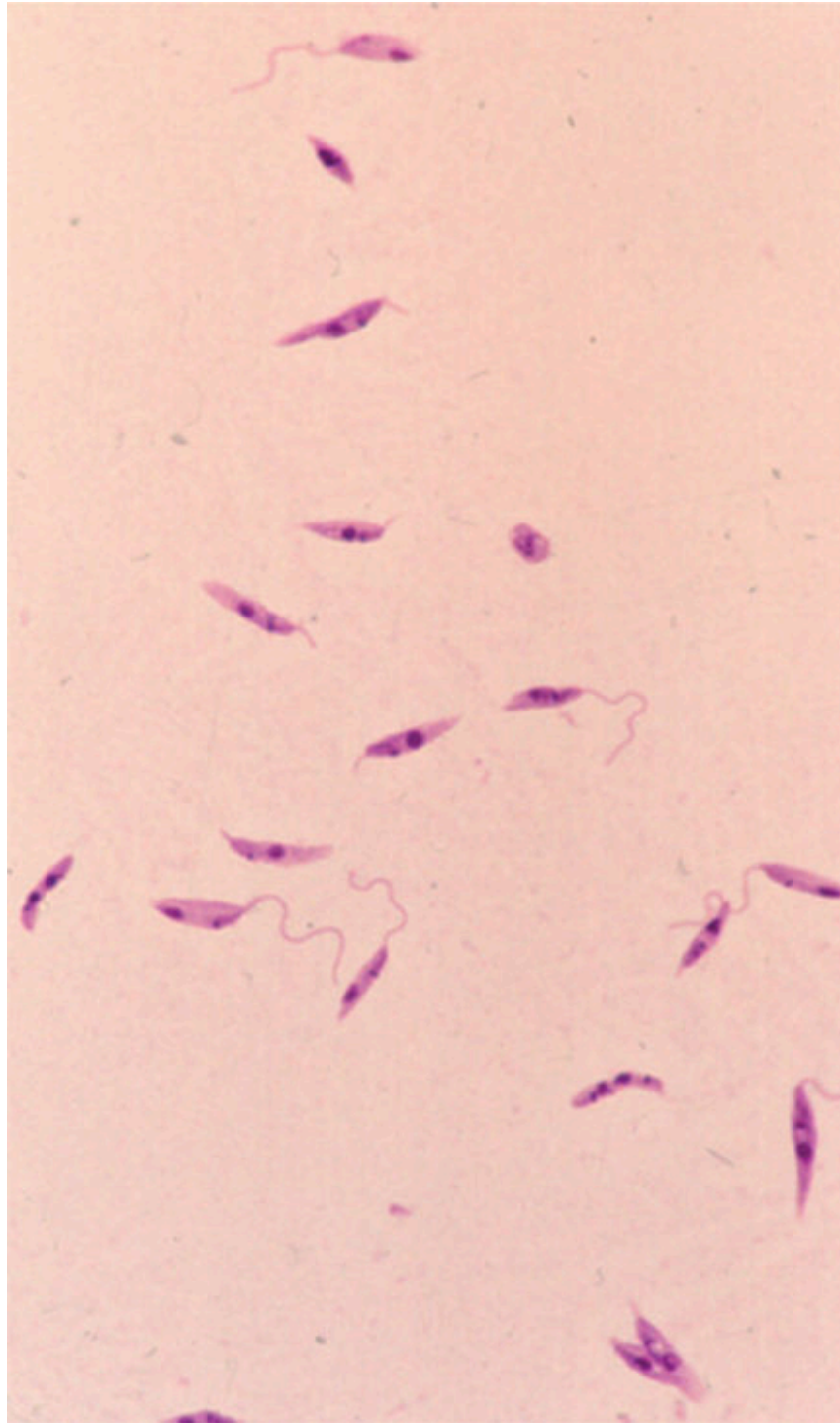


Figure 2. *Leishmania* promastigotes (Giemsa stain, 1,000×). Photo courtesy of Luis Cardoso, taken from the book *Leishmaniosis: una revisión actualizada* (Solano-Gallego *et al.* , Servet, 2013).

Transmission

In geographic areas in which CanL caused by *L. infantum* is present, dogs appear to serve as the primary reservoir of the parasite for humans and other animals. However, infections have also been described in many domestic and wild mammals, including cats, horses, wild canids, rodents, and rabbits. Some of these mammals, such as cats, rats, hares, and wild rabbits can act as a source of infection for sandflies, but their role as reservoirs requires further investigation (Millan *et al.* , 2014).

The main route of *Leishmania* transmission is the bite of infected sandflies, which are the only proven vectors of this protozoan. Vector populations have a continuous life cycle in the tropics and subtropics, and a seasonal life cycle (spring to autumn) in the Mediterranean region. In Europe, several species of the genus *Phlebotomus* are strongly implicated in the transmission of *L. infantum* in dogs (Cardoso and Solano-Gallego, 2013).

Epidemiology

Prevalence of infection and disease

In areas in which CanL is endemic, infection with *L. infantum* does not necessarily imply disease, given the high prevalence of subclinical infections. Thus, in an endemic area the percentage of infected dogs is high (a prevalence of nearly 60 %) compared with that of dogs with actual leishmaniasis (incidence, 5 %–10 %). Clinical disease is only the tip of the iceberg in endemic areas, where most of the canine population is exposed and is infected without developing clinical manifestations or serum antibodies against *Leishmania* (Cardoso and Solano-Gallego, 2013).

The northward spread of infection in some parts of Europe has been linked to changes in the distribution and abundance of vectors caused by global warming, as well as increased movement of infected dogs from the Mediterranean region. Moreover, CanL prevalence has increased in northern latitudes where the sandfly is absent or present in very low densities. Most dogs infected in these regions come from endemic areas, or have travelled

with their owners to these areas, although in some cases transmission may be autochthonous (Cardoso and Solano-Gallego, 2013).

Can this infection be transmitted without the participation of the sandfly?

Non-vector mediated modes of transmission, although less common, have also been demonstrated. These include infection via the transfusion of blood or derivatives from infected donors and vertical and venereal transmission. Other possible routes of transmission, which have not yet been demonstrated, include transmission by other vectors such as ticks and fleas and transmission via direct contact between dogs (Cardoso and Solano-Gallego, 2013).



Figure 3. *Phlebotomus* spp. (CDC / Prof. Frank Hadley Collins, Dir., Cntr. for Global Health and Infectious Diseases, Univ. of Notre Dame).

Risk factors

Whether infection in a dog is controlled or progresses to clinical disease depends on many factors. The immune system and the genetic background are probably the most important risk factors, although age, sex, nutritional status, coinfections or concomitant diseases, states of immunosuppression, parasitic load, virulence of the parasite, prior infections, and the mode of transmission also play important roles (Solano-Gallego *et al.* , 2009). The mechanisms underlying resistance or susceptibility to infection by this parasite in dogs remain unknown. However, whether a dog is resistant or susceptible depends on the capacity of its immune system to deal with *L. infantum* infection. Moreover, states of susceptibility or resistance are not permanent. Any stressor, drug treatment, or concomitant immunosuppressive disease can affect the immune system, such that a dog that was previously able to control the infection may develop clinical disease (Solano-Gallego *et al.* , 2009).

Other risk factors have also been identified. In Europe, dogs in rural and peri-urban environments appear to be more exposed, although infection is also becoming more prevalent in urban areas.

Living or sleeping outdoors is an important risk
factor for infection
with *L. infantum* (Cardoso and Solano-Gallego,
2013).

Most studies show no significant differences in disease incidence between sexes. While dogs can become infected at any age, seroprevalence shows a bimodal pattern, with higher levels of infection in animals of less than 3 years and more than 8 years of age (Cardoso and Solano-Gallego, 2013).

Boxers, German shepherds, and Rottweilers appear to be more vulnerable to developing the disease, whereas disease incidence in poodles and Yorkshire terriers is significantly lower as compared with reference populations. Differences in the prevalence of *Leishmania* infection described between

breeds may be due mainly to the habits and activities of dogs, and their resulting exposure to sandflies. However, it is accepted that certain indigenous breeds such as the Ibizan hound are more likely to develop protective cellular immunity and rarely show clinical signs of leishmaniasis (Cardoso and Solano-Gallego, 2013).

Pathogenesis

As mentioned above, not all dogs develop clinical disease, and a large proportion of those exposed develop subclinical infections. The immune response plays a very important role in the infection versus disease dichotomy.

The two opposite ends of the spectrum of clinical manifestation are as follows:

- 1. Apparently healthy infected dogs:** characterised by a mild humoral response and the presence of specific cellular immunity against the parasite that protects against and limits infection (Th1-type).
- 2. Seriously ill infected dogs:** characterised by an exaggerated humoral response and a reduced or absent specific cellular response, which is not protective and results in a high parasitic load (Th2-type) (Baneth *et al.* , 2008).

The pathogenic effects of typical clinical leishmaniasis are due mainly to the humoral response of the host to the parasite. The continuous antigenic stimulation caused by the high parasite load, as well as an exaggerated, nonprotective antibody response, trigger several immune mechanisms including immune complex deposition. These immune complexes can cause glomerulonephritis, vasculitis, polyarthritis, uveitis, and meningitis (Baneth *et al.* , 2008).

Clinical presentation

Clinical stages	Serology	Clinical signs	Laboratory abnormalities
I Mild disease	Negative or low antibody levels.	Mild clinical signs such as peripheral lymph node enlargement and papular dermatitis.	Clinical/pathological changes not usually observed. Normal renal profile: ■ Creatinine <1.4 mg/dl. ■ No proteinuria: UPC <0.5.
II Moderate disease	Antibody levels ranging from low to high.	In addition to the signs listed for clinical stage I, dogs may present with the following signs: ■ Symmetrical or diffuse cutaneous lesions, such as exfoliative dermatitis or onicogriphosis. ■ Ulcerations on the nasal plane, footpads, bony prominences, and mucocutaneous junctions. ■ Anorexia. ■ Weight loss. ■ Fever. ■ Epistaxis.	Clinical/pathological abnormalities such as mild nonregenerative anaemia, hyperglobulinaemia, and hypoalbuminaemia Can be classified into substages: ■ Normal renal profile (without proteinuria creatinine <1.4 mg/dl; UPC <0.5). ■ Altered renal profile (proteinuria): creatinine <1.4 mg/dl; UPC = 0.5–1.
III Severe disease	Antibody levels ranging from medium to high.	In addition to the clinical signs listed for stages I and II, dogs may show signs caused by lesions resulting from immune complex deposition: ■ Vasculitis. ■ Arthritis. ■ Uveitis. ■ Glomerulonephritis.	Clinical and pathological alterations listed for clinical stage II, together with those specific for CKD: ■ IRIS stage I (UPC >1). ■ Stage II (creatinine: 1.4–2 mg/dl).
IV Very severe disease	Antibody levels ranging from medium to high.	In addition to the clinical signs listed for stage III, dogs present with: ■ Pulmonary embolism. ■ Nephrotic syndrome and end stage renal disease.	Clinical and pathological alterations listed for stage III, together with those specific for CKD: ■ IRIS stage III (creatinine: 2–5 mg/dl). ■ IRIS stage IV (creatinine >5 mg/dl). ■ Nephrotic syndrome: high proteinuria (UPC >5).

UPC: urine protein/creatinine ratio; CKD: chronic kidney disease; IRIS: International Renal Interest Society.

Clinical signs

The clinical signs of CanL are described in Box 1 (Solano-Gallego and Miró, 2013). Below is a list of skin and eye lesions, which are the most common clinical manifestations of this disease.

The associated cutaneous disorders are both clinically and histopathology diverse. Typical cutaneous clinical signs include:

- Exfoliative dermatitis (Fig. 4).
- Ulcerative dermatitis affecting bony prominences (Fig. 5).
- Papular dermatitis.
- Onychogriphosis.

Atypical clinical features include:

- Dermatitis of the nasal plane (Fig. 6).
- Mucocutaneous ulcerative dermatitis.
- Ulcerative dermatitis in areas subject to trauma.
- Ulcerative dermatitis in areas of skin covering the extremities.
- Multifocal alopecia.
- Cutaneous and mucocutaneous nodular dermatitis.
- Pustular dermatitis.
- Nasodigital exfoliative dermatitis (Ordeix and Fondati, 2013).

The ocular presentations most frequently associated with canine leishmaniasis are:

- Blepharitis (Fig. 7).
- Conjunctivitis.
- Blepharoconjunctivitis.
- Keratitis.
- Keratoconjunctivitis sicca (KCS) (Fig. 8).
- Anterior uveitis.
- Glaucoma.
- Posterior uveitis.
- Panuveitis.
- Panophthalmitis.

Less common clinical presentations include:

- Orbital cellulitis.
- Granuloma of the nictitating membrane.
- Solitary iridic granulomas.
- Scleritis.
- Nodular granulomatous episcleritis/episclerokeratitis.

In most cases, ocular signs are bilateral. Unilateral signs, if observed, are associated with early diagnosis of the illness (Peña *et al.* , 2013).

Box 1. Clinical signs of canine leishmaniasis. Adapted from Solano-Gallego and Miró, 2013.

- » Generalised lymphadenopathy
- » Skin lesions
- » Body weight loss
- » Decreased or increased appetite
- » Lethargy
- » Pale mucous membranes
- » Eye lesions
- » Splenomegaly
- » Polyuria and polydypsia
- » Pyrexia
- » Vomiting
- » Diarrhoea
- » Epistaxis
- » Nodular or ulcerative lesions in mucocutaneous junctions or mucous membranes (oral, genital, and nasal)
- » Lameness (erosive or nonerosive polyarthritis, osteomyelitis, polymyositis)
- » Atrophic myositis of masticatory muscles
- » Vascular alterations and imbalances (systemic vasculitis, arterial thromboembolism)
- » Neurological disorders



Figure 4. Symmetrical exfoliative dermatitis affecting the periocular skin and dorsal part of the nose of an adult male Belgian shepherd. Photo courtesy of Laura Ordeix, taken from the book *Leishmaniosis: una revisión actualizada* (Solano-Gallego *et al.* , Servet, 2013).



Figure 5. Ulcer with neat borders, caused by *L. infantum*, located on the tarsus of an adult male Rottweiler. Photo courtesy of Laura Ordeix, taken from the book *Leishmaniosis: una revisión actualizada* (Solano-Gallego *et al.*, Servet, 2013).



Figure 6. Nasal plane dermatitis, characterised by depigmentation accompanied by erosions, ulcers, and crusting, which in this leishmaniasis patient partially affects the nasal plane. Photo courtesy of Laura Ordeix.



Figure 7. Bilateral blepharitis of the medial canthus in a miniature Schnauzer with leishmaniasis. Image courtesy of Veterinary Ophthalmology Service, Autonomous University of Barcelona (Dr. Teresa Peña and Dr. Marta Leiva), taken from the book *Leishmaniosis: una revisión actualizada* (Solano-Gallego *et al.*, Servet, 2013).



Figure 8. Mucopurulent discharge, conjunctival congestion, neovascularisation, corneal oedema, and chronic periocular alopecia in a dog with keratoconjunctivitis sicca caused by leishmaniasis. Image courtesy of Veterinary Ophthalmology Service, Autonomous University of Barcelona (Dr. Teresa Peña and Dr. Marta Leiva), taken from the book *Leishmaniosis: una revisión actualizada* (Solano-Gallego *et al.*, Servet, 2013).

Laboratory abnormalities

It is important to note that there are no pathological alterations pathognomonic of CanL. Accordingly, a differential diagnosis should always be performed based on clinical signs and laboratory results corresponding to a given patient.

The most common abnormalities in dogs with clinical leishmaniasis (stages II, III, and IV) are described in Box 2 . The most common blood disorder is mild normocytic and normochromic nonregenerative anaemia. This mild

nonregenerative anaemia develops as a result of a decrease in erythropoiesis due to chronic disease, or, in a smaller proportion of cases, due to chronic kidney disease. Moderate normocytic and normochromic nonregenerative anaemia is less common. In rare cases the anaemia may become complicated by blood loss, exacerbating the clinical picture, and resulting in moderate to severe regenerative anaemia. Immune-mediated haemolysis is very rare in this disease. Thrombocytopaenia and leukopaenia are relatively uncommon (Solano-Gallego and Miró, 2013).

Pathologic findings observed in histology or cytology in cases of CanL include macrophagic, neutrophilic/macrophagic, neutrophilic, and lymphoplasmacytic inflammation and reactive hyperplasia of lymphoid organs (Solano-Gallego and Miró, 2013).

Box 2. Laboratory abnormalities in canine leishmaniasis. Adapted from Solano-Gallego and Miró, 2013.

Serum proteins and protein electrophoresis

- » Hyperglobulinaemia: betaglobulinaemia and/or polyclonal gammaglobulinaemia
- » Hypoalbuminaemia
- » Reduced albumin/globulin ratio

Haemogram

- » Nonregenerative anaemia (mild to moderate)
- » Leukocytosis, lymphopaenia

Biochemical profile and urinalysis

- » Proteinuria (mild to severe)
- » Renal azotaemia
- » Increased liver enzyme activity

Diagnosis

Diagnosis of CanL is very complex and difficult due to the broad spectrum and nonspecific nature of the associated clinical and pathological alterations.

Diagnosis of *L. infantum* infection is established for the following reasons:

- To confirm the presence of clinical disease.
- To determine the presence of infection in clinically healthy dogs living in endemic areas, including blood donor dogs, dogs due for vaccination, and dogs that may be progressing towards disease; prevent importation of infected dogs in nonendemic areas; and monitor the response to treatment.

For these reasons, it is very important to differentiate between *Leishmania* infection and actual disease and to apply distinct diagnostic techniques in each particular case (Solano-Gallego *et al.* , 2009).

Several strategies are used for the diagnosis of CanL. It is recommended to combine clinical diagnosis, based on clinical signs and laboratory abnormalities, with specific techniques for the diagnosis of CanL. The following techniques are used for the detection of *L. infantum* infection in dogs:

- Parasitological diagnosis: cytology, histology, and immunohistochemistry.
- Molecular diagnosis: conventional, nested, and real time polymerase chain reaction (PCR).
- Serological methods: qualitative and quantitative tests.
- Tests to detect a cellular immune response.

Other techniques with less clinical applicability include culture, delayed hypersensitivity tests, and xenodiagnostic techniques (*Phlebotomus* infection) (Solano-Gallego and Villanueva-Saz, 2013a). Cytological or histological diagnosis, with or without immunohistochemistry, can be established using samples of any tissue or biological fluid. Aspirates of lymphatic organs, bone marrow, or skin lesions are the most commonly used sample types.

Serological diagnosis

Detection of serum antibodies should be performed using quantitative serological techniques, such as IIF and ELISA. Drawbacks of serology include crossreactivity with other pathogens, such as other species of *Leishmania* or *Trypanosoma* , and antibodies elicited by vaccination against CanL. The presence of high levels of antibodies is associated with moderate to very severe disease if the dog has not been recently vaccinated. However, the presence of lower levels of antibodies is not necessarily indicative of disease and it is essential to confirm CanL diagnosis using other diagnostic

methods such as cytology, histopathology, or PCR (Solano-Gallego *et al.* , 2009).

Molecular diagnosis

PCR has improved the sensitivity of diagnosis of *Leishmania* infection in dogs. Several methods using the nuclear genome or kinetoplast DNA (kDNA) have been developed. Methods that use kDNA appear to be more sensitive for direct detection in infected tissues. Quantitative PCR using kDNA is an advanced technique that can detect extremely low parasitic loads (Francino *et al.* , 2006) and is therefore more sensitive than conventional PCR (Miró *et al.* , 2008).

Serology in vaccinated dogs

To date, there are no serological tests that can distinguish between vaccine-induced antibodies and those induced by natural infection. Furthermore, it has been reported that vaccinated dogs show a significant increase in antibody levels between 8 and 12 weeks after the third dose of vaccine, and can remain seropositive for several months (Moreno *et al.* , 2014). For these reasons, it is recommended to be cautious when interpreting serological results from unvaccinated dogs, and in cases of positive results to always perform additional confirmatory diagnostic tests (Roura *et al.* , 2015).

PCR can be performed by extracting DNA from different tissues such as blood, biological fluids, and even histological or cytological material. Bone marrow, lymph nodes, spleen, and skin are most appropriate tissues for diagnosis by PCR. Blood and urine samples provide lower sensitivity. PCR analysis of lymph node or bone marrow samples is more sensitive than direct observation of amastigotes in smears or parasitological culture (Solano-Gallego and Villanueva-Saz, 2013a).

The analysis of conjunctival swabs, which can be acquired noninvasively, appears to allow high sensitivity detection of *L. infantum* in seropositive dogs with clinical leishmaniasis (Strauss-Ayali *et al.* , 2004). Recent studies have described the use of other noninvasively acquired samples including oral, vaginal, nasal, and otic swabs (Ferreira Sde *et al.* , 2013; Hernández *et al.* , 2015; Lombardo *et al.* , 2012) and hair samples (Belinchón-Lorenzo *et al.* , 2013) for the diagnosis of disease in dogs. These noninvasively acquired samples appear to provide moderate to good sensitivity, although relatively

few studies have been performed, sometimes with conflicting results. The diagnostic utility of these samples should be confirmed in future studies.

Information provided by PCR should be assessed in conjunction with data obtained from the clinicopathological examination and serological tests, and all results combined to ensure a complete evaluation. It is essential to understand the basis and limitations of each diagnostic test and to appropriately interpret the resulting data (Solano-Gallego *et al.* , 2009).

Treatment and prognosis

To correctly treat the affected animal it is necessary to clinically evaluate the patient and establish a classification based on the severity of clinical signs and pathologic alterations. This will allow selection of the most appropriate type of intervention (Miró, 2013; Solano-Gallego *et al.* , 2009).

The combination of leishmanicidal molecules with allopurinol (a leishmaniostatic compound) is the current treatment of choice for dogs with leishmaniasis (clinical stages II, III); pentavalent antimonials are considered the most effective treatment, followed by miltefosine (Miró, 2013). Table 2 outlines the therapeutic protocols corresponding to each clinical stage, while Table 3 lists the dosage and adverse effects of indicated treatments.

The future of therapy for this disease is the combination of parasitocidal/parasitostatic compounds with immunomodulators to combat the parasite on the one hand and to provoke an adequate immune response on the other (Miró, 2013).

Currently, the prognosis for diseased dogs in which anti-*Leishmania* treatment has been instituted is generally favourable, especially if severe kidney disease is absent. The prognosis varies depending on the clinical stage of each patient (Table 2). In general, the prognosis depends on the severity of the clinical and pathological changes observed when leishmaniasis treatment begins, and in particular on the severity of kidney damage, which should be assessed objectively in accordance with IRIS recommendations (IRIS, 2015a and b); the individual response of each dog to treatment and the development of

treatment resistance (Yasur-Landau *et al.* , 2016); and any comorbidities (Miró, 2013).

Table 2. Prognosis and treatment based on clinical stage of canine leishmaniasis. Adapted from Solano-Gallego *et al.* , 2009.

Clinical stage	Treatment	Prognosis
I Mild disease	<ul style="list-style-type: none"> ■ Monitoring without treatment. ■ Treatment of short duration (1–3 months) including any anti-<i>Leishmania</i> drug and/or immunomodulator (e.g. domperidone). 	Good.
II Moderate disease	<ul style="list-style-type: none"> ■ N-methylglucamine antimonate + allopurinol ■ Miltefosine + allopurinol. 	Good to guarded.
III Severe disease	Same as stage II plus IRIS treatment recommendations.	Guarded to unfavourable.
IV Very severe disease	Same as stage II plus IRIS treatment recommendations.	Unfavourable.

Table 3. Drugs for treatment of canine leishmaniasis: dosage and adverse effects. Adapted from Solano-Gallego *et al.* , 2009.

Active substance	Dosage	Adverse effects
N-methylglucamine antimonate	50–100 mg/kg SC every 12–24 hours for 4–8 weeks.	Potential nephrotoxicity, skin abscesses/cellulitis.
Miltefosine	2 mg/kg PO every 24 hours for 1 month.	Digestive disorders (dysorexia, vomiting, diarrhoea).
Allopurinol	10–20 mg/kg PO every 12 hours for a minimum of 8–12 months.	Xanthinuria, renal mineralisation, xanthine urolithiasis.
Domperidone	0.5 mg/kg every 24 hours for 4 weeks.	Galactorrhea.

Prevention

Disease prevention requires a comprehensive approach that includes disease control in individual dogs and populations, as well as actions targeting the environment in which the vector perpetuates (Otranto and Dantas-Torres, 2013).

In recent decades, considerable effort has been expended to develop new cost-effective strategies to tackle this disease in endemic areas. Vaccination is considered one of the most promising strategies for CanL control. In the field of immunology, research has evolved from the development of vaccines based on crude lysate extracts of the parasite to the use of recombinant DNA. Currently, second generation CanL vaccines are available in Europe and Brazil, and show varying rates of effectiveness in terms of reducing disease incidence (Otranto and Dantas-Torres, 2013).

Domperidone (a D₂ dopamine receptor antagonist) has shown efficacy in reducing seroconversion, i.e. it appears to reduce the production of antibodies in dogs in areas in which leishmaniasis is endemic. It is registered in some European countries, including Spain, Portugal, and Italy, as a preventive measure against CanL (Sabate *et al.* , 2014).

Various compounds with repellent activity (pyrethroids) have also proven useful as a cost-effective means of reducing the risk of transmission of *L. infantum* among dog populations, applied either via pipette (*spot-on*) or in a collar (Otranto and Dantas-Torres, 2013).

Given that the complex mechanisms of transmission of this parasite make CanL control a real challenge, one of the most successful strategies for disease control will be the combined use of new generation vaccines and repellents, which will also reduce the risk of infection of humans and other mammals (Otranto and Dantas-Torres, 2013).

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Canine babesiosis

Laia Solano Gallego

Canine babesiosis

Definition

Canine babesiosis encompasses a variety of protozoal diseases that are caused by different species of *Babesia* and transmitted mainly by ticks. In dogs, these infections have variable presentations, ranging from subclinical infection to severe systemic disease. The most common clinical and pathological findings are haemolytic anaemia and thrombocytopaenia.

Aetiology, biological cycle, and transmission

Aetiology

Babesia species are protozoa that infect the erythrocytes of a wide variety of domestic and wild animals, as well as humans, and are primarily transmitted by ticks. *Babesia* species belong to the phylum Apicomplexa, class Piroplasma, order Piroplasmida.

Some *Babesia* species that infect dogs have a worldwide distribution, while others are restricted to specific biogeographical zones. Historically, infection with *Babesia* spp. was identified based on the morphological appearance of the protozoan within the erythrocyte upon microscopic examination of a blood smear. All large forms of *Babesia* were identified as *Babesia canis*, while all small forms were considered *Babesia gibsoni* (Boozer and Macintire, 2003). With the development of molecular identification methods, it has since been demonstrated that dogs can be infected by other small *Babesia* species such as *Babesia conradae* (Kjemtrup and Conrad, 2006) and *Babesia vulpes* (previously known as *B. microti*-like or *Theileria annae*) (Baneth *et al.*, 2015). Multiple large *Babesia* species have been identified, including *Babesia rossi*, *B. canis*, and *Babesia vogeli*. These are considered distinct species based on the marked differences in their clinical and

pathological effects, geographical distribution, and vector specificity. However, they were previously considered subspecies of *B. canis* , as they are morphologically identical (Solano-Gallego and Baneth, 2011). Table 1 lists the species that most commonly infect dogs in different biogeographic regions.

Biological cycle

Sporozoites are injected into the skin of the dog via the saliva of the tick. The parasites invade the erythrocytes and appear as ring-shaped structures known as trophozoites. Next, the parasites replicate within the erythrocytes and form merozoites. In some *Babesia* species these appear as paired, pear-shaped structures. Merozoites can divide to form eight or more parasites within a single erythrocyte, resulting in destruction of the erythrocyte and liberation of the parasites into the bloodstream where they can invade other erythrocytes. The ingestion by ticks of merozoite-infected blood allows the sexual cycle of the parasite to continue in the gut of the tick. This is followed by sporogony in other tissues. Parasites ultimately reach the salivary glands of the tick, from where transmission to dogs occurs.

Transmission

Transmission of *Babesia* occurs mainly through tick bites. Table 1 shows the different ticks implicated in the transmission of *Babesia* species that commonly infect dogs. Other routes of transmission demonstrated for *B. gibsoni* include blood transfusion, transplacental transmission, and direct contact between dogs (wounds, saliva, or ingestion of blood) (Solano-Gallego and Baneth, 2011). Possible vertical transmission has also been documented for *B. canis* (Mierzejewska *et al.* , 2014) and *B. vulpes* (Simoes *et al.* , 2011).

Epidemiology

The prevalence of infection with *Babesia* spp. depends on the habitat of the tick species involved. The geographical distribution of different *Babesia* species is shown in Table 1 . To date, infection with *B. rossi* has only been described in sub-Saharan Africa and infection with *B. canis* only in Europe,

while infections caused by *B. vogeli* and *B. gibsoni* have a wider distribution. *B. canis* infection is observed in parts of Europe with cold, wet climates, and its prevalence is higher in central and northern Europe than in the Mediterranean basin. By contrast, *B. vogeli* infection is diagnosed in parts of the Mediterranean basin and surrounding areas. *B. vulpes* infection has been documented in several European countries, and in Spain is especially prevalent in Galicia and Asturias (Camacho-Garcia, 2006).

Pathogenesis

The pathological effects of *Babesia* infection in dogs vary considerably depending on species, age, coinfections, and concomitant diseases. The severity of the disease also depends on the immune status of the individual, and their response to infection. For this reason immunosuppressive treatments, debilitating diseases, and other clinical conditions that cause immunosuppression can aggravate these infections (Solano-Gallego and Baneth, 2011).

The spleen plays an important role in controlling immune infection. Rapid active multiplication of the parasite can be observed in splenectomised dogs, leading to clinical babesiosis and high levels of parasitaemia.

Splenectomy is an important risk factor for the
development of babesiosis in dogs (Solano-
Gallego and Baneth, 2011).

In general, *Babesia* species cause haemolytic anaemia and the development of systemic clinical signs of varying intensity. They also induce a series of immune responses that can give rise to severe lesions (Ayoob *et al.* , 2010). Haemolytic anaemia can be intravascular or extravascular. The mechanisms underlying the destruction of erythrocytes can vary considerably. One such mechanism is the direct destruction of erythrocytes following replication of

the intracellular parasites (intravascular haemolysis). However, other mechanisms have been described, including activation of the response upon antibody binding to the cell surface and subsequent complement activation; the production of sera containing haemolytic factors; erythrocyte oxidation; vascular stasis; increased phagocytosis of erythrocytes; and increased osmotic fragility of erythrocytes. Anti-erythrocyte antibodies have been detected in dogs infected with *B. gibsoni* and *B. vogeli* but not *B. canis* .

Intense haemolysis can give rise to haemoglobinaemia, haemoglobinuria, bilirubinaemia, and bilirubinuria (Solano-Gallego and Baneth, 2011). However, in some cases thrombocytopaenia is the only laboratory abnormality observed, suggesting a link with the immune response (immune-mediated thrombocytopaenia), possibly due to splenic sequestration or increased platelet consumption caused by haemolysis- or vascular damage-induced coagulation. Severe alterations in secondary haemostasis are not commonly observed during *Babesia* infection (Solano-Gallego and Baneth, 2011).

Tissue hypoxia contributes to the production of many of the clinical signs caused by most *Babesia* species (Solano-Gallego and Baneth, 2011) and primarily affects the central nervous system, kidneys, and muscles (Jacobson, 2006).

The causes of hypoxia include:

- Anaemia.
- Distributive shock.
- Vascular stasis.
- Excessive production of endogenous carbon monoxide.
- Destruction of haemoglobin by the action of the parasite.
- Decreased ability of haemoglobin to transport oxygen in erythrocytes infected with *Babesia* (Ayoob *et al.* , 2010).

Hypoxia appears to be more important than
haemoglobinuria as a cause of kidney damage in

dogs with babesiosis (Solano-Gallego and Baneth, 2011).

Tissue hypoxia, distributive shock, multiple organ failure, and death have all been recorded in cases of *B. rossi* infection (Jacobson, 2006). Infection by this species can manifest with an acute presentation or even as a hyperacute and fatal syndrome, with massive haemolysis, renal insufficiency, and disturbances in acid-base balance. The release of oxygen free radicals and the mechanisms underlying the harmful effects of cytokines have been associated with increased vascular permeability and vascular endothelial injury, which can give rise to pulmonary oedema due to disseminated intravascular coagulopathy (DIC). Cerebral babesiosis, caused by the sequestration of parasites in cerebral capillaries, has also been described in *B. rossi* infections (Jacobson, 2006).

The clinical manifestations of other *Babesia* species are generally less severe and range from subclinical infection to mild or moderate disease in the case of *B. vogeli* , and moderate to severe infections in the case of *B. canis* , *B. vulpes* , *B. gibsoni* , or similar (Solano-Gallego and Baneth, 2011).

Clinical presentation

The main clinical features (anamnesis and clinical history), as well as major clinical signs and laboratory abnormalities for different *Babesia* species that infect dogs are described in Table 1 .

Clinical signs

The most common clinical signs for many of these *Babesia* species are fever, lethargy, anorexia, weakness, pale or jaundiced mucous membranes, and dark coloured urine (pigmenturia). Some differences are observed depending on the species of *Babesia* involved, as shown in Table 1 . For example, in cases of *B. gibsoni* infection lymph node enlargement, splenomegaly, or weight loss may be the only clinical signs observed. It is important to note that patients

with *B. rossi* infection may present with clinical babesiosis without complications, similar to that described for other species such as *B. canis* and *B. vogeli* . However, other patients can develop a more severe clinical disease with multiple complications.

Laboratory abnormalities

The most common laboratory abnormalities observed for all species of *Babesia* are thrombocytopaenia, mild to moderate haemolytic and regenerative anaemia, bilirubinuria, and bilirubinaemia. However, some differences are observed between species, as shown in Table 1 .

Table 1. Geographical distribution, implicated tick species, clinical signs and characteristics, laboratory abnormalities, treatment, and prognosis of the main *Babesia* species that infect dogs.
Adapted from Solano-Gallego and Baneth, 2011.

Species	Biogeographical distribution	Ticks involved	Clinical characteristics	Clinical signs and laboratory abnormalities	Treatment and response	Prognosis	
Large <i>Babesia</i> species	<i>Babesia canis</i>	Europe (from Portugal to northern and eastern Europe)	<i>Dermacentor reticulatus</i>	■ Young and adult hunting, grazing, or guard dogs (German shepherd and Komondor) living outdoors. ■ Cases are observed in autumn and spring.	■ Fever, lethargy, anorexia, pale or jaundiced mucous membranes, and pigmenturia. ■ Mild to moderate thrombocytopaenia, nonregenerative anaemia, regenerative anaemia (less common), neutropenia, and bilirubinaemia.	Good	
	<i>Babesia vogeli</i>	Mediterranean basin and neighbouring regions	<i>Rhipicephalus sanguineus</i>	Puppies, adult dogs, and elderly dogs with concomitant illnesses.	■ Fever, lethargy, anorexia, pale or jaundiced mucous membranes. ■ Thrombocytopaenia, immune-mediated haemolytic anaemia, nonregenerative anaemia, leukocytosis, and leukopenia.	Good	
	<i>Babesia bigemina</i> -like	North America (east coast)	Unknown	Splenectomised or immunocompromised dogs.	■ Fever, lethargy, and anorexia. ■ Thrombocytopaenia and nonregenerative mild anaemia.	Good	
	<i>Babesia rossi</i>	South Africa, Nigeria, Sudan	<i>Haemaphysalis elliptica</i>	Young and adult dogs.	■ Uncomplicated clinical presentation: similar to that described for <i>B. canis</i> and <i>B. vogeli</i> . ■ Complicated clinical presentation: hypotension, acute respiratory distress syndrome, pancreatitis, myalgia, rhabdomyolysis, ascites, pulmonary oedema, brain and kidney damage (acute kidney failure [anuria]), and shock. Metabolic acidosis and respiratory alkalosis, renal azotaemia, coagulopathy, DIC, and hypoglycaemia.	From good to guarded	
Small <i>Babesia</i> species	<i>Babesia gibsoni</i>	North America, Central America, Asia, Australia, and Europe ¹	■ <i>Haemaphysalis longicornis</i> ■ <i>H. bispinosa</i> ? ² ■ <i>R. sanguineus</i> ? ³	Fighting dogs such as the pit bull terrier and Tosa Inu.	■ Fever, lethargy, anorexia, pale or jaundiced mucous membranes, pigmenturia, lymphadenopathy, splenomegaly, and weight loss. ■ Regenerative haemolytic anaemia (sometimes immune-mediated), bilirubinaemia, bilirubinuria, neutropenia, and thrombocytopaenia.	■ Atovaquone (13.5 mg/kg PO every 8 hours) and azithromycin (10 mg/kg PO every 24 hours) for 10 days. ■ Moderate to poor response with frequent relapses.	Guarded to poor
	<i>Babesia vulpes</i> (previously <i>B. microti</i> -like)	United States and Europe ¹	■ <i>Ixodes hexagonus</i> ? ¹ ■ <i>I. canisuga</i> ? ²	■ Young or young adult dogs. ■ Hunting or guard dogs living outdoors.	■ Fever, lethargy, weakness, and pigmenturia. ■ Moderate to severe regenerative haemolytic anaemia, thrombocytopaenia, azotaemia, and proteinuria.		
	<i>Babesia conradiae</i>	United States (southern California)	<i>R. sanguineus</i> ? ³	Cubs and young and adult dogs.	■ Lethargy, pale mucous membranes, vomiting, and lymphadenopathy. ■ Immune-mediated regenerative haemolytic anaemia and thrombocytopaenia.		

¹ *B. gibsoni* infection documented in Germany, Croatia, Italy, Spain, UK, Slovakia, and Serbia.
² *B. vulpes* infection documented in Spain, Portugal, Italy, France, Croatia, Serbia, and Sweden.
³ Vectorial capacity not demonstrated to date. Involvement is assumed based on epidemiological association.

Diagnosis

Parasitological and molecular diagnosis

Cytological analysis of blood smears stained with Wright’s, Giemsa, or Diff-Quik stain has been the standard method for the diagnosis of babesiosis for

many years. This method is sufficiently sensitive in the presence of moderate to high parasitaemia.

Two types of *Babesia* species can be observed by light microscopy:

- **Large species** , which range in size from 2.5 μm to 5 μm (Fig. 1).
- **Small species** , which range in size from 1 μm to 3 μm (Fig. 2).

Observation using an optical microscope is very useful for the diagnosis of dogs infected with *B. canis* , as most of these patients present with large forms of *Babesia* that are easily detectable in a blood smear. Although the diagnostic utility of smear cytology in dogs infected with *B. vogeli* is less clear, in cases involving moderate to high parasitaemia parasites can be easily detected under a microscope. Small babesia species such as *B. gibsoni* and *B. vulpes* are more difficult to observe by optical microscopy given their smaller size, but can be visualised in blood smears if parasitaemia levels are sufficiently high. As such, the observation of blood smears should be the first diagnostic method used for patients with suspected babesiosis. However, a negative result does not rule out *Babesia* infection, as the sensitivity of blood smear analysis is lower than that of molecular techniques. Furthermore, the species of *Babesia* involved cannot be identified based on morphological observation. This requires molecular techniques such as PCR.

Smears should always be performed using fresh blood. Babesias appear to be more abundant in capillary blood and blood obtained from the pinna, and thus can be more easily visualised in smears using these types of blood samples (Irwin, 2009).

PCR is particularly useful for babesia detection in animals with low levels of parasitaemia, both in disease states and subclinical infections. Several different molecular techniques can be used for the identification and differentiation of different *Babesia* species, including PCR with internal

primers (“semi-nested”), reverse line blotting (RLB), and restriction fragment-length polymorphism (PCR-RFLP). The tissues of choice are the blood and spleen (Solano-Gallego and Baneth, 2011). These molecular techniques allow identification of the species involved in the infection, helping the clinician to both establish a diagnosis and provide a more accurate prognosis. This is also a very useful technique for treatment monitoring.

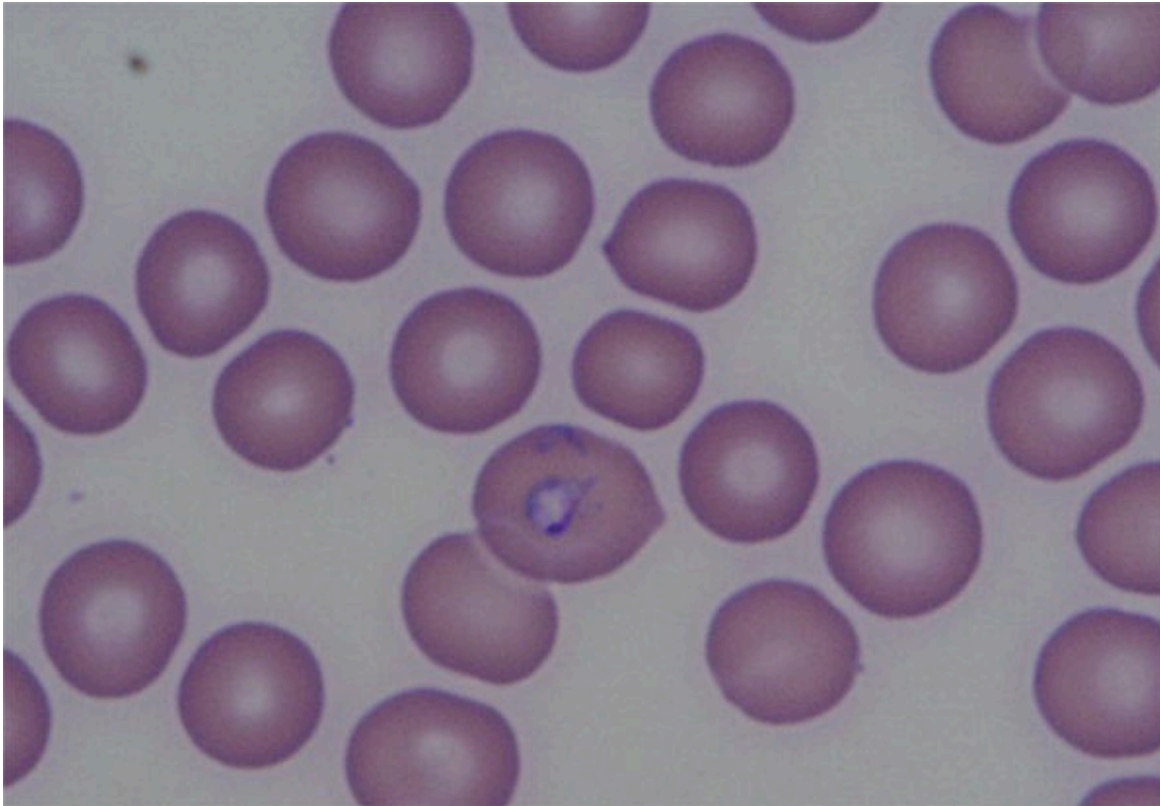


Figure 1. *Babesia* species inside an erythrocyte. Erythrocytes with mild anisocytosis and an absence of platelets are observed (100×).

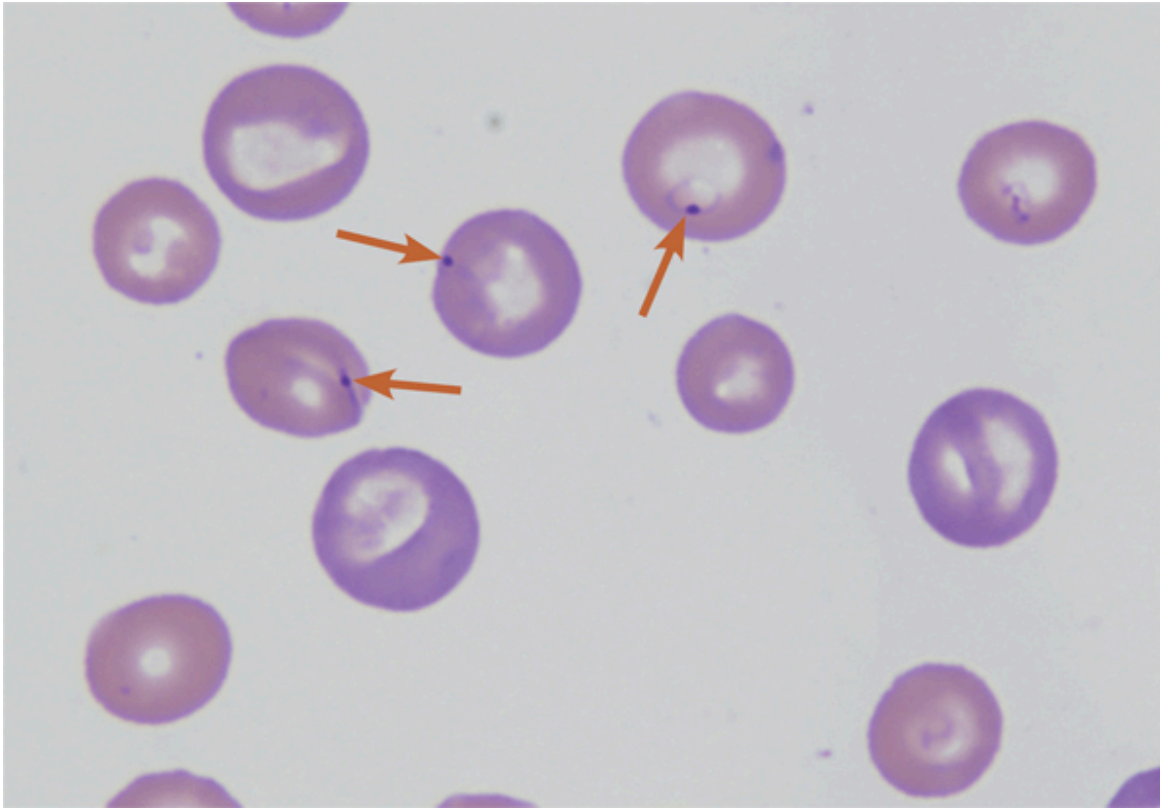


Figure 2. Small *Babesia* species inside several erythrocytes (arrows). Moderate polychromasia and anisocytosis are observed (100×).

Serological diagnosis

Serology can indicate a persistent, present, or past infection. The most commonly used serological technique is indirect immunofluorescence (IIF). However, crossreactivity between different species, such as *B. canis* and *B. gibsoni*, has been described. Moreover, infections with certain species such as *B. canis* and *B. rossi* are characterised by acute or hyperacute disease courses. Consequently, the results of serological tests may be negative. In such cases, it is necessary to perform antibody titres during the convalescent phase in order to demonstrate acute infection. Given its inability to evaluate seroconversion, serology is not the test of choice for canine babesiosis. Furthermore, serology cannot identify the specific species of *Babesia* involved in a given infection (Solano-Gallego and Baneth, 2011).

Treatment and prognosis

Large *Babesia* species are usually treated with imidocarb dipropionate, with good clinical response. By contrast, small *Babesia* species are more difficult to treat and are refractory to conventional treatments that are effective against larger forms. Usually, treatments for infections with small *Babesia* species do not result in clinical and parasitological cure, and relapses are common. The treatment of choice in these cases appears to be the combination of atovaquone and azithromycin (Solano-Gallego and Baneth, 2011).

Table 1 describes the different active ingredients used as well as the corresponding dose, duration, and treatment response, depending on the *Babesia* species with which a dog is infected.

Clinical management of both large and small *Babesia* species may require supportive treatment, including intravenous fluid administration, transfusions of blood or blood products, and anti-inflammatory therapy (Ayoob *et al.* , 2010).

Large *Babesia* species such as *B. vogeli* and *B. canis* have a good prognosis if treatment with an effective anti-*Babesia* compound such as imidocarb dipropionate is instituted. Dogs recover clinically 24 to 48 hours after beginning treatment. However, small *Babesia* species such as *B. gibsoni* and *B. vulpes* have a worse prognosis, ranging from guarded to poor. The prognoses for different *Babesia* species that infect dogs are listed in Table 1 .

Prevention

The prevention of babesiosis is based primarily on the use of individually applied acaricide treatments, administered either topically (Jongejan *et al.* , 2015; Otranto *et al.* , 2010) or orally (Beugnet *et al.* , 2014; Taenzler *et al.* , 2015), in order to reduce exposure to ticks and pathogen transmission.

Periodic prophylactic treatment with imidocarb dipropionate does not appear to be effective in preventing *Babesia* infection. Currently, the use of this drug for prophylaxis of babesiosis is not recommended.

Because these protozoa can be transmitted through blood transfusions, routine analysis of blood donors for infection is recommended, and dogs that test

positive in PCR analyses of blood samples should be ruled out as potential donors (Wardrop *et al.* , 2005).

Commercial vaccines against *B. canis* containing soluble parasite antigens are available in some European countries, including Spain. These vaccines induce partial protection against disease caused by *B. canis* and reduce the severity of clinical signs, parasitaemia, and the duration of the disease (Schetters *et al.* , 2009). In dogs, the vaccine can be administered from 6 months of age, with revaccination 3 to 6 weeks later and subsequently every 6 months.

In recent years, progress has been made in the development of vaccines against other *Babesia* species, such as *B. gibsoni* (Goo and Xuan, 2014).

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Giardiasis

Giardiasis

Definition

Diarrhoeal disease caused by *Giardia duodenalis* and one of the most frequent parasitic diseases of dogs.

Aetiology and incidence

Giardia duodenalis is a protozoan with a worldwide distribution. Currently, seven different genotypes are described, of which A, B, C, D, and E can infect canine species. In addition to these intraspecies genetic differences, differences in cyst morphology are also observed between genotypes. The most prevalent genotypes in dogs are C and D. Genotyping is based on a mutation in the triosephosphate isomerase gene.

G. duodenalis has an active and mobile form, the trophozoite, which localises in the intestinal epithelium where it attaches to the villi. It measures $15\ \mu\text{m} \times 8\ \mu\text{m}$ and has a teardrop-like shape. It contains two nuclei in the anterior third, separated by the axoneme bundle, and four pairs of flagella. In the dog, trophozoites are found from the duodenum to the ileum. Trophozoites divide by binary fission until a cyst is formed (Figs. 1 and 2). The cyst is shed in the faeces and is resistant to the external environment. Ingestion by the host of the cyst form, following faecal contamination of water or food, results in the release of two trophozoites from each cyst in the duodenum in response to the action of gastric and pancreatic secretions, thus completing the parasite's life cycle.

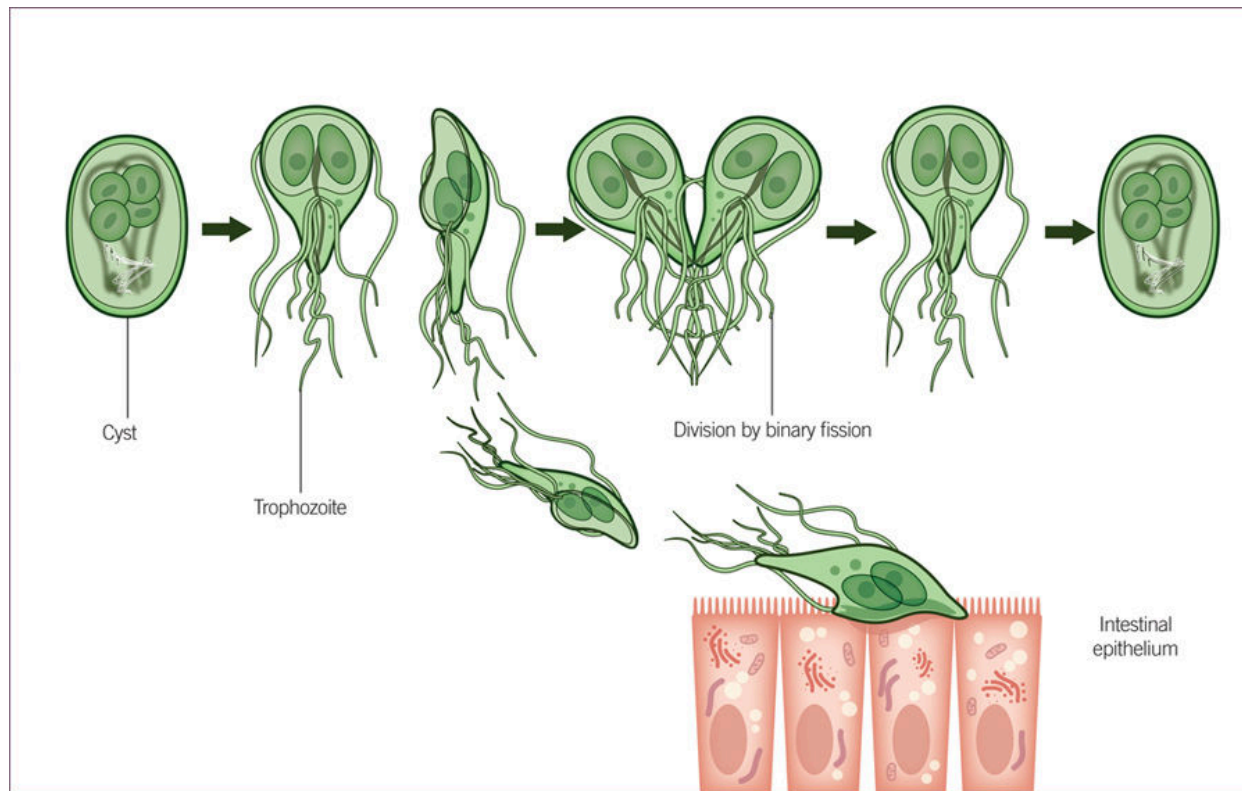


Figure 1. *Giardia duodenalis* trophozoites and cysts.

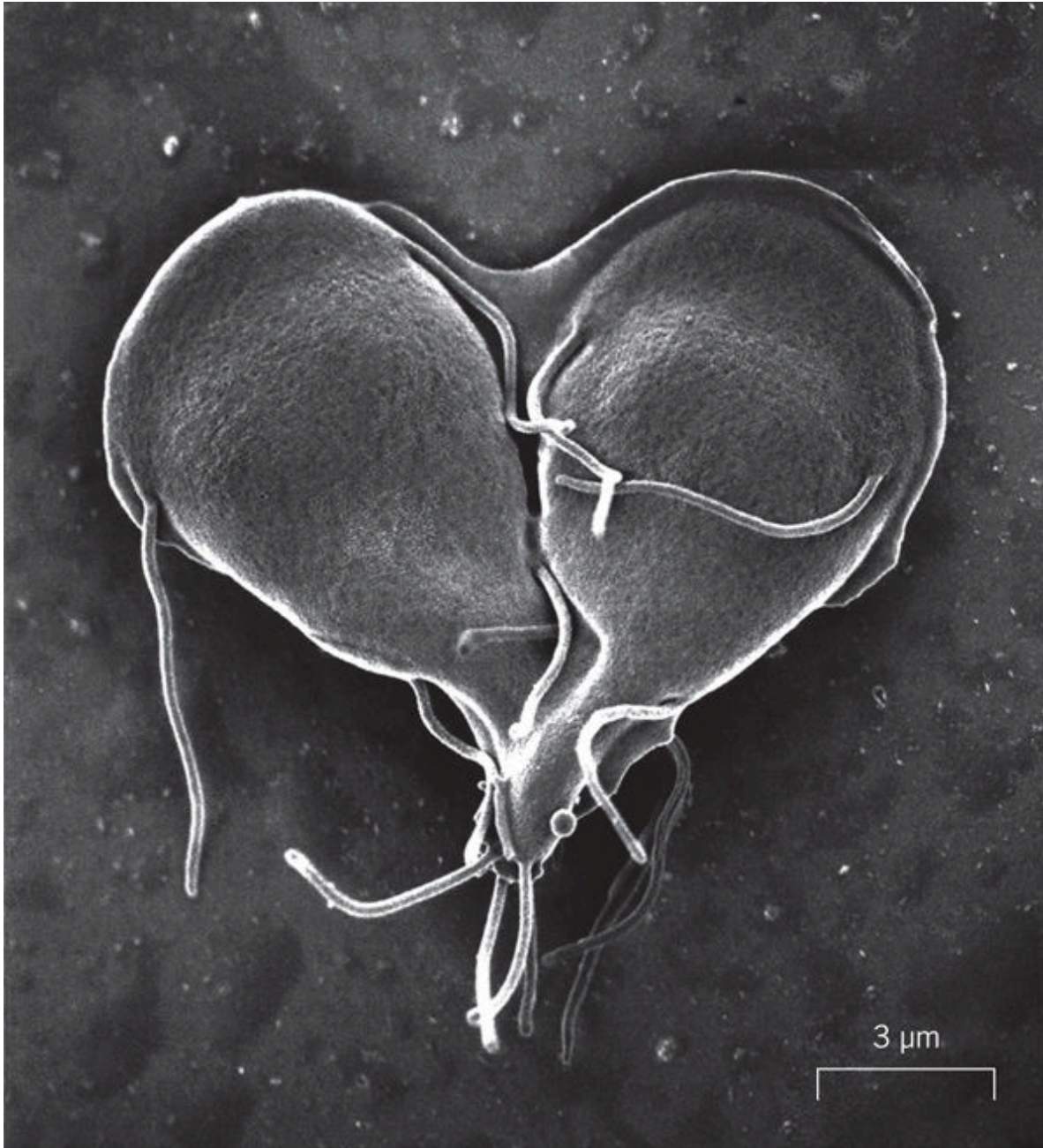


Figure 2. Late stage of binary fission of a *Giardia* trophozoite. Image obtained by scanning electron microscopy (CDC / Dr. Stan Erlandsen).

Epidemiology

The prepatent period of canine giardiasis ranges from 4 to 12 days (mean duration, 10 days). Puppies can shed an estimated 2,000 cysts per gram of

faeces. Cysts are shed in the faeces for about a month (27–35 days) in self-limiting infections, although in some patients this process can last several months.

In an epidemiological study of 56 puppies, although 18 had no diarrhoea and 22 showed no significant clinical signs of gastrointestinal disease, *G. duodenalis* was the most common parasite detected. Specifically, samples positive for the following species were found: *Toxocara canis* (n = 29), *Giardia duodenalis* (n = 35), *Cystoisospora* (n = 22), *Escherichia coli* (n = 47), and *Clostridium perfringens* (n = 20).

The overall prevalence of giardiasis in dogs, based on the results of 127 published studies, is 2.61 %, with a combined prevalence rate of 15.2 %.

The prevalence rate in dogs in Spain is 16.4 %.

The prevalence rate is affected by multiple factors: geographical region, the presence of diarrhoea, age of less than 6 months, and diagnostic method used. As regards the latter, studies using PCR methods detect higher prevalences than those using optical microscopy.

In essence, the prevalence of *G. duodenalis* in dog populations depends on the population studied and ranges from 10 % in pets, and 30 % to 50 % in puppies, up to 100 % in shelters and colonies.

Pathogenesis

Infection of the small intestine by *G. duodenalis* results in malabsorption, caused by attachment of the protozoan to the brush border of the enterocytes, accompanied by secretory diarrhoea. *G. duodenalis* damages the glycocalyx and the intestinal epithelial microvilli, inhibits digestive enzymes, and

triggers an inflammatory response. Parasitism induces apoptosis in enterocytes, consequently increasing intestinal permeability.

Infected animals progress from asymptomatic carriers to develop acute or chronic diarrhoea with malabsorption.

Route of infection and disease spread

Infection occurs through the ingestion of cystic forms released into the environment in the faeces of infected animals (faecal contamination). The mean prepatent period is 7 to 10 days.

Clinical presentation

Most dogs that shed *G. duodenalis* cysts are asymptomatic. Clinical giardiasis is more common in puppies, immunodeficient animals, and those that live in crowded conditions (Fig. 3).

The main clinical signs are diarrhoea and weight loss (Box 1). Malabsorption results in profuse, fetid, mucosal diarrhoea with steatorrhaea, without bleeding or vomiting. Giardiasis is not associated with any laboratory abnormalities.

In cases of severe clinical presentations, additional causes should be investigated, as coinfections are common.



Figure 3. Puppy with weight loss due to giardiasis.

Box 1. Clinical signs in giardiasis.

- » Diarrhoea
- » Steatorrhea
- » Weight loss
- » Tenesmus
- » Anorexia
- » Lethargy/depression

Diagnosis

Clinical diagnosis

Clinical diagnosis is very nonspecific as most parasitised dogs are asymptomatic. Those exhibiting clinical signs present with acute diarrhoea or chronic diarrhoea combining signs of small and large intestine diarrhoea.

Diagnostic imaging

Given the presence of trophozoites throughout the small intestine, duodenoscopy allows the collection of samples in which *G. duodenalis* can be identified. However, neither the sensitivity nor the complexity of this invasive procedure justify its application for the diagnosis of giardiasis.

Direct diagnosis

While optical microscopy allows the observation of *G. duodenalis* trophozoites in fresh stool samples (Fig. 4), the sensitivity of this technique is very low and poor specificity means that *G. duodenalis* can be confused with other protozoa (e.g. genus *Tritrichomonas*). The sensitivity of this method can be increased by using stool samples that have been concentrated by centrifugation and sucrose (or zinc sulfate) gradient flotation, and observing cystic forms (Fig. 5). The problem with this method is that cystic forms are not shed continuously. Detection sensitivity can thus be further increased by analysing three stool samples collected over the space of a week. Stool analysis is the most commonly used diagnostic method in the clinic, and enables detection of other intestinal parasites in addition to *G. duodenalis* . Furthermore, it is the most appropriate technique to assess the response to treatment.

Cystic forms can be identified by direct immunofluorescence. Despite its technical complexity, this technique offers high sensitivity and specificity, and coproscopy using direct immunofluorescence is considered the reference method for the identification of *Giardia* cysts.

A commercially available ELISA kit for the detection of *G. duodenalis* in faecal samples offers sensitivity and specificity that are comparable to those of the stool test.

The best way to achieve greater diagnostic efficacy in dogs with a diarrhoeal presentation is

to perform both techniques, i.e. a coprological examination and a faecal sample ELISA.

PCR assays are suitable for distinguishing between genotypes of *G. duodenalis*, which may be of interest for the assessment of zoonotic risk, as only genotypes A and B are known to parasitise humans. The high degree of sensitivity and specificity of these tests may call into question the use of direct immunofluorescence coproscopy as the reference method.

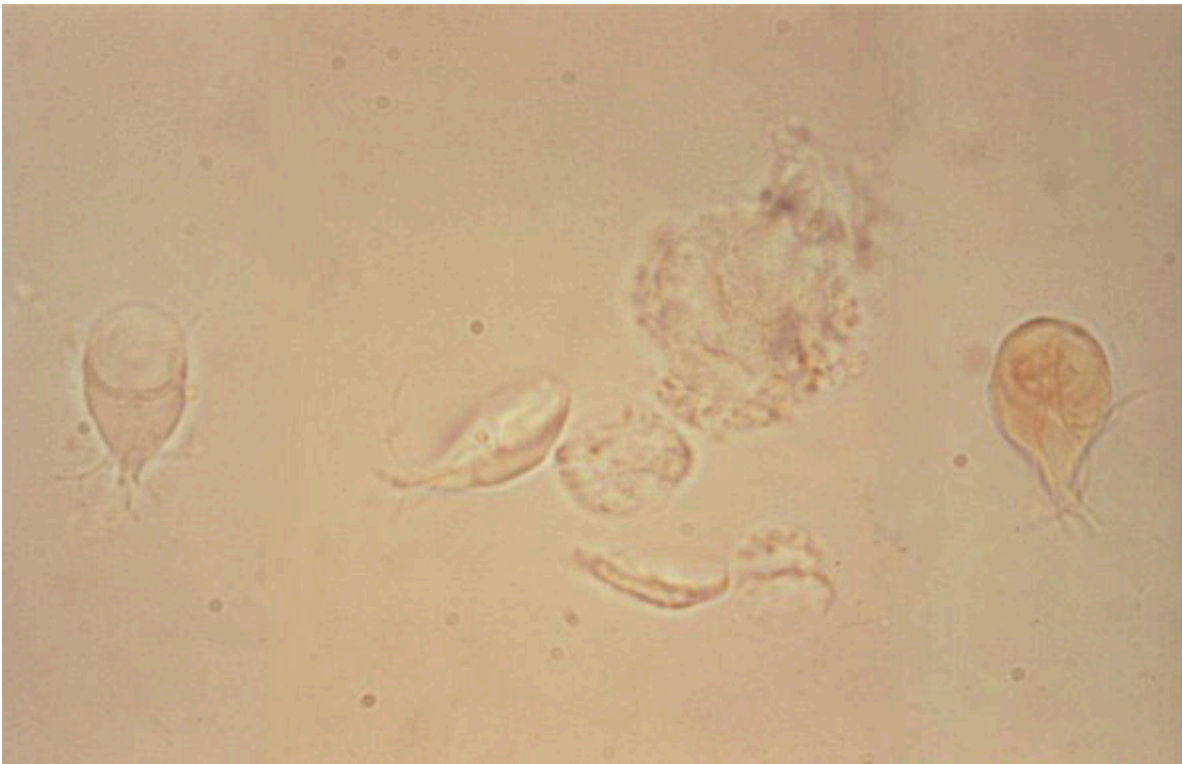


Figure 4. Trophozoites of *G. lamblia* (*G. duodenalis*) obtained from the intestine of an infected host (CDC / Dr. Mae Melvin).



Figure 5. *G. lamblia* (*G. duodenalis*) cyst visualised by iodine staining (CDC / Dr. Mae Melvin).

Treatment

Treatment is based on maintaining a proper diet and the administration of antiprotozoal compounds, generally nitroimidazoles. The most commonly used drugs are:

- **Metronidazole:** recommended dose, 25 mg/kg PO every 12 hours for 5 days. An advantage of this treatment is that it also controls potential bacterial overgrowth. The disadvantage is that the recommended dose is very high.
- **Fenbendazole:** recommended dose, 50 mg/kg PO every 24 hours for 5 days, although administration for 3 days is effective in most cases. An advantage of this drug is that it is a broad-spectrum antiparasitic capable of eliminating metazoans.
- **Albendazole:** recommended dose, 25 mg/kg PO every 12 hours for 2 days. Although effective, this drug is associated with potential myelosuppression.

- Combination of **febantel** (25 or 56.5 mg/kg) **pyrantel** (5 or 11.3 mg/kg), and **praziquantel** (5 or 11.3 mg/kg), administered PO for 3 or 5 days, with variable results, probably due to the availability of multiple formulations of this combined antiparasitic. One study concluded that it is necessary to bathe the patient and perform environmental disinfection in order for the treatment to be effective.
- **Tinidazole**: 44 mg/kg PO every 24 hours for 6 days.
- **Iprnidazole**: 126 mg/l in drinking water for 7 days.
- **Quinacrine**: 9 mg/kg PO every 24 hours for 6 days.
- **Silymarin**: 3.5 mg/kg PO every 24 hours, administered in combination with metronidazole or fenbendazole. Can improve recovery in symptomatic animals, but does not itself possess any antiparasitic activity.
- **Probiotics**: a study of the administration for six weeks of the probiotic *Enterococcus faecium* (strain SF68) in dogs with chronic giardiasis reported no effects on local immunity or faecal shedding.

Prevention

The key to disease prevention is cleaning and disinfection. Failure to eliminate *G. duodenalis* cysts results in re-exposure and reinfection. It is thus necessary to establish a cleaning protocol, in addition to the administration of antiparasitics, to avoid reinfection. Contaminated animal faecal material must be eliminated from objects, cages/kennels, and fabrics.

At the turn of the century, a *Giardia* vaccine designed to stimulate humoral immunity (IgA and IgG) was developed for oral and parenteral administration. The aim was to reduce the zoonotic potential of this protozoan and resolve cases of chronic giardiasis resistant to standard treatments with metronidazole and fenbendazole. Vaccination significantly reduced the elimination of viable cysts. A *Giardia* vaccine containing inactivated trophozoites has been developed, but is not widely used or available in all countries.

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Aspergillosis

Aspergillosis

Definition

Aspergillus and *Penicillium* are opportunistic pathogenic fungi. As such, the development of infection depends on host immunocompetence. These fungi can cause sinonasal, systemic, or respiratory infections.

Aetiology and incidence

Aspergillus and *Penicillium* are ubiquitous saprophytic fungi. Species are determined based on the morphological criteria of their asexual spores and, more precisely, by genetic analysis.

Rhinosinusitis caused by aspergillosis is much more common than that caused by penicilliosis (the latter is unusual and poorly characterised). Species are distinguished by examination of their conidiophores or by genetic analysis. The use of both techniques, particularly genetic analysis, has demonstrated that the following species can cause rhinosinusitis: *Aspergillus fumigatus* (most common), *A. niger*, *A. nidulans*, *A. flavus*, *A. tubingensis*, and *A. uvarum*.

Disseminated aspergillosis is caused by *A. terreus*, *A. deflexus*, *A. flavipes*, and *A. fumigatus*. The first two species are the most common.

Epidemiology

Respiratory disease usually occurs in young or middle aged (3-3 years) dolichocephalic and mesocephalic dogs.

Most cases of systemic aspergillosis are observed in (predominantly female) German shepherd dogs of 2 to 8 years of age (mean age of onset, 4.5 years). Immunosuppressive states are thought to predispose to systemic aspergillosis. Predisposing factors thus include leukaemias, leukocytic diseases that cause phagocyte dysfunction, neutropaenia, immunosuppressive chemotherapy, and chronic antibiotic therapy.

The prevalence of rhinitis due to aspergillosis is high: 12 % to 34 % of dogs with nasal secretions have aspergillosis.

Pathogenesis

Conidia of *Aspergillus* spp. are abundant in the environment and enter the host via the airborne route. Immunocompetent animals with appropriate cellular immune responses eliminate these conidia via innate immune mechanisms. Alternatively, they can adhere to and penetrate the respiratory epithelium. In order to do this they must be capable of resisting phagocytosis. *A. fumigatus* produces a gliotoxin that inhibits macrophage phagocytosis and causes immunosuppression. Fungal invasion causes cell death, resulting in osteolysis. The growth of *Aspergillus* in the mucosa and the subsequent inflammatory response destroys the nasal turbinates and even the cribriform plate and the palatine and orbital bones. The resulting inflammatory lesions are usually pyogranulomatous and lymphoplasmacytic.

Some German shepherds develop chronic cavitary lung lesions without actually developing a systemic infection.

In the case of systemic aspergillosis, infection is acquired via the airborne route, and spreads haematogenously. Circulating fungi localise in the intervertebral discs, renal glomeruli, and uvea. Less commonly, they reach the parenchymal organs, muscles, and long bones.

Disease progression

In normal conditions, the immune response eliminates conidia that enter the organism via the aerogenous route. Alternatively, these conidia can adhere to and invade the respiratory epithelium, resulting in a confined infection that manifests as rhinosinusitis. In other cases they reach the lung tissue and produce chronic lesions in the parenchyma. In some German shepherds, the mycosis is disseminated haematogenously after aerogenic penetration, resulting in systemic aspergillosis.

Immune response

The sinonasal mucosa is an important interface with the environment and represents a dynamic system for innate defence of the host. In addition to constituting a barrier to the entry of pathogens, epithelial cells lining the airways can recognise pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs), which are found in epithelial and dendritic cells, may play a crucial role in distinguishing between commensal and pathogenic flora (Fig. 1). Single nucleotide polymorphisms (SNPs) in TLR genes influence the immune response to microbial antigens, and are thus implicated in the onset and development of infections. TLRs recognise a wide variety of pathogens (viruses, bacteria, parasites, and fungi). TLR2, TLR4, and TLR9 recognise fungi such as *Candida albicans* and *Aspergillus fumigatus*. Some SNPs in these TLRs have been associated with an increased risk of fungal disease in man, but not in dogs.

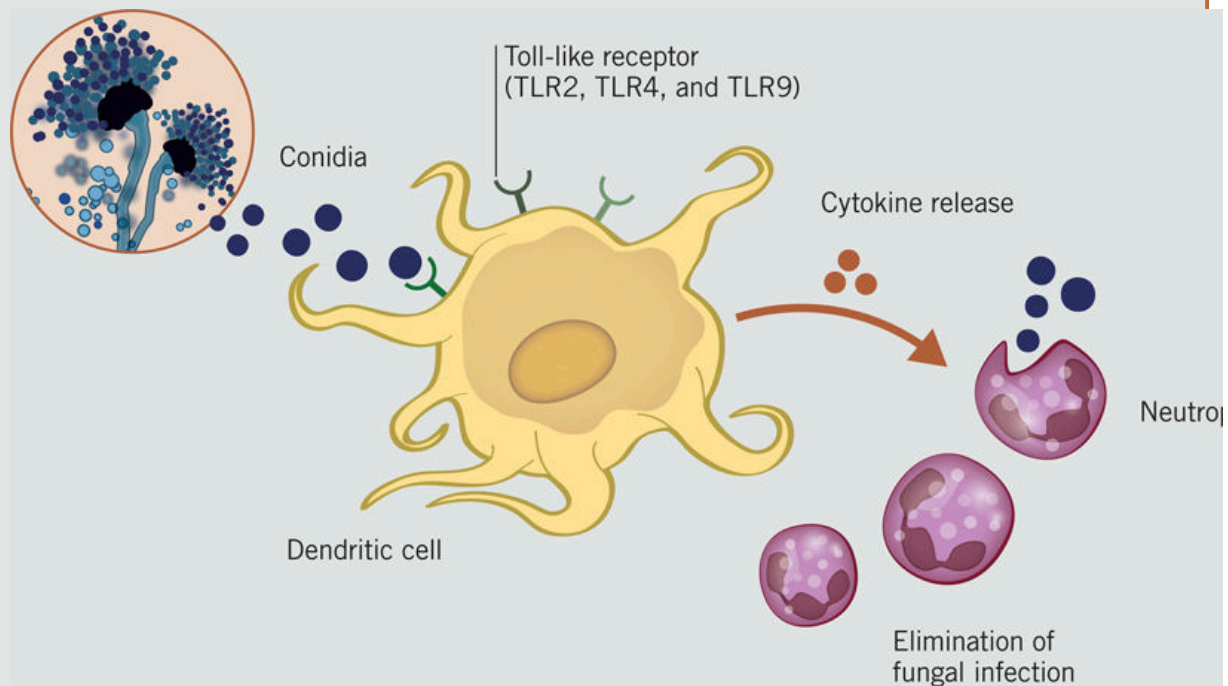


Figure 1. Action of Toll-like receptors in the defence against fungi.

Clinical presentation

Nasal form

Affected dogs show mucopurulent nasal discharge (bilateral or initially unilateral), epistaxis, sneezing, pain or discomfort in the nasal region, anorexia, lethargy, depigmentation of the nose, ulceration of the nasal passages, and inspiratory dyspnoea (Fig. 2). Aspergillosis with sinusitis and orbital involvement is characterised by craniofacial swelling, nasolacrimal duct obstruction, lacrimation, exophthalmos, and strabismus. Neurological signs such as convulsions and stupor may be observed if the fungus crosses the cribriform plate (Box 1).



Figure 2. Nasal aspergillosis with mild hyperkeratosis of the nose. No external injuries are evident. This animal has been anaesthetised for imaging tests.

Box 1. Clinical signs of rhinosinusitis.

- » Mucopurulent nasal discharge
- » Epistaxis
- » Sneezing
- » Pain or discomfort in the nasal region
- » Anorexia
- » Lethargy
- » Depigmentation of the nose
- » Ulceration of the nostrils
- » Inspiratory dyspnoea
- » Craniofacial swelling
- » Epiphora
- » Exophthalmos
- » Strabismus
- » Seizures
- » Stupor

Pulmonary form

The pulmonary form presents with cough and haemoptysis. In rare cases it presents with rupture of cavitary lesions, pneumothorax, pleural effusion, or pericardial effusion, in which case the predominant clinical sign is dyspnoea (Box 2).

Box 2. Clinical signs of pulmonary aspergillosis.

- » Cough
- » Haemoptysis
- » Dyspnoea
- » Pleural effusion
- » Pneumothorax

Systemic form

Systemic aspergillosis is a chronic disease that progresses slowly. Intervertebral disc involvement is characterised by pain and evolves to paraparesis and paraplegia. Lameness, local inflammation, and fistulas may be observed. It is accompanied by nonspecific systemic signs such as anorexia,

weight loss, emaciation, fever, weakness, lethargy, and vomiting. Lymph node enlargement, cutaneous oedema, pyometra, and uveitis may be observed. The following neurological signs have also been described: vestibular syndrome, hemiparesis, circling behaviour, and seizures. Peritoneal effusion, bloating, gastrointestinal signs and pericarditis are rare (Box 3).

Systemic aspergillosis may be accompanied by haemogram alterations including neutrophilic leukocytosis, monocytosis, and eosinophilia. Changes observed in serum biochemistry and urinalysis depend on organ involvement, but the most common are azotaemia, hyperproteinaemia, hypoalbuminaemia, hyperglobulinaemia, hypercalcaemia, isosthenuria, and proteinuria.

Box 3. Clinical signs of systemic aspergillosis.

- » Spinal pain
- » Paraparesis
- » Paraplegia
- » Lameness
- » Fistulas
- » Anorexia
- » Wasting
- » Fever
- » Weakness
- » Lethargy
- » Vomiting
- » Lymphadenomegaly
- » Oedema
- » Pyometra
- » Uveitis
- » Peritoneal effusion
- » Pericardial effusion

Diagnosis

Clinical diagnosis

Physical examination can detect signs of rhinitis or sinusitis, but diagnostic tests are required to identify aspergillosis.

The differential diagnosis of aspergillosis includes:

- Rhinitis.
- Nasal tumour.
- Foreign body.

Systemic aspergillosis is a chronic disease that progresses slowly. As a result, diagnosis is often established too late.

Diagnostic imaging

This technique is essential when dealing with cases of rhinosinusitis. Simple radiography, computed tomography (CT), and magnetic resonance imaging (MRI) can locate lesions and determine the extent of damage. However, rhinoscopy is necessary to visualise fungal material and to obtain a sample to confirm the diagnosis. Best practice is to perform rhinoscopy after CT or MRI.

Simple radiography allows evaluation of the destruction of the turbinates and the radiopacity of the content in the nasal area and paranasal sinuses. CT allows detection of bone lesions with greater detail and accuracy. Enhancement of the nasal epithelium following contrast administration reveals destruction of the turbinates, osteolysis, and inflammatory reaction. In the MRI the most reliable sign is moderate to severe destruction of the nasal turbinates, with hyperintense lesions in the musculature in T1-weighted images, a phenomenon not observed in lymphoplasmacytic rhinitis (hypointense or isointense lesions in T1-weighted images). This technique provides no advantages over CT (Fig. 3).

One challenge of rhinoscopy is accessing the frontal sinus in some patients. It is possible that fungal colonies are exclusively located within the sinus. Prior radiological tests may indicate that, in cases involving lesions, trepanation is necessary to access the sinus for rhinoscopy.

In the case of pulmonary aspergillosis, a simple thoracic radiograph reveals an interstitial or alveolar pulmonary pattern.

Simple radiography and CT are useful for diagnosing discospondylitis. Abdominal ultrasound may reveal kidney damage.

These techniques can facilitate the selection of tissue samples for biopsy in order to establish diagnosis. Ultrasound or CT can also facilitate biopsy collection.

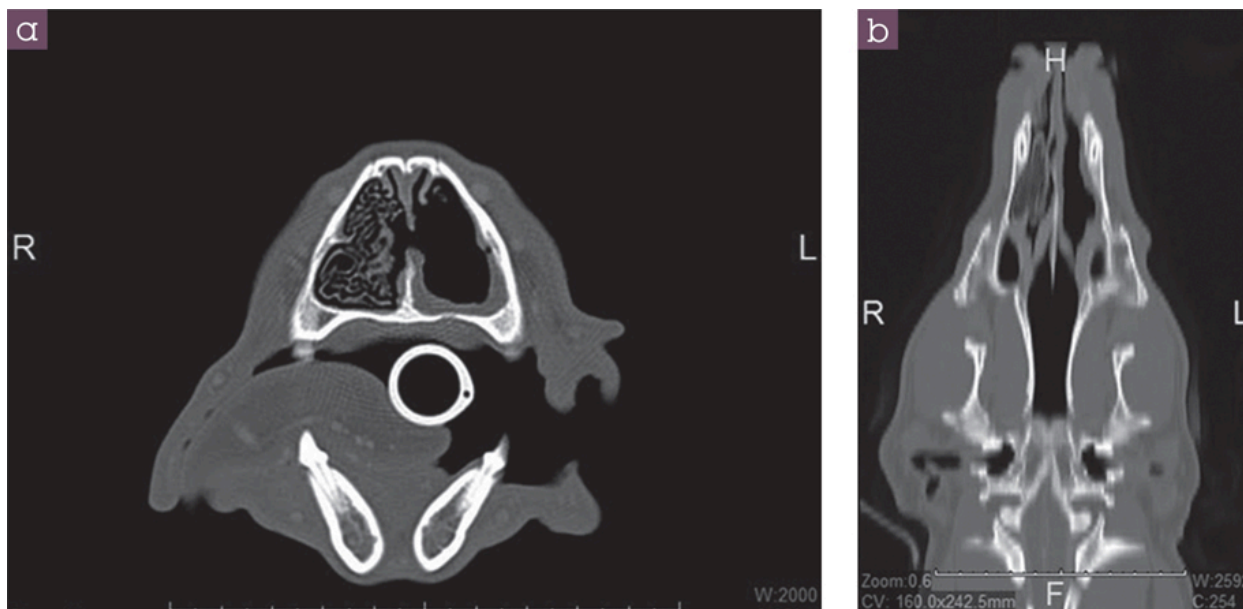


Figure 3. Bone algorithm computed tomography of the nasal showing marked destruction of the turbinates of the left nasal cavity in transverse plane (a) and dorsal reconstruction (b).

Direct diagnosis

Hyphae can be observed by optical microscopy in biopsy samples obtained by rhinoscopy (Fig. 4). These samples can also be used to identify the species involved either by culturing or using PCR. Samples can be obtained by collecting nasal discharge or by blind introduction of a swab, although these methods are less effective, particularly in cases of sinusitis. According to one published study, fungal structures are only observed in 20 % of samples obtained by nasal swabbing or direct collection of nasal discharge. The

samples of choice for diagnosis of the disease are those obtained by endoscopy (biopsy or scrapings).

One of the advantages of PCR is that it can be used to analyse multiple sample types, e.g. blood, urine, cerebrospinal fluid, cavity effusions, or bronchoalveolar lavage fluid. This technique facilitates the diagnosis of systemic and pulmonary aspergillosis.

ELISA for the detection of *Aspergillus* galactomannan antigen is used for the diagnosis of human systemic aspergillosis. This test detects the galactomannan antigen released into body fluids by *Aspergillus* spp. and is considered sensitive and specific for the diagnosis of aspergillosis (sensitivity, 40 %–71 %; specificity, 53 %–89 %). This test is insensitive for the diagnosis of nasal aspergillosis. When used for diagnosis of canine systemic aspergillosis, it has a sensitivity and specificity of 92 % and 86 % in serum and 88 % and 92 % in urine, respectively. False negatives are observed in dogs with localised pulmonary aspergillosis. The ELISA result is expressed as the galactomannan index (GI), and is considered positive at values of 0.5 and higher. By applying a cutoff value of 1.5 to the GI, specificity increases to 93 % (in serum and urine samples), with no loss of sensitivity for the diagnosis of disseminated aspergillosis in dogs.

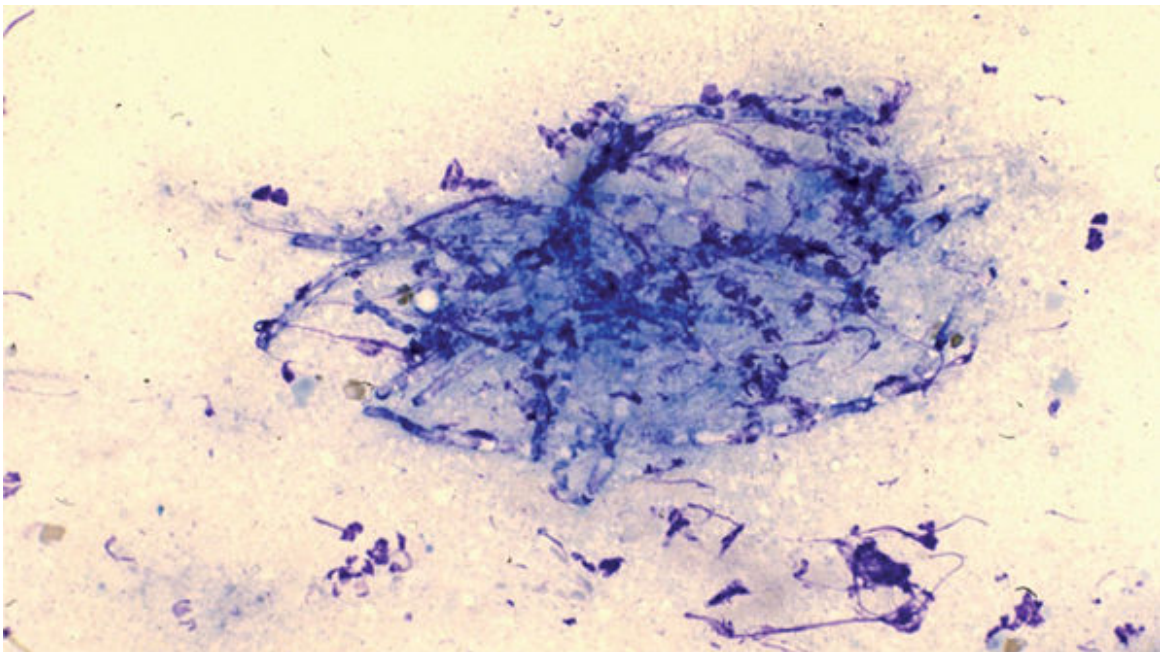


Figure 4. Nasal aspergillosis: cytological sample obtained by rhinoscopy in which fungal colonies are observed.

Serological diagnosis

Titres of *Aspergillus* antibodies can be determined by agar gel double diffusion (AGDD), counterimmunoelectrophoresis (CIE), and ELISA. Although AGDD and CIE are considered more sensitive and specific than ELISA, they also produce false negatives. Culture is more sensitive and has equivalent specificity. ELISA has high sensitivity but poor specificity, and can produce false positives.

Histopathological diagnosis

The definitive diagnosis of aspergillosis is based on histopathology, the diagnostic value of which is greater than that of fungal culture. The problem with this approach is that the collection of biopsy samples is highly invasive, and can result in persistent epistaxis. Nasal aspergillosis cannot be ruled out by fungal culture or AGDD. AGDD has been shown to produce false positives in dogs with lymphoplasmacytic rhinitis. False positives were also reported in cultures from dogs with neoplasia in a 1981 study, but not in a later 2007 study by Pomrantz et al.

Treatment

Systemic treatment of rhinosinusitis

This involves long-term oral administration of antifungal drugs (triazoles) (Table 1). Itraconazole and fluconazole are recognised as the most effective drugs, but are expensive and commonly cause adverse effects.

Table 1. Treatment of nasal aspergillosis.

Drug	Dose	Administration period
Thiabendazole	10 mg/kg every 12 hours	6–8 weeks
Ketoconazole	5 mg/kg every 12 hours	6–18 weeks
Itraconazole	5 mg/kg every 12 hours	10 weeks
Fluconazole	2.5-5 mg/kg every 12 hours	10 weeks

Terbinafine	30-40 mg/kg every 24 hours	Until resolution
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Local treatment of rhinosinusitis

Local administration of antifungals requires scraping, lavage, and suction of fungal material by rhinoscopy or sinoscopy prior to treatment application. The cure rate is higher than that associated with systemic treatment in cases of *Aspergillus* rhinosinusitis.

Local administration can be achieved by surgical implantation of infusion and irrigation tubes or by using two Foley catheters (20 Fr, 30-ml balloon), introduced orally into the nasopharynx, which is occluded upon inflation of the balloons, and two additional Foley catheters (12 Fr, 5-ml balloon) introduced into each nostril.

Summary

Published rates of sensitivity and specificity and negative predictive values:

Test	Sensitivity	Specificity	Negative predictive value
Serological test	67 %	98 %	93 %
Culture	81 %	100 %	90 %

Enilconazole and clotrimazole preparations are used as topical antifungals. Implantation of a nasal or sinus catheter is required for their administration under general anaesthesia. Clotrimazole is infused over the course of an hour. A single treatment is sufficient in some patients. The viscosity of the creamy preparation promotes greater persistence of the antifungal compound in the frontal sinus compared with propylene glycol solutions, ensuring prolonged contact with fungal colonies. Moreover, movement of the animal and sneezing help disperse the cream throughout the nasal cavity. In some cases, repeated rhinoscopy-guided nasal or sinus infusion of clotrimazole or enilconazole is required. The procedures are performed at three-week intervals, after performing a control CT.

Local administration is not indicated when aspergillosis has crossed the cribriform plate.

Treatment of pulmonary aspergillosis

Lobectomy followed by antifungal therapy with itraconazole for several months has been tested for the treatment of pulmonary aspergillosis.

Treatment of systemic aspergillosis

Treatment of systemic aspergillosis is not usually effective, but can prolong patient survival in some cases. Clinical remission can be induced with oral itraconazole at doses of 5 to 10 mg/kg every 12 hours, indefinitely (up to 3 years), or with oral terbinafine (5 mg/kg every 12 hours, indefinitely).

One of the obstacles to treating human aspergillosis is the resistance of fungi to azole drugs. However, a recent dog study reported no such problem in cultures treated with itraconazole, posaconazole, voriconazole, fluconazole, or ketoconazole.

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Cryptococcosis

Cryptococcosis

Definition

Cryptococcosis is a fungal disease that affects humans, cats, and dogs, with the highest incidence observed in cats.

Aetiology and incidence

Cryptococcosis is caused by *Cryptococcus neoformans* and *C. gattii*. While *C. albidus*, *C. carnescens*, *C. laurentii*, *C. luteolus*, *C. magnus* , and *C. uniguttulatus* are considered saprophytic species, rare infections caused by *C. albidus*, *C. flavescens* , and *C. laurentii* have been described. These are dimorphic, basidiomycetous fungi. The life cycle of these fungi in the environment includes both asexual and sexual reproduction, a strategy that allows the fungus to adapt to environmental conditions and develop virulence. Sexual reproduction involves fusion of the a mating type, while asexual reproduction involves fusion of the α -type. Fusion of the a mating type results in the generation of dikaryotic filaments (known as the perfect state). The filaments produce basidia, which in turn generate basidiospores. Cells of the α type can undergo asexual reproduction or haploid fruiting, which occurs in conditions of nitrogen deficit, in the absence of pheromones, or in response to desiccation. Based on the molecular differences between fungi isolated from hosts, they have been renamed as *C. neoformans* and *C. gattii* complexes. Basidiospores are the infective form, while the filamentous form is the vegetative form. The filamentous form is a member of the genus *Filobasidiella*. Basidiospores are rounded and are surrounded by a protective polysaccharide capsule.

No sexual predisposition has been described for cryptococcosis, and the average age of presentation in dogs is less than 4 years. While geographic variations in breed susceptibility have been described, the most commonly

affected breeds are the American cocker spaniel, great Dane, German shepherd, boxer, and Doberman.

Epidemiology

Cryptococcosis is distributed worldwide. The infection affects several species of mammals and birds, both domestic and wild.

The prevalence of infection is eight times higher
in feline versus canine species.

Infection is produced by spores found in the environment, usually in bird droppings (pigeons), but also in decaying plant debris from certain trees (e.g. eucalyptus). Spores can remain viable for long periods of time in bird guano (up to two years).

Infection occurs naturally through the inhalation of spores, but can also occur following the entry of spores via wounds in the skin or through ingestion.

Pathogenesis

After they are inhaled, basidiospores invade the nasal mucosa or reach the alveoli of the lungs. Invasion of the nasal mucosa can result in granuloma formation or asymptomatic colonisation. Alternatively, basidiospores can be disseminated haematogenously. The evolution of the disease depends on three factors:

- The quantity of spores inhaled.
- The strain of *Cryptococcus*.
- Host immunocompetence.

The polysaccharides of the capsule are released into the extracellular fluids of the host, and although the capsule stimulates neutrophil chemotaxis, it is also capable of inhibiting phagocytosis.

Pulmonary infection results in haematogenous spread to other tissues via macrophages, usually reaching the central nervous system (CNS). This situation also occurs in cases of nasal cryptococcosis involving osteomyelitis with destruction of the cribriform plate. In this case the fungus penetrates the olfactory bulb, frontal sinus, middle ear, or eye. This can ultimately result in meningoencephalitis, with the presence of fungus detectable in the cerebrospinal fluid.

Multifocal skin lesions in a case of cryptococcosis are indicative of haematogenous spread. In other cases, disseminated abdominal cryptococcosis may be observed. This presentation is associated with infection caused by the ingestion of spores by dogs with possible immunosuppression.

Clinical presentation

In 80 % of cases, canine cryptococcosis presents with involvement of multiple anatomic locations. Half of all cases involve lesions in atypical sites. Cryptococcosis presents with weight loss, lethargy, and anorexia, and in some cases gastrointestinal signs (Box 1 , Fig. 1). Infection of the CNS and eyes is common. For this reason, affected animals often present with stupor, head tilt, nystagmus, anisocoria, facial palsy, paresis, paraplegia, quadriplegia, ataxia, circling behaviour, seizures, fibrillations, inability to open the mouth, or cervical hyperaesthesia. Neurological signs are the most common clinical signs in canine cryptococcosis (Box 2). Eye involvement is characterised by chorioretinitis and optic neuritis. Retinal detachment, mydriasis, and blindness, as well as uveitis and conjunctivitis, may be observed. In cases involving rhinosinusitis affected animals present with sneezing, mucopurulent nasal discharge, and presence of an intranasal mass. Deformation of the cranionasal bone is rare in dogs, as are skin lesions such as papules, nodules, and ulcers.

Haemogram and serum biochemistry often reveal nonspecific alterations. Nonregenerative anaemia (microcytic and hypochromic, due to chronic gastrointestinal bleeding), thrombocytosis, lymphopaenia, leukocytosis, neutrophilia, monocytosis, and eosinophilia may be observed. Alterations in serum chemistry depend on the affected organs (pancreas, gastrointestinal system, liver, or kidney). Analysis of the cerebrospinal fluid of patients with cryptococcal disease with CNS involvement usually reveals protein increases and pleocytosis. The latter is usually mixed, consisting of neutrophils, mononuclear cells, and in some cases eosinophils.

Box 1. General clinical signs of cryptococcosis.

- » Weight loss
- » Lethargy
- » Anorexia
- » Diarrhoea
- » Abdominal distension



Figure 1. Weight loss and abdominal distension due to peritoneal effusion in a case of disseminated abdominal cryptococcosis.

Box 2. Neurological signs of cryptococcosis.

- » Stupor
- » Head tilt
- » Nystagmus
- » Anisocoria
- » Facial palsy
- » Paresis
- » Paraplegia
- » Quadriplegia
- » Ataxia
- » Circling behaviour
- » Seizures
- » Fibrillations
- » Mandibular trismus
- » Cervical hyperaesthesia

Diagnosis

Clinical diagnosis

Clinical diagnosis constitutes the first step in the diagnostic process. The location of the infection is highly variable; clinical signs merely provide an indication of whether the clinical picture is respiratory, ophthalmologic, neurologic, gastrointestinal, renal, systemic, or dermatological. Until imaging techniques have been performed and lesions sampled, the infectious agent cannot be identified nor a definitive diagnosis established.

Diagnostic imaging

Diagnostic imaging techniques are essential to identify the location of lesions (typically granulomas). Simple radiography, computed tomography

(CT), and magnetic resonance imaging (MRI) enable the detection of lesions and evaluation of the extent thereof. In the case of rhinosinusitis lesions, rhinoscopy is required to obtain the samples necessary to confirm diagnosis. Best practice is to perform rhinoscopy after CT or MRI.

MRI and the analysis of cerebrospinal fluid (provided samples can be safely obtained) are the most suitable tests for dogs with neurological signs. MRI reveals multifocal lesions in the parenchyma, meningeal thickening, and hyperintensity in the cerebellum, cerebrum, and cervical spinal cord. Lesions usually vary in intensity in T1-weighted images, are uniformly heterogeneous, display contrast enhancement, and are hyperintense in T2-weighted images.

Thoracic radiography reveals a multifocal interstitial lesion pattern, widening of the cranial mediastinum caused by mediastinal lymphadenopathy, pleural effusion, hilar masses, and a multifocal alveolar pattern.

Abdominal ultrasound may reveal intestinal lesions with alterations in echogenicity and loss of stratification, lymphadenopathy, nephromegaly, pancreatitis, and peritoneal effusion (Fig. 2). Ultrasound can be used to guide biopsy to subsequently establish diagnosis.

Monostotic lesions have been described in the spine, with bone lysis in the vertebral body and bone proliferation of the spinous process. This in turn causes extradural compression, which can be detected by CT and myelotomography.

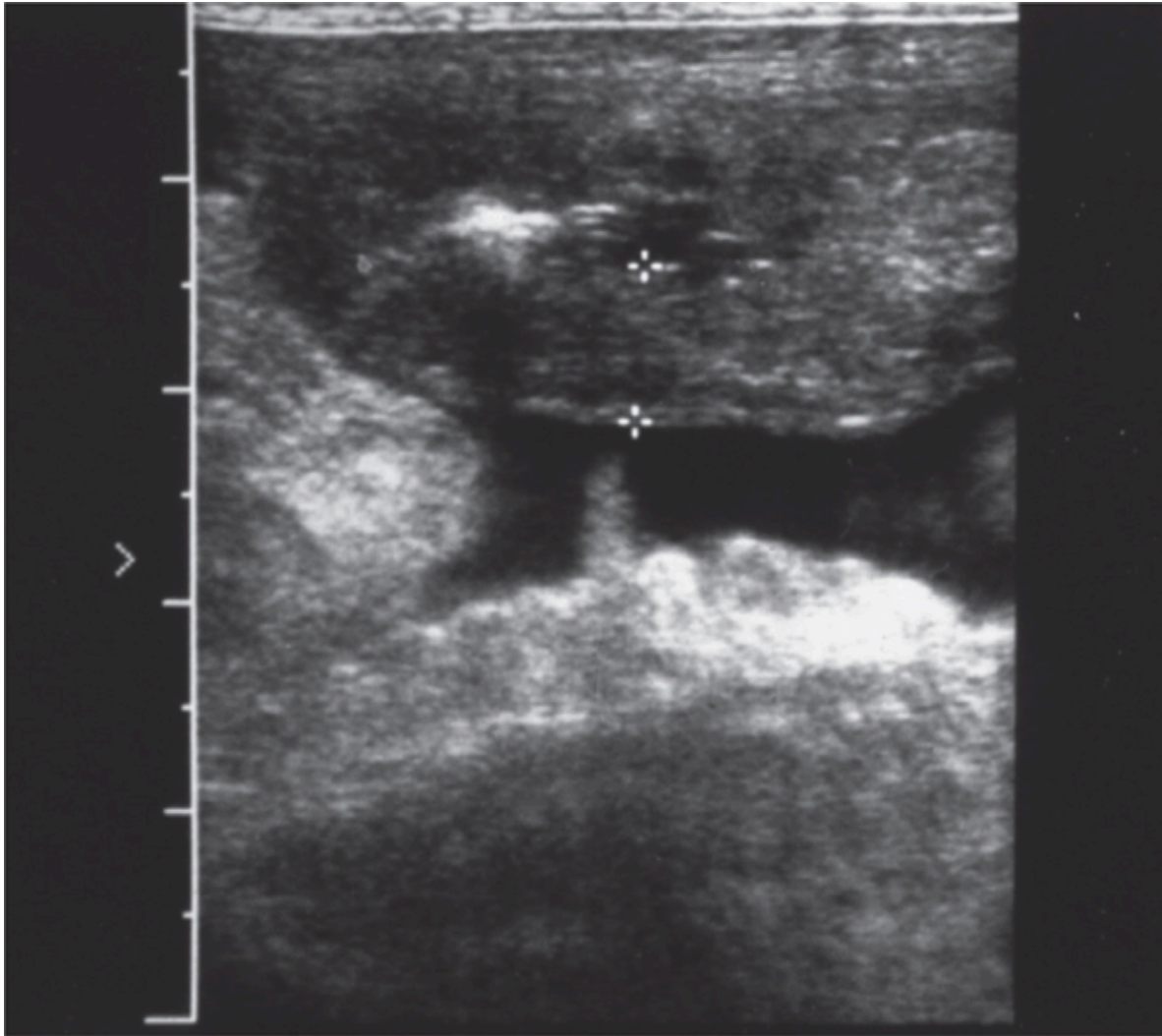


Figure 2. Ultrasound of an intestinal granuloma in a case of cryptococcosis.

Direct diagnosis

Samples can be obtained by nasal swab, nasal lavage, rhinoscopy, lymph node aspiration, ultrasound-guided aspiration of granulomas in abdominal organs (Fig. 3), bronchoalveolar lavage, thoracentesis, extraction of cerebrospinal fluid, or urine sampling.

Observation by optical microscopy of cytological preparations allows visualisation of spores. This is a simple and specific method, but frequently produces false negatives. Histopathology of fungal granulomas provides better results than cytology. Immunohistopathology can be used to confirm the species of the fungus involved. Samples can be cultured in order to

identify the fungal species involved and its susceptibility to antifungal agents. PCR can also be used, but is more common in research environments than in clinical practice. PCR techniques have enabled the distinction of at least eight molecular forms of *C. neoformans* and *C. gattii* complex.

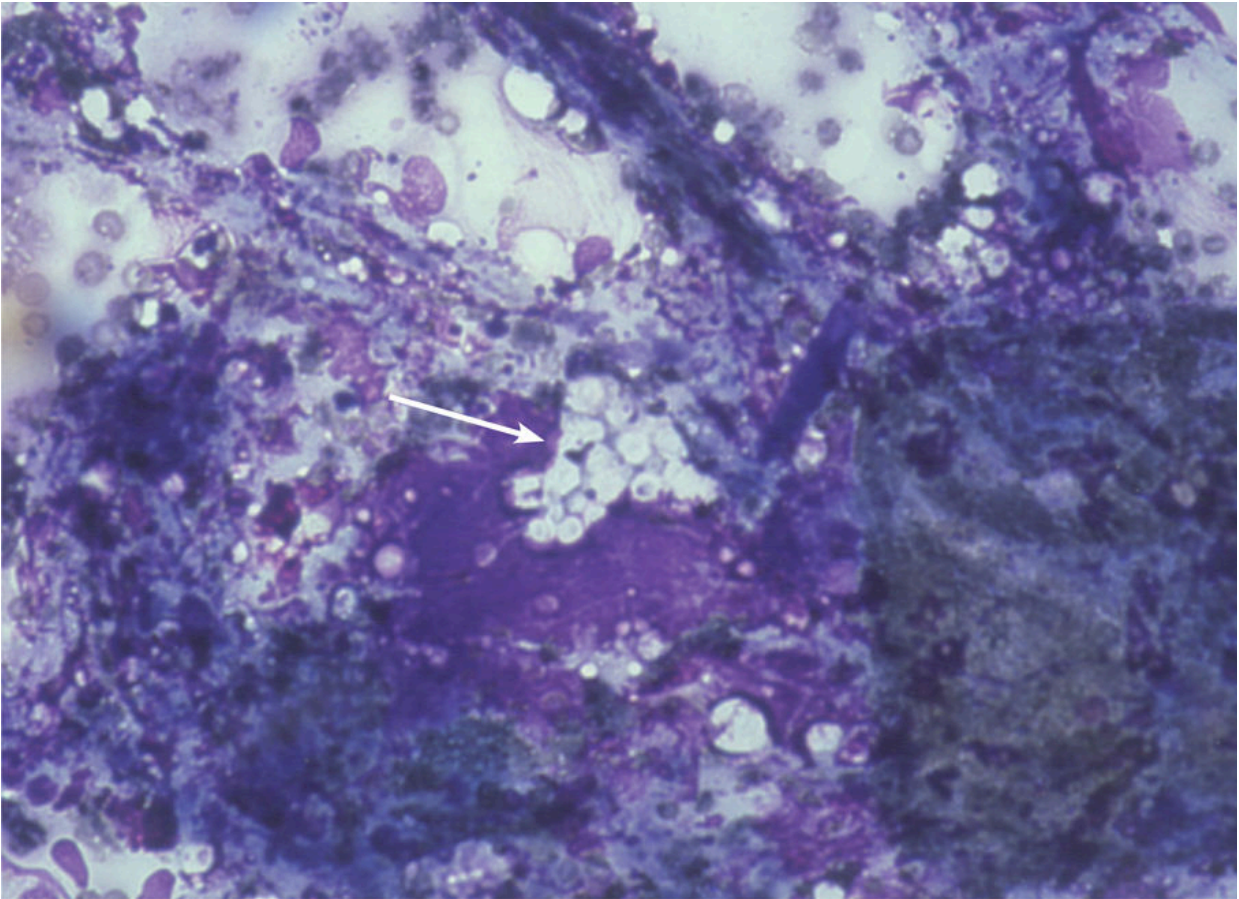


Figure 3. Ultrasound-guided aspiration cytology of mesenteric lymphadenitis in a case of cryptococcosis. Granulomatous inflammation, necrosis, and the presence of cryptococci (arrow).

Serological diagnosis

Latex agglutination allows detection of antibodies against the *Cryptococcus* spp. capsule antigen. Serum, urine, and cerebrospinal fluid samples can be used for these serological tests. False negatives can occur in cases involving lesions of the CNS, eyes, and skin. False positives are very rare in dogs. Positive antibody titres are highly variable at the moment of diagnosis; titres are generally considered positive if higher than 1/2. However, monitoring of antibody titres is useful when assessing the response to treatment.

Latex agglutination is a sensitive technique, but should not be used exclusively for the diagnosis of cryptococcosis.

Histopathological diagnosis

Histopathology allows definitive diagnosis of cryptococcosis, as it is a more sensitive and specific diagnostic method than serology. Both biopsy and necropsy in cases of cryptococcosis have revealed the presence of fungal granulomas in the following locations: skin, nasal cavity, sinuses, tonsils, lungs, kidneys, pancreas, myocardium, liver, stomach, intestine, spleen, peritoneum, thyroid, tongue, prostate, pleura, joints, mediastinum, urinary bladder, and adrenal glands (Fig. 4).

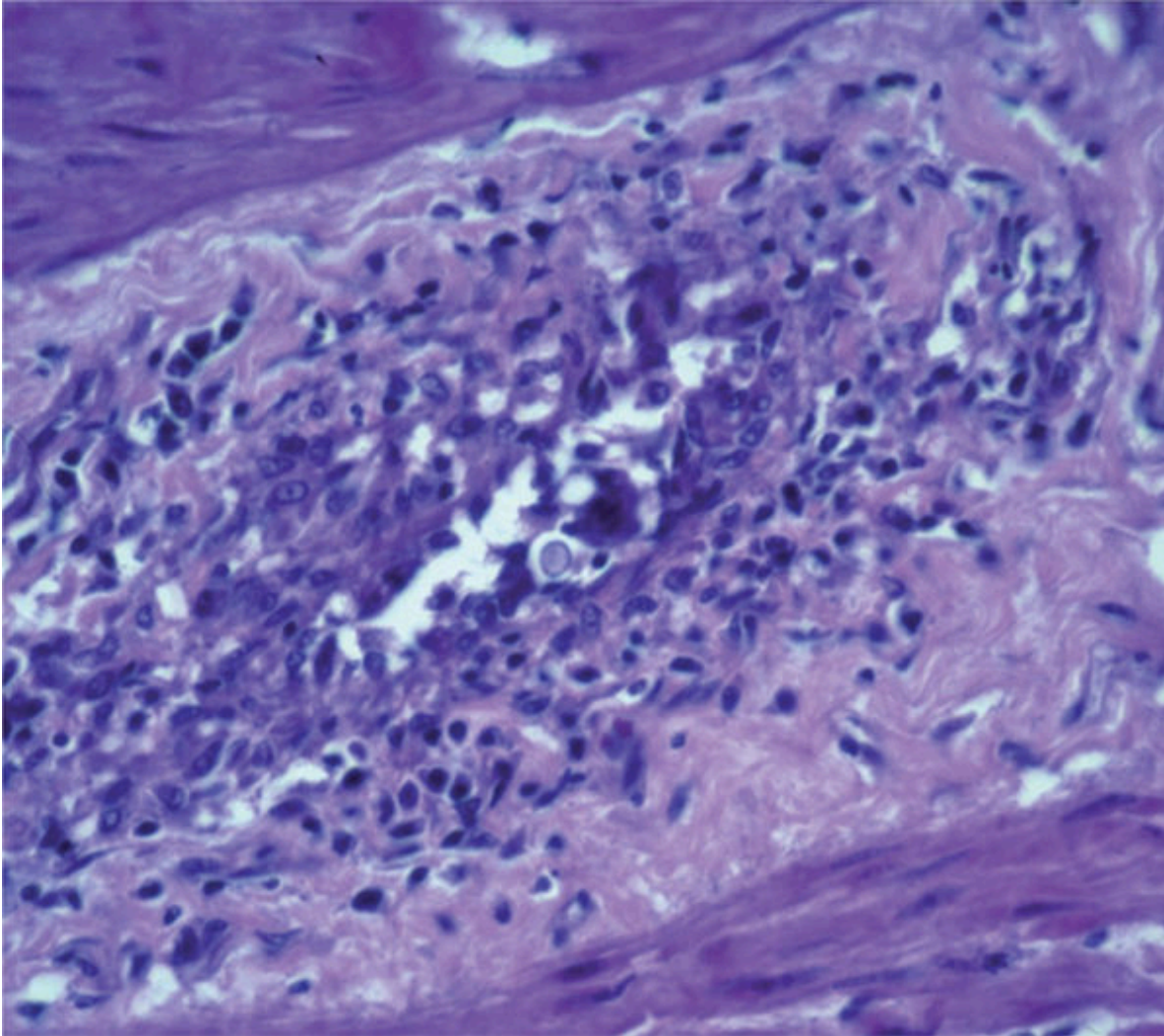


Figure 4. Histological section of an intestinal granuloma in a case of cryptococcosis.

Treatment

The treatment of cryptococcosis is protracted (over six months), complicated, and expensive. Excision of granulomas from the nasal cavity or lymph nodes upon commencing treatment may be beneficial. Treatment can be unsuccessful due to recurrences or the persistence of infection. Resolution rates for treated dogs range from 20 % to 55 %.

Sensitivity to antifungals such as fluconazole, posaconazole, voriconazole, flucytosine, and itraconazole appears to vary depending on the molecular

variation of *C. neoformans* and *C. gattii* species:

- **Flucytosine:** administration with another antifungal is recommended. Crosses the blood brain barrier, but side effects such as skin reactions, gastrointestinal signs, and myelosuppression are common. Is administered orally at doses of 50 mg/kg to 75 mg/kg every 8 hours for several months (up to one year).
- **Amphotericin B:** considered the most effective antifungal for the treatment of cryptococcosis. Has difficulty crossing the blood-brain barrier, and is thus usually combined with flucytosine. Is administered intravenously over 2 to 6 hours at a dose of 0.5 mg/kg diluted in 300 to 1,000 ml of 5 % dextrose, 3 times per week, or subcutaneously at doses of 0.5 mg/kg to 0.8 mg/kg, diluted in 500 ml (body weight <20kg) or 1000 ml (body weight >20 kg) isotonic glucosamine solution, 2 or 3 times a week. Liposomal amphotericin B is less nephrotoxic and is administered intravenously at a dose of 2 mg/kg to 3 mg/kg, 3 times per week.
- **Itraconazole:** more effective than ketoconazole and has fewer adverse effects. Has limited ability to cross the blood-brain barrier. Administered orally at doses of 10 mg/kg every 24 hours for several months (up to over a year).
- **Ketoconazole:** tends to be prescribed for economic reasons, but is not recommended. Is prescribed orally at doses of 10 mg/kg every 12 to 24 hours for several months (up to over a year).
- **Voriconazole:** effective, and crosses the blood-brain barrier. There is very little data on this drug. The proposed dose is 4 mg/kg to 5 mg/kg PO every 12 hours.
- **Posaconazole:** effective, and crosses the blood-brain barrier. Should be administered with food. A single dose (PO, with food) of 5 mg/kg to 10 mg/kg every 12 to 24 hours is suggested.
- **Terbinafine:** effective treatment of one case of abdominal cryptococcosis following oral administration at doses of 30 mg/kg once daily, indefinitely, has been described.

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Appendix

Recommendations and consensus for vaccination of dogs

Recommendations and consensus for vaccination of dogs

Vaccination guidelines or recommendations outline three regimens for commercially available vaccines:

- Recommended vaccinations (Table 1).
- Optional vaccinations (Table 2).
- Non-recommended vaccinations

The presence of endemic diseases can result in a usually optional vaccine being recommended for animals in a particular geographical area. Moreover, in certain situations vaccination is legally required by the health authorities of certain countries, or for the transportation of animals.

During the first weeks of life, puppies depend on passive immunity acquired from the mother. After weaning, between 8 and 12 weeks of age, animals can be actively immunised by vaccination. Accordingly, it is usually recommended to begin vaccine protocols during the eighth or ninth week of life, and to revaccinate 3 or 4 weeks later. A third vaccination may even be recommended at 14 to 16 weeks of age.

In all cases, regardless of whether the puppy has received 2 or 3 revaccination shots, a booster shot should be administered around one year after the last vaccination.

In the case of adult dogs, if vaccination has been correctly performed using live modified vaccines, revaccination can be performed at intervals of three or more years, although certain legal restrictions may apply. It may also be necessary to revaccinate more frequently against specific diseases. In practice, animals are examined annually and the appropriate recommended or optional vaccines administered each year.

Serological tests can be performed to monitor a dog's immunity before proceeding with revaccination, at least in the case of distemper virus (CDV),

canine parvovirus (CPV-2), canine adenovirus type 2 (CAV-2), and rabies. A serological test for rabies virus antibodies is required prior to transporting dogs to certain countries.

Table 1. Vaccines recommended by the WSAVA (World Small Animal Veterinary Association), 2015 (parenteral administration).

Vaccine	Primary vaccination (<16 weeks)	Primary vaccination (>16 weeks)	Revaccination
» CPV-2 (MLV) » CDV (MLV) » CDV (r) » CAV-2 (MLV)	At 6–8 weeks of age; revaccination every 2–4 weeks up to 16 weeks.	Two doses 3–4 weeks apart or a single dose with r or MLV.	At 6–12 months of age, and subsequently every 3 years.
Rabies (IV)	Single dose from 12 weeks of age. Second dose 12 weeks later if animal has been previously vaccinated.	Single dose.	Revaccinate every 1–3 years, depending on relevant regulations.

CPV-2, canine parvovirus; CDV, distemper virus; CAV-2, canine adenovirus type 2; MLV, modified live virus; r, recombinant virus vaccine; IV, inactivated virus.

Table 2. Optional vaccines according to the WSAVA (World Small Animal Veterinary Association), 2015.

Vaccine	Primary vaccination (<16 weeks)	Primary vaccination (>16 weeks)	Revaccination
Parenteral CPiV (MLV)	At 6–8 weeks of age; revaccination every 2–4 weeks up to 14 weeks of age.	Two doses 3–4 weeks apart or a single dose.	About 1 year of age, and again every 3 years
Intranasal CPiV (MLV)	Single dose at 3 weeks of age.	Single dose.	Annual.
Intranasal <i>Bordetella bronchiseptica</i> (attenuated vaccine)	Single dose at 3 weeks of age.	Single dose.	Annual.
Parenteral <i>Bordetella bronchiseptica</i> (inactivated vaccine)	At 6–8 weeks of age; second dose at 10–12 weeks of age.	Two doses 2–4 weeks apart.	Annual.
<i>Leptospira</i> spp. (inactivated vaccine)	At 8 weeks of age; second dose 2–4 weeks later.	Two doses 2–4 weeks apart.	Annual.
Parenteral CIV (IV)	>6 weeks of age; second dose 2–4 weeks later.	Two doses 2–4 weeks apart.	Annual.
Parenteral <i>Borrelia burgdorferi</i> (inactivated or recombinant vaccine)	At 12 weeks of age; second dose 2–4 weeks later.	Two doses 2–4 weeks apart.	Annual.
<i>L. infantum</i> excreted and secreted proteins (LiESP)	Not applicable.	Seronegative dogs can be vaccinated from 6 months of age. Quantitative serological tests are recommended. Administration of 3 doses at 3-week intervals.	Annual.

CPiV, canine parainfluenza virus; CIV, canine influenza virus; MLV, modified live virus; IV, inactivated virus.